EDITORIAL

LIVER BIOPSY – IS STILL AN OPTION?

Why this question? Maybe, because many papers which have appeared in hepatological literature in recent times have assessed different modalities for the evaluation of liver diseases. Here we refer to diffuse illnesses starting with the "classical" liver biopsy and progressing to non-invasive tests, such as biological tests, where blood results are used together for evaluation, or elastographic methods such as ultrasound based techniques.

These present times can be confusing for many people as we are in a transitional period. There are still many physicians that believe that the liver biopsy is the only method that can give a correct answer concerning the severity of a hepatic disease. However, a cohort of researchers have found that biological tests or elastographic methods can replace the liver biopsy, offering the same accuracy.

Let's take a look at this controversy. Liver biopsy was the first method for evaluation in the hepatological field and for more than 50 years was the gold standard. The severity of a disease was diagnosed by this method and also the prognosis and decision of the treatment was decided. This was the standard of care in these times and possibly the patient was not always the first consideration! Criticism of this method was the invasivity, the pain for the patient and sometime complications with rare fatalities. However, is it not normal to have some fatalities when you are conducting an initial research, not just trying to cure? Secondly, we must examine the performance of a liver biopsy (or performance in the "intent to diagnose"): how often do we have a very correct morphologic diagnosis in which we can trust? For a long time, this was not a real preoccupation of the physician, but when the competition of non-invasive methods appeared, the performance began to count. And here the statistical results are not very good. From the past we know that a liver biopsy can miss liver cirrhosis in approx. 20% of cases. More recently, in large cohort of patients, we found that an optimal morphological fragment is obtained in percutaneous liver biopsy in only approx. 40% of cases. Other methods of histologic evaluation (tranjugular liver biopsy) offered less hepatic tissue while additional factors such as interobserver variability between pathologists are well known. Some pathologists demonstrated that short liver specimens can underestimate the severity of the lesions, finally resulting in an incorrect histological appreciation. As a result of this and on account of the invasivity of the liver biopsy, researchers started to search for non-invasive modalities for liver evaluation, in patients with diffuse liver diseases.

Advantages: first for patient! No pain, no stress before this evaluation, repetitivity, not very high cost! The development of these methods was made first in Europe and France was the leader in this field. They developed biological tests, well known, like FibroTest-ActiTest (for activity and fibrosis evaluation) and later FibroMax (activity, fibrosis, steatosis, nonalcoholic and alcoholic steatohepatitis evaluation). In the field of ultrasound based elastography, FibroScan (Transient Elastography) was developed in France too. Many publications concerning the non-invasive evaluation of liver in comparison with liver

biopsy came from France (Poynard, Castera and others). From France came the first recommendation in the guidelines for the use of non-invasive modalities for liver evaluation as an alternative to liver biopsy. Later, the EASL (European Association for the Study of the Liver) Guideline proposed non-invasive modalities like an alternative to liver biopsy. During time, non-invasive modalities for liver evaluation were spread in other European countries (but, not only European). Others biological test were developed (like ELF, Hepascore, Lok, others) and other ultrasound based elastographic methods appeared and started to be evaluated (in comparison with liver biopsy and in the last time with Transient Elastography), like ARFI (Acoustic Radiation Force Impulse Elastography) or 2D-SWE (2D-Shear Wave Elastography, SuperSonic Imagine).

These non-invasive modalities for liver evaluation have spread everywhere in the world, from Japan, China and Korea to Canada and Brazil. Increasing number of patients are evaluated with these methods now, decreasing in the real world, the number of liver biopsies. Only in the USA there is still a delay for this introduction, maybe in connection with the severity of FDA regulations for the approval of such methods.

In this moment, many meta-analyses or large cohorts of patients have demonstrated very good results of these non-invasive methods for the evaluation of fibrosis (elastographic methods) or inflammation, steatosis and fibrosis (in biological tests such as FibroMax). These methods are good for the "intention to diagnose" a case, the failure rate being quite low and can be repeated as often as is necessary while being excellent for establishing the diagnosis of moderate or severe fibrosis (accuracy more then 85-90%). On the other hand, in some diseases, such as C chronic hepatitis, we have now very potent drugs, than can cure the vast majority of patients, irrespective of the degree of fibrosis. If we consider prioritising these patients, non-invasive methods are quite good.

Therefore, in the future, everywhere we shall see a decreasing number of liver biopsies (including in more conservative countries such as the USA). Probably, in a percentage of patients, the liver biopsy is still necessary (maybe in autoimmune hepatitis, colestatic diseases, B chronic hepatitis or others), or maybe for some academic studies.

For focal liver lesions (which is not the topic of our discussion), probably we shall see an increasing need for biopsy (echoguided), regarding the molecular medicine perspective or personalized treatment for every patient.

Finally to answer the question in the title: liver biopsy - is it still an option? Yes, is a good option, but in many cases it can be now replaced by non-invasive modalities of liver evaluation. Biological tests or elastographic methods or a combination of these two modalities (this combination can increase confidence) will replace many liver biopsies. The comfort and the safety for patients, their reasonable price and the excellent accuracy of these methods are the main arguments in the future for this new direction of evolution, from an invasive procedure (liver biopsy) to the non-invasive modalities of evaluation.

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IN MEMORIAM

PROFESSOR GEORGETA DATCU, M.D., Ph.D.

(27 August 1939-10 August 2015)

The academic community of "Grigore T. Popa" University of Medicine and Pharmacy Iasi expressed by the voice of 1962 promotion its deep regret for unexpected loss of our distinguee colleague Professor Doctor Georgeta Datcu, teacher and head of the Ist Medical Clinic for more than four decades.

Originating in Bacau in a family of intellectuals, his father dr. Demostene Teja being a renowned radiologist and her mother being pharmacist, she naturally oriented toward medical career. High school graduating with honors she benefited a merit bachelor being admitted without examination and between 1956-1962 pursued the General Medical faculty courses distinguishing herself for intellectual qualities and passion of perfection in medical profession. As colleague and chief of our group she proved modesty and diplomacy, never wanting social roles, her politics being only school performance.

Due to its endowment but also to the great generation of masters we have benefited: I Iancu, I. Nițulescu, Gh. Chipail, I Enescu, Vl. Butureanu, Maria and O. Franke, Th. Economu i.e., she graduated with one of the highest accolades.

She became by competition apprentice (1960) and *house officer* (1962) carrying out her training in prestigious clinics mainly in internal medicine services.

In 1962 she sustained the licence thesis intitled *"Pseudartrosis of the rachisyntesis graft in Pott's disease in children"* conducted in Pediatric Surgery Clinic and appreciated with maximum qualifier.

In the Ist Medical Clinic she was spotted and guided by Professor Constantin Negoiță, known for the rigor of its staff selection who was her mentor and role model for entire career, dr. Georgeta Teja being the first in a series of valuable graduates, four of which becoming later heads of clinics. Therefore in 1965 she became jr assistant of this clinic where will activate for 44 years being successively promoted assistant (1968), lecturer (1983), associate professor (1990) and professor (1994). She became also registrar (1968) and consultant in internal medicine (1978) and cardiology (1983).

She led the clinic for 15 years until her retirement with professionalism, strictness and balance maintaining its excellence and reputation.

As practician outside her encyclopedic knowledge and holistic approach of the suffering, Professor Datcu had particular clinical feel destined only to "true doctors" always surprising by the finesse and accuracy of diagnosis and optimal therapeutic solution

Although her priority was competence in cardiology, like her illustrious predecessors Professors Bacaloglu, Niculescu, Tudoranu and Negoiță, Professor Datcu

demonstrated broad knowledge in other specialties of internal medicine.

She gave equal importance to teaching activity through clinical internships and demonstrations filled of attainments, dedication and patience stored in the memory of students but also of young colleagues, residents or physicians in training, all of them decisive benefiting in their education and perfection.

Her lectures have excelled by sober style without pedantry, logic and systematized, oriented to practice, all dressed in an elegant, worship language, always appropriate to the audience. Like her mentor, Professor Datcu had an impressing scientific activity not by the number but by the value of its works. Most of publications realized together with Professor Negoiță and collaborators from the clinic include clinical trials or casuistry published in specialty literature. As a tragic coincidence our last common work treating cardiothyreosis was printed quite in her dying day.

In 1984 she sustained her doctoral thesis having as theme the *"Study of capacitant vessels in systemic arterial hypertension"* headed by Professor Negoiță completing an original research spread over several years enjoying unanimous appreciations. In 1996 Professor Datcu became Ph.D. superviser in internal medicine and cardiology directing with her proverbial competence and exigency a number of theses.

Its main medical writings still remain chapters included in the treaty of Medical Clinic under the editorship of Professor Negoiță in 1995 (bronchiectasis, bronchial asthma, bronchopulmonary carcinoma) and those under editorship of her colleague and husband Professor Mihai Dan Datcu *"Clinical confrontations - commented cases"* 2003 and *"Controversies and diagnostic errors in cardiology"* 2009, all of great use and practical interest for students, residents but also specialized doctors.

She also participated actively and consistently with papers, reports or in quality of chairman at numerous scientific meetings with topics of internal medicine and cardiology, either autochthonous - some organized in Iasi – either abroad, established itself as one of the most prominent contemporary personalities of specialty Colleague and friend for almost sixty years I could appreciate the intelligence and culture not only professional of Professor Georgeta Datcu, her great character full of frankness, honesty, loyalty and altruism, all wrapped in a mantle of humor and Moldavian kindness.

Her disappearance - the first female professor of internal medicine in our country - is a heavy loss both for the Romanian medical world and for collaborators, colleagues and its friends.

God rest her soul….

Dr. Mihai Radu Diaconescu,
Professor of Surgery

EDITORIAL

CELL-FREE FETAL NUCLEIC ACIDS (cffNA) – NEW NON-INVASIVE PRENATAL DIAGNOSIS

Prenatal screening and diagnosis are routinely offered in antenatal care and are considered to be important in allowing women to make informed choices about the continuation of pregnancies affected by genetic conditions. Diagnostic testing currently requires removal of a sample of fetal cells directly from the uterus for genetic analysis, using either chorionic villus sampling between 11 and 14 weeks gestation or amniocentesis after 15 weeks. However, these invasive procedures carry a risk of miscarriage of around 1% (Mujezinovic and Alfirevic, 2007).

Cell-free fetal nucleic acids (cffNA) can be detected in the maternal circulation during pregnancy, potentially offering an excellent method for early non-invasive prenatal diagnosis (NIPD) of the genetic status of a fetus. Using molecular techniques, fetal DNA and RNA can be detected from 5 weeks gestation and are rapidly cleared from the circulation following birth.

Intact fetal cells circulating in maternal blood present an attractive target for NIPD, particularly for the diagnosis of fetal sex and chromosomal abnormalities by simple karyotyping. Although the existence of fetal cells in maternal blood has been known for more than a century, isolation of intact fetal nucleated red blood cells for the purpose of prenatal diagnosis was not achieved until 1990 (Bianchi et al., 1990). Since then, the isolation and detection of fetal cells from maternal blood has been extensively investigated and various methods of fetal cell enrichment developed. However, results to date have been disappointing, due to the scarcity of intact fetal cells in the maternal circulation (around one cell per ml of maternal blood), low efficiency of enrichment and difficulties with chromosomal analysis associated with abnormally dense nuclei in some cells (Babochkina et al., 2005). Although certain fetal cells (specifically nucleated red blood cells) have a relatively short lifespan in maternal blood (Lurie and Mamet, 2000), other fetal cell types can persist in the maternal circulation for decades following pregnancy (Bianchi et al., 1996), potentially causing false-positive results in subsequent pregnancies. The majority of recent research has focused on cell-free fetal DNA (cffDNA) in the maternal blood, of which there is significantly more present (by a factor of almost 1000).

Fetal DNA originates from apoptotic placenta cells (trophoblasts) derived from the embryo and comprises around 3 –6% of the total cell-free DNA in maternal circulation during early and late pregnancy, respectively (Lo et al., 1998b) (the other 94 – 97% being maternal cell-free DNA). Unlike cellular DNA, circulating cffDNA consists predominantly of short DNA fragments rather than whole chromosomes, of which 80% are, 193 base-pairs in length (Chan et al., 2004). Fetal DNA can be detected from the 4th week of gestation (Illanes et al., 2007), though only reliably from 7 weeks, and the concentration increases with gestational age-from the equivalent of 16 fetal genomes per milliliter of maternal blood in

the first trimester to 80 in the third trimester (Birch et al., 2005)-with a sharp peak during the last 8 weeks of pregnancy (Birch et al., 2005). In contrast to fetal cells, cffDNA is rapidly cleared from the maternal circulation. cffDNA may be detectable for several days following termination of a pregnancy (Wataganara et al., 2004a).

Additionally, cell-free fetal mRNA (cffRNA) has also been detected in the maternal circulation in 2000 (Poon et al., 2000), derived from genes that are uniquely actively expressed in the placenta. Although the majority of work to date has focused on cffDNA, both types of cffNA, i.e. DNA and RNA, could potentially be used for the NIPD of specific genetic characteristics of the fetus.

There are a number of general problems associated with detecting cffDNA in the maternal circulation: (1) the concentration of all cell-free DNA in blood is relatively low; (2) the total amount of cell-free DNA varies between individuals; (3) cffDNA molecules are outnumbered 20:1 by maternal cell-free DNA molecules; (4) the fetus inherits half its genome from the mother.

The most common technique currently used for detection and identification of specific cffDNA sequences is polymerase chain reaction (PCR). A number of different types of PCR have been explored, of which the most popular is real-time quantitative PCR, as it combines high sensitivity with a closed detection system, thereby minimizing the risk of contamination. Higher precision of measurement may be possible through the application of digital PCR, which allows the exact number of original template DNA molecules to be counted (Fan and Quake, 2007; Lo et al., 2007a). Another sensitive technique that has recently been applied to the identification of cffDNA is mass spectrometry (Ding et al., 2004), in which the precise mass of each DNA fragment is analysed to determine the genetic sequence, and hence detect fetal-specific alleles that differ from the maternal sequence by as little as a single base.

Owing to the difficulties with isolating cffDNA from the maternal circulation, its diagnostic potential at present is limited to known differences between fetal and maternal genomes. It is important to emphasize that complete fetal genotyping is not conceivable using cffDNA in the maternal circulation and that the genetic information derived from cffDNA is entirely restricted to the specific DNA sequence (or chromosome) detected. However, given modern high-throughput technologies, such as DNA microarrays (Ge et al., 2006), thousands of different sequences could potentially be detected simultaneously if required for multiple diagnoses. There are a number of quite discrete clinical applications of cffNA analysis in prenatal screening and/or diagnosis, based on distinct and detectable differences between fetal and maternal genomes:

(1) sex determination - by detecting cffDNA sequences on the Y chromosome. NIPD of sex using cffDNA is applicable to any sex-linked disease, to date it has been published within the context of haemophilia, Duchenne muscular dystrophy, X-linked mental retardation, adrenoleukodystrophy, Alport's syndrome, X-linked severe immunodeficiency, retinitis pigmentosa, X-linked hydrocephalus, anhidrotic ectodermal dysplasia, Hunter's syndrome, Menke's syndrome and Lesch–Nyhan syndrome (Costa et al., 2002), as well as some endocrine disorders including congenital adrenal hyperplasia (Chitty et al., 2007).

(2) single gene disorders (autosomal

dominant diseases: Huntington's disease, Acondroplasia, Myotonic dystrophy) or autosomal recessive diseases (Cystic fibrosis, Hemoglobinopathy, Congenital adrenal hyperplasia (21-hydroxylase deficiency) - by detecting a paternally inherited allele in cffDNA.

(3) pregnancy-related disorders - by detecting either the presence of a working copy of the Rhesus gene or an elevation in the absolute concentration of cffDNA. Numerous studies have shown conclusively that the level of cffDNA (usually measuring Y chromosome DNA of male pregnancies) is elevated by 2 – 3-fold before the onset of preeclampsia and 2 – 14-fold during preeclampsia (Hahn et al., 2005). A number of other pregnancy-related disorders have been linked to an elevated concentration of cffDNA: preterm labor, *hyperemesis gravidarum*, invasive placentation, intrauterine growth restriction, feto-maternal hemorrhage and polyhydramnios.

(4) aneuploidy - by detecting an abnormal concentration of a particular chromosome, potentially using cffRNA specific to the fetus and chromosome of interest. For trisomy 21 and other common aneuploidies, we found that cfDNA testing had a higher sensitivity and specificity in a general prenatal screening population. The false positive rate of cfDNA testing was nearly 100 times lower than that of standard screening.

However, maternal serum and nuchal translucency screening can identify risk for a broad array of abnormalities that are not detectable on cfDNA testing.

Although these data support the use of cfDNA testing in women regardless of age or risk status, further cost utility studies are warranted. As emphasized by professional societies, the use of cfDNA testing and other genetic tests requires an explanation of the limitations and benefits of prenatal test choices to the patient.

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EDITORIAL

THERAGNOSTICS - A SILENT REVOLUTION

Medicine has evolved over time based on two distinct components: new concepts on how to integrate and interpret the various data available and the continuous technological evolutions that provided more precise data concerning patient's status. While this second part is constantly viewed and publicized in the media as driving effectiveness of health care this is in fact not entirely the case and major changes in the modalities to view the disease concept have driven major changes.

Medicine started as an art of healing based on philosophical consideration about health status and the possible modalities to reverse disease status to health. Hippocratic medicine has introduced the direct and systematic observation of clinical signs and the theory of the equilibrium of humors and marked a rupture with the previous concepts of the divine nature of disease. Hippocrates had a deterministic view of the health and disease status and that both could be influenced by concrete actions of man. The following 2,000 years were marked by a better understanding of basic principles of anatomy and physiology in parallel of the development of the techniques of macroscopically and microscopically examinations but without major developments in terms of therapeutic options. The next step in our understanding of illness was the development of the anatomo-clinical method proposed by Giovanni Morgagni and developed by Xavier Bichat, René Laennec and Jean-Martin Charcot. For them every disease and clinical sign had an anatomo-pathological correspondent and therefore it was sufficient to carefully know these correlations in order to proper diagnose a disease. This approach led to the identification of most of the major nosologic groups known today. In parallel, the introduction of Claude Bernard principles of scientific experimentation resulted in major understanding of the disease process and massive advancement in term of available efficient therapeutic interventions. However this approach resulted in an archetypal understanding of disease and each patient was fitted in a global model of the pathological process without clear possibilities to explain differences in presentation, evolution and response to treatment between patients. Progressively, the understanding of individual variability due to a better understanding of human genetics and its interactions with environmental variables based on the advances in molecular biology and epidemiology set the foundation for *personalized medicine*, the next evolutive step in medical science.

Until then a relatively homogenous group of patients as defined by various clinical, biochemical and pathological features will receive the "standard" best treatment available and afterwards interventions were adapted based on response to treatment and toxic effects of therapy. This result in three groups of patients: those who benefit from therapy and improve / cure patients who do not benefit from therapy and patients with toxic effects from therapy with some possibilities of overlap between these groups. Using this approach the critical issue was to correctly name the disease process without

any possibility to predict in advance how our patient will respond, this being usually inferred from a post hoc analysis. Eventually, treatment strategy was modulated based on heuristics resulting from previous experience of the clinician.

Advances in the understanding of genetic background of disease and patients enable us now to predict to some extent which disease will respond to a particular treatment and which patients will have toxic effects from therapy. Thus major progresses were possible concerning treatment: we know expect to be able to predict the effects of therapy both in term of efficacy and toxicity thus maximizing benefit of therapy.

Still, such an approach is not without difficulties. First we need to have a good understanding of the disease process at the molecular level. This represents an important effort which should be done for every disease process we know or that is newly identified. The actual experimental approach is not sufficient for this since we need to cover most if not all disease subtypes. For this to be successful, we first need to obtain biological samples at every level (biopsies, blood and other bodily fluids) store them in large biobanks and to have access to a complete clinical and therapeutic profile of individual patients. The development of robust statistical methods for analysis of these *"Big Data"* collections is also a challenge since classical statistical analyses are not applicable.

Second we need to completely change our way to develop drugs. Instead of testing *"blindly"* a large number of drug in preclinical or animal models and then in clinical trials we need to create (in parallel to drug development) a companion diagnostic test than is sufficiently predictive for drug efficacy. This necessity is not only imposed by the need to maximize drug benefit but mainly for diminishing toxicity and to limit the cost of treatment. Indeed the costs of drug development are rising fast and consequently innovative drugs have very high prices despite of a limited increase of the production costs. Thus it is no more possible to administer drugs to all patients and then evaluate their efficacy because such an approach is not sustainable from the payer's perspective.

This whole new domain called *theragnostics* is rapidly imposing itself as the standard approach to disease and today most developments of drugs follow this path. Despite being promising we still need global analyses both from clinical trials and from day to day practice that prove that such an approach will both improve treatment success and keep costs at a tolerable level.

Professor Bogdan Dragoş Grigoriu, M.D., Ph.D.

EDITORIAL

PAIN MANAGEMENT: STRATEGIC GUIDELINES

„Pain does represent a major health problem all over the world. Although acute pain is more often a symptom of an injury or disease, chronic and recurrent pain is a specific health problem, being a disease itself" (1, 2).

As a counselor of the European Federation of the local chapters of the International Association for the Study of Pain (IASP) I have expressed in 2001 my support for the statement „Chronic Pain a Major Healthcare Problem, a Disease on its Own Right", launched in European Parliament, in Brussels. The text of the Statement (from which I quoted in the introduction) was conceived by the professors David Niv and Marshall Devor and was previously discussed and approved by the Council of the European chapters of IASP, only after each branch has provided the relevant informations concerning the different aspects of the study and management of pain in the respective membership countries.

If symptomatic simple, acute pain is easier to manage, the chronic pain, by its duration and intensity is responsible for the drastically limitation of life quality, affecting work, social and private life. For these reasons, pain should be considered among the top priorities of the healthcare system.

The above mentioned document shows that the approach of the adequate prevention and therapy of the chronic pain should be ruled from different perspectives, which must include: modern diagnostic techniques availability, accurate information of the patients, the right to benefit of the most modern invasive and non-invasive therapy methods, as well as a satisfactory monitoring. Like this were drawn main strategic guidelines in the management of the chronic pain.

In the following years, in some countries were conducted efforts in order to optimize pain management through training specialists, facilitating the availability of powerful analgesics or some legislation initiatives. However, notable differences did perpetuate, between southern European and northern European countries, and especially between eastern and western European countries.

Several countries did recognize chronic pain as a disease itself; unfortunately this is not the case in our country.

2001 European initiative had a positive impact all over the world, also generated a more wide and generous approach of the problem by IASP, which supported the idea that pain management is part of the human fundamental rights.

This gave birth to another initiative, which was brought alive on the occasion of XIIIth World Pain Congress in 2010, under the name of „Declaration of Montreal". The document is stating the will of more than 260 pain specialists from 80 countries; after stressing the inadequate access of patients to different therapeutical methods used in the treatment of acute and chronic pain, the lack of specialists, the barriers in what concerns the availability of opiate analgesics, was proposing a set of measures destined to implement national strategies in the field of pain (Romanian Algesiology Association was among first organizations which expressed their support for the initiative) (4).

An outcome of the Declaration of Montreal was the European Parliament Pain Symposium organized by the European chapters of IASP, in Brussels in May 2011, entitled „Societal Impact of Pain". On the occasion professor Giustinno Varasi (ex-president of the European Federation of IASP) was restating that pain should be considered a major health problem by the governments of all European countries, since patients had the right to benefit of the most adequate invasive and non-invasive therapeutic methods and a satisfactory monitoring (1, 6).

European statistics shows that about 25% of the European population has suffered articular, muscular or vertebral column pain, with duration of more than three months. This suffering is resulting in enormous social costs, which should determine political and decisional factors to adopt adequate strategies and to provide resources able to solve this difficult problem.

In our country were achieved some initiatives, such as the development of palliative care centers, in some locations; unfortunately these initiatives are drastically limited by the chronic underfinanced medical system.

2011 European Document is underlying the differences between its members, in what concerns the opportunity to take good care of their health. Significant statistical data are thus demonstrating that populations with low educational level are more often affected by pain suffering than those with a consistent educational level, especially because higher social and economic status is providing a better access to healthcare means. On the other hand, the growth of the average life duration is leading to an increase of the risk for development of chronic pain, with approximately 30-40 % in the following 20 years. Chronic pain is often associating anxiety and depression, thus being reflected in mental health of the individuals, which is further increasing the costs of the chronic pain.

We must understand that chronic pain is not only a disease itself, but it is generating problems which are involving the health system (diagnosis, hospitalization, medical prescriptions), ensuring companies (overspending), as well as the community (work incapacity, inefficiency, disabilities, low life quality).

At a review of the recent statistical data (2009-2013) regarding the powerful analgesics consumption in Europe, considered as main marker of the therapy of severe pain, we can notice embarrassing discrepancies (as we have been signaling previously) between the consumption level in the western and eastern European countries. From the table made by the author, form data provided by *Pain & Policy* studies Group and published in *Pain Europe* review, in which is presented the total morphine equivalent (in mg/capita), we see values which are confirming the above observation (3, 5).

Country	**Total Morphine Equivalence mg/capita**
Germany	395
Norway	274,12
Great Britain	253
France	210
European mean[x]	135
Bulgaria	68,8
Ukraine[x]	11,9
Moldavia[x]	8,75
Russia[x]	2,04

[x] In some countries the reported data are incomplete

From the table, data regarding Romania are missing, since they were not reported by our country to the international charged authority, and the efforts of the author to

obtain those data from the Health Department or from National Health Insurances House lead to inconclusive results.

Otherwise, from the discussions with colleagues, specialists in the field from eastern and southern European countries emerged the idea of a persistent „catastrophic approach", regarding morphine and derivatives prescription. Even in the cases with a more permissive legislation (Romanian Law No.339/2005), ***physicians do fear to prescribe, pharmacists to stock and patients to accept a therapy with morphine or other potent opiate substances.***

On the occasion of „World Medicine Park", which took place at the beginning of may 2014 in Minorca island (Spain), was stressed the importance of improvement of patients perceptions in what concerns the opiate analgesics, since it was estimated that each year, more than 5.5 million patients with terminal cancer do not receive the adequate therapy for their suffering (7).

Taking into consideration all the above elements and trying to adapt them to specific conditions of our country, we can draw the following main strategically guidelines:

- ✓ the need to organize medical centers and clinics exclusively destined for the management of the chronic pain as a disease itself;
- ✓ placement of the diagnosis, and when is possible of the therapy of the chronic pain at the primary level of the medical care, which is that of family medicine physicians;
- ✓ the need for continuous education on the modern means of pain diagnosis and therapy, in view of preventing the evolution of such cases toward the estate of „chronic patients";
- ✓ actual and potential patients should be thoroughly informed to know how to avoid those conditions which could generate or enhance their pain problem at workplace;
- ✓ we should identify significant financial resources, (not from the Health Department, only), for continuous translational researches in the field of the pain therapy and for the discovery of less expensive and more efficient new analgesics.

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Professor Dr. Ostin C. Mungiu

INFECTIOUS COMPLICATIONS IN PATIENTS WITH LIVER CIRRHOSIS

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INFECTIOUS COMPLICATIONS IN PATIENTS WITH LIVER CIRRHOSIS (Abstract): Liver cirrhosis is the end stage of any chronic liver disease. Complications occurring in patients with liver cirrhosis may be specific to this pathology and to gastroenterology (upper gastrointestinal bleeding, hepatic encephalopathy) or may interfere with other specialties (hepatorenal syndrome, spontaneous bacterial peritonitis, and other localized infectious complications). Over the past few decades, major efforts have been made to increase survival in patients with cirrhosis, but unfortunately, few therapeutic methods have been proven effective. Bacterial infections are frequent and serious complications of liver cirrhosis, resulting in high morbidity and mortality, especially in hospitalized patients, despite significant progress in health care for those with advanced liver disease. **Keywords:** LIVER CIRRHOSIS, INFECTIOUS COMPLICATIONS, BACTERIAL, MORTALITY.

EPIDEMIOLOGY

The estimated incidence of infectious complications in hospitalized patients without liver diseases is 5-7%, significantly lower than in cirrhotic patients (30-40%) (1).

Intrahospital mortality in cirrhotics with infectious complications is approximately 15%, significantly higher than in those without infectious pathology, while mortality from infectious complications is 20 times higher in patients with cirrhosis than in general population (2).

The most frequent infectious complications in cirrhotics are spontaneous bacterial peritonitis (25%), infections of the urinary tract (20%), pneumonia (15%), bacteremia (usually after therapeutic procedures - 12%), and cellulitis (1.2%) (1, 3).

Diagnosis and initiation of therapy are often delayed in infectious complications of cirrhosis due to negative bacteriology findings (culture and antibiogram). Blood cultures and cultures from ascitic fluid are positive in only 50-70% of cases (1).

Bacteria responsible for infectious complications are mostly Gram negative bacilli, especially *Escherichia coli*, accounting for almost 60% of infectious complications; Gram positive bacilli account for 30-35% of infectious complications, while bacterial association is confirmed in 5-10% of cases. Together with *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecali* and *Streptococcus pneumoniae* are the bacterial agents that cause infectious complications in patients

with liver cirrhosis.

Among infections occurring as complications during hospital stay, over 60% are caused by Gram positive cocci (4).

PATHOGENESIS

Patients with liver cirrhosis are exposed to infectious complications, but the mechanisms of an increased susceptibility to infection in these patients have not been fully established yet. Several mechanisms and favoring factors are suggested: qualitative dysfunctions of the reticuloendothelial system, increased intestinal permeability, presence of systemic shunts, systemic inflammatory response syndrome (5).

Immunodeficiency

Liver cirrhosis is characterized by a dysimmunitary status of multifactorial etiology, in which occur: decrease in the bactericidal activity of phagocytes, lower opsonic activity, low complement and protein C levels, alteration of the reticuloendothelial system, the main defensive system against hematogenously acquired infections (alteration of Kupffer liver cells activity and the existence of porto-systemic shunts result in deficient purge of systemic bacteria and endotoxins) (6).

Bacterial translocation is an important factor in the pathogenesis of infectious complications in cirrhotic patients; this mechanism is one of the key elements in the pathogenesis of spontaneous bacterial peritonitis and spontaneous bacteremia in liver cirrhosis. In conditions of sympathetic hyperactivity, in cirrhosis the reduction of intestinal motility favors stasis and bacterial overgrowth (7). In its turn, portal hypertension causes edema of submucosa and a change in intestinal permeability, thus favoring the translocation process (8).

Systemic inflammatory response syndrome (SIRS)

Sepsis is the systemic inflammatory response syndrome to infection. Other causes, such as acute pancreatitis, burns, and traumatisms, may also cause the same *systemic inflammatory response syndrome (SIRS),* but without infection as an etiological agent. The exact definitions of these syndromes cannot always be applied as such in liver cirrhosis, as patients may have leucopenia due to hypersplenism, increased basal heart rate (HR) due to hyperdynamic syndrome, hyperventilation caused by encephalopathy, or absence of fever syndrome.

Patients with liver cirrhosis present numerous factors that favor the occurrence of infectious complications, mostly with progression to septic shock, multiple organ failure (MSOF) and, eventually, death (9). One of the pathogenic mechanisms of SIRS is the abnormal, exaggerated response of cytokines (TNF-α, IL-6, IL-1). Endotoxins stimulate the production and release of nitric oxide (NO), a vasodilator agent whose growth in a cirrhotic patient (by definition with vasodilator status) has disastrous effects. At the same time, nitric oxide metabolites increase cellular necrosis and platelet aggregability. Proinflammatory aggression factors are numerous, while the level of "protective" anti-inflammatory factors, such as protein C, is low in liver cirrhosis (3).

Impact of infectious complications on the progression of cirrhosis

The association of an infectious complication, sometimes common in other patients, may increase mortality 4 times in patients with liver cirrhosis in comparison with cirrhotic patients without bacterial/fungal infections (10, 11). A retrospective study of more than 11,000 patients

with cirrhosis suggests that there are some predictive factors for death in patients with infectious complications such as: severe liver failure, shock/multiple organ failure, digestive hemorrhage, hepatic encephalopathy, hepatocellular cancer, nosocomial infections (10). Acute renal insufficiency (hepatorenal syndrome, as a particular form of functional renal insufficiency) occurs in 27-34% of patients with decompensated liver cirrhosis, severe liver failure (12, 13); an infectious complication associated to hepatorenal syndrome (preexistent or precipitated by a septic status) increases the mortality rate to 40-50% in liver cirrhosis (10, 12).

Statistical data show an intrahospital mortality of 20-50% for cirrhotic patients with bacterial superinfections, twice higher than in cirrhotics without infections; infections are directly responsible for 30-50% of deaths in liver cirrhosis (14).

Bacterial infections in patients with cirrhosis can be asymptomatic or paucisymptomatic, reason why they must be suspected in any cirrhotic patient with sudden unaccountable deterioration of liver function. Any suspicion of infectious complication requires close monitoring of vital functions: temperature, heart rate, respiratory rate, arterial pressure, physical exam of thorax and abdomen, presence of cutaneous lesions. Mandatory investigations in these cases include hemoleukogram, blood cultures, urine test and urine culture, thorax X-ray, sputum cultures, ascitic fluid/pleural fluid cultures, and abdominal ultrasound.

Spontaneous bacterial peritonitis

Spontaneous bacterial peritonitis (SBP) is the infection of ascitic fluid without organ perforation or abdominal inflammations such as abscesses, acute colecystitis, acute pancreatitis etc. SBP is a frequent and severe complication of cirrhosis, with an incidence of 7-25% in hospitalized cirrhotic patients.

CLINICAL FEATURES

Clinical symptoms are extremely variable in asymptomatic patients (in 30% of cases) progressing to severe deterioration of general status, with the occurrence of hepatoportal encephalopathy, hepatorenal syndrome or death from toxicoseptic shock without obvious cause (1).

The diagnosis of SBP is made by diagnostic paracentesis. This must be performed upon any clinical suspicion of ascitic fluid infection (refractory ascites, abdominal pain, fever etc.) in all admitted patients after the first episode of liver decompensation.

The main SBP diagnostic criteria are based on ascitic fluid microscopy findings (*polymorphonuclear neutrophils* (PMN) count – cut-off value: 250/ml) and *microbial cultures*. Ascitic fluid examination should reveal PMN>250/mm^3 and positive cultures (Gram negative bacilli in 60-80% of cases: *Echerichia coli, Klebsiella Pneumoniae*) (6).

There are two forms of ascitic fluid infection which do not meet all SBP diagnostic criteria, but must be treated similarly:
- neutrocytic ascites with negative culture
- monomicrobial non neutrocytic bacter ascites

Neutrocytic ascites with negative culture – the diagnostic criteria are similar to SBP, but cultures are negative. It is compulsory to rule out other causes outside cirrhosis: peritoneal tuberculosis, acute pancreatitis, and peritoneal carcinomatosis.

Course and prognosis are similar to SBP and, consequently, treatment is identical.

Monomicrobial nonneutrocytic bacter ascites (without neutrophils) is characterized by positive cultures, but with less than 250 PMN/mm^3 in fluid. It may signal the contamination of the ascitic fluid, but also an early form of SBP; clinical course depends on the presence or absence of signs of systemic infection; in patients with signs of infections, prognosis and treatment are similar to SBP.

SBP TREATMENT must be initiated early, immediately after fluid collection and empirically, without waiting for culture results. According to the present standard, 2g of Cefotaxim should be administered every 8h i.v. for 5 days, which is highly effective in approximately 85% of cases, and does not require dose adjustment depending on the degree of liver or renal insufficiency. Intravenous alternatives with similar efficacy are Ceftriaxone 2g every 24h, for 5 days, and Amoxicilin associated to Clavulanic acid 1.2g every 6-8 h, for 2 days (1). In patients who do not present other complications (SDH, encephalopathy, ileus, shock) fluoroquinolons may be administered p.o. for 8 days. The antibiotic must be changed according to the result of the antibiogram.

In cases marked by the occurrence of the hepatorenal syndrome, adjuvant treatment is recommended, some studies showing that associating albumin (1.5g/Kg in the first day, followed by 1g/Kg for another 72 h) with antibiotic is more efficient than the antibiotic administered alone (15).

Administration of early and efficient treatment has allowed us to reach a resolution rate of 90% in spontaneous bacterial peritonitis, with 70-90% intrahospital survival rate.

SBP PROPHYLAXIS

Prophylaxis is recommended in patients at increased risk for asctic fluid infection: upper gastrointestinal bleeding, previous bacterial peritonitis or hypoproteic ascitis (<1g/dl) associated to severe circulatory dysfunction/insufficiency, when administration of Norfloxacin reduces SBP occurrence risk from 61% to 7% (16). In superior digestive hemorrhage, prophylaxis is of short duration and consists of either Norfloxacin p.o./naso-gastric tube for 7 days or a 3rd generation cephalosporine administered i.v. (Ceftriaxone/Cefotaxim). In this context, SBP prophylaxis reduces infectious complication rate, hemorrhage relapse rate, and consequently mortality rate (15% vs. 24%) (16).

In patients with no previous SBP episode the relapse risk is extremely high, reaching up to 70% in the first year, and requires permanent prophylaxis with Norfloxacin 400mg daily. Prophylaxis has considerable results: relapse reduction in the first year from 68% to 20%, with an even more spectacular improvement if only infections caused by Gram negative germs are present (from 60% to 3%) (14).

URINARY TRACT INFECTIONS

In terms of frequency, the infections of the urinary tract are the second most common location, after SBP, for infectious complications in patients with cirrhosis. Their incidence is higher in patients with urinary catheter and in females; also, severe liver insufficiency is often associated with this kind of infections (11).

In non-cirrhotic patients, the etiological spectrum is comparable with that of urinary

infections: Gram negative bacilli and coagulase negative staphylococci (3). The most commonly isolated microorganisms are *Escherichia coli, Klebsiella pneumoniae* and *Enterococcus sporulatus*, but the prolonged use of antibiotic prophylaxis with fluoroquinolons during the last years has determined a significant increase in infections with Gram-positive bacteria, especially in nosocomial infections. Also, an increase in the prevalence of bacterial resistance has been recorded in the case of nosocomial infections (69%) as well as in community-acquired infections (22%) (3). Standard empirical treatment in cirrhotics with urinary infection includes 3rd generation cephalosporins or amoxicillin-clavulanic acid, avoiding fluoroquinolons.

RESPIRATORY INFECTIONS

Microorganisms involved in *community-acquired pneumonia* in patients with cirrhosis are generally the same as in general population, except that in cirrhotics there has been noticed an increased rate of bacteremia, multilobar affectation, renal insufficiency, and septic shock; the course is fatal in almost 15% of cases, mortalitaty being significantly higher than in general population.

The most common causative agent of pneumonia is *Streptococcus pneumoniae*, but other bacteria, normally present in the oral cavity, especially anaerobes, such as *Haemophilus influenzae, Klebsiella pneumoniae, Mycoplasma pneumoniae* or *Legionella* are often involved. In these patients, empirical antibiotic therapy consists in the administration of macrolides (erythromycin) combined with one of the following: cefotaxim, ceftriaxone, amoxicillin-clavulanic acid or quinolons active on pneumococci (17).

Nosocomial pneumonia is caused mainly by Gram-negative bacilli and staphylococci. Patients at increased risk for aspiration pneumonia are mostly those in the ICU, with upper gastrointestinal bleeding (especially those with mechanical tamponade with Blackmore catheter), hepatic encephalopathy or orotracheal tube, situations which favor tracheobronchial aspiration, as well as orotracheal intubation, esophageal tamponade and hepatic encephalopathy. Empirical antibiotic therapy must include 3rd generation cephalosporins (cefotaxim i.v.), carba-penems or piperacillin-tazobactam plus ciprofloxacin or levofloxacin and vancomycin or linezolide (3).

Cirrhotic patients with hydrothorax may develop *spontaneous bacterial empyema* (by analogy with SBP), which occurs in the absence of a pneumonic infection or other obvious cause for empyema) (18,19). Spontaneous pleural empyema occurs in 10-20% of cirrhotic patients with hydrothorax, the main risk factor being SBP (17). The initiation of antibiotic therapy (3rd generation cephalosporins) is indicated even in the absence of bacteriological confirmation, if the pleural fluid contains over 250 PMN/mm^3 (3).

SKIN AND SOFT TISSUE INFECTIONS

Cellulitis and lymphangitis are other infections frequently occurring in patients with cirrhosis as compared to those without cirrhosis. Incriminated risk factors are thin fragile skin, edemas, poor hygiene, malnutrition, frequent hospitalizations, and invasive maneuvers. The involved germs are often *Staphylococcus aureus, Streptococcus pyogenes* and Gram-negative enteric

bacteria, while the incidence of Gram-negative bacilli, such as *E.coli, Klebsiella spp. or Pseudomonas aeruginosa,* has increased (3).

Although skin infections may seem common at a first glance, they may progress to severe cellulitis and necrotizing fasciitis, complications associated with an extremely high mortality, up to 76% (20, 21).

Antibiotic therapy is efficient in most cases with mild forms of cellulites, still with a high degree of recurrence. Antibiotic therapy must be initiated on suspicion of skin infection, as further delay will definitely result in severe forms, where therapeutic intervention is limited, and mortality high.

Standard empirical treatment includes amoxicillin plus clavulanic acid or cephalosporin and ofloxacin, with 83% efficiency in community-acquired infections, but only 50% efficiency in the nosocomial ones (20). Surgical debridement of necrotized tissue is mandatory in necrotizing fasciitis.

MENINGITIS

As with other infectious complications, meningitis has a higher incidences and severity in cirrhotic patients; mortality may reach 50-65% and even exceeds these values in elderly patients or in those with alcoholic cirrhosis (3).

While community-acquired meningitis is mostly caused by pneumococcus and meningococcus, in cirrhotics an increase in the number of meningites caused by *E. coli* and *Listeria* has been observed (3). Another characteristic of meningitis in cirrhosis is the increased risk of recurrence.

Diagnosis of meningitis in cirrhotics is often more difficult, especially in those with hepatic encephalopathy, where neuropsychic symptoms, apparently justified, may confuse and delay diagnosis. Physical examination of the cirrhotic patients with fever syndrome, cephalalgia and neuropsychic disorders must not exclude meningitis. Early diagnosis (it is estimated that in cirrhotic patients this is delayed by 4 times compared to other patients) and empirical antibiotic therapy (with antibiotics that cross the blood-brain-barrier – vancomycin combined with 3rd generation cephalosporins and ampicillin) can help reduce mortality (11).

INFECTION WITH *CLOSTRIDIUM DIFFICILE*

The incidence of *Clostridium difficile (CD)* infection has increased in the past years, while patients admitted to gastro-hepatoenterology units, especially those with liver cirrhosis, show favoring conditions for this complication (3).

What seems intriguing is that this infection occurs less frequently in patients with superior digestive hemorrhage or hepatic encephalopathy, who usually receive antibiotic treatment (an important risk factor in CD infection); this peculiarity may be accounted for by the fact that lactulose (constantly administered in cirrhotic patients with such complications) causes reduction in the production of short chain fatty acids and suppresses CD proliferation (22).

TUBERCULOSIS IN LIVER CIRRHOSIS

Bacillus infection occurs frequently in immunocompromised patients, in chronic alcoholics; by definition, cirrhosis is an important risk for the occurrence of pulmonary or extrapulmonary tuberculosis (23).

Bacillus peritonitis may often be mistaken for SBP (refractory ascites, abdominal

pain etc.), but it usually occurs in patients without significant liver insufficiency. Peritoneal biopsy is often necessary for differential diagnosis, showing multiple disseminated white-yellow nodules, inflammation predominantly with lymphocytes and granulomas.

Cirrhotics with tuberculosis usually have a satisfactory response to tuberculostatic treatment, but with more severe hepatotoxic adverse effects and significantly decompensated liver functions (10).

CONCLUSIONS

Bacterial infections represent an important cause of morbidity and mortality in patients with cirrhosis as a consequence of their immunocompromised status. All patients with liver cirrhosis must be monitored for early diagnosis of infectious complications. Over the past few years, prompt diagnosis and correct antibiotic therapy have determined a significant reduction in mortality due to bacterial infections in patients with liver cirrhosis.

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NEWS

MEASLES VACCINATION IN THE PRESENCE OR ABSENCE OF MATERNAL MEASLES ANTIBODY: IMPACT ON CHILD SURVIVAL

Measles vaccine (MV) has a greater effect on child survival when administered in early infancy, when maternal antibody may still be present. Abay et colab. reanalyzed data from 2 previously published randomized trials of a 2-dose schedule with MV given at 4–6 months and at 9 months of age. In both trials antibody levels had been measured before early measles vaccination. In trial I (1993–1995), the mortality rate was 0.0 per 1000 person-years among children vaccinated with MV in the presence of maternal antibody and 32.3 per 1000 person-years without maternal antibody. In trial II (2003–2007), the mortality rate was 4.2 per 1000 person-years among children vaccinated in presence of maternal measles antibody and 14.5 per 1000 person-years without measles antibody. Possible confounding factors did not explain the difference. In a combined analysis, children who had measles antibody detected when they received their first dose of MV at 4–6 months of age had lower mortality than children with no maternal antibody, the MRR being 0.22 (95% CI, .07–.64) between 4–6 months and 5 years. In conclusion, child mortality in low-income countries may be reduced by vaccinating against measles in the presence of maternal antibody, using a 2-dose schedule with the first dose at 4–6 months (earlier than currently recommended) and a booster dose at 9–12 months of age.(Abay P, Martins CL, Garly ML et al, Measles vaccination in the presence or absence of maternal measles antibody: impact on child survival *Clin Infect Dis*. (2014) 59 (4):484-492.

Ecaterina Enache

EXTRA INTESTINAL MANIFESTATIONS AND COMPLICATIONS IN INFLAMMATORY BOWEL DISEASE

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EXTRAINTESTINAL MANIFESTATIONS AND COMPLICATIONS IN INFLAMMATORY BOWEL DISEASE (Abstract). Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), doesn't affect only the intestinal tract, but also involve other organs such as: eyes, skin, joints, liver and biliary tracts, kidneys, lungs, vascular system. It is difficult to differentiate the true extraintestinal manifestations from secondary extraintestinal complications. The pathogenetic autoimmune mechanisms include genetic susceptibility, antigenic display of autoantigen, aberrant self-recognition and immunopathogenetic autoantibodies against organ-specific cellular antigens shared by colon and extra-colonic organs. An important role is owned by microbes due to molecular mimicry. This paper reviews the frequency, clinical presentation and therapeutic implications of extraintestinal symptoms in inflammatory bowel diseases. **Keywords:** INFLAMMATORY BOWEL DISEASE, ULCERATIVE COLITIS, CROHN'S DISEASE, EXTRAINTESTINAL MANIFESTATIONS

Ulcerative colitis (UC) and Crohn's disease (CD) are systemic disorders that often involve other organs such as joints, skin, eyes, lungs, biliary tract or urinary system . The etiology is unknown. The main characteristic is an increase of pro-inflammatory cytokines levels: IL-1,-6,-8, tumor necrosis factor (TNF)-α, a decrease of anti-inflammatory interleukins: IL-6,-10 and an increased recruitment of leukocytes to the area of inflammation.

Ulcerative colitis has a chronic intermittent course with periods of remission and relapse. Inflammation is limited to the colon mucosal layer and affects the mucosa in a retrograde and continuous way. An immune mediated mechanism determines an inappropriate immune response against intraluminal antigens affecting individuals who are genetically predisposed. According to the distribution of the inflammation at the mucosal layer, the Montreal Classification was made (tab. I).

TABLE I

Montreal classification of UC

E1	Proctitis (inflammation limited to rectum)
E2	Left-sided disease/procto-sigmoiditis (colorectum distal to splenic flexure)
E3	Extensive disease/pancolitis (proximal to splenic flexure)

Crohn's disease is a chronic relapsing IBD. It is characterised by transmural granulomatous inflammation often involving the ileoceacal part of the gut and leading to a stricturing or even fistulising disease. The ethiology is unknowm, but it was seen an inadequate response of the immune system to the gut microbiota in genetically predisposed people.

The Montreal classification divides CD in three categories according to the localization of the inflammatory process (tab. II).

TABLE II

Montreal classification of CD

Age	Localization	Course of the disease
< 16 years	ileal	Inflammatory
17-40 years	colonic	Stricturing
> 40 years	Ileo-colonic	Penetrating

Prevalence of extraintestinal manifestations (EIM) oscillates between 6-47% of IBD patients (1). In most of the cases they follow the clinical course of gut inflammation, but also may not correlate with disease activity. Some studies suggested an autoimmune reaction regarding an isoform of tropomyosin (Tropomyosin related peptide) detected in skin, joints, eye, biliary epithelium and gut (2) and a common genetic background, HLA system being one of the major genetic markers associated with IBD and EIM. There is a correlation between CD and HLA-A2,-DR1, DQW5 and between UC and HLA-DRB1*0103, B27, B58 (3, 4). Also the genetic ground can guide about the risk of extraintestinal manifestations (tab. III) (3, 5, 6).

TABLE III

HLA system and the risk of EIM

HLA- B8, DR3	primary sclerosing cholangitis
HLA- DRB1*0103 (DR103)	ocular, articular manifestations
HLA B27, B58	uveitis
HLA B27	ankylosing spondylitis
1031 C TNF-α	erythema nodosum
CARD 15, NOD2 gene	sacroiliitis

TABLE IV

Classification of EIM

Extraintestinal immune-related manifestations of IBD	• arthritis • erythema nodosum • pyoderma gangrenosum • aphtous stomatitis • iritis/uveitis	• the same pathogenic mechanism • associated with intestinal inflammatory activity
Autoimmune disorders associated to IBD	• ankylosing spondylitis • primary sclerosing cholangitis • polymyositis • primary biliary cirrhosis • alopecia areata • thyroid disease	• major susceptibility to autoimmunity • independent of IBD
Extraintestinal complications	• osteoporosis • biliary/urinary lithiasis • anemia • tromboembolic events • amyloidosis • fatty liver	• metabolic or anatomical abnormalities due to IBD • inflammatory activity • medication use • poor nutrient intake/absorption

Extraintestinal manifestations can be devided in three categories: extraintestinal immune-related manifestations of IBD, autoimmune disorders associated to IBD and extraintestinal complications according to the pathogenic mechanism and the relation between intestinal inflammatory activity and the presence of this secondary disorders (tab. IV) (7).

1. **Musculoskeletal manifestations**

Inflammatory arthropathies are the most common extra-intestinal manifestations of IBD and take part of a wider group named spondylarthropathies. Joint manifestations can precede, be synchronous or can manifest afterward IBD.

Peripheral arthropathies appear can pre-date the onset of the gut clinical signs. Their prevalence in UC is smaller (5-10%) than in CD (10-20%) (8). The main characteristic is their non-erosive, non-deforming pattern. *Type1***:** pauci-articular (less than five joints) is defined by migratory non-deforming and non-erosive large-joint arthropathy predominantly of the lower limbs. It is associated with HLA B27, -B35, HLA-DRB1-0103 in up to 65% of the patients. The acute episode is self limiting (mean duration is 5 weeks) and runs in parallel with the IBD. *Type 2***:** polyarticular (more than five joins affected) characterised by symmetrical persistent small joint poly-arthropathy. The course is independent of the underlying IBD and can last for several months (8). The genetic background is strongly correlated with HLA B44, MHC class I chain-like gene A (on chromosome 6) (4, 9).

The etiology is based on genetic predisposition and the exposure to luminal bowel contents. It has been demonstrated a dysbiosis with a decreasing quantity of bifidobacteria, lactobacilli, *Escherichia coli*, an increase of facultative flora (*Staphylococcus, Klebsiella*, *Proteus*), a modification of the colonocyte's cell receptor maturity of mucus (decreased staining intensity by lectins) and a cytokine imbalance with an increase of proinflammatory markers (IL-1,6,8, TNF α) (10).

Axial arthropaties are not correlated with disease activity of IBD. Sacroiliitis is non-progresive and is not associated with HLA B27. This form is associated with enthesitis, tenosynovitis, dactylitis even in the absence of arthritis (11). Bacteria and bowel inflammation plays a decisive role in the pathogenesis of IBD-associated ankylosing spondylitis, fact demonstrated by ileocolonoscopy in patients with idiopathic spondylarthropathies (12).

Osteoporosis is an extraintestinal complication of IBD. The main role in the ethiopathogenesis is assumed to the connection between OPG-RANKL-RANK (13). Osteoclastogenesis is induced by a surface receptor (RANK) located on osteoclasts. Its ligand (RANKL) is induced by proinflammatory cytokines. On the other hand, osteoblasts produce osteoprotegerin (OPG) which prevents ligation of RANKL to RANK, so the bone loss is stoped. It has been proved that alendronate improved spine bone density in IBD patients (14).

Osteomalacia is also a complication of IBD. It is determined by a prolonged and severe vitamin D deficiency. Often appear after multiple intestinal resections in severe cases of CD (15).

2. **Hepatobiliary manifestations**

It can or cannot be related to immunological disorders or may depend on side effects of therapy used for IBD.

Primary sclerosing cholangitis (PSC) is a chronic, slowly progressive cholestatic

disorder characterized by inflammation and fibrosis of intrahepatic / extrahepatic bile ducts. 2-7% of patients with UC develop PSC. It is a premalignant condition for cholangiocarcinoma and determine a higher risk of colonic dysplasia/carcinoma due to folate deficiency, long periods and asymptomatic colitis or changes in bile salts (16, 17). High titres of autoantibodies to neutrophils (pANCAs) were found in the serum of these patients (18) and also they have, in a proportion of 70%, HLA DR3, B8 haplotype.

Hepatobiliary complications include: cholelithiasis, more frequent in CD and caused by the malabsorption of bile salts from the inflamed terminal ileum, fatty liver due to steroid therapy or poor nutrition, liver abcesses, portal vein thrombosis, suppurative pylephlebitis.

3. **Mucocutaneous manifestations**

Mucocutaneous manifestations can be divided in three classes of injuries relaying on the ethiopathogenic mechanism (tab. V).

TABLE V

Classification of mucocutaneous manifestations

Granulomatous disorders	• perianal/peristomal ulcers and fistulas • metastatic CD • oral granulomatous ulcers
Reactive skin manifestations of IBD	• aphthous stomatitis • pyoderma gangrenosum • erythema nodosum • Sweet's syndrome
Nutritional- deficient cutaneous manifestations	• acrodermatitis enteropathica

Granulomatous lesions and IBD have the same histological pattern. Perianal / peristomal ulcers and fistulas frequently appear in patients with CD (50%) and can be external or internal (entero-cutaneous) (19). In the group of external manifestations we can find: perianal erythema, abscesses and perianal complex fistulae. Internal fistulas are more serious and require a prompt systemic medication and even surgery. Metastatic CD are represented by subcutaneous nodules or ulcers which appear especially at the lower limbs (20). Oral lesions are aphthous ulcers which appear in 10% of patients with UC and in 20-30% in those suffering from CD.

Reactive skin manifestations of IBD usually are diagnosed in parallel with intestinal disease activity. *Aphthous stomatitis* follows the colonic localization of IBD. The histological pattern show a lymphohistiocytic infiltrate located in the lower derme. The disease can be associated with arthritis and correlated with IBD acuteness.

Pyoderma gangrenosum may run an independent course of the underlying IBD activity. Commonly involves the skin with a predilection for the lower limbs, but also may occur around surgical stomata or in any area of the skin. Macroscopically it is represented by discrete pustules which coalesce and form ulcerations with central necrosis and with erythematous or violaceous edges. Histologicaly there is a deep suppurative folliculitis with diffuse neutrophilic infiltration and dermolysion. It can exist in association with other extraintestinal manifestations such as arthritis, ery-

thema nodosum (21). *Erythema nodosum* appears as raised, warm, tender erythematous nodules tipically in the pre-tibial areas. Histologicaly it is a septal panniculitis due to immune complex deposition. It was proved a genetic association between erythema nodosum and a certain HLA region of chromosome 6 (HLA B) (22). Flares occur concurrently with gut exacerbations. The disease normaly heals without ulcerations and resolve spontaneously within 3-6 weeks. *Sweet's syndrome* is a neutrophilic dermatosis which appears as painful erythematous nodules or plaques. It is often associated with fever and leukocytosis.

Nutritional - deficient cutaneous manifestations include acrodermatitis enteropathica determined by zinc deficiency. It has a clinical pattern as a psoriasis erythema.

4. **Ocular manifestations**

There are two kinds of manifestations depending on their immunopathogeny. Immune-related conditions such as: episcleritis, scleritis, uveitis, corneal disease or related to drug exposure: cataract, glaucoma (table VI). A high risk for developing these conditions is the presence of genotypes HLA B27, B58, DRB 10103. Usually are flaring and subsiding at the same time as the bowel inflammation (22, 23) (tab. VI).

TABLE VI
Classification of eye disorders

immune-related ocular manifestations	• episcleritis • scleritis • uveitis • corneal disease
ocular manifestations related to drug exposure	• cataract • glaucoma

Episcleritis is the most common extraintestinal manifestation, mainly in CD patients. It appears at the same time with active gut disease. Clinically we can find acute redness, irritation, burring, tender to palpation. The treatment refers to topical steroids and also the therapy of the underlying intestinal condition.

Uveitis appears more common in females and more often bilaterally. It may not parallel gut disease activity and sometimes can precede the diagnosis of IBD. It need a prompt therapy because can lead permanent damage to the eye.

Corneal disease includes ***conjunctivitis*** which is quite frequent.

Use of steroids can lead to ***cataract*** and the malabsorption of vitamin A can determine ***keratopathy*** or ***night blindness***.

5. **Pancreatic manifestations**

Pancreatic manifestations can take two forms: acute or chronic pancreatitis. Acute pancreatitis is induced by drugs such as salycilates, azathioprine, 6-mercaptopurine. Usually it has a mild course with a remission of the inflammatory processes after discontinuing the therapy. In 40% of CD patients were found pancreatic autoantibodies (24). Chronic pancreatitis is characterized by a decreased exocrine function due to circulating inflammatory mediators or to autoantibodies against pancreatic tissue (25, 26). Patients with IBD have modifications of intrapancreatic duct (26).

6. Thromboembolic events

Thromboembolic events have an incidence between 1.2% to 6.1%, but some necropsy studies found out that this incidence is much higher being up to 30% (27). The pathogenesis refers to hyperhomocysteinemia due to the deficiency of vitamin B_6, B_{12} (malabsorption) or folate (malabsorption, use of folate-inhibiting drugs such as methotrexate/ sulfasalazine) (28). One study found a significant higher prevalence of factor V Leiden in the group of IBD patients (29). Other events which participate at the procoagulatory status are: an increase frequency of antiphospholipid antibodies, higher levels of lipoprotein (A) (27).

7. **Anemia**

Anemia is considered to have many pathogenic ways: chronic intestinal bleeding, iron malabsorption in duodenum and upper jejunum, vitamin B12 malabsorption, folate deficiency due to malabsorption or to side effects of drugs. Also the high level of proinflammatory cytokines can make a diversion of iron traffic to reticuloendothelial system (30). A lot of drugs used for the treatment of the current gut disease can have direct myelosuppressive affect (sulfasalazine, azathioprine, 6-mercaptopurine) (30).

8.**Urinary system manifestations**

IBD patients present quite frequent minimal, subclinical, glomerular inflammatory changes. Interstitial nephritis and tubular proteinuria is often attributed to CD (31). Glomerulonephritis can lead to nephrotic syndrome and renal failure and is directly related to the activity of the bowel disease. Urinary complications include nephrolithiasis and urinary tract fistulas. Nephrolithiasis appear more frequent in CD than in UC (32). The most common are calcium-oxalate stones due to fat malabsorption that cause a lack of free calcium and increased oxalate absorption.

Renal amyloidosis appers more often in ileal CD. The ethiology is unclear, but is related to chronic inflammation and acute phase reaction proteins (33).

9. **Pulmonary manifestations**

Usually pulmonary disease parallels the bowel intestinal activity. The most common manifestation is bronchial inflammation and suppuration with or without bronchiectasis. The etiopathogeny includes: the same embryological origin of the lung and gastrointestinal system by ancestral intestine (34), similar immune systems in the pulmonary and intestinal mucosa (35), the presence of circulating immune complexes and auto-antibodies.

Half of patients with CD have ***subclinical alterations*** of the lungs without pulmonary symptoms (36). Abnormal pulmonary function tests are found in 42% of IBD patients without respiratory symptoms or radiograph findings (37). Lung inflammation correlates with bowel disease activity.

Airway diseases include upper-airway obstruction, tracheobronchitis, chronic bronchitis, granulomatous bronchiolitis, bronchiectasis, asthma and acute respiratory failure. The most frequently appear bronchiectasis (37). Bronchoscopy shows diffuse inflammation of the trachea and bronchi and the biopsy reveals metaplastic disorders in the epithelium and granulomatous infiltration.

Lung parenchymal diseases include bronchiolitis obliterans with organizing pneumonia (BOOP), unspecified interstitial lung disease, noncaseating granulomatous inflammation and fibrosis, parenchymal nodules and granulomata, alveolitis and alveolar consolidation. The lung biopsy shows acute alveolitis, granulomatous lymphocytic infiltration of the interstitium and

of the walls of small arteries with slight interstitial fibrosis.

Pleural diseases include pneumothorax, pleural thickening, pleuritis and pleural effusion (38). There is an association between pleural effusion and pericarditis (39). The pleural complications of CD may run an independent course and may be present at the time of inactive bowel disease.

Drug-related lung disease can be interpreted as complications. Sulfasalazine and mesalamine can induce interstitial disease, eosinophilic pleuritis/ pneumonia or even asymptomatic lung injury(40). After the use of azathioprine or 6-mercaptopurine has been reported interstitial pneumonitis, BOOP, chronic pneumonitis /fibrosis and pulmonary edema (41). Methotrexate can cause interstitial pneumonitis, granuloma formation and bronchiolitis (42). Biological therapy with anti TNF drugs (infliximab, adalimumab, certolizumab) is associated with the presence of opportunistic infections due to the suppression of T cell-mediated immunity or can directly affect the lung parenchyma causing acute respiratory distress syndrome, diffuse alveolar hemorrhage, nonbronchiolitis inflammatory nodular pattern of the lung and interstitial lung disease. Frequently appear or is reactivated latent tuberculosis (43).

10. **Cardiovascular manifestations**

Heart involvement is rare. It is seen more frequently in men and in those with ulcerative colitis. These manifestations are not related to the activity of the bowel disease. Cardiac involvement may present as pericardial effusion, myopericarditis and conduction defects. Pericarditis is the most common manifestation in CD.

Other patients with Crohn's disease or ulcerative colitis suffer from vasculitis. There is a relation between Takayasu's arteritis and Crohn's disease due to cross-reacting antibodies against gut mucosa and aortic tissue. Some patients developed thrombotic complications by activating the coagulation system and it manifest like atrial thrombi, embolism of the pulmonary arteries, myocardial infarction and disseminated intravascular coagulopathy. Furthermore, a few cases were reported about atrio ventricular blocks, amyloidosis of the heart, dilative cardiomyopathy and endomyocardial fibrosis in patients with chronic inflammatory bowel disease (44).

11. **Neurological manifestations**

It includes central and peripheral nervous system involvement. Peripheral neuropathy is one of the most common complications. It has also been found an inflammatory myopathy. Cranial neuropathies include the Melkersson-Rosenthal syndrome, optic neuritis, and sensorineural hearing loss (45-47). Ischemic stroke depends on few mechanisms, including large/small artery disease, paradoxical embolism, endocarditis, vasculitis. Thrombosis of the dural sinus and cerebral veins are as frequent as arterial stroke in IBD. Multiple sclerosis has been frequently associated with IBD (48). Almost 50% of IBD patients have asymptomatic white matter lesions. Other central nervous system manifestations are represented by: a slowly progressive myelopathy, epidural and subdural spinal empyema, seizures and encephalopathy (49, 50).

CONCLUSIONS

Beside the classic gastrointestinal manifestations, a large number of IBD patients present with extraintestinal manifestations which can affect every site of the body. Extraintestinal manifestations may produce greater morbidity than the underlying intes-

tinal disease and may even be the initial presenting symptoms of the IBD. Some of these disorders are more frequent associated with active colitis (joint, skin, ocular and oral manifestations), others are related to small bowel dysfunction (cholelithiasis, nephrolithiasis, and obstructive uropathy) and few are nonspecific manifestations (osteoporosis, hepatobiliary disease, and amyloidosis). The distinction between disease and treatment side effects can be extremely difficult and may sometimes be impossible. Early recognition of these extraintestinal manifestations should help guide therapy that will reduce overall morbidity in affected patients.

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ALOPECIA – A CHALLENGE FOR DERMATOLOGISTS

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ALOPECIA - A CHALLENGE FOR DERMATOLOGISTS (Abstract): Alopecia is a loss of hair in the areas where it normally grows. It has to be distinguished from atrichia, the congenital absence of hair due to the absence of hair follicles, and hipotrichosis, scarcity or absence of hair in some congenital diseases. Alopecia is either scarring, when the skin appears atrophic, scaling, and smooth and the hair follicles are absent, or nonscarring, when hair loss is not accompanied by the destruction of hair follicles. This paper is a review of all types of alopecia and their features in an attempt to make them easier to identify and differentiate.
Keywords: ALOPECIA, ANDROGENIC ALOPECIA, ALOPECIA AREATA.

Hair is an esthetic element with important psychosocial impact in both women and men, regardless of age. All hair disorders are actually hair follicle disorders.

Alopecia is a loss of hair in the areas it normally grows. It has to be distinguished from atrichia, the congenital absence of hair due to the absence of hair follicles, and hipotrichosis, scarcity or absence of hair in some congenital diseases.

Alopecia is either scarring (tab. I), when the skin appears atrophic, scaling, smooth, and the hair follicles are absent, or nonscarring, when hair loss is not accompanied by the destruction of hair follicles; nonscarring alopecia may be localized or diffuse (1, 2, 3) (tab.II).

Sometimes, alopecia and hypotrichosis together with other skin and adnexal changes are part of a congenital syndrome.

The growth cycle of the hair follicle differs depending on body area; thus, scalp hair has 3 phases of unequal duration: anagen (3-5 years), catagen (2-3 weeks) and telogen (3-4 months). Approximately 85% of hair follicles are in the growth phase (anagen) and 70-100 hairs are shed daily (telogen). Hair follicles of the eyebrows, trunk and limbs have a similar duration of anagen and telogen phases (6 months). The growth cycle of the hair follicle is influenced by a number of inhibitory and stimulatory factors.

The onset of anagen phase recapitulates the molecular events during hair follicle development, and insulin like growth factor 1 (IGF-1) and fibroblast growth factor 7 (FGF-7) have important roles in their development and cycling. The length of hair is directly proportional to the duration of

anagen cycle, and the cessation of this cycle is controlled by fibroblast growth factor 5 (FGF- 5), expressed in the hair follicle prior to the end of this phase, and also by the system of the epidermal growth factor receptors (EGF) .

The catagen phase is the phase during which the hair follicle goes through a highly controlled process of involution, reflecting the stimulation of apoptosis in the majority of follicular keratinocytes and in some follicular melanocytes. Thus, follicular melanogenesis ceases. Dermal papillae condense and move upward, coming to rest underneath the hair follicle bulge and ceasing its cycle. There is a programmed destruction of a few hair follicles by the action of an inflammatory cell infiltrate.

During the telogen phase the hair shaft matures which is eventually shed from the follicle. The percentage of hair follicles in telogen phase varies substantially according to the body region. Later, the hair follicles reenter the anagen phase and the cycle is repeated (4).

TABLE I
Classification of alopecia

Nonscarring alopecia	**Scarring alopecia**
- androgenic alopecia - alopecia areata - telogen effluvium - drug-induced alopecia - toxic alopecia - postradiation therapy alopecia - infectious disease - collagenoses - renal failure - deficiency disorders - dysthyroidism	- primary lymphocytic: • chronic discoid lupus erithematosus • lichen planopilaris • classic pseudopelade of Brocq • keratosis folicularis spinulosa decalvans • alopecia mucinosa - primitive neutrophilic: • folliculitis decalvans • dissecting cellulitis/folliculitis - primitive mixed: • keloid acne of the neck • pustular erosive dermatitis - secondary • defective development • primitive or secondary malignant neoplasms • infections • physical factors

Nonscarring alopecia

Androgenetic alopecia (AGA) is the most common form of nonscarring alopecia, accounting for 95 % of all cases of alopecia. It affects 50% of men by age 50 years, 13% of premenopausal women, and 37% of menopausal women. It is characterized by progressive, genetically determined hair loss, and occurs in most men with hairline recession at the temples and vertex, and also in some women (5). It begins in the frontotemporal region or on the vertex with a 10 time-decrease in hair diameter and hair diameter variations. In men, there is a family

history of AGA in the first or second degree relatives, while in women AGA is associated with micropolycystic ovary syndrome (MPCOS), hormonal treatments, or ovarian tumors. AGA is progressive and the rate of hair loss is unpredictable.

In female, Ludwig classification is used to differentiate the three clinical forms of AGA (6, 7):

1. diffuse thinning in midline part;
2. progressive thinning in midline part with preservation of frontal hairline;
3. oval patch of hair loss with antero-posteior long axis, surrounded by a round band of normal density hair.

In women, vortex alopecia, male-pattern baldness or diffuse alopecia are not very common (5).

TABLE II

Classification of alopecia

Temporary/reversible alopecia	**Permanent/irreversible alopecia**
- diffuse acute: • acute telogen effluvium • drug-induced alopecia • toxic alopecia • postradiation therapy alopecia • acute anemia - diffuse chronic • androgenetic alopecia • nonandrogenetic alopecia - chronic telogen effluvium - drug-induced alopecia - renal and hepatic insufficiency, dysthyroidism - circumscribed: • alopecia areata • traumatic: trichotillomania • postinfectious: syphilis • in chronic dermatites: - chronic eczema, psoriasis	- primitive limphocytic: • chronic discoid lupus eritematosus • lichen planopilaris • classsic pseudopelade of Brocq • keratosis folicularis spinulosa decalvans • alopecia mucinosa - primitive neutrophilic: • folliculitis decalvans • dissecting cellulitis/folliculitis - primitive mixed: • keloid acne of the neck • pustular erosive dermatitis - secondary • defective development • primitive or secondary malignant neoplsams • infections • physical factors

In males, it is used Hamilton-Norwood classification (8).

Laboratory investigations to be performed are: hormone testing (free testosterone, dehydroepiandrosterone sulphate, prolactin, luteinizing hormone); ovarian and adrenal gland ultrasound; serum iron and ferritin; complete blood test; trichogram: hair strands uneven in diameter; scalp biopsy; trichoscanning; phototrichogram.

In men a systemic treatment with 5-alpha-reductase inhibitors such as Finasteride 1 mg/day, 3-6 months, or Dutasteride, and in women a systemic antiandrogen treatment with Diane 35, 6-12 months alone or associated with Androcur, 6 months, spironolactone, flutamide may be

initiated.

In both cases a series of topical solutions can also be used: Minoxidil 2% or 5%, Dercos-Aminexil, Chronostim; shampoos: Anaphase, Dercos; in severe cases hair implant and camouflaging techniques are recommended.

Alopecia areata (AA) is a nonscarring inflammatory alopecia of unknown cause, with an unpredictable course, a prevalence of 0.1% among the population, and twice more common in women.

Etiological hypotheses:

- psychosomatic factors: emotional stress (9);
- infectious factors: cytomegalovirus;
- genetic factors: there is a 55% concordance rate in monozygotic twins, compared to no concordance in dizigotic twins (10);
- endocrine factors: association of myxedema, diabetes mellitus type 1;
- chemical factors: interferon alfa, ribavirin, biological agents;
- association with atopy in 10% of cases;
- AA is considered a T-lymphocyte mediated autoimmune disease.

Hair strands, path gnomonic for AA, have an "exclamation mark" appearance, 1 cm long, the distal end is darker and rough, and proximal shaft is depigmented, narrow, with small, poorly formed root. Their presence suggests active phase of the disease, being absent on old alopecia areata patches (11).

Clinical forms

AA is clinically characterized by the presence of round non-inflammatory hair loss areas that may enlarge and form bizarre patterns. Recent patches sometimes are discretely erythematous or present depression in the skin, but usually the skin is normal in appearance, but hypotonic (Jacquet sign) .

Ophiasis AA is characterized by hair loss patches that begin in the occipital region and then extend along the edge of the hairy scalp, and partially or completely wrap the scalp (fig. 1).

Fig. 1. Ophiasis alopecia areata

AA of the beard appears either independently or associated with that of the scalp.

Alopecia totalis affects the entire scalp and *alopecia universalis* covers the entire body.

Laboratory investigations to be performed are: trichogram: dystrophic hairs; hormonal and immunological tests for the detection of a possible autoimmune thyroid disease; tests for the detection of associated atopy; histopathology: atrophic hair follicle and the presence of perifollicular lymphocytic infiltrate; antibody determination: antinuclear, antithyroid, antiparietal cell, anti-endothelial.

Although there is no causal treatment, a range of therapies with beneficial effects can be used.

The goals of treatment are a cosmetically acceptable hair regrowth and a long-term

therapeutic effect without severe side effects (12,13,14). Therefore, the following can be used: immunosuppressive therapy: corticosteroids (inhibit the production of proinflammatory cytokines), cyclosporine A (specific inhibitor of CD4+ lymphocyte activation by its action on calcineurin), macrolide immunosuppressants (inhibition of calcineurin), photochemotherapy; topical immunomodulatory therapy which induces production of contact dermatitis (DNCB = dinitrochlorobenzeon, SADBE = squaric acid dibutylester, DPCP = diphenylcyclopropenone); minoxidil; dithranol(15).

Telogen effluvium (TE) is characterized by temporary diffuse hair loss as a result of shortened hair cycle and an increased number of hairs are in telogen phase. Thinning of hair on the scalp is diffuse, sometimes being predominant in the parietal area. It is noted when 20-25% of the scalp hair has falled out.

Of the causative factors we mention: iron deficiency anemia, hyper- or hypothyroidism, delivery, inadequate diet, oral contraception or its discontinuation, medications: hormones lipid-lowering agents, chemotherapy, antihypertensive drugs, psychotropic drugs, anticonvulsants, accidental exposure to toxic substances, chronic renal insufficiency, secondary syphilis (11).

Headington has described 5 fuctional types of ET:

- immediate anagen release and telogen entry: psychological stress;

- delayed anagen release: after pregnancy (during pregnancy - anagen is prolonged);

- short anagen phase: idiopathic shortening of anagen;

- immediate telogen release: minoxidil shortens the normal telogen to stimulate hair follicles to cycle into anagen;

- delayed telogen release - prolonged telogen followed by transient anagen (usually in animals, occasionally in humans).

Laboratory investigations to be performed are: trichogram, blood tests, total body iron, liver tests, kidney tests, thyroid tests, tricoscanninge.

As part of treatment, avoidance of stress and systemic exposure to toxic substances is recommended. Systemic treatment includes zinc, biotin, cysteine, essential fatty acids supplements and correction of existing hormonal imbalances. The use of topical hair loss products is also recommended (16).

Scarring alopecia

Primitive lymphocytic scarring alopecia

Chronic discoid lupus erythematosus is two times more common in women over 40 years. It is characterized by erythematous, squamous plaques with irregular extension on the scalp and pruriginous scars. In the colored people dyschromias and, more rarely, calcification at plaque level may appear. With time, these plaques may undergo malignant transformation to squamous cell carcinoma (17). As treatment, systemic and topical corticosteroids, synthetic antimalarials, acitretin, dapsone, cyclophosphamide, cyclosporine, and methotrexate may be used.

Classic pseudopelade of Brocq has a higher frequency in women aged 30-50 years. Darier and Civatte talk about this condition as about a folliculitis, while Rabut, Duperre, Leclerq support the theory of pseudopelade state according to which there can be many causes (lichen planus, lupus) inducing the same effect. Initially, there are small isolated atrophic plaques that progressively enlarge, become irregu-

lar, and have a tendency to confluence, giving them the "footprints in the snow" appearance. The plaques have polycyclic or scalloped edges. Alopecia plaque is white, smooth, clean, slightly depressed, scarring, and hairs fall out imperceptibly, without prior skin lesions (fig. 2). Disease progression is slow, and often remits spontaneously. The treatment is usually ineffective. Autografting from unaffected areas and scar reduction with tissues expansion can be performed.

Fig. 2. Classic Brocq pseudopelade

Lichen planus is an idiopathic inflammatory disease affecting the skin, hair and nails. There are 3 clinical variants of lichen that can cause alopecia: lichen planopilaris, frontal fibrosing alopecia, Graham Little syndrome.

Recent lichen planopilaris lesions are purple erythematous-squamous papules that, over time, are covered by follicular hyperkeratotic plugs that eventuate into scars. Subsequently, plugs shed and leave a white, smooth, atrophic scar. In the hair loss area the follicular orifices are absent, and at its margin the hait pull test is positive. In the presence of inflammation, the treatment implies the use of high-potency topical corticosteroids.

Hydroxychloroquine, acitretin, and cyclosporin can also be administered.

Postmenopausal frontal fibrosing alopecia is characterized by the loss of follicular orifices, occurrence of erythema and follicular hyperkeratosis located in the frontal region, and a gradual progression.

Graham Little syndrome occurs in women aged 30 to 70 years, and is characterized by scarring alopecia of the scalp and nonscarring axillary and pubic alopecia. A few months later follicular keratosis develops on the trunk and limbs. The therapeutic choice is the administration of cyclosporin (18).

Neutrophilic primitive scarring alopecia

The etiology of folliculitis decalvans of the scalp is unknown; it may occur around the onset of puberty. Typically, the bacteriological examination of pustular secretions identifies *Staphylococcus aureus*. Clinically, initially seen are follicular pustules that evolve and heal with the formation of alopecia plaque surrounded by scales and new follicular pustules. Treatment consists in antibiotic therapy to eradicate *Staphylococcal aureus*.

Dissecting cellulitis of the scalp usually occurs in black men aged 18 to 40 years. Its cause is unknown. It is characterized by the occurrence of perifolliculitis capitis with the formation of superficial and deep abscesses in the dermis that have the tendency to fistulizing and healing with extensive scarring (fig. 3). It can be associated

Fig. 3. Dissecting cellulitis of the scalp

with two other diseases that cause pilosebaceous system obstruction: hidradenitis suppurativa hydradenitis and acne conglobata. In time, it can transform into squamous cell carcinoma. Treatment with systemic retinoids, general corticotherapy, and later local cortico-therapy with antibiotics is recommended.

Mixed primitive scarring alopecia

Erosive pustular dermatosis occurs in the elderly and is of unknown etiology. Grattan conducted a study on a group of 12 patients with erosive pustular dermatosis and found the presence of some common factors: local trauma and sun exposure. It can be associated with androgenetic alopecia. Initially, a small area of the scalp becomes erythematous, covered by crust and then superficial pustules occur on the area that became erosive. Active areas coexist with areas of scarring. The literature mentions the likelihood of malignant transformation to squamous cell carcinoma. Initial treatment relies on high-potency topical corticosteroids and later on moderate-potency ones, the use of sunscreen, zinc supplements, calcipotriol.

Keloid acne the neck is characterized by chronic perifollicular inflammation, more intense in the isthmus and infundibulum, with sebaceous gland loss. In advanced stages there is total destruction of the pilosebaceous system; it heals with scarring.

Secondary scarring alopecia may occur due to:

- as a result of the action of some physical factors: radiodermatitides, mechanical trauma, postsurgery, thermal/electrical burns, traction alopecia;
- diseases characterized by marked skin fibrosis: scleroderma, lichen sclerosus, porphyria cutanea tarda, chronic graft-versus-host disease;
- granulomatoses: sarcoidosis, necrobiosis lipoidica, granulomatous infections;
- infectious causes: bacterial: folliculitis, carbuncle/boil; fungal: kerion celsi, scab, tinea capitis (very rare); viral: herpes zoster, chickenpox, HIV; protozoa: leishmaniasis; treponema: syphilis; mycobacteria: tuberculosis;
- benign tumors: cylindromas;
- primitive malignancies: basal cell carcinoma, squamous cell carcinoma, cutaneous T-cell lymphoma;
- malignancies secondary to: kidney, lung, gastrointestinal tumors, lymphomas, leukemias (4,19).

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NEWS

LOW VITAMIN C LINKED TO INTRACEREBRAL HAEMORRHAGE

A group of researchers leaded by Dr. St Vannier, from Pontchaillou University Hospital, Rennes, France, shows that vitamin C depletion was more common among intracerebral haemorrhage (ICH) cases than controls. The case-control study was performed on 135 subjects, with a mean plasma vitamin C concentration of 45,8 μmol/L and showed that the vitamin C level was significantly lower in 65 patients with ICH than in 65 controls. Also, the study found that hypertension ($p = 0,008$), alcohol consumption ($p = 0,023$), and overweight ($p = 0,038$) were the most important risk factors for ICH. The researchers presented that vitamin C deficiency may play a role in the aetiology of high blood pressure and may increase the risk for atherosclerosis and heart diseases. The study reveals this vitamin contributes to collagen biosynthesis and regulation and the deficiency of vitamin C is responsible for unstable and dysfunctional collagen which may lead to haemorrhages. The authors show that the hospitalization length was significantly shorter for patients with a normal concentration of vitamin C than for those with a lower level, and the risk for complication/related infections was higher for those with vitamin C deficiency. (Anderson P. Low Vitamin C linked to intracerebral haemorrhage. *Medscape*. February 14, 2014).

Mioara Matei

ASPECTS OF MOLECULAR MECHANISMS IN MYOCARDIAL HYPERTROPHY, PARTICULAR MORPHOLOGICAL CHANGES AND CELL BIOENERGETIC CHARACTERISTICS IN PATIENTS WITH DILATED CARDIOMYOPATHY

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ASPECTS OF MOLECULAR MECHANISMS IN MYOCARDIAL HYPERTROPHY, PARTICULAR MORPHOLOGICAL CHANGES AND CELL BIOENERGETIC CHARACTERISTICS IN PATIENTS WITH DILATED CARDIOMYOPATHY (Abstract): **Aim:** To present the particular morphological aspects of hypertrophied myocardium by determining protein expression profile, the role of creatine kinase in cardiac cell function and mitochondrial respiration using endomyocardial biopsy samples obtained at cardiac catheterization in patients with dilated cardiomyopathy (DCM). **Material and methods:** The study included 43 DCM patients (37 men and 6 women, mean age 34±8.6 years). Protein content in myocardial samples was determined by quantitative immunoblotting and electrophoresis, morphometric indices were evaluated with a JEM-100C electron microscope, and mitochondrial respiratory parameters were determined with a Clark electrode in an oxigrtaphic cell. **Results:** The study of the expression of hypertrophic protein markers showed increases in the amount of tubulin on average by 42%,desmin by 33%, and skeletal myosin beta-isoform by 20%, and of smooth muscle proteins – alpha-smooth muscle actin and SM22 on average by 1.5 times. In all DCM patients C-reactive protein (CRP) level was on average 52% and that of myosin light-chain kinase (MLCK) 70% higher. The morphometric study showed variable characteristics of cardiomyocytes in the entire patient group, particularly bizarre and oversized nuclei, determining us to divide the patients into two groups. The electrophoresis analysis of total creatine kinase fraction revealed significant alterations in the expression of creatine kinase isoenzymes. Mitochondrial respiration rate after addition of creatine was very low. **Conclusions:** We found a high content of myosin light-chain kinase and CRP in the myocardium of patients with DCM, proving their role in hypertrophy; and this can be used in the differential diagnosis of myocardial hypertrophy as a consequence of cardiomyocyte hypertrophy and a result of the fibrotic process. Energy disturbances are significantly more pronounced in patients with DCM, with a more significant cardiomyocyte hypertrophy and an obvious increase in the size of nuclei. **Keywords:** CARDIOMYOPATHY, HYPERTROPHIC CARDIOMYOCYTES, MITOCHONDRIA

In DCM, disease accompanied by dilatation of cardiac chambers and severe myocardial contractility disturbances, cardiomyocytes are subjected to a substantially increased mechanical load (1, 2) The changes in myocardial structure include a

percentage of cardiomyocyte apoptosis, impaired segmentation and coordination of myocyte distribution in the ventricular walls, interstitial fibrosis and hypertrophy of the remaining functional muscle cells (3). Lately, there have been huge efforts towards establishing the molecular mechanisms of cardiac hypertrophy. The core element of cardiomyocyte hypertrophy (CMC) is the forming of the contractile apparatus – sarcomerogenesis (4, 5). It is assumed that smooth muscle and nonmuscle proteins play the key role in the forming of presarcomere structures, expressed in CMC, especially myosin light-chain kinase (MLCK) and the alternative product of MLCK genetic locus -myosin- CRP agglutinated protein (4, 6). Experimental models showed that MLCK fermentation activity is required for myofibrillar assembly in the hypertrophic response of cardiomyocytes (CMC).

Existing data advocate that the multiple products of MLCK genetic locus are involved in the forming of contractile structures of CMC in animals. It is logical to assume that similar processes also occur in the human heart, in cardiac hypertrophy, and especially in cardiomyopathies, but alas, papers on this topic in the literature are lacking. Valuable information on the probable mechanisms of CMD evolution at sub cellular level has been acquired by using different biochemical research methods. Changes in the activities of key myocardial cell enzymes in patients with DCM, including respiratory chain enzymes, creatinine kinase system isoenzymes were detected. The decreased phosphocreatine (PCr) content, redistribution of creatine kinase isoenzymes and of myosin was demonstrated (9). However, in the literature, there are only isolated works focused on studying the role creatinine kinase isoenzymes and cellular respiration parameters in the pathogenesis and clinical course of CMD.

MATERIAL AND METHODS

The study included 43 DCM patients (37 men and 6 women, mean age 34±8.6 years). Protein content in myocardial samples was determined by quantitative immunoblotting and electrophoresis. Morphometrically were assessed: volume fraction of fibrous tissue; myocyte size, nuclear area, nucleus/sarcoplasm ratio; and nucleus irregularity coefficient. Tissue respiration rate was measured with a Clark electrode in an oxigraphic cell.

RESULTS

The study of the expression of the proteins markers of cardiac hypertrophy showed increased content of tubulin by an average of 42%, desmin by 33% and skeletal myosin beta-isoform by 20%. Expression of protein smooth muscle proteins-markers of embryonic myocytes – alpha-smooth muscle actin and SM22, was increased on average by 1.5 times. In all DCM patients CRP and MLCK levels were increased on average by 52% and 70%, respectively. Thus, in the myocardium of DCM patients a significant part of cardiomyocytes were hypertrophied and there was a partial regression to the fetal protein expression, accompanied by increased expression of CRP and MLCK. The morphometric study showed variable characteristics of cardiomyocytes (tab. I, II).

The alteration in cell characteristics and their area varies broadly in the entire patient series. A few patients presented a characteristic pattern of oversized and bizarre nuclei with markedly high levels of

membrane convolutions. The patients from which these sample were collected formed group I (n = 13), and the remaining patients group II (n = 30). Myocyte size and nuclear area in first group were significantly greater than in group II (tab. III).

TABLE I

Clinical data of the 43 patients with dilated cardiomyopathy

Patients	Sex (M/F)	Mean age (years)	NYHA Class I II III IV	Disease duration (months)
Group I (13)	12 / 1	31 ± 8,4	0 1 6 6	38 ± 21
Age (years)		19 - 46		2 - 144
Group II (30)	25 / 5	36 + 9.0	2 7 13 8	28 ± 24
Age (years)		21 - 56		3 - 120
Total (43)	37 / 6	34 ± 8.6	2 8 19 14	33 ± 22

TABLE II

Histology data from the biopsy samples collected from the 43 patients with dilated cardiomyopathy

	Group I (n=13)%(n)	Group II (n = 30) %(n)	Total (n=43) %(n)
Abnormal nuclei	100/13	0	31/13
Cardiomyocyte hypertrophy:			
mild	15/2	56/17	44/17
moderate	15/2	16/5	16/7
severe	69/9	3/1	23/10
Fibrosis:			
diffuse	38/5	63/19	56/24
focal	31/4	68/18	51/22
Endocardiac changes:			
fibrosis	69/9	80/24	79/34
elastosis	15/2	23/7	21/9
Thrombi	23/3	27/8	25/11
Smooth muscle cells	46/6	27/8	32/14

TABLE III

Morphometric indices, mitochondrial respiratory parameters and hemodynamic data in the DCM patients

Parameters	Group I	Group II
Myofibril diameter (mcm)	30±4*	23±3
Nuclear area (mcm^2)	57±5*	42±6
Nuclear shape index	2.25±0.13*	1.75±0.11
Nucleus/sarcoplasm ratio	0.045±0.004*	0.028±0.003
% - Cr-stimulated respiration	36±4*	90±12
FE VS	0.23±0.03*	0.4±0.08
IVTD VS (ml/m2)	231±59*	121±29
PTD VS (mm Hg)	23±7*	10±4

Also, the nucleus/sarcoplasm ratio and the nuclear shape index were higher in group I. Degenerative alterations of nuclei – breaking of chromatin and lysis of the nucleolema - were observed in 4 group I patients. In 5 patients in the same group, mitochondrial surface area was found to be increased by more than 50% of the myocyte surface area and dominated the myofibrilar surface area, while interstitial fibrosis was similar in both groups.

The electrophoretic analysis of total creatine kinase fraction revealed significant alterations in the expression of creatine kinase isoenzymes. The diseased myocardium had diminished average creatinine kinase content and a higher MB content compared to healthy myocardium (fig. 1).

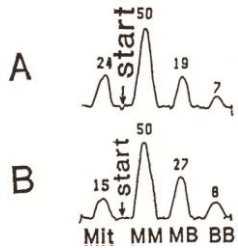

Fig. 1. Spectrum of creatine kinase isoenzyme in normal (A) and dilated (B) human myocardium

This alteration suggests that the insufficient energy transformation due to defective activity of mitochondrial creatine kinase can occur in diseased myocardium. In fact, a very low respiration rate after addition of creatine was found in group I compared to group II (tab. III). There was no significant difference between age-matched groups (tab. I); however, patients in group I had a significantly longer duration of illness and almost all belonged to NYHA classes III-IV. Important differences were noted between groups at cardiac contractile function assessment. The hearts of group I patients showed end diastolic left ventricular pressure (EDLVP) and volume index (VI) significantly higher at the same time with a decrease in ejection fraction (EF) (tab. III). Also, the contractile function parameters and morphometric indices suggestive of cellular hypertrophy correlated with reduced creatine-stimulated respiration.

DISCUSSION

In the present paper, we approached the biochemical aspects of cardiomyocyte hypertrophy in DMC, namely the changes in protein composition of these cells. In mature cardiac tissue, the mechanical load on myocytes causes the release by cardio-myocytes of growth factors and the autocrine stimulation of the expression of a number of genes that initiate thehypertrophic phenotype. Activation of expression of structural and motor proteins is the compensatory response of the cell, which requires strengthening of the cytoskeleton and contractile apparatus. However, an increased synthesis of a series of cytoskeleton proteins may lead over time to pathological changes. Higher level expression of tubulin isoforms, the main constituents of microtubules and intermediate filament protein desmin in animals initially leads to increased stiffness and resistance of microfilaments that bear mechanical load, and is an adaptive process. As these proteins accumulate in the cell, the viscosity of the sarcoplasm that surrounds the functional actomyosin structures increases. As a result, the advancement of myosin filaments relative to actin filament becomes more difficult and contraction efficiency decreases.

Thus, a substantial increase in tubulin and desmin content found by us in our DCM patients can partially influence the maladaptive nature of cardiomyocyte hypertrophy, when, despite the increasing size of functional fibers contraction efficiency is found

to be low. Recently, the presence of marked unusually shaped cardiomyocyte and macronuclei hypertrophy has been reported (7, 8). The authors believe that these changes are characteristic of dilated cardiomyopathy and able to distinguish it from secondary forms. These particular features were used in our research to form a separate study group and to study the particularities of DCM clinical manifestations, depending on the nature and degree of changes in myocardial energy metabolism. We have found that in that group of patients, the relative content of mitochondrial creatine kinase was two times lower, indicating extensive changes in the biosynthesis of various isoferments / isoenzymes in this disease.

With the decrease in mitochondrial creatine kinase the efficacy of phosphocreatine pathway for energy transport decreases considerably, as demonstrated by the sharp drop in creatine-stimulated respiration and aerobic phosphocreatine synthesis in mitochondria. Concomitantly with the reduction of mitochondrial creatine kinase activity in the heart the MB isoenzyme content increases, demonstrating a change of creatine kinase gene expression in the heart with cardiomyopathy. The diminished mitochondrial function of creatine kinase can be explained by the altered expression of the enzyme (9) and sarcoplasmic accumulation of Pi (inorganic phosphate) (10) due to insufficient ATP formation. However, the activity of the enzyme can be associated to the same extent with the low myofibrilar ATPase activity in the diseased heart (10). This supposition is confirmed by the reduction to up to 57% of creatine content in patients with DCM.

Energy transport system disturbances probably lead to a significant increase in diastolic blood pressure and a decrease of ejection fraction. End-diastolic pressure increase in DCM can be largely related to increased myocardial stiffness, due to the increase in myofibrillar ADP as a result of a decreased rate of ADP rephosphorylation because of the diminished efficiency of phosphocreatine pathway for energy transport. As known, ADP is bound to myosin strong enough, with concomitant inhibition of ATPase activity and dissociation of cross-bridges. This may be due to increased end-diastolic pressure and lower ejection fraction in DCM patients presenting the above mentioned features.

CONCLUSIONS

The high myosin light chain kinase and CRP content in the myocardium of patients with DCM demonstrates their involvement in hypertrophy, and can be used in the differential diagnosis of myocardial hypertrophy as a consequence of cardiomyocyte hypertrophy and a result of a predominantly fibrotic process. Energy disorders are significantly more marked in DCM patients in which cardiomyocyte hypertrophy is more pronounced and nuclei are larger in size.

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NEWS

ARM LYMPHEDEMA AFTER AXILLARY DISSECTION

Arm lymphedema is an important complication after surgery intervention for breast cancer. It causes functional and physical defects as well as psychological maladjustments, thereby reducing patient's quality of life. Early diagnosis and treatment is considered important for successful management of breast cancer related arm lymphedema. Patients who have undergone a mastectomy with axillary lymph node dissection have a greater arm-volume and more commonly report symptoms of lymphedema, as compared to patients who have received a sentinel lymph node biopsy. A retrospective review of 57 patients with unilateral arm after an axillary dissection for breast cancer was performed between January 1, 2001 and October 30, 2011. The patients, treated with two weeks of complete decongestive therapy (CDT) and self-administered home therapy, were followed for 24 months. Arm volume was serially measured by using an optoelectronic volumeter prior to and immediately after CDT; and there were follow-up visits at 3, 6, 12, and 24 months. Patients were divided into two groups according to the percent excess volume (PEV) prior to complete decongestive therapy: group 1, PEV<20% and group 2, PEV≥20%. In group 1, mean PEV before CDT was 11.4±5.0% and 14.1±10.6% at 24 months after CDT with no significant difference. At the end of CDT, PEV was 28.8±15.7% in group 2, which was significantly lower than the baseline (41.9±19.6%). The reduction of PEV was maintained for 24 months in group 2. The long-term effects of CDT were well-maintained for 24 months, but there was a difference in progression of PEV between the two groups. The patients with more initial PEV showed significant volume-reducing effects of CDT. In patients with less initial PEV, the severity of lymphedema did not progress to higher grades. (Hwang JM, Hwang JH, Kim TW, et al. Long-term effects of complex decongestive therapy in breast cancer patients with arm lymphedema after axillary dissection. *Ann Rehabil Med. 2013*;37(5):690-677.)

Alina Manole, Roxana Livadariu

THE INFLUENCE OF ANTIBIOTIC TREATMENT ON THE DYNAMICS OF OXIDATIVE STRESS IN SPONTANEOUS BACTERIAL PERITONITIS

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THE INFLUENCE OF ANTIBIOTIC TREATMENT ON THE DYNAMICS OF OXIDATIVE STRESS IN SPONTANEOUS BACTERIAL PERITONITIS (Abstract): Bacterial infections play an important role in liver cirrhosis complications, being together with variceal bleeding and hepatic encephalopathy an important cause of morbidity and mortality in cirrhotic patients. Spontaneous bacterial peritonitis (SBP) is a major complication of liver cirrhosis, with a significant mortality. Recent studies have demonstrated the involvement of oxygen free radicals in the pathogenesis of liver cirrhosis, but the role of oxidative stress in the development of SBP is not very clear yet. **Purpose**: This study aims to evaluate the role of oxidative stress in the pathogenesis of spontaneous bacterial peritonitis and its changes after therapy. **Material and Methods**: The study is a prospective case-control, which included 33 patients divided into 3 groups: group I – 10 patients with decompensated cirrhosis and SBP, group II – 17 patients diagnosed with decompensated liver cirrhosis with ascites, and group III – 6 patients with compensated liver cirrhosis. The control group consisted of 19 healthy subjects recruited from hospital staff, adapted to patients by age and sex. Malonildyaldehida (MDA), a product of lipid peroxidation, was dosed in the blood and ascitic fluid of patients by assay thiobarbituric acid reactive substances (TBARS). **Results**: Serum MDA significantly increased in the group with decompensated cirrhosis and SBP compared with the control group. MDA levels in ascitic fluid showed a statistically significant increase in the SBP group compared with patients without SBP. There was a decrease of MDA after 6 months of antibiotic treatment compared with the initial stage, while MDA values increased in the absence of treatment. **Conclusions**: The study demonstrates the increased oxidative stress markers in the blood and ascitic fluid of cirrhotic patients with SBP, which can be considered a predictor of SBP and also a marker of treatment response. **Keywords**: SPONTANEOUS BACTERIAL PERITONITIS, OXIDATIVE STRESS, ANTIBIOTIC TREATMENT, PROPHYLAXIS.

Bacterial infections play an important role in liver cirrhosis complications, being together with variceal bleeding and hepatic encephalopathy an important cause of morbidity and mortality in cirrhotic patients (1). Compared with the general population, the incidence of infections such as peritonitis, pneumonia, urinary tract infections or meningitis is 10 times higher in cirrhotic patients and the mortality is 20 times high-

er, pointing out that liver cirrhosis is a status of acquired immune deficiency (2).

Spontaneous bacterial peritonitis (SBP) is a major complication of liver cirrhosis, with a significant mortality. The incidence of SBP in hospitalized patients with cirrhosis is between 7% and 23%. The probability for a patient with cirrhotic ascites to develop the first episode of SBP is 10% per year, but it increases in ascites with low protein concentration (below 1g / 100ml) and severe hepatic impairment (1, 2).

In most cases, SBP is a monobacterial infection of the ascitic fluid; its culture reveals a single microorganism, the number of neutrophils being over 250/mmc.

SBP probably develops as a consequence of ineffective defense mechanisms against infection, seen in patients with liver cirrhosis. Although the key steps in the pathogenesis of SBP are not fully elucidated, it is clear that the main source of infection is the bacterial gut, while the altered intestinal motility promotes the bacterial translocation, a key element in the pathogenesis of SBP (3).

Despite all protrective measures, such as early diagnosis and prophylactic antibiotic therapy, as well as the introduction of new antibiotics, albumin infusions, the prognosis of these patients remains poor, with a mortality rate of 20-40% (4). The installation of an episode of spontaneous bacterial peritonitis reduces the survival rate to about 30% per year and 20% at 2 years.

The literature shows a mortality rate of 50% during hospitalization; 70% of SBP relapse, and 80% of patients die within the first year after the initial infectious episode (5). In this context, the identification of patients with increased risk of death is extremely important for improving their prognosis.

Early establishment of an appropriate antibiotic therapy is critical in most cases of SBP.

Knowing the risk factors for SBP is important not only in order to identify patients who could benefit from the preventive therapy, but also in understanding the pathogenesis of the disease.

Identifying risk factors for SBP is important in the development of a safe and cost-effective prophylactic strategy.

A new concept much-discussed lately is oxidative stress. It is defined as the imbalance between oxidants and antioxidants in the favor of oxidants, with destructive and pathogenic potential. The oxidants are normally formed during aerobic metabolism and their amount increases under certain conditions. In such circumstances, physiological antioxidant mechanisms that inactivate reactive oxygen species and repair several molecular lesions may prove insufficient. The imbalance between the oxidant and antioxidant capacity of the body is called oxidative stress and is the origin of various diseases with a high prevalence in modern medicine, such as proliferative diseases, atherosclerosis, glomerulonephritis, pulmonary fibrosis and cirrhosis (6).

Recent studies have demonstrated the involvement of oxygen free radicals in the pathogenesis of liver cirrhosis, but the role of oxidative stress in the development of SBP is not very clear yet. However, recent studies have shown the important role of oxidative stress in increased intestinal permeability, an important element in the pathogenesis of SBP. In patients with liver cirrhosis there is an intestinal motility disorder, which promotes intestinal bacterial overpopulation. Bacterial translocation is possible due to intestinal barrier alteration. In the experimental conditions of lipid

peroxidation, infiltrations with neutrophils were observed in the intestinal mucosa, lesions which are correlated with bacterial translocation and portal hypertension, major factors in the development of SBP (7).

The free radicals, also called pro-oxidants, are compounds of circulating blood, very active, biochemically and biologically virulent, acting on membranes, nuclei and cell cytoplasm, producing and maintaining an intense oxidative stress (8). Antioxidant defense is achieved by the endogenous antioxidants and, where these systems are overcome, by means of the exogenous antioxidants. Antioxidants are designed to neutralize or remove reactive oxygen and nitrogen species and products of lipid peroxidation. It is always desirable that the level of pro-oxidants (free radicals) should be below the capacity of the antioxidants. When free radicals exceed the antioxidants (oxidative stress), phenomena of wear and early cellular aging appear (9).

Early establishment of an appropriate antibiotic therapy is critical in most cases of SBP. Empirical antibiotic treatment should be initiated prior to receiving the result of ascitic fluid cultures and blood cultures if the clinical picture is pathognomonic and the PMN number is above 250/mmc. The results of the cultures allow subsequent adaptation of the treatment regimen (10).

For over two decades, third generation cephalosporins were the antibiotics of choice in the treatment of SBP. Felisart et al. in 1985 have validated the use of cefotaxime as an empiric antibiotic choice in the treatment of SBP, further studies determining the optimal dose and duration of therapy. A recent study by Navas et al. demonstrated that oral therapy with ofloxacin is as effective as that with cefotaxime iv in patients with uncomplicated SBP (11).

Prophylactic antibiotic treatment aims to selective decontaminate the gastro-intestinal tract in order to reduce the risk of developing spontaneous bacterial peritonitis. Given the increased cost and risk of developing resistant organisms, antibiotic prophylaxis is indicated in three cirrhotic groups: those with a history of SBP, those hospitalized for upper gastrointestinal bleeding, those with low-protein ascitic fluid.

MATERIAL AND METHODS

The study is a prospective case-control, which included 33 patients divided into 3 groups: group I – 10 patients with decompensated cirrhosis and SBP, group II – 17 patients diagnosed with decompensated liver cirrhosis with ascites, and group III – 6 patients with compensated liver cirrhosis. The control group consisted of 19 healthy subjects recruited from hospital staff, adapted to patients by age and sex. Malonildyaldehida (MDA), a product of lipid peroxidation, was dosed in the blood and ascitic fluid of patients by assay thiobarbituric acid reactive substances (TBARS). MDA levels were statistically analyzed using one-way analysis of variance (ANOVA). The results are expressed as mean ± SEM. The values of F for which $p < 0.05$ were considered statistically significant.

RESULTS

Serum MDA levels, a marker of lipid peroxidation, have experienced significant growth both in the group with decompensated cirrhosis and SBP ($F(1.24) = 53$, $p = 0.0000001$) and in the group with decompensated cirrhosis without SBP ($F(1.31) = 30$, $p = 0.000005$) in comparison with the control group (fig. 1).

Regarding MDA levels in ascitic fluid,

there was also a statistically significant MDA increase in the group with SBP compared with patients without SBP (F (1.20) = 5, p = 0.047) (fig. 2).

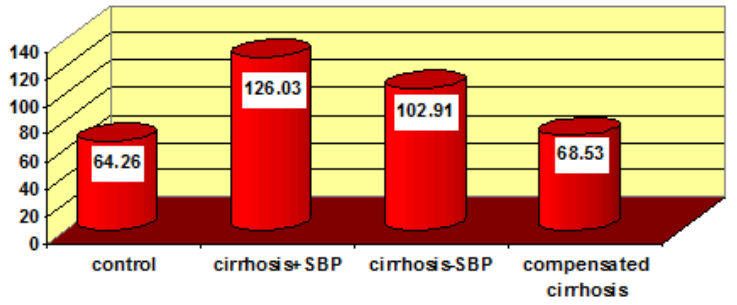

Fig. 1. MDA levels in serum in our research groups

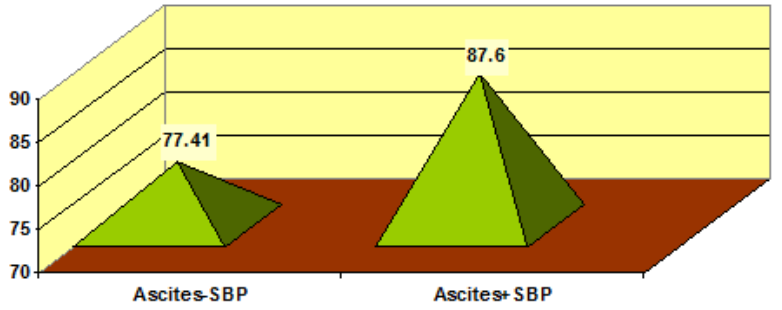

Fig. 2. MDA levels in ascitic fluid in our research groups

Regarding the evolution of MDA in the context of prophylactic antibiotic treatment, there was a decrease in MDA after 6 months of antibiotic treatment compared to the initial stage, whereas MDA values increased in the absence of treatment (fig. 3).

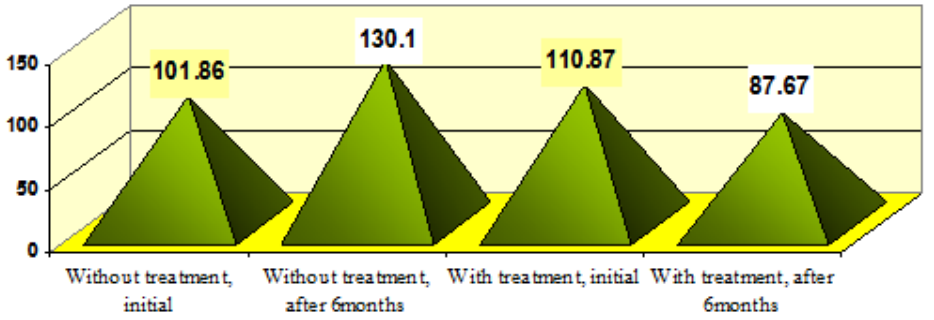

Fig. 3. The evolution of MDA in patients with and without treatment

TABLE I

Average values of oxidative stress markers in patients with and without treatment, initially and after 6 months

Oxidativ stress marker	Without treatment (3 cases)				With treatment (6 cases)			
	Before		After		Before		After	
	Average	Ab. st.	Average	Ab. St.	Average	Ab. st.	Average	Ab. st.
MDA	101.86	18.383	130.10	24.673	110.87	19.351	87.67	19.989

DISCUSSION

The results obtained showed the presence of oxidative stress, increased markers of lipid peroxidation, suggesting the involvement of oxidative stress in SBP and its dynamics within the context of antibiotic therapy.

A recent study by S Bhandari et al. demonstrated the role of oxidative stress in worsening the severity of cirrhosis, assessed by Child-Pugh score, by determining the levels of pro-oxidant substances (serum levels of MDA) and of the antioxidant (superoxide dismutase and glutathione peroxidase) in cirrhotic patients (12).

Previous studies on rats with experimental model of liver cirrhosis induced by alcohol and iron found elevated levels of MDA in the liver. A significant increase in plasma MDA levels was also observed (13).

A recent study published by Shaden M et al., demonstrated the involvement of oxidative stress in SBP (14). Oxidative stress was evidenced by elevated MDA and by significantly reduced antioxidant components SOD, GPx. After antibiotic treatment, MDA, NO and TNF expression significantly decreased and there was significant increase in antioxidant elements. Also, in a study by Natarajan SK (15), a significant increase of ascitic MDA was observed in patients with SBP compared to controls. This study demonstrated the presence of oxidative stress in the ascitic fluid from patients with SBP and showed that the nitrate in the ascitic fluid may be a marker for diagnosis of SBP and an useful index to determine the therapeutic response to treatment with antibiotics.

Understanding this is important because the interaction between NO and superoxide may cause extremely damaging reactive species, such as Peroxynitrite.

Randomized trials have demonstrated the efficacy of intravenous cefotaxime, which is the antibiotic of choice in SBP (16).

In determining the therapeutic response in patients with SBP, Natarajan et al. measured the parameters of oxidative stress in relation to the number of neutrophils in the ascitic fluid. The study showed a decrease of NO in the ascitic fluid, in concordance with the number of neutrophils. Park et al demonstrated no significant change in nitrate from ascites of patients with SBP after treatment with antibiotics. However, the nitrate levels in ascites per se were altered in patients with SBP in this study, in contrast to data from other studies (17).

A Geetha et al. (12) demonstrated that in cirrhosis red cells undergo severe oxidative stress with significant changes in membrane properties. Oxyhemoglobin and methemoglobin levels were analyzed in blood red cells. Markers of oxidative stress, such as lipid peroxides, lipid hydroperoxides, as well as nitric oxide, were deter-

mined with enzymatic anti-oxidants. Methemoglobin level was significantly higher ($p < 0.001$) in erythrocytes of patients with liver cirrhosis complicated with bleeding compared to patients without bleeding. Levels of oxidative stress markers, including nitric oxide has been shown to be higher in these patients.

Enzymatic antioxidants levels were low. Gines, in his study, showed that norfloxacin, a selective quinolone for gram-negative bacilli with low absorption in dose of 400mg/day reduces the risk of SBP recurrence in the first year from 68% to 20% (18).

Five randomized trials have shown the net benefit of using short-term antibiotic prophylaxis in cirrhotic patients admitted with variceal bleeding. A meta-analysis showed a significant decrease in the rate of infection in patients receiving antibiotic treatment on admission (19).

Grange et al. (20), in a randomized placebo-controlled trial evaluating norfloxacin as primary prophylaxis in cirrhotic patients with low ascitic fluid protein, demonstrated a decreased incidence of SBP in the group that received antibiotics (2%) compared with the placebo group (17%) and also decreased mortality rates at 6 months (19% vs. 36%).

CONCLUSIONS

Our results demonstrate the presence of increased oxidative stress in patients with decompensated cirrhosis and SBP, compared to those without SBP and those with compensated liver cirrhosis. This was shown by increased MDA, a marker of lipid peroxidation.

Thus, measurement of this parameter of oxidative stress may play an important role in the diagnosis and follow up of this important liver pathology

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NEWS

PHARMACOLOGICAL ACTIVITY OF MYRTUS NIVELLEI ESSENTIAL OIL

Sahara myrtle is a medicinal plant traditionally used for dermatosis treatment. In order to justify the use of this plant, Bouzabata *et al.* aimed to elucidate the composition of *Myrtus nivellei* essential oil extracted by hydrodistillation of aerial parts, its antifungal properties and the cytotoxicity on keratinocytes. Chemical analysis using chromatographic and spectroscopic techniques elucidated the structure of two new compounds and showed a different composition of *Myrtus nivellei* oil from that of *Myrtus communis*. Broth macrodilution method was used to evaluate the activity against yeasts and filamentous fungi. Determination of MICs showed that the oil was more active against *Cryptococcus neoformans* (MIC = 0.16µL/mL), followed by dermatophytes (MICs of 0.64 and 1.25µL/mL). Evaluation of cell viability in HaCaT keratinocytes revealed no cytotoxicity at concentrations up to 1.25µL/mL. Thus, appropriate doses of *Myrtus nivellei* oil could be found, having antifungal activity and very low cytotoxicity on keratinocytes. (Bouzabata A, Bazzali O, Cabral C, Gonçalves MJ, Cruz MT, Bighelli A, *et al*. New compounds, chemical composition, antifungal activity and cytotoxicity of the essential oil from Myrtus nivellei Batt. & Trab., an endemic species of Central Sahara. *J Ethnopharmacol.* 2013 Jul 31. pii: S0378-8741(13)00487-X. doi: 10.1016/j.jep.2013.06.054.)

Teodora Vremera

ZENKER'S DIVERTICULUM, A RARE CAUSE OF UPPER GASTROINTESTINAL BLEEDING

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ZENKER'S DIVERTICULUM, A RARE CAUSE OF UPPER GASTROINTESTINAL BLEEDING (Abstract): The most common complication of Zenker's diverticulum is aspiration pneumonia, compression of the trachea and esophageal obstruction with large diverticulum, and increased risk of development of carcinoma. Thus bleeding occurs rarely, can be massive and life threatening, with ulceration being the most common cause. **Material and methods:** We describe a patient with sever upper gastrointestinal bleeding as a result of a Zenker's diverticulum. A 75 year-old woman was referred to the emergency room and hospitalized for hematemesis, melena, asthenia and total dysphagia. In this particular case we preferred open technique because of the diverticulum dimensions and bleeding episode. Left cervicotomy was practiced on the anterior edge of the sternocleidomastoid muscle, being known that Zenker diverticulum extend into the left neck 90% of the time, fact also confirmed by radiology in this case. **Results:** Postoperatively, the patient showed a complication free recovery. Five days after treatment the patient resumed nourishment. Several days later our patient was able to return home. Follow-up at 12 months after the operation showed complete recovery. Ulcer of the basis of Zenker's diverticulum is a rare entity and, only a few cases were reported in the literature to date. Omitting thecricomyotomy predisposes to fistula or diverticulum recurrence due to the persistence of a high pharyngeal intraluminal pressure that acts on the posterior wall just proximal to the upper esophageal sphincter. **Conclusions:**Zenker's diverticulum is an unusual site of origin for clinically significant upper gastrointestinal hemorrhage and differential diagnosis must include other more frequent causes of upper gastrointestinal bleeding. In our opinion, classicalsurgical therapy is indicated when distal esophageal imaging cannot be obtained during endoscopic examination, there is a large diverticulum or in an emergency setting when fast control over the bleeding source is required. **Keywords:** ZENKER'S DIVERTICULUM, GASTROINTESTINAL BLEEDING, CERVICOTOMY

Zenker's diverticulum is a pulsion-type diverticulum of the hypo pharynx, the result of herniation of the esophageal mucosa and submucosa through the area of natural weakness known as Killian's triangle.

It is hypothesized that abnormal activity in the cricopharyngeus muscle results in an uncoordinated swallowing mechanism, which, coupled with increased intraluminal pressure, results in the slow, progressive distention and herniation of the pharynx mucosa (1). The incidence of Zenker's Diverticulum is estimated at 2 per 100,000, with highest prevalence rates in the 7th and

8th decades of life. Risk factors for development of Zenker'sdiverticulum include presence of hiatal hernia and/or gastro esophageal reflux disorder (GERD), male gender and age (1, 2).

The most common complication of Zenker's diverticulum is aspiration pneumonia, compression of the trachea and esophageal obstruction with large diverticulum, and increased risk of development of carcinoma (3). Thus bleeding occurs rarely, can be massive and life threatening, with ulceration being the most common cause.

Since its description by Zenker and von Ziemssen in 1877, numerous surgical techniques have been developed to treat the diverticulum. Initially, transcervical resection of the diverticular pouch has been the procedure of choice (4). However, the diverticular sac is in fact a consequence of the high pharyngeal pressure secondary to a lack of compliance of the cricopharyngeus muscle in relation to local fibrosis. Consequently, due to unacceptably high rate of recurrence, cricopharyngeal myotomy (CPM) was recommended for the management of the physiologic abnormality (5, 6).

Endoscopic therapy can be applied in two forms, either by the application of diathermic or laser coagulation, or stapling technique, which allowed an endoscopic suture of the cut margins (7, 8, 9, 10) (tab. I).

Size of the diverticulum determines which procedure to carry out (11, 12) (tab. II).

TABLE I

Endoscopic and open techniques

Endoscopic techniques	Open techniques
Endoscopic stapled diverticulostomy	CPM
CO_2 laser / Electrocautery	CPM with diverticulectomy
	CPM with diverticulopexy

TABLE II

Criteria of the Morton and Bartley

Criteria	Surgical technique
I. Small sacs are less than 2 cm in length	CP myotomy alone is sufficient
II. Intermediate sacs are 2-4 cm in length	Endoscopic or open procedure can be used
III. Large sacs are greater than 4 cm in length	Open surgery

We describe a patient with sever upper gastrointestinal bleeding as a result of a Zenker's diverticulum. A 75 year-old woman was referred to the emergency room and hospitalized for hematemesis, melena, asthenia and total dysphagia. She was known to have endured various respiratory tract infections in the previous several months and had difficulty to swallow solid foods for a few months. During the previous few days the ingestion of fluids had become impossible.

The clinical exam reveals pallor, evidence of dehydration and a 5 by 3 cm palpable tumor on the left side of the neck. Patient was tachycardic but with normal blood pressure. Blood exams shows moderate anemia. Esophagography with barium revealed a medium to voluminous pharyngo-esophageal diverticulum.

Surgical treatment was decided. In this particular case we preferred open technique because of the diverticulum dimensions and bleeding episode. Left cervicotomy was practiced on the anterior edge of the sternocleidomastoid muscle, being known that Zenker diverticulum extend into the left neck 90% of the time, fact also confirmed by radiology in this case.

The omohyoid muscle was cleaved and the superior thyroidian artery and vein were ligated and cleaved, in order to obtain suitable diverticulum exposure. The recurrent laryngeal nerve was identified and protected.

The diverticulum, with a diameter of approximately 3-4 cm, is identified and dissected (fig. 1). A Faucher probe was passed with some difficulty into the distal esophagus. Esophageal and crico-pharyngeus muscles are longitudinally divided for 3-4 cm. Mechanical suture at the base of the diverticulum is practices with aid of a TA 30/3.5 stapler (fig. 2). The diverticulum is sectioned close to the stapler (fig. 3).

Fig. 1. The diverticulum, identified and dissected

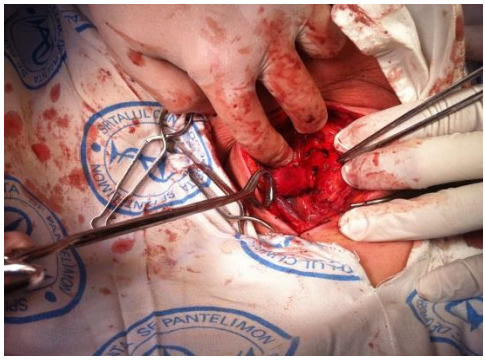

Fig. 2. Mechanical suture at the base of the diverticulum

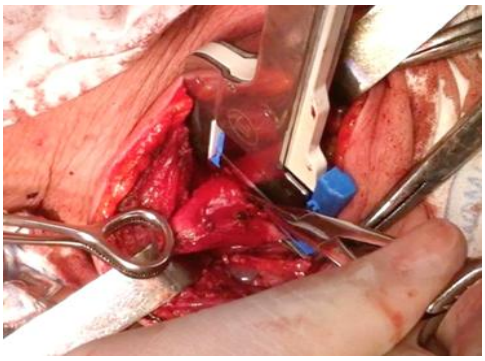

Fig. 3 The diverticulum is sectioned close to the stapler

Final aspect after removing stapler and control of suture sealing and hemostasis (fig. 4). The excised diverticulum containing traces of barium from previous examination (fig. 5). Paraesophageal drainage through counter incision was preferred ostoperatively, the patient showed a complication free recovery. Five days after

treatment the patient resumed nourishment. Several days later our patient was able to return home. Follow-up at 12 months after the operation showed complete recovery.

Fig. 4. Final aspect after removing stapler

Fig. 5. The excised diverticulum

DISCUSSION AND CONCLUSIONS

As a summary, the case is rare complication of Zenker's diverticulum that was initially misdiagnosed as having esophageal varices. Ulcer of the basis of Zenker's diverticulum is a rare entity and, only a few cases were reported in the literature to date (1, 5, 6). The diverticulum had a deep ulceration that was the source of the bleeding. The cause of the ulceration is unknown but it is possible that the ulcer has been caused by chronic NSAID or other ulcerogenicdrugs prescribed for prior respiratory infections. Chronic alcohol abuse was the other contributing factor, known to predispose ulcer formation by facilitating the development of gastroesophageal reflux and stimulation of acid secretion (7, 8, 9).

Zenker's diverticulum is an unusual site of origin for clinically significant upper gastrointestinal hemorrhage and differential diagnosis must include other more frequent causes of upper gastrointestinal bleeding (10, 11).

In our opinion, classical surgical therapy is indicated when distal esophageal imaging cannot be obtained during endoscopic examination, there is a large diverticulum or in an emergency setting when fast control over the bleeding source is required.

Omitting thecricomyotomy predisposes to fistula or diverticulum recurrence due to the persistence of a high pharyngeal intraluminal pressure that acts on the posterior wall just proximal to the upper esophageal sphincter (12, 13).

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NEWS

MALIGNANT MESOTHELIOMA: NONASBESTOS-RELATED ASSOCIATIONS

It should be realized that although there is a strong link between the development of malignant mesothelioma (MM) and asbestos exposure, not all cases are etiologically related to asbestos exposure. The proportion of cases attributable to asbestos exposure varies between the sexes and country, according to occupation and use of amphibole asbestos. A number of other agents have been implicated in the causation of MM but the best evidence appears to relate to another mineral fiber (erionite) and to irradiation. However, there is strong experimental, epidemiologic and molecular evidence to suggest a possible carcinogenic or cocarcinogenic role of viruses such as simian virus 40 (SV40), a monkey polyoma virus, in the induction of MM. Malignant mesothelioma has followed 20 years after plombage therapy with lucite spheres for tuberculosis. Additional cases have been associated with chronic empyema, chronic peritonitis, induced pneumothroax for tuberculosis, and familial Mediterranean fever. (Jasani B, Gibbs A. Mesothelioma not associated with asbestos exposure. *Arch Pathol Lab Med*. 2012;136:262–267).

Doina Butcovan

STABILITY AND CHANGE IN THE CLINICAL COURSE OF SCHIZOAFFECTIVE DISORDER

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STABILITY AND CHANGE IN THE CLINICAL COURSE OF SCHIZOAFFECTIVE DISORDER (Abstract): Schizoaffective disorder currently raises several questions, one of them being related to the stability of the clinical diagnosis over time. The **aim** of this study is to identify the clinical and evolutional particularities in the longitudinal course of schizoaffective disorder. **Material and methods:** 44 subjects with a current diagnosis of schizoaffective disorder have been assessed prospectively. Following parameters were analyzed: socio-demographic (age at onset, gender, educational, professional and marital status at onset) and clinical (total duration of evolution, diagnosis at onset, duration of the evolution until the switch to the schizoaffective disorder diagnosis). **Results and discussion:** Socio-demographic parameters are similar to those in literature and the clinical assessment revealed that schizoaffective disorder is present as a diagnosis along with the longitudinal course of other types of psychosis. **Conclusions:** Schizoaffective disorder appears as a heterogeneous pathology in terms of the longitudinal course. **Keywords:** SCHIZOAFFECTIVE DISORDER, DIAGNOSIS STABILITY, LONGITUDINAL COURSE.

We have addressed in this study one of the controversial topics of today's psychiatry, namely the concept of schizoaffective disorder (1). Generally, current concerns that regard schizoaffective disorder are linked to the question whether schizoaffective disorder is a nosological entity distinct from schizophrenia or from affective disorders? From a clinical point of view, another question is related to the stability of the diagnosis of schizoaffective disorder in terms of its clinical longitudinal course.

The circumscription of schizoaffective disorder varies in scientific literature between the following guidelines: schizoaffective disorder with only schizoaffective episodes , schizoaffective disorder that also presents along with its course schizophrenia episodes, purely delusional episodes or even a combination of both, schizoaffective disorder that has mainly schizophrenia episodes to which schizoaffective episodes are added and schizoaffective disorder that has mainly affective episodes to which schizoaffective episodes are added. Generally, the interferences between schizophrenia, schizoaffective disorder and affective disorders can widely vary and their clarifi-

cation can be achieved through longitudinal studies. The results of these studies are controversial, varying from results that show that there are differences between these disorders to the ones that show no differences.

The aim of this study is to identify clinical and evolutional particularities in the longitudinal course of schizoaffective disorder. One of the specific features of this study is the minimum of 15 years of psychotic pathology evolution of the subjects accepted in this research.

MATERIAL AND METHODS

The research was conducted on a total of 44 subjects who were admitted for the first time in Timisoara Psychiatric Clinic between 1985 and 1995. These subjects have been diagnosed during their first admission with a first episode of psychosis and currently, in the year 2011, have a schizoaffective disorder diagnosis. Since we have considered a clinical population, the sample does not claim to be statistically representative and we have not used random or quota sampling techniques, but we have applied inclusion/exclusion criteria.

Inclusion criteria: diagnosis of schizoaffective disorder (F25 according to the ICD-10 criteria) at the assessment, meaning the current diagnosis , at least 15 years of evolution of the pathology from the first episode of psychosis, age under 60 years at assessment, subjects who are able and willing to cooperate with the assessor - we have obtained the informed consent of the subjects that have participated in this study and outpatients of Timisoara Mental Health Centre and of the ambulatory psychiatric surgeries from Timisoara. *Exclusion criteria*: the presence of other associated psychiatric disorders: mental retardation, alcoholism, personality disorders. We mention that where necessary we have used the conversion tables of ICD 9/ICD 10, because the onset diagnosis was made according to ICD 9.

The study sample has been assessed based on the data from the existent research project of Timisoara Psychiatric Clinic, through direct discussions with the subjects but also with the physicians who are regularly seeing the patients in ambulatory. Following parameters were analyzed: social and demographic (age at onset, gender, educational status at onset, professional and marital status at onset) and clinical and evolutionary (total duration of evolution, diagnosis at onset, and duration of the evolution until the switch to the schizoaffective disorder diagnosis).

RESULTS

For the subjects included in the study the overall average evolution is of 21.5 years (std. dev. 3.67 years), with a minimum of 15 years of evolution and a maximum of 30 years.

The diagnostic assessment from the onset was performed using the existing records from our clinic and also with the help of the conversion tables from ICD-9 to ICD-10 diagnosis (fig. 1).

Since it is of great interest to determine the average time required for the stabilization of the symptoms and for the framing of the disease into the nosological category of schizoaffective disorder, we took into account the period of time (in years) for the subjects we have investigated, from the onset to the establishment of the schizoaffective disorder diagnosis. Therefore, for establishing the diagnosis of schizoaffective disorder for the subjects included in the study sample 5.98 years (std. dev. 3.23)

were needed, with a minimum of 1 year and maximum of 14 years. The average length of time for the change of the diagnosis varies according to the diagnosis at onset (fig. 2).

TABLE I

Socio-demographic characteristics of the sample

Socio-demographic characteristics	Schizoaffective disorder sample (N=44)
Average age at onset	25.45 (std.dev. 6.17 years)
Gender:	
male	18
female	26
Educational status :	
10 grades	3
High school	41
Average years of school	11.7
Professional status :	
Employed	32
Unemployed	2
High school/college student	10
Marital status:	
Married	17
Single	21
Divorced	6

TABLE II

The distribution on groups of years of evolution

Evolution years at assessment	Schizoaffective disorder sample			
	Number	Cum.number	Percentage	Cum.percentage
15-19 years	15	15	34,09091	34,0909
20-24 years	23	38	52,27273	86,3636
25-30 years	6	44	13,63637	100,0000

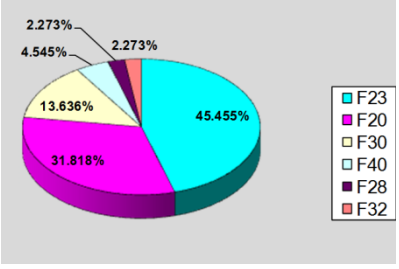

Fig. 1. Distribution of the sample according to the diagnosis at onset

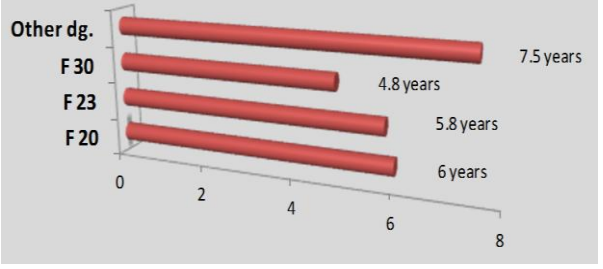

Fig. 2. The diagnosis at onset / the average of time for the change of the diagnosis

DISCUSSION

Generally, the assessment of the socio-demographic parameters is similar to that illustrated in the scientific literature (2). The average age at onset is one that corresponds to the young adult period and the gender distribution shows that schizoaffective disorder prevails among female, this is also shown in the majority of the studies (3, 4). To note that the study we conducted has no epidemiological value since we have not used any statistical methodology for the inclusion of the subjects in the sample. The average length of schooling is about the same having a value of 11 years which corresponds to a medium tuition. From the point of view of the profession we observe that most subjects are performing an activity (72.7%), whether they were or they are enrolled in schools (20.4%). Family status shows that most are unmarried (47%) - a predictable percentage since their young age at onset.

The evolution course is very long when comparing with most clinical research. It can be observed that most subjects (n=23) have an evolution of 20 to 24 years. Regarding the diagnosis of onset we notice that no subjects had a schizoaffective disorder diagnosis - this diagnosis has been placed somewhere along the course of the disease. The diagnoses palette is diversified, but with the observation that a large number of subjects has a diagnosis of acute and transient psychotic disorder (45.5%), followed by schizophrenia (31.8%). There were also a reduced number of subjects included who experienced at onset an affective pathology (manic or depressive).The average length of time necessary for the change of the diagnosis varies depending on the diagnosis at onset, the shortest being for the subjects who had an affective pathology at onset (4.8 years) and the longest for those with a diagnosis of schizophrenia (6 years). In this research we have also included subjects who had at onset a diagnosis of other psychotic disorders (according to ICD 10) or even of neurosis. For this subgroup the time until the change of the diagnosis was higher (7.5 years). This is however correlated with the diversity of the clinical diagnoses.

Schizoaffective disorder is a nosological category for which establishing a diagnosis from onset is rarely possible, more often it is only possible to identify psychotic features and a great variability of psychotic symptoms. The establishment of the diagnosis of schizoaffective disorder is to be made after a period of evolution of the pathology (5).

CONCLUSIONS

Schizoaffective disorder appears as a heterogeneous pathology in terms of longitudinal course, meaning that most cases that reach this diagnosis also have other diagnoses during their evolution, especially in the period that follows the onset. From the clinical point of view it is important to distinguish between schizoaffective disorder and schizoaffective episode, nuance which is not currently achieved by the diagnostic systems.

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NEWS

NEW INSIGHT INTO MOLECULAR EPIDEMIOLOGY OF MRSA STRAINS IN ROMANIA

A recent study by Székely and co. brings new informations regarding methicillin-resistant *Staphylococcus aureus* strains (MRSA) circulating in Romania. The authors used pulsed-field gel electrophoresis (PFGE), *spa* typing and SCC*mec* typing to describe the clonal relations and virulence profiles of MRSA isolates from patients admitted to intensive care and surgical units, during 2010, in Mures County Emergency Clinical Hospital. The study identified 25 pulsotypes clustering into 4 major clonal groups, named from A to D. The majority of MRSA isolates, included into clonal group A (82%), were associated with the successful HA-MRSA clone, spa type t030, which carried SCC*mec* type III and enterotoxin A genes. Group B was represented by 15 % of the strains, which were assigned to spa types t127, t015 and t321. These strains harboured SCC*mec* type IV and enterotoxin genes. Two strains that belonged to spa types t044 and t582 were included into clonal groups C and D, respectively. They were PVL-producing strains (Panton-Valentine leukocidin) and had genes encoding for enterotoxin G and SST-1 (toxic shock syndrome toxin). The remaining two strains were non-typeable by PFGE and belonged to spa type t034, which is characteristic for livestock-associated MRSA (LA-MRSA). These strains were isolated from community onset infections, harboured SCC*mec* type V and were negative for the searched exotoxins. The study concluded that most MRSA strains were clonally related and suggested an intrahospital origin, but also pointed out the danger of new emerging LA-MRSA strains. (Székely E, Man A, Mare A, Vas KE, Molnár S, Bilca D, *et al*. Molecular epidemiology and virulence factors of methicillin-resistant *Staphylococcus aureus* strains in a Romanian university hospital. *Rev Rom Med Lab*. 2012; 20: 370-382).

Teodora Vremeră

RELATIONSHIP BETWEEN BONE MINERAL DENSITY, WEIGHT, AND ESTROGEN LEVELS IN PRE AND POSTMENOPAUSAL WOMEN

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RELATIONSHIP BETWEEN BONE MINERAL DENSITY, WEIGHT, AND ESTROGEN LEVELS IN PRE AND POSTMENOPAUSAL WOMEN **(**Abstract): Bone loss in postmenopausal women is mainly due to estrogen deficiency affecting the balance between osteoclast resorption and bone formation controlled by osteoblasts. **Aim:** To determine the relationship between bone mineral density (BMD) in pre and postmenopausal Caucasian women, and estrogen levels. **Material and methods:** Cross-sectional study including six groups of 8 to 15 pre- and postmenopausal healthy volunteers with different weights, body mass index (BMI) (normal or underweight < 25 kg/m^2, overweight 25-30 kg/m^2, and obese > 30 kg/m^2), not exposed to antiosteoporotic therapy. Lumbar bone mineral density (BMD) and body composition (BC) were evaluated by dual X ray absorptiometry (DXA, Hologic), while serum estradiol and estrone were measured by ELISA. **Results**: BMD in postmenopausal women is lower than in premenopausal women irrespective of body weight ($p<0.05$). Estradiol and estrone are positively correlate with bone mass in premenopausal women, but not in postmenopausal women (R2 0.3209, R2 0.2579, respectively). **Conclusions**: It is very important to identify the risk factors for osteoporosis, especially in postmenopausal women, as we will show that aromatization of androgens into estrogens in adipose tissue appears not to have a significant role in postmenopausal women bone protection. **Keywords**: BODY COMPOSITION, MENOPAUSE, ESTRONE, ESTRADIOL.

Osteoporosis is a chronic disease of the skeletal system characterized by a decrease in bone mass and abnormalities of bone tissue microarchitecture resulting in bone fragility and risk for fracture. It is a public health issue, solely in Romania being approximately 1.5 million patients with osteoporosis, hence the interest in better understanding the factors that determine the occurrence of osteoporosis (1, 2).

MATERIAL AND METHODS

Cross-sectional study on 6 groups of about 8 women each. Age dstribution: 31 premenopausal women and 37 postmenopausal (at least two years of menopause). BMI: normal and underweight (BMI <25 kg/m^2), overweight (BMI between 25-30 kg/m^2) and obese (BMI> 30-40 kg/m^2). Age-group and BMI distribution: premenopausal women: 16 normal and underweight, 9 overweight, and 6 obese; postmenopausal women: 8 normal and underweight, 11 overweight, and 17 obese. All women volunteered to participate in this study, and were recuited into

the study from patients, students and staff of Iasi „St. Spiridon" County Emergency Hospital. A signed informed consent was obtained from all women.

Exclusion criteria were: diabetes mellitus, osteoporosis under treatment, hormone replacement therapy, severe liver or kidney disease, hyperthyroidism, primary hyperparathyroidism, hypo-gonadism, growth hormone deficiency, Cushing's syndrome, corticotherapy for over a year, anorexia nervosa, malabsorption syndrome, genetic syndromes. A fasting blood sample was collected, centrifuged, and stored in a refrigerator (-30°C) for estrone and estradiol determinations. Then, for all volunteers height and weight were measured, and subjected to osteo-densitometry by dual X-ray absorptiometry (DXA). A certified technician measured bone mineral density (BMD) of the lumbar spine on Hologic DXA. Total fat and lean masses were expressed in grams. Lumbar BMD was expressed in g/cm^2. Leptin (ng / ml), adiponectin (mg / l), IGF-1 (µg / l) were determined using commercial ELISA kits (Diagnostic Automation Inc., Calabasas, CA) The results were statistically processed using NCSS 2007 software, simple Pearson correlation, student t test, the correlation being considered significant when $p < 0.05$. Multivariate regression (ANOVA) was used to exclude systematic biases.

RESULTS

Bone mineral density was significantly lower in postmenopausal compared with premenopausal women ($p < 0.05$) (fig. 1).

The relation between various body compartments and total body bone mass was followed in all volunteers. BMI positively correlated with lumbar BMD, T score, and Z score. The best correlation was with Z score, because this parameter excludes age-related influence on bone (fig. 2).

Given the heterogeneity of the study group we used Z score, which compares BMD with age-matched mean BMD.

Body compartments correlate differently to lumbar Z score. Thus, in our study series Z score was correlated with total body weight expressed as BMI, the correlation being positive and significant in both pre- and postmenopausal women. (fig. 3).

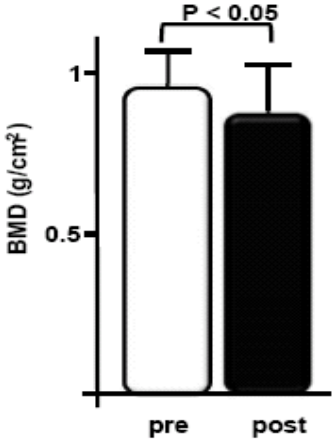

Fig. 1. Bone mineral density in pre and

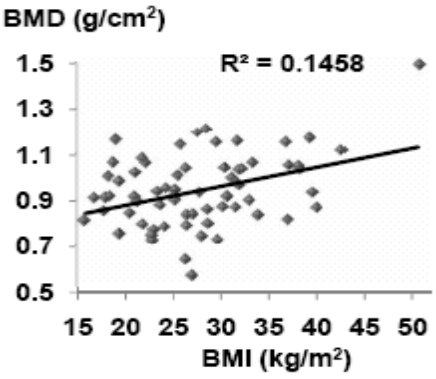

Fig. 2. Correlation between BMD and BMI

postmenopausal women

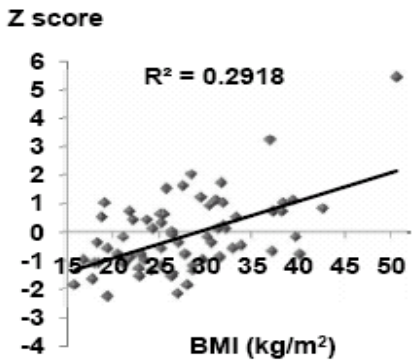

Fig. 3. Correlatona between lumbar Z score and BMI in the entire group

Adipocyte can synthesize trough aromatization estrogens from androgen precursors (3), being an important source of estrogen production and thus protecting the bone mass in postmenopausal women. In our study group we found that both estradiol (predominat estrogen secreted by ovaries) and estrone (produced by aromatization of androgens into estrogens in adipose tissue) correlated with bone mass in premenopausal women but not in the postmenopausal ones, suggesting that the alleged adipose tissue role as additional postmenopausal estrogen reservoir is negligible in keeping an adequate bone mass.

in all study women

Both estradiol and estrone positively correlated with Z score only in premenopausal women (fig. 4).

If the correlation is positive in premenopause, the same is not true in postmenopause (fig. 5).

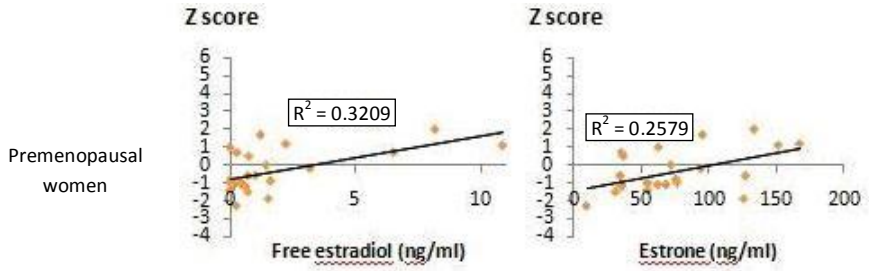

Fig. 4. Correlation between între estradiol and estrone levels and Z score in premenopausal women

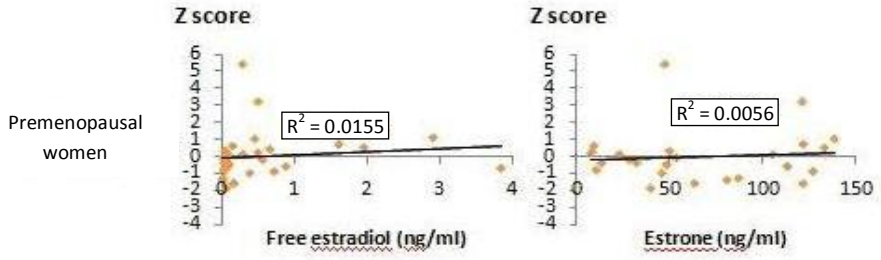

Fig. 5. Correlation between între estradiol and estrone levels and Z score in postmenopausal women

DISCUSSION

Bone mineral content is strongly influenced by menopause and estrogen depletion. Trabecular bone is characterized by high turnover, the vertebrae being the first bones in which after menopause mineral loss becomes significant (3, 4).

Body mass also influences the bone mineral content (5). Low body weight is known to be an independent risk factor for osteoporosis in pre- and post menopausal women (6). Data on adipose tissue and bone are even more conflicting, some authors suggesting that fat mass appears to be more important in bone mass prediction, especially in postmenopausal women (7).

This effect may be mediated by adipocitokine (8) or extragonadal estrogen reservoir represented by adipocytes (9). Adipocyte can synthesize through aromatization estrogen from androgen precursors (10), and thus can be an important source of estrogen for bone mass protection in postmenopausal women (11). In our study group we found that both estradiol, predominant estrogen secreted by ovaries, and estrone, produced by aromatization of androgens into estrogens in adipose tissue correlated with bone mass in premenopausal women but not in the postmenopausal ones, suggesting that the alleged role of additional postmenopausal estrogen reservoir of the adipose tissue is negligible in maintaining a suitable bone mass.

CONCLUSIONS

This clinical study shows that bone mass correlates to body weight in pre- and postmenopausal Caucasian women, most likely through a direct mass effect caused by gravitational stress. Increased body weight prevents bone loss in postmenopausal women, normal or underweight postmenopausal women having a lower BMD than premenopausal women ($p < 0,05$).

Aromatization of androgens into estrogens in the adipose tissue seems to play a significant role in osteoporotic protection of postmenopausal women ($R^2 = 0.0056$).

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NEWS

EFFECTIVENESS OF SELF-ADHESIVE LUTING CEMENTS IN BONDING TO CHLORHEXIDINE-TREATED DENTIN

Chlorhexidine has a broad spectrum of antibacterial action and it is claimed to serve as a potent inhibitor of endogenous collagenolytic/gelatinolytic activity of the matrix metalloproteinases, preserving the bonding integrity in adhesive restorative techniques. These properties recommend chlorhexidine as a cavity disinfectant prior to the application of restorative materials and, more recently, as a root canal irrigant. The aim of this study was to investigate the influence of dentin pre-treatment with different concentrations of chlorhexidine solutions on the microtensile bond strength and failure pattern of self-adhesive luting cements. Resin-composite (Filtek Z250, 3 M ESPE) discs (12 mm in diameter, 6.0 mm thickness) were cemented to the smear layer-covered dentin using RelyX, 3 M ESPE; Multilink Sprint, Ivoclar Vivadent, after dentin pretreatment with 0.2% or 2.0% chlorhexidine solutions. Results were compared with the control, untreated dentin. The specimens were tested in tension with a crosshead speed of 0.5 mm/min in a universal testing machine, and the failure patterns were classified. Two-way ANOVA and Tukey's tests were performed. Additionally, teeth were subjected to energy-dispersive X-ray spectroscopy (EDS) analysis and micromorphology characterization of the smear layer-covered dentin and 0.2% or 2.0% chlorhexidine-treated dentin surfaces. The microtensile bond strength obtained for both control groups were significantly higher, regardless of the chlorhexidine concentration and/or the cement used. Bond strengths were significantly higher for RelyX than for Multilink Sprint, except when 2.0% chlorhexidine was applied. Fractographic analysis indicated that most failures in the control groups occurred cohesively in the self-adhesive luting cements. Pre-treated dentin with 0.2% and 2.0% chlorhexidine solutions presented higher incidences of adhesive failures. EDS/SEM analysis exhibited varied concentrations of chlorine ions and crystal-shaped precipitates, depending upon the chlorhexidine concentration. These results suggest that pre-treatment of dentin with 0.2% or 2.0% chlorhexidine adversely affects the bonding efficacy when associated with the self-adhesive luting cements tested, probably because of the chlorine residues on the dentin surface. The benefits of application of chlorhexidine solutions prior to cementation of indirect restorations using self-luting cements should be reconsidered (Vinicius Di Hipólito, Flávia Pires Rodrigues, Fabiana Bárbara Piveta, Lídia da Cunha Azevedo, Roberta Caroline Bruschi Alonso, Nick Silikas, Ricardo Marins Carvalho, Mario Fernando De Goes, Paulo Henrique Perlatti D'Alpino. Effectiveness of self-adhesive luting cements in bonding to chlorhexidine-treated dentin. *Dental Materials* 2012; 28: 495-501).

Gianina Iovan

LONG-TERM PROGNOSIS OF POLYVASCULAR PATIENTS WITH ASSOCIATED CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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LONG TERM PROGNOSIS OF POLYVASCULAR PATIENTS WITH ASSOCIATED CHRONIC OBSTRUCTIVE PULMONARY DISEASE (Abstract): **Objective**: To evaluate by various tools the prognosis of the polyvascular patients (defined as the presence of more than one affected vascular bed), who also associate chronic obstructive pulmonary disease. **Material and methods**: Fifty-eight patients discharged after an episode of acute cardiorespiratory failure were examined at 3 month-intervals for 1, 2 and 3 years (2010-2012). The following were performed: physical examination, biochemical and hematological tests, spirometry, electrocardiography, transthoracic echocardiography, brain computer tomography or magnetic resonance imaging. All patients in our study were smokers with chronic pulmonary obstructive disease. Treatment relied on the European recommendations for cardiac pathology and associated medical conditions. **Results:** A favorable clinical course was noticed in compliant patients. Patients with metabolic syndrome and/or old stroke, and peripheral arterial disease have a poor prognosis. A strong link seems to exist between systolic function of the right ventricle and cardiovascular mortality. The association of this condition to ischemic heart disease modifies the right ventricle hemodynamics. **Conclusions:** Polyvascular patients in acute cardiorespiratory failure have a mortality of 36% in the first 3 weeks. After 3 years, 86% of the patients survive. The modern methods of diagnosis and treatment allow improving the quality of life and increasing its duration. **Key words:** MYOCARDIAL ISCHEMIA, STROKE, PERIPHERAL ARTERIAL DISEASE.

Polyvascular disease is currently defined as a simultaneous clinical impairment of two or three arterial territories (coronary, cerebral or peripheral). As PRISMA survey shows, despite the educational measures aimed at changing lifestyle and therapeutical attitude, polyvascular patients still have a poorer control of serum cholesterol levels (1). In REACH registry (2) 68,000 vasculopathic patients were monitored for two years. Investigators found that in the polyvascular patients the carbohydrate and lipid metabolism were more difficult to control. Also, 2-year cardiovascular events were twice as frequent in polyvascular as monovascular patients. Importantly, only one of six patients had symptoms, hence the need for proper assessment of vascular damage. The same study shows that in polyvascular patients, revascularization rate was two

times higher.

Our study followed for three years (2010, 2011, and 2012) the disease course of a group of 58 polyvascular patients admitted to hospital in 2009 in critical condition after an episode of acute cardiorespiratory failure on the background of chronic respiratory disease. The systolic dysfunction of right ventricle was associated with more frequent cardiovascular events.

MATERIALS AND METHODS

The study included 58 patients, 34 males and 24 female. Patients' age at the beginning of the study ranged between 52 and 82 years. We performed a spirometry on all patients, when included in the study. This method established the stage of the chronic obstructive pulmonary disease (according to the GOLD criteria – Global Initiative for Chronic Obstructive Lung Disease). The recommended treatment for chronic respiratory disease consisted of: inhaled parasympaticolytics (Tiotropium 18μg), association of inhaled sympathomimetics and corticosteroids (50 μg Salmeterol / Fluticasone 125-500 μg), Theophylline retard 350mg and oxygen therapy at home, 4-6 l / min, 6-8 hours / day, intermittently if the patient is GOLD stage IV.

All patients were subjected to cardiovascular assessment: immediate blood pressure measurement in supine position in both arms and in standing position, and 5 minutes later in orthostatic position, cardiac auscultation, palpation and auscultation of carotid and subclavian arteries, abdominal aorta, renal, femoral, and popliteal arteries, pulmonary auscultation, jugular inspection, detection of hepato-jugular reflux and lower limbs edema.

Resting electrocardiogram was performed with a Heart Screen HS60G device for all patients, every 3 months during the three follow up years. Right and posterior chest leads were also used. Transthoracic echocardiography was performed with a Fukuda Denshi UF-850 XTD echocardiograph. Peripheral arterial damage was confirmed by arterial Doppler, while the evaluation of cerebral complications was studied with brain computer tomography or magnetic resonance imaging.

For cardiovascular diseases, the patients received dual antiplatelet therapy (Aspirin 75mg and Clopidogrel 75 mg) as recommended by CHARISMA study (3,4), statins (Atorvastatin 40-80 mg, dosage varying according to patient's tolerance, muscle mass and the degree of nitrogen retention), cardioselective beta-blockers (5-10 mg Nebivolol, Carvedilol 12.5 mg), angiotensin converting enzyme (Perindopril 5-10 mg) or sartan (Telmisartan 40-80 mg), fixed drug combinations (Perindopril 5 mg /Indapamide 1.5 mg), combination of diuretics (Furosemide 20 mg + Spironolactone 50 mg) - in congestive heart failure.

RESULTS

In regards to chronic obstructive pulmonary disease, seven patients (12%) were in GOLD stage II, 14 patients (24%) in GOLD stage III, and 37 patients (62%) in GOLD stage IV.

Forty-seven patients (82%) were diagnosed with arterial hypertension, 6 patients (10%) with orthostatic hypotension, and the latter requiring special therapeutic attention upon prescribing hemodynamically active medication. Baseline congestive heart failure features were found in 20 patients (34%). Pathological heart murmurs were detected in 45% of patients (26 patients).

Biochemical test results revealed that the polyvascular patients more frequently

presented mixed dyslipidaemia (higher total serum cholesterol and triglycerides levels), this feature being due to the frequent association of metabolic syndrome in the patients with this condition (2).

The most common electrocardiographic finding was ST depression and negative T waves, consistent with changes suggesting trivascular coronary disease (37 patients, 35%). The remaining patients showed electrocardiographic features characteristic of myocardial necrosis (myocardial infarction in chronic stage). Right ventricular ischemic injury was revealed for 80% of the patients by the changes in right chest leads, and supported by the echocardiographic changes. Special attention was given to echocardiographic exploration of the right ventricular damage (right ventricular parietal kinetics, M-mode tricuspid annulus systolic motion, systolic pulmonary flow acceleration time, pulmonary artery systolic pressure). Perhaps, the more common association of right heart ischemia in polyvascular patients is due to hemodynamic overload induced by chronic obstructive pulmonary hemodynamic.

Echocardiographic examination of the left ventricle revealed ischemic dilated cardiomyopathy in 35 patients (60%), segmental parietal kinetic changes in 20 patients (35%), and isolated diastolic dysfunction in 3 patients (5%). Cardiac murmurs, detected at physical examination in 26 patients (25%) were confirmed echocardiographically as being mild or moderate, mitral and/or aortic valvular disease.

Peripheral arterial Doppler detected critical stenosis in 40 patients (70%), and features of atheromatosis without stenosing lesions in 15% of patients (9 patients). Cerebral arterial damage - old ischemic stroke - was detected in 38 patients (65%).

Interventional and/or surgical myocardial revascularization was required by 30% of patients (17 patients); 45% of patients underwent aorto-femoral bypass.

Vascular pathology in the study group was associated with 2 or 3 arterial territories (fig. 1).

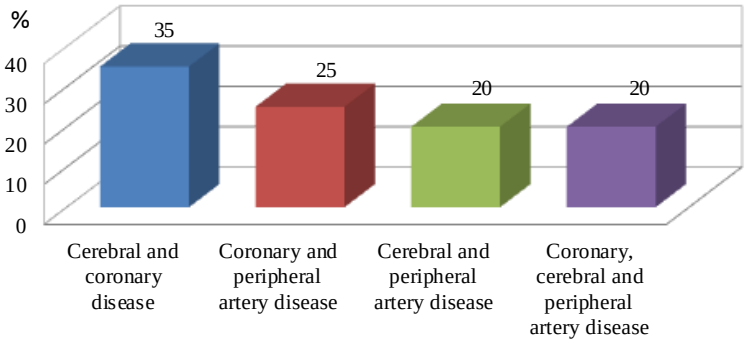

Fig. 1. Bi- or trivascular disease of the patients

Long-term prognosis was marked by an increased rate of cardiovascular events in patients with peripheral arterial disease (23 patients, 41%) after the 3 follow up years.

It is worth mentioning that arterial, myocardial, or peripheral revascularization was required at baseline by 16 patients; the other 10 patients underwent revascularization 3 to 25 months from baseline.

The incidence of metabolic syndrome in the study patients was observed in 49 patients (85%); diabetes mellitus was diagnosed in 46% of patients (26), and diabetic nephropathy was confirmed in 27% of patients (15). These three conditions associated with polyvascular disease in our study patients fed a poor prognosis (fig. 2).

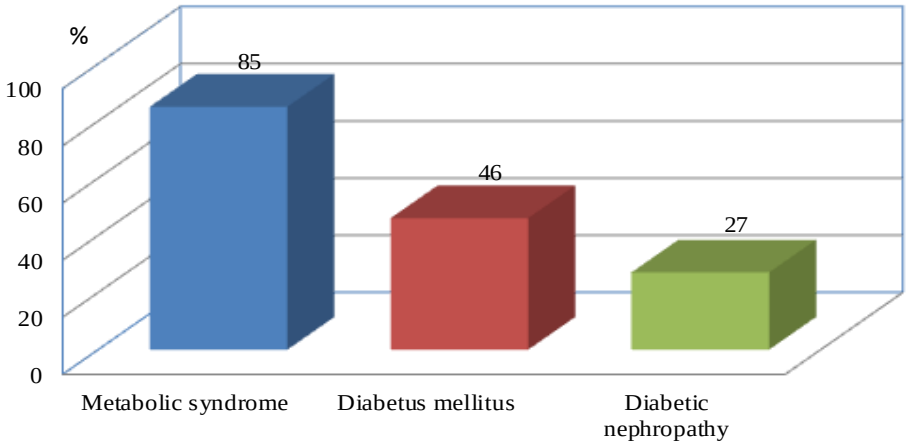

Fig. 2. The incidence of unfavorable prognostic factors in polyvascular patients

At the end of the 3 year follow-up, the cardiovascular mortality was of 14% (8 patients: 5 by acute myocardial infarction and 3 by stroke).

DISCUSSION

As noted by Criqui et al (5), peripheral arterial disease is an early indicator of cardiovascular mortality. In our study, the polyvascular patients with peripheral arterial disease presented an increase in the severity of coronary or cerebral ischemic pathology, requiring sustained therapeutic intervention.

In 2009, Deepak et al (6) published the results of CRUSADE registry, which enrolled 111,972 patients with polyvascular disease. They reported that polyvascular patients have a higher risk for cardiovascular events compared with monovascular patients. It is necessary that we continue this study by comparing the frequency of cardiovascular events in monovascular versus polyvascular patients.

Steg et al (7) participated in REACH study. They noted, like Deepak, that polyvascular patients have a higher risk for cardiovascular events than monovascular patients, since the first year of medical monitoring.

CONCLUSIONS

Our study concluded that polyvascular patients with systolic dysfunction of the right ventricle (80%) had a worse long-term prognosis than those without this dysfunction (20%). Even affected by right ventricle disease, our patients had a survival rate of 86%, 3 years after the study. The high survival rate is due to the modern

methods of diagnostics and treatment, which were applied on our patients for both cardiorespiratory diseases (medical, interventional and surgical). In addition, we remarked an improvement of the quality of life for the patients in our study.

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NEWS

SMOKING AND THE RISK FOR DEMENTIA

Smoking is a possible risk factor for dementia. A new cohort study examined the association between smoking history and cognitive decline in early old age. The cognitive function was investigated using a series of 5 tests (memory, vocabulary, executive function composed of 1 reasoning and 2 fluency tests). The assessment has been made regularly for a 10 years period. For middle-aged men, the results show that the faster cognitive decline (in global cognition and executive function) was observed among current smokers compared with never smokers. For ex-smokers, with at least a 10-year cessation, the decline in executive function was similar to that among never smokers. The rate of cognitive decline among women was not affected by smoking. These new research showed that middle-aged male smokers are more likely to have a faster cognitive decline (Séverine Sabia, Alexis Elbaz, Aline Dugravot et al. Impact of Smoking on Cognitive Decline in Early Old Age The Whitehall II Cohort Study. *Arch Gen Psychiatry*. 2012; 69(6):627-635).

Cătălina Luncă

CERCETĂRI PENTRU EVALUAREA UNOR ELEMENTE DE ORIENTARE A DIAGNOSTICULUI ÎN CANCERUL GASTRIC

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INVESTIGATIONS FOR ASSESSING OF SOME ELEMENTS SUGGESTIVE FOR A DIAGNOSIS OF GASTRIC CANCER (Abstract). **Aim:** Based on new data, this investigation aimed at emphasizing the importance and the medical and socio-economic implications of an early diagnosis in gastric cancer. **Material and methods:** The study included a series of 77 patients with gastric cancer admitted and treated at the surgical department of the Moineşti Municipal Emergency Hospital in the interval 2000-2010. Patients' data were obtained by clinical-epidemiological inquiry, laboratory and imaging investigations, and official statistical reports. Epidemiological and statistic-mathematical methods were used to process and interpret the collected data. **Results and conclusions:** Hemoglobin and hematocrit levels as well as the presence of anti-*Helicobacter pylori* antibodies were determined by laboratory tests. Barium sulfate X-ray examination was performed in 52% of our patients; endoscopy concurrent with the histo-pathological examination of gastric biopsy samples showed that most tumors were of vegetative and ulcerative type, being prevalently located in the antropyloric area and lesser gastric curvature. Computer tomography proved to be the method of choice for tumor dissemination and staging. The late diagnosis of gastric cancer, with aggressive forms and poor prognosis, suggests the need of preventive population screening programs. **Key words:** GASTRIC CANCER, TUMOR MARKERS, ENDOSCOPY, CANCER SCREENING

Cancerul gastric (CG), ca şi alte forme de neoplazie, ridică importante obstacole în stabilirea precoce a unui diagnostic care să favorizeze reducerea impactului medical şi socio-economic, adesea sever (1, 2).

Simptomatologia clinică, necaracteristică, este tolerată şi ignorată perioade lungi de timp şi, astfel, adresabilitatea la medic este tardivă.

În acest context, mai ales pentru persoanele cu risc crescut, explorările paraclinice, între care cele imagistice, au un rol esențial pentru depistarea precoce a CG într-un stadiu favorabil intervențiilor terapeutice.

MATERIAL ŞI METODE

Studiul se referă la un lot de 77 pacienți cu cancer gastric asistați în secția de chirurgie a Spitalului Municipal de Urgență Moinești, județul Bacău, în perioada 2000-2010.

Pentru caracterizarea pacienților incluşi

în studiu s-au acumulat informaţii printr-o anchetă clinico-epidemiologică special concepută, precum şi cele oferite de evidenţele statistice oficiale ale spitalului.

Evaluarea diagnosticului paraclinic s-a realizat prin investigarea valorilor hemoglobinei (Hb), hematocritului (Ht), prezenţa anticorpilor anti-*Helicobacter pylori*, markerii tumorali (CA 19-9; AFP; CA 125; TPSA; CA 15-3) şi rezultatele examenului imagistic (radiologic, endoscopic, eco-endoscopic, computer tomografic şi cel pentru stadiile invaziei ganglionare).

Prelucrarea şi interpretarea datelor s-a realizat prin metode epidemiologice (ancheta, tabularea, incidenţa, prevalenţa, reprezentările grafice) şi statistico-matematice – aplicaţia *Epi Info* (regresia liniară, diferenţa standard, t-Student, χ^2).

REZULTATE

1. Investigaţiile de laborator au evidenţiat că valorile hemoglobinei (Hb) au variat între 4,19-14g/dl cu o medie de 9,77g/dl; hematocritul (Ht) a înregistrat un nivel de 13,1-52% cu o medie de 32,39% (tab. I).

TABELUL I

Limitele valorilor hemoglobinei şi hematocritului la bolnavii cu cancer gastric incluşi în studiu

Parametrii	Nivelurile		
	Minim	Mediu	Maxim
Hemoglobina (g/dl)	4,19	9,77	14,00
Hematocritul (%)	13,1	32,39	52,00

Aceşti indicatori pot marca intensitatea modificărilor determinate de procesul neoplazic şi astfel, orientează natura evoluţiei şi a prognosticului (3,4).

Implicarea *Helicobacter pylori* în favorizarea declanşării carcinogenezei la nivelul mucoasei gastrice poate fi luată în discuţie ca urmare a evidenţierii prezenţei anticorpilor specifici la 17 (22%) bolnavi din lotul studiat (5).

În studiul nostru, pentru diagnosticul şi prognosticul bolii s-au analizat şi următorii markeri tumorali: CA 19-9: Antigenul carbohidrat CA 19-9; AFP: alfa-fetoproteina; CA 125: Antigenul carbohidrat CA 125; TPSA: Antigenul prostatic specific si CA 15-3: Antigenul carbohidrat CA 15-3 (tab. II).

TABELUL II

Valorile unor markeri tumorali evidenţiaţi la 12 pacienţi cu cancer gastric

Marker	Lotul de studiu			
	Nr.	% (x)	Valori	
			Normale	Crescute
CA 19-9	1	1,3	1	-
AFP	2	2,6	2	-
CA 125	3	3,9	3	-
TPSA	3	3,9	1	2
CA 15-3	3	3,9	3	-

(x) Pentru valorile mai mici de 10, proporţiile sunt orientative

2. Investigațiile imagistice.

2.1. Examenul radiologic s-a efectuat la 40 (52%) pacienți, cel mai frecvent investigarea a folosit sistemul dublu contrast, cu o compresiune moderata şi ingestie de sulfat de bariu cu densitate mare. În stadiul incipient, modificările radiologice au fost minime, iar în forma avansată s-au înregistrat schimbări ale reliefului mucoasei şi ale peristalticii gastrice (6,7,8).

2.2. Examenul endoscopic a permis evaluarea aspectului macroscopic şi localizarea leziunilor mucoasei gastrice. Astfel, s-a constatat că zona antro-pilorică a înregistrat în peste o treime din cazuri proliferări epiteliale neoplazice maligne. Date similare au fost semnalate şi de alți autori (8,9,10) (tab. III).

TABELUL III

Distribuția pe zone anatomice a leziunilor din cancerul gastric, la lotul inclus în studiu

Tipul leziunii	Zona anatomică	Cazuri	
		Nr.	% (x)
Primitivă	Eso-cardială	6	7,8
	Corp gastric+Fornix	13	16,9
	Mica curbură	21	27,2
	Marea curbură	3	3,9
	Antro-pilorică	**32**	**41,6**
Total		75	97,4
Recidivă	Bont gastric	1	1,3
	Gura de anastomoză	1	1,3
Total		2	2,6

(x)Pentru valorile mai mici de 10, proporțiile sunt orientative

În peste 80% din cazurile studiate, s-a evidențiat endoscopic localizarea tumorii neoplazice la un singur segment gastric. La ceilalți bolnavi, tumora s-a extins în ordine descrescătoare, spre corpul gastric, mica curbură şi în regiunea eso-cardială (tab. III).

2.3. Examenul ecoendoscopic a fost efectuat la 81,8% dintre pacienți şi a permis evaluarea gradului de invazie a peretelui gastric (T), afectarea ganglionilor regionali (N) şi invazia la distanță (M). A rezultat că 49,2% din cazuri s-au încadrat în faza a III-a de invazie, cu invadarea integrală a peretelui gastric fără a depaşi seroasa peritoneală (tab. IV).

TABELUL IV

Distribuția în raport cu nivelurile invaziei peretelui gastric evidențiată ecoendoscopic, la 63 bolnavi cu cancer gastric

Tip de invazie (T)	Cazuri	
	Nr.	% (x)
Tipul I	4	6,3
Tipul II	11	17,5
Tipul III	31	**49,2**
Tipul IV	17	27
TOTAL	63	100

(x)Pentru valorile mai mici de 10, proporțiile sunt orientative

Investigația ecoendoscopică a dovedit a avea o valoare limitată în a identifica diseminarea la distanță a procesului canceros. În studiul nostru, numai 6 (9,5%) pacienți din sublotul de 63 au prezentat diseminări secundare, la nivel hepatic şi a seroasei peritoneale (9,10,11).

2.4. Examenul computer-tomografic a fost practicat la 18 (23,4%) pacienți şi a evidențiat, pentru 2/3 din cazuri, extinderea procesului tumoral în structurile din vecinătatea peretelui gastric. A rezultat că 78% din tumori se încadrează în stadiile III şi IV de invazie, 75% cu determinări ganglionare şi 34 % cu metastaze evidente (tab. V).

TABELUL V

Distribuția celor 18 cazuri de cancer gastric examinate computer-tomografic, în relație cu stadiul de gravitate (T= afectarea peretelui gastric; N= interesarea ganglionilor regionali; M= diseminarea la distanță)

Stadiul afectării (TNM)	Nr.	% (x)
I A	1	5,56
I B	4	22,22
II A	1	5,56
II B	2	11,11
III A	3	16,66
III B	1	5,56
IV	**6**	**33,33**
TOTAL	18	100

(x)Pentru valorile mai mici de 10, proporțiile sunt orientative

3. Investigații morfopatologice.

3.1. Aspectele macroscopice au evidențiat pe toate piesele de exereză chirurgicală, elemente semnalate şi de alți autori (11). Astfel, ponderea localizării proximale a CG a fost de numai 5% pentru întregul lot care a beneficiat de examen endoscopic, şi de aproximativ 8% în cazul pacienților supuşi intervenției chirurgicale. Extensia tumorilor la mai mult de un segment gastric a fost prezentă, în lotul nostru, la aproximativ 20% din cazuri, comparativ cu alți autori care au găsit valori de până la 6% (12).

Distribuția cazurilor de CG studiate în raport cu diferitele localizări şi aspecte macroscopice au evidențiat valori similare cu cele semnalate în Europa, dar diferite de cele din Asia, unde există un risc mai semnificativ de apariție a acestui tip de cancer (13).

3.2. Aspectele microscopice studiate, folosind piesele rezultate din actele operatorii, au fost analizate în raport cu elementele sistemului de clasificare OMS şi au evidențiat că forma tubulară a fost prezentă în 41,6% din cazuri, incluzând, îndeosebi, CG moderat diferențiate.

Folosind sistemul de clasificare Laurén, peste 50% din tumori s-au încadrat în categoria "difuză" şi 35% în cea de tip "intestinal" (14) (tab. VI).

TABELUL VI

Distribuția cazurilor de cancer gastric, în lotul studiat, în relație cu aspectul microscopic

Tipuri ale aspectului microscopic	Cazuri	
	Nr.	% (x)
Tubular	32	41,6
Papilar	7	9
Mucinos	13	16,9
Cu celule în "Inel cu pecete"	19	24,7
Nediferențiat	6	7,8
TOTAL	77	100

(x)Pentru valorile mai mici de 10, proporțiile sunt orientative

DISCUȚII

Explorările de laborator, imagistice şi anatomopatologice aduc un aport asociativ esențial în evaluarea cazurilor de CG, atât în formele inițiale cât şi in cele avansate, permițând creşterea eficientei stabilirii diagnosticului, prognosticului si a conduitei terapeutice.

Continua sa aibă prioritate examenul radiologic gastric cu sulfat de bariu, care poate evidenția triada: nişa, lacuna, infiltrație (1,2).

Endoscopia digestiva superioara poate vizualiza un CG inițial, permite prelevarea biopsiilor de mucoasa gastrica, supravegherea in dinamica a stărilor precanceroase: gastrita atrofică cu metaplazie intestinala, anemia pernicioasa, polipii gastrici, stomacul rezecat, displazia gastrica (9).

Endoscopia gastrica cu magnificație asociază examenului endoscopic clasic un proces de mărire a imaginii de pana la 150 ori prin utilizarea unui sistem optic care se comportă ca un microscop de înaltă rezoluție. În aceste condiții pot fi evaluate, cu acuratețe, modificările macroscopice identificate la examenul endoscopic (11).

Ecoendoscopia combină endoscopia digestivă superioară cu ecografia prin ataşarea la vârful endoscopului a unui transductor ultrasonografic, fiind astfel posibilă vizualizarea peretelui gastric şi a structurilor adiacente (10,11).

Computer tomografia gastrică are o acurațe mai redusă în ce priveşte CG inițial, în special tipul plat dar este superioară celorlalte metode imagistice pentru diagnosticul formelor avansate şi permite evaluări ale prognosticului, contribuind la realizarea stadializarii TNM (T- profunzimea tumorii primare; N- afectarea ganglionilor regionali; M- metastazele la distanță) (10).

CONCLUZII

Investigațiile de laborator au evidențiat date privind nivelurile hemoglobinei, hematocritului, markerilor tumorali, prezența anticorpilor anti-*Helicobacter pylori*. Pentru 52% dintre pacienții incluşi în studiu s-a efectuat examenul radiologic, cu ingestia sulfatului de bariu cu densitate mare, cu o compresiune moderată sau dublu contrast reuşindu-se astfel evidențierea modificării reliefului mucoasei şi a peristalticii, fiind posibilă stabilirea diagnosticului sau a suspiciunii de CG.

Examenul endoscopic combinat cu cel histopatologic al prelevatelor prin biopsie gastrică a permis conturarea unui profil morfologic particular al lotului de pacienți evaluați. S-a stabilit că tumorile studiate au

fost, în general, de tip vegetant şi ulcerat, cu localizare predilectă la nivelul regiunii antro-pilorice, a micii curburi şi a feţelor anterioară şi posterioară ale corpului gastric, cu caracter extensiv şi cu predominanţa formelor slab şi moderat diferenţiate.

Investigaţia gastrică computer tomografică s-a dovedit a fi metoda de elecţie pentru evaluarea diseminării la distanţă a procesului tumoral, dar pentru completa caracterizare a CG s-a impus şi efectuarea unui examen endoscopic combinat cu cel histopatologic al fragmentelor de mucoasă gastrică prelevate prin biopsie şi stadializarea tumorii în sistemul TNM.

Predominanţa unui diagnostic tardiv al CG, existenţa unor forme agresive şi a unui prognostic sever sugerează necesitatea continuării unor preocupări asociativ multidisciplinare pentru elaborarea şi realizarea de programe pentru un *screening* populaţional prevenţional.

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ETIOLOGIA PNEUMONIEI ASOCIATĂ VENTILAȚIEI MECANICE LA CLINICA DE CHIRURGIE CARDIOVASCULARĂ DIN IAŞI

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ETIOLOGY OF VENTILATOR-ASSOCIATED PNEUMONIA AT THE CARDIO-VASCULAR SURGERY CLINIC OF IASI (Abstract): **Aim**: We want to assess the etiology of ventilator-associated pneumonia (VAP) and emergence of resistant phenotypes for greater efficiency of the first-line antimicrobial treatment post-cardiac surgery. The study focuses on patients with VAP post-cardiac surgery at the Institute of Cardiovascular Surgery between April 2nd 2002 and August 27th 2008, divided in two groups, before and after June 14th, 2005. **Material and methods**: The tracheal aspirates were examined quantitatively (microscopy, culture). The isolates with clinical significance (≥ 106 CFU/mL) were identified by standard methods and tested by disk difussion or with the breakpoints method for susceptibility to antibiotics. **Results**: Among the 1527 operated patients, 28 of them were diagnosed with VAP through the clinical pulmonary infection score ≥ 6. The 9 most common pathogens in VAP etiology were Pseudomonas aeruginosa 23.81%, Acinetobacter baumannii 19.05%, Stenotrophomonas maltophilia 11.91%, Candida albicans 9.53%, while Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Corynebacterium striatum/amycolatum, Pneumocystis jirovecii amounted 4.76% each. Acinetobacter baumannii and S.maltophilia appear starting with June 15th, 2005. From that moment on we isolated with a higher frequency the gram-negative bacilli which produce extended-spectrum beta-lactamases 15.62%, with multidrug resistance (MDR) 46.86%. The MDR phenotype was more frequent at A.baumannii (15.62%), S.maltophilia (15.62%) and P.aeruginosa (12.50%) compared to K.pneumoniae (3.12%) or E.coli (0%). Methicillin-resistant S. aureus was isolated with a 6.25% frequency. **Conclusions**: The most common etiologic agents of ventilator-associated pneumonia post-cardiac surgery are Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia and Candida albicans. The large use of antibiotics selects infectious agents with intrinsic resistance and multiresistant phenotypes. **Key words**: ETIOLOGY, VENTILATOR-ASSOCIATED PNEUMONIA, CARDIAC SURGERY.

Pneumonia asociată ventilației mecanice (PAV) este o infecție nosocomială majoră la pacienții din unități de terapie intensivă (UTI), iar agenții diferă pentru fiecare unitate (1, 2). În UTI post-chirurgie cardiacă, PAV reprezintă cea mai frecventă infecție nosocomială (3), cu mortalitate care poate ajunge la 76% (2).

În acest studiu am evaluat etiologia PAV și emergența microorganismelor antibioticorezistente în UTI a clinicii de chirurgie cardiovasculară din Institutul de Boli

Cardiovasculare din Iaşi (IBCVI) pentru a creşte eficienţa tratamentului antimicrobian de primă intenţie la pacienţii suspectaţi de PAV post-operator.

MATERIAL ŞI METODE

Studiul retrospectiv s-a realizat pe un lot de 28 pacienţi diagnosticaţi cu PAV post-chirurgie cardiacă cu *by-pass* cardiopulmonar (BCP) la IBCVI în perioada 2002-2008. Am analizat comparativ două intervale egale, marcate prin data izolării în UTI a primei tulpini multirezistente de *Acinetobacter baumannii*.

Eligibili au fost pacienţii cu vârsta ≥ 18 ani, cu VM ≥ 48 ore şi PAV confirmată microbiologic. Studiul a fost aprobat de Comisia de Etică a IBCVI.

Aspiratele traheale (AT) au fost examinate cantitativ (microscopie şi cultură) (4). Identificarea izolatelor a fost realizată prin metode standard şi sistemul comercial API bioMérieux. Sensibilitatea la antibiotice am testat-o difuzimetric (5) sau prin metoda punctelor de ruptură pe galerii API cu citire spectrofotometrică (mini API - bioMérieux).

Pneumonia asociată ventilaţiei mecanice (un episod) a fost diagnosticată prin scorul clinic de infecţie pulmonară (SCIP) ≥ 6 (6). Cazurile de PAV au fost confirmate microbiologic prin izolarea cantitativă a cel puţin unui microorganism condiţionat patogen cu pragul cantitativ $\geq 10^6$ UFC/mL (7) ce s-a regăsit semnificativ asociat celulelor inflamatorii în culturile din AT (4). Hemoculturile pozitive cu aceeaşi tulpină izolată din AT au confirmat etiologia PAV. Semnificaţia clinică pentru *Pneumocystis jirovecii*, fung necultivabil, a fost stabilită de asocierea semnificativă a chiştilor şi formelor trofice cu celulele inflamatorii pe frotiul Giemsa din AT (4).

Rezistenţa multiplă la antibiotice (RMA) este definită doar pentru bacilii Gram-negativ (BGN) ca rezistenţa la cel puţin o cefalosporină (ceftriaxonă/cefotaxim/ceftazidim), un aminoglicozid (amikacină/tobramicină) şi ciprofloxacin (8).

Doze zilnice prescrise (DZP) reprezintă frecvenţa cumulată a dozelor zilnice de agenţi antimicrobieni prescrişi după natura acestora (9).

Variabilele cantitative le-am analizat cu testele χ^2 şi exact Fisher. Variabilele continui le-am exprimat prin medie ± deviaţie standard. Programele utilizate au fost Excel 2007 şi SPSS 16 (pentru validare). Semnificaţia statistică a fost stabilită pentru $p < 0.05$. Pentru fiecare episod PAV a fost consemnată data apariţiei şi tipul agentului patogen implicat; apoi, tratând timpul ca o variabilă statistică continuă am calculat distribuţia temporală (utilizând o distribuţie Student) pentru fenotipurile rezistente. În acest caz variabila temporală utilizată este purtătoarea informaţiei utile, intervalul de încredere (determinat pentru $p = 0.05$) reprezentând perioada de emergenţă în UTI a acelui tip de microorganism (caracterizată de un moment de apariţie, un moment de dispariţie din UTI, şi un maxim al probabilităţii de incidenţă). Dacă intervalul de încredere depăşeşte perioada analizată, am calculat probabilitatea de eroare totală rezultată.

REZULTATE

În cei 6 ani au fost operaţi 1527 de pacienţi, 673 înainte de 14.06.2005, iar 854 după această dată. Postoperator, 28 au fost confirmaţi cu PAV.

Din datele demografice, vârsta medie a celor 28 de pacienţi studiaţi a fost 57,4 ± 15,4 ani. Dintre comorbidităţi, insuficienţa cardiacă (IC) a fost prezentă la 26 (92,86%) pacienţi. Douăzeci şi şase (92,86%) din 28 de pacienţi au fost spitalizaţi ≥ 5 zile la IBCVI până la debutul PAV. Cinci pacienţi din 28 (17,86%) au primit terapie antibac-

teriană înainte de operație (în ultimele 90 de zile anterioare intervenției). Hemoculturi pozitive, cu același microorganism identificat în aspiratul traheal, au avut 5 (17,86%) dintre pacienți.

Cei 28 de pacienți au avut 35 episoade de PAV care au fost monomicrobiene 28 (80%) și posibil mixte 7 (20%). Am izolat 42 de tulpini, iar cei mai comuni 9 patogeni ai PAV au fost: *P. aeruginosa* 23,81%, *A. baumannii* 19,05%, *S. maltophilia* 11,91%, *C. albicans* 9,53%, iar *K. pneumoniae*, *E. coli*, *S. aureus*, *C. striatum/amycolatum*, *P. jirovecii* fiecare în procent de 4,76% (tab. I).

TABELUL I

Etiologia PAV în perioada 02.04.2002-27.08.2008

Agenții etiologici ai PAV	**Înainte de 14.06.2005**	**După 14.06.2005**	**Numărul total**	**% total**
P.aeruginosa	5	5	10	23.81
A.baumannii	0	8	8	19.05
S. maltophilia	0	5	5	11.91
C. albicans	2	2	4	9.53
K pneumoniae	0	2	2	4.76
E. coli	0	2	2	4.76
S.aureus	0	2	2	4.76
C.striatum/amycolatum	2	0	2	4.76
P. jirovecii	0	2	2	4.76
C.glabrata	0	1	1	2.38
C.krusei	0	1	1	2.38
Candida sake	0	1	1	2.38
Corynebacterium jeikeium	0	1	1	2.38
Geotrichum capitatum	1	0	1	2.38
TOTAL	**10**	**32**	**42**	**100**

Cu o frecvență de 23,81% fungii ocupă locul 2 după BGN nefermentativi 54,77%. *Acinetobacter baumannii* și *S.maltophilia* au apărut începând cu 15.06.2005. După această dată am izolat cu frecvență crescută BGN care produc β-lactamaze cu spectru extins (BLSE) 15,62% și cu RMA 46,86%. Fenotipul RMA a fost mai frecvent la *A. baumannii* (15,62%), *S.maltophilia* (15,62%) și *P.aeruginosa* (12,5%) comparativ cu *K.pneumoniae* (3,12%) sau *E.coli* (0%). *Staphylococcus aureus* meticilino-rezistent a fost izolat cu frecvență de 6,25% (fig. 1).

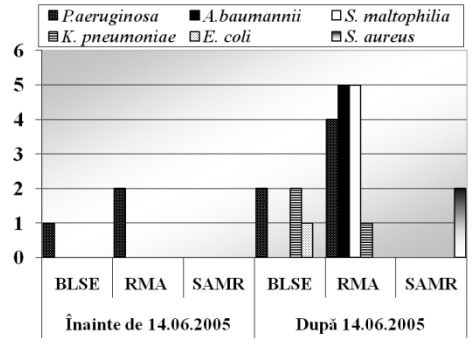

Fig. 1. Emergența speciilor de *A.baumannii* și *S.maltophilia*, creșterea prevalenței fenotipurilor rezistente în etiologia PAV

Am evaluat statistic implicarea fenotipurilor rezistente în etiologia PAV (fig. 2, tab. II, tab. III).

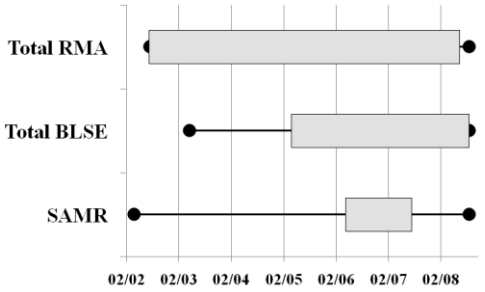

Fig. 2. Distribuția Student

Cei 28 de pacienți au primit antibiotice (1168 DZP): *preoperator*, 5 pacienți (17,86%) au primit tratament antibiotic (116 DZP – 92 DZP antibacteriene şi 24 DZP antifungice); *intraoperator* şi *postoperator* toți pacienții au primit antibiotice până la debutul PAV (1052 DZP – 803 DZP antibacteriene şi 249 DZP antifungice).

DISCUȚII

Etiologia PAV în majoritatea episoadelor include organisme oportuniste ale fondului microbian de spital. Pentru tratamentul antibiotic de primointenție este esențial să fie cunoscut spectrul etiologic local şi sensibilitatea la antibiotice (1, 2).

Prin acest studiu retrospectiv am furnizat informații medicilor intensiviști cu privire la modificarea fondului microbiologic, prin emergența speciilor de *A.baumannii, S.maltophilia* şi creşterea prevalenței fungilor.

TABELUL II

Repartiția unui fenotip rezistent în UTI (02.04.2002-14.06.2005 *versus* 15.06.2005-27.08.2008)

Fenotip	Test Fisher	Test Pearson		Test Yates	
		χ^2	p	χ^2	p
BGN nefermentativi R˚	0.46	1.21	0.27	0.54	0.46
BGN nefermentativi BLSE[1]	1.00	0.12	0.73	0.12	0.73
BGN nefermentativi RMA[2]	0.26	2.22	0.14	1.25	0.26
Enterobacteriaceae R˚	0.56	1.08	0.30	0.12	0.73
Enterobacteriaceae BLSE[3]	1.00	0.12	0.73	0.12	0.73
Enterobacteriaceae RMA[4]	0.26	2.22	0.14	1.25	0.26
SAMR	1.00	0.70	0.40	0.00	1.00
Total BLSE	1.00	0.19	0.66	0.00	1.00
Total RMA	0.16	2.28	0.13	1.3	0.20
Total BGN R˚	0.16	2.1	0.14	1.27	0.27

BGN, bacili Gram-negativ;
˚, Rezistent;[1], *P. aeruginosa*; [2],*P.aeruginosa, A.baumannii, S.malthophilia*;
[3], *E.coli, K.pneumoniae*; [4], *K. pneumoniae*.

TABELUL III

Probabilitatea de apariție a unui fenotip rezistent în UTI aferentă secției de chirurgie de la IBCVI

Fenotip	Primul caz	Ultimul caz	Distribuție Student		
			Limita inferioară	Limita superioară	p
BGN nefermentativi R˚	07/02	06/08	01/03	08/08*	0.08
BGN nefermentativi BLSE[1]	04/05	02/06	10/03	08/07	0.05
BGN nefermentativi RMA[2]	07/02	06/08	11/02	08/08*	0.09
Enterobacteriaceae R˚	12/05	08/08	04/02*	08/08*	0.23
Enterobacteriaceae BLSE[3]	12/05	08/08	04/02*	08/08*	0.23
Enterobacteriaceae RMA[4]	12/05	12/05	-	-	-
SAMR	04/06	07/07	04/02*	08/08*	0.21
Total BLSE	04/05	08/08	04/03	08/08*	0.08
Total RMA	07/02	06/08	01/03	08/08*	0.09
Total BGN R˚	07/02	08/08	05/03	08/08*	0.09

BGN, bacili Gram-negativ;
˚, Rezistent; [1],*P.aeruginosa*; [2], *P.aeruginosa, A.baumannii, S.malthophilia*;
[3], *E.coli, K.pneumoniae*; [4], *K. pneumoniae*; *, limitat de perioada analizată (02.04.2002-27.08.2008).

La IBCVI, bacilii Gram-negativ (64,29%) s-au dovedit a fi cel mai frecvent implicați în PAV, ceea ce se corelează cu rezultatele obținute de Chastre şi Fagon (2) - 48,1% sau Rello et al. (10): Montevideo (62,04%), Barcelona (58,73%), Paris (51,87%) şi Sevilla (52,71%). În contrast, distribuția şi prezența acestora (tab. IV), dar şi a celor-lalți agenți etiologici ai PAV este diferită pentru fiecare UTI (10). Toate aceste date susțin afirmația: fiecare UTI are un spectru etiologic propriu (1, 2). În studiul nostru, fungii (23,81%) ocupă locul 2 în etiologia PAV. Datele noastre sunt similare cu cele obținute de Wójkowska et al (11) în 2009: cei mai comuni agenți etiologici ai PAV după chirurgie coronariană sunt BGN (52,2%) şi fungii (14,6%).

TABELUL IV

Variația distribuției agenților etiologici în PAV

Agenții etiologici	M* (10)	B* (10)	P* (10)	S* (10)	IBCVI
P.aeruginosa %	13,28	52,44	15,91	14,54	23,81
A.baumannii %	18,69	0	8,98	27,27	19,05
S.maltophilia %	6,5	0	2,49	0	11,91
Enterobacteriaceae %	23,57	6,29	24,49	10,9	9,52
S. aureus (%)	17,89	11,18	21,22	18,18	4,76

* Montevideo, Barcelona, Paris, Sevilla.

Incidența în creştere a BGN în etiologia PAV la IBCVI, de la 50% în prima perioadă la 68,75% în a doua perioadă, a fost însoțită de emergența tulpinilor rezistente şi multirezistente la antibiotice: pentru tulpinile producătoare de BLSE de la 10% la 15,62%, iar pentru RMA de la 20% la 46,86%. *Pseudomonas aeruginosa* a fost singurul BGN prezent în prima perioadă: BLSE 10% şi RMA 20%. După 14.06.2005, odată cu apariția speciilor de *A. baumannii* şi *S. malthophilia* am constatat creşterea prevalenței fenotipului RMA (46,86%), fiind mai frecvent la *A.baumannii* (0%-15,62%), *S.maltophilia* (0%-15,62%) față de *P.aeruginosa* (20%-12,5%), *K.pneumoniae* (0%-3,12%) şi *E.coli* (0%-0%). În a doua perioadă fenotipul BLSE (15,62%) a fost mai frecvent la *K.pneumoniae* (0%-6,25%) şi *E.coli* (0%-3,12%) comparativ cu *P.aeruginosa* (10%-6,25%), iar SAMR a crescut de la 0% la 6,25%.

Majoritatea pacienților (92,86%) au fost spitalizați în IBCVI cel puțin 5 zile înainte de operație şi au prezentat IC. Toți au avut intervenții cu BCP şi au primit antibiotice până la debutul PAV.

Terapia antimicrobiană în ultimele 90 de zile, spitalizarea actuală de cel puțin 5 zile şi imunosupresia, sunt factori de risc pentru implicarea unui patogen cu rezistență la antibiotice (1). Pacienți imunodeprimați sunt şi cei cu IC (12), iar operațiile efectuate cu BCP determină o supresie temporară a imunității începând din ziua 1 până în ziua 3-5 postoperator (13). În plus, terapia cu antibiotice are un rol major în selectarea microorganismelor cu rezistență intrinsecă (*P.aeruginosa*, fungi) sau dobândită (2).

Emergența speciilor de *A.baumannii*, *S.maltophilia* a condus la creşterea prevalenței tulpinilor cu fenotipul RMA în UTI post-chirurgie cardiacă de la IBCVI.

Rezultatele testelor χ^2 şi exact Fisher nu pot preciza semnificativ statistic ($p<0.05$) apartenența *strict* la una din perioade a niciunui fenotip implicat în etiologia PAV. Rezultatele arată totuşi tendința de creştere a numărului de apariții după 14.06.2005 a fenotipurilor RMA şi, ca o consecință totalitatea BGN rezistenți (Total BGN R), datorită valorilor crescute pentru χ^2 şi corespunzător coborâte pentru p (Fisher) (tab. II).

Prin analiza distribuției Student, se pot identifica uşor fenotipurile pentru care nu se poate pune în evidență o evoluție în sensul "apariției" respectiv "dispariției" din UTI a fenotipului respectiv deoarece limitele intervalului de încredere ($p = 0.05$) depăşesc limitele perioadei analizate (tab. III). Consecința imediată este obținerea unei probabilități de eroare mai mari în specificarea intervalului ($p > 0.05$). În cazul mai multor fenotipuri se poate identifica totuşi un moment de apariție semnificativ statistic (limită inferioară ulterioară datei de debut a perioadei analizate - 04/2002) ceea ce semnifică probabilitate foarte mică ($p < 0.025$) de apariție a fenotipului corespunzător înainte de data calculată (tab. III).

În prezent, conform datelor din literatură, UTI post chirurgie cardiacă se confruntă cu emergența fenotipurilor rezistente, multirezistente până la panrezistente, mai ales pe seama BGN nefermentativi (3, 14). De Santo et al (3), în 2008 a constatat că 61,7 % dintre bacteriile Gram-negativ implicate în infecțiile nosocomiale după intervențiile pe cord au fost rezistente la antibiotice.

CONCLUZII

La Institutul de Boli Cardiovasculare din Iaşi în unitatea de terapie intensivă a clinicii de chirurgie cardiovasculară cei mai comuni agenți etiologici ai pneumoniei asociată ventilației mecanice post-chirurgie

cardiacă majoră sunt, în ordine descrescătoare, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* şi *Candida albicans*. Unitatea de terapie intensivă s-a confruntat după 14.06.2005 cu creşterea prevalenței fenotipurilor bacteriene multirezistente şi emergența speciilor cu rezistență intrinsecă la numeroase antibiotice. Prin utilizarea pe scară largă a antibioticelor UTI este placa turnantă pentru emergența, amplificarea şi răspândirea patogenilor rezistenți la antibiotice. Apare necesar un efort multidisciplinar de a limita acest fenomen prin supravegherea igienizării activităților cotidiene, procedurilor aseptice şi îngrijirii pacienților, dar şi printr-o țintire şi utilizare judicioasă a antibioticoterapiei.

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ROLUL ENDOSCOPIEI DIGESTIVE SUPERIOARE ÎN EVALUAREA HIPERTENSIUNII PORTALE

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THE ROLE OF UPPER GASTROINTESTINAL ENDOSCOPY IN EVALUATION OF PORTAL HYPERTENSION (Abstract): Portal hypertension is the main cause of complications of hepatic diseases determining portosystemic collateral circulation, ascites and gastroesophageal varices. **Aim** of the study is to review current opinions and to correlate clinical and endoscopic signs determined by portal hypertension. **Material and methods:** We performed a retrospective study on 150 patients diagnosed and treated for diseases associated with portal hypertension at the Institute of Hepatology and Gastroenterology from Iasi between 2007 and 2009. **Results and discussion:** Most of the patients presented cirrhosis (112 cases) mainly of toxic (63 cases) and viral (31 cases) etiology. In order to appreciate bleeding from esophageal varices we applied Forrest criteria and identified type Ia bleeding in 26,6% of cases and type Ib bleeding at 73,4% of patients. In 15% of cases, variceal bleeding represented the first symptom of slowly progressing hepatic cirrhosis. **Conclusions:** The objective of subsequent evaluation of small varices is detecting size increase, an important prognostic and therapeutic index. **Key words:** PORTAL HYPERTENSION, ESOPHAGEAL VARICES, UPPER GASTROINTESTINAL TRACT ENDOSCOPY.

Endoscopia constituie cea mai sensibilă metodă de identificare şi clasificare în funcţie de risc a dilataţiilor varicoase, motiv pentru care se constituie şi într-o procedură de elecţie în diagnosticul şi tratamentul statusului variceal asociat HTP. Diagnosticul endoscopic al varicelor esofagiene se constituie într-un factor de prognostic relevant în ceea ce priveşte riscul predictiv individual de hemoragie, corelat direct cu gradul de dilatare şi diametrul varicelor esofagiene, gastropatia portală, apartenenţa la clasa Child (1, 2).Scopul acestui studiu este a trece în revistă opiniile moderne cu privire la complicaţiile hipertensiunii portale şi de a analiza comparativ aspectele clinice şi endoscopice evidenţiate la pacienţii cu hipertensiune portală.

MATERIAL ŞI METODĂ

Cercetarea de faţă realizează un studiu retrospectiv comparativ pe o perioadă de 3 ani, cuprinsă între 2007 şi 2009 pe un număr de 150 pacienţi diagnosticaţi, investigaţi endoscopic şi trataţi în cadrul Institutului de Gastroenterologie şi Hepatologie pentru complicaţii asociate hipertensiunii portale.

Baza de date a studiului de faţă a fost realizată folosind informaţiile obţinute din foile de observaţie clinică ale pacienţilor selectaţi şi înregistrările din protocoalele investigaţiilor endoscopice. Datele obţinute au fost analizate şi exprimate procentual, iar asocierile dintre diferiţi factori, au fost interpretate cu ajutorul testelor de corelaţie. Prelucrarea statistică a fost realizată prin intermediul programului Statistica 8.0.

Criteriile de includere a pacienţilor în loturile de studiu au asociat date de identificare (vârstă, sex, mediu de proveniență, durata spitalizării, traseul interclinic), motivele internării, diagnosticul de internare; comorbidităţi, antecedente medicale şi chirurgicale.

Criteriile de excludere a pacienţilor din lotul de studiu au asociat pacienţi care au refuzat indicaţia diagnostică sau includerea în lotul de studiu, pacienţi cu vârsta sub 16 ani.

REZULTATE

Din cei 150 de pacienţi incluşi în studiu, 106 au fost de sex masculin, iar 44 de sex feminin. Vârsta medie a pacienţilor cuprinşi în lot a fost de 54,32±9,70 ani, cu valori extreme de 34 şi 68 de ani. Majoritatea pacienţilor (112 cazuri) au prezentat afectare hepatică de tip cirotic, predominant de etiologie toxică (63 de pacienţi), urmată de etiologia virală (31 de pacienţi), intricarea celor două mecanisme etiopatogenice fiind identificată în 18 de cazuri.

Prognosticul pacienţilor conform clasificării Child este redat în tabelul I.

Varice esofagiene de diferite grade au fost decelate în cazul a 65,5% pacienţi de sex masculin şi a 78,3% pacienţi de sex feminin (tab. II). Varice gastrice au fost observate numai în cazul pacienţilor de sex masculin (6,2%).

TABELUL I

Încadrarea pacienţilor din lot în clasificarea Child

	Masculin	**Feminin**
Child A	41,5%	43,18%
Child B	45,28%	47,72%
Child C	13,22%	9,1%

TABELUL II

Clasificarea pacienţilor în funcţie de severitatea varicelor esofagiene

Varice absente	29,28%
Varice gradul I	13,81%
Varice gradul II	25,66%
Varice gradul III	31,25%

Hemoragia digestivă superioară (HDS) a fost prezentă în antecedentele a 33,6% pacienţi de sex masculin şi a 35,1% pacienţi de sex feminin. HDS, uşoară în majoritatea cazurilor, exteriorizată sub formă de hematemeză şi melenă de peste 60% dintre pacienţi.

Cei mai mulţi dintre pacienţi (46,7%) nu au primit tratament HDS anterior internării curente, iar beta-blocantele, selective şi neselective, au reprezentat terapia de elecţie (tab. III) în majoritatea situaţiilor.

TABELUL III

Tratamentul de prevenire al HDS în lotul studiat

Măsura terapeutică	
Beta-blocante neselective	25,98%
Beta-blocante + nitraţi	6,9%
Beta-blocante + IPP	14,8%
Beta-blocante + nitraţi + IPP	2,3%
Nitraţi	3,32%
Fără tratament	46,7%

Endoscopia digestivă superioară (EDS) s-a efectuat prin folosirea unui fibroscop de

tip Olympus GIF tip XQ20, dotat cu o înaltă flexibilitate şi elasticitate. Varicele esofagiene au fost diagnosticate la aproximativ 40% din pacienţii compensaţi şi la 60% din cei care prezintă ascita ca simptom principal în momentul stabilirii diagnosticului de ciroză. Evaluarea endoscopică a varicelor esofagiene s-a efectuat în funcţie de clasificarea Sarin (tab. IV)

În 15% din cazuri, hemoragia variceală a fost prima manifestare clinică a cirozei hepatice cu evoluţie latentă.

TABELUL IV

Repartiţia pacienţilor examinaţi endoscopic în funcţie de severitatea varicelor esofagiene

Varice izolate	42%
Varice tip I	16,67%
Varice tip IIa	22,67%
Varice tip IIb	11,33%
Varice tip III	7,33%

În vederea aprecierii activităţii hemoragiilor variceale au fost aplicate criteriile Forrest, identificându-se hemoragie în jet (Forrest Ia) la 26,6% din pacienţi, iar hemoragie Forrest Ib la 73,4% din cazuri (fig. 1).

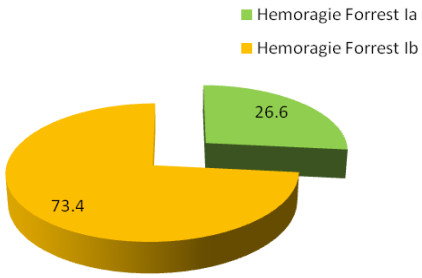

Fig. 1. Repartiţia pacienţilor în funcţie de severitatea hemoragiei variceale

DISCUŢII

Incidenţa varicelor esofagiene la pacienţii cirotici se estimează a fi de 5% pe an, motiv pentru care tendinţa actuală este de a practica endoscopia ca metodă de screening la toţi pacienţii cu suspiciune de HTP. La pacienţii care nu prezintă varice la endoscopia iniţială, trebuie efectuată o evaluare secundară în vederea identificării dezvoltării varicelor înainte ca acestea să sângereze, consensul general fiind de a practica o a două endoscopie după 2-3 ani la toţi aceşti pacienţi. La pacienţii care prezintă varice mici la endoscopia iniţială, obiectivul evaluărilor subsecvente îl constituie detectarea progresiei de la varice mici la varice mari, ca mijloc important de prognostic şi terapie (3, 4).

Rata de progresie a dimensiunilor varicelor este de 10-15% pe an, motiv pentru care se consideră că endoscopia trebuie repetată la fiecare 1-2 ani la pacienţii care prezintă varice mici la examenul iniţial. În vederea interpretării corecte a rezultatelor studiilor clinice este utilă standardizarea aspectului endoscopic al varicelor (evaluarea prezenţei şi a dimensiunilor în plenitudine completă înaintea îndepărtării endoscopului) şi obţinerea de imagini digitale care pot fi revăzute şi comparate cu cele din literatura de specialitate. De asemenea, este foarte importantă ameliorarea acceptabilităţii procedurii prin realizarea unei sedări corecte cu droguri care nu modifică semnificativ HTP (5, 6).

Endoscopia evaluează caracteristicile varicelor gastroesofagiene, caracteristici extrem de importante prin valoarea lor prognostică în ceea ce priveşte hemoragia.

Ambii factori descrişi anterior şi gradul afectării hepatice sunt cuantificaţi în clasa Child-Pugh, şi constituie cel mai utilizat index prognostic al sângerărilor variceale (6) (tab. V).

Din punct de vedere practic, pacienţii cu varice esofagiene peste 5 mm în diametru prezintă risc major de sângerare variceală, mai ales dacă sunt încadraţi în clasele B sau C.

TABELUL V
Clasificarea Child-Pugh a bolilor hepatice cronice

Parametru	1 punct	2 puncte	3 puncte	Unitate de măsură
Bilirubina totală	<34 (<2)	34-50 (2-3)	>50 (>3)	μmol/l (mg/dl)
Albumina serică	>35	28-35	<28	g/l
INR	<1.7	1.71-2.20	>2.2	-
Ascită	Absent	Reversibilă medicamentos	Refractară	-
Encefalopatie portală	Absent	Gradele I-II (sau reversibilă medicamentos)	Gradele III-IV (sau refractară)	-

Puncte	Clasa	Supraviețuire la 1 an	Supraviețuire la 2 ani
5-6	A	100%	85%
7-9	B	81%	57%
10-15	C	45%	35%

Identificarea varicelor gastrice la endoscopie poate fi realizată cu unele dificultăți tehnice care, momentan, au fost depăşite prin introducerea endosonografiei. Astfel, se pot identifica varicele gastrice ca fiind structuri anecogene, rotunde, localizate majoritar în submucoasa gastrică. Recent a fost introdus un indice endoscopic care combină dimensiunea varicelor cu prezența gastropatiei hipertensive portale şi a varicelor gastrice. Valoarea sa în evaluarea HTP rămâne încă discutabilă.

CONCLUZII

Datorită mortalității crescute a varicelor esofagiene este importantă efectuarea endoscopiei la fiecare pacient cu HTP. Prezența varicelor gastroesofagiene confirmă diagnosticul deşi absența lor nu exclude HTP. Există situații în care varicele esofagiene sunt diagnosticate incidental în cazul unor pacienți la care se practică endoscopie pentru alte afecțiuni gastroesofagiene, situație în care etiologia HTP trebuie imediat identificată. În vederea interpretării corecte a rezultatelor studiilor clinice este utilă standardizarea aspectului endoscopic al varicelor (evaluare prezenței şi a dimensiunilor în plenitudine completă, înaintea îndepărtării endoscopului) şi obținerea de imagini digitale care pot fi revăzute şi comparate.

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SINDROMUL CARDIORENAL – O ENTITATE INCOMPLET DEFINITĂ

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CARDIORENAL SYNDROME (Abstract): Cardiac and renal disease are common and frequently coexist, resulting in increased risk of mortality, morbidity and cost of care. The interaction between heart and kidney is refered to as cardiorenal syndrome, but a consensus definition has not been established. Also, there are limited data about the true incidence, the pathophisiology is poorly understood and standardized diagnostic criteria are lacking. Because the process is complex, treatment can be a challenge despite the novel therapies. This paper addresses all the complex interactive aspects of the cardiorenal relationship, from pathophysiology to epidemiology, diagnosis and treatment, to have more clear perspectives on the future therapeutic approaches to this deadly association. **Key words**: CARDIORENAL SYNDROME, HEART FAILURE, RENAL DYSFUNCTION, DIURETICS, DIURETIC RESISTANCE.

IMPORTANŢA PROBLEMEI: PREVALENŢA REALĂ NECUNOSCUTĂ

Relaţia între boala renală cronică (BRC) şi insuficienţa cardiacă (IC) este dovedită de numeroase trialuri şi studii clinice. Relaţia este bidirecţională, se manifestă din stadii precoce de afectare a unuia din cele două organe şi are impact asupra prognosticului cardiovascular.

Incidenţa afectării cardiorenale are un trend ascendent evident din cauza creşterii ponderii populaţiei vârstnice, a hipertensiunii arteriale, diabetului zaharat, obezităţii şi altor factori de risc cardiovascular. Primele date epidemiologice consistente sunt oferite de trialul Acute Decompensated Heart Failure National Registry (ADHERE) care a înrolat 105.388 de pacienţi neselecţionaţi spitalizaţi pentru IC acută (1). Trialul raportează că 1 din 3 pacienţi au diagnostic concomitent de BRC. Metaanaliza lui Smith confirmă frecvenţa crescută a afectării renale în IC (63% din pacienţi, dintre care 20% cu afectare moderată şi severă) precum şi relaţia cu severitatea IC (clearence de creatinină < 30 ml/min la 39% din cazurile de IC clasa NYHA IV şi 31% din cazurile de IC clasa NYHA III) (2). Interesant este că BRC este prezentă în egală măsură în IC cu fracţie de ejecţie scăzută sau păstrată (2, 3, 4).

Studiile ulterioare atrag atenţia asupra *semnificaţiei prognostice a BRC la pacientul cu IC*. Deteriorarea funcţiei renale se produce la 27-45% din cazurile de IC acută, în general în faza iniţială a spitalizării. La aceste categorii de pacienţi o creştere cu 0,3 mg % a creatininei determină o creştere a duratei spitalizării şi a mor-

talităţii pe termen scurt şi lung (5). Trialul ESCAPE subliniază că episoadele de decompensare a IC sunt asociate frecvent cu disfuncţia renală care necesită doze crescute de diuretice (6).

Implicaţiile prognostice trebuie privite şi din perspectiva *rolului IC asupra afectării renale*. Problema este importantă atât din cauza incidenţei crescute a BRC (în SUA peste 8 milioane de indivizi au rata de filtrare glomerulară < 60 ml/min conform raportului NHANES III), cât şi a riscului asociat înalt pentru infarct miocardic, IC, mortalitate de cauză cardiovasculară (2, 7). Metaanaliza lui Tonelli arată că indivizii cu BRC primară au risc de mortalitate cardiovasculară de 10-20 de ori mai mare (8), iar IC este o prezenţă comună în stadiile avansate, atingând rate de până la 33% (9).

Problema actuală din punct de vedere epidemiologic *este uniformizarea definirii disfuncţiei renale*. Cu alte cuvinte, care este parametrul standard şi gradul de modificare necesar pentru definirea SCR? Metaanaliza lui Smith pe 16 trialuri (80.000 de pacienţi) arată că afectarea renală este definită variabil ca rată de filtrare glomerulară estimată (RFGe) < 90 ml/min, creatinină > 1 mg %, cystatin C > 1,03 mg % etc (2). Pe de altă parte, relaţia disfuncţiei renale cu riscul de morbiditate şi mortalitate prin IC este raportată diferit. De exemplu, în studiul Enhanced Feedback for Effective Cardiac Treatment (EFFECT) ureea serică şi hiponatremia sunt considerate predictori independenţi ai prognosticului cardiovascular, care contribuie la creşterea cu 20% a mortalităţii prin IC (10). Owan demonstrează că riscul de mortalitate prin IC creşte cu 15% pentru fiecare creştere cu 0,5 mg % a creatininei (3). Hillege subliniază că descreşterea cu fiecare 10 ml/min a RFGe duce la creşterea cu 7% a mortalităţii cardiovasculare (11). Aspectul este important deoarece, conform National Kidney Foundation, definirea standard a BRC se face în funcţie de valorile RFGe, parametru cu valoare dovedită de predictor independent al mortalităţii în IC (1, 12, 13) superior chiar fracţiei de ejecţie (14).

Problema rămâne deschisă dar este esenţială pentru stabilirea incidenţei reale a sindromului cardiorenal (SCR) în trialuri prospective randomizate. La ora actuală putem afirma doar că SCR este o prezenţă comună în practica clinică.

DEFINIŢIA SINDROMULUI CARDIORENAL: O EVALUARE ÎN PROGRES

Natura complexă şi insuficient clarificată a SCR nu a permis elaborarea unei definiţii universal acceptate. În 2004 grupul de lucru al National Heart, Lung, and Blood Institute defineşte SCR ca "rezultatul interacţiunii între rinichi şi alte compartimente circulatorii care cresc volumul circulant şi agravează simptomele de IC şi progresia bolii. În forma avansată terapia de ameliorare a simptomelor de IC congestivă este limitată de agravarea consecutivă a funcţiei renale" (15). Ulterior sunt propuse diverse definiţii:

- "o condiţie fiziopatologică în care disfuncţia combinată cardiacă şi renală amplifică progresia insuficienţei fiecăruia din organe şi conduce la creşterea morbi-mortalităţii" (16)
- "prezenţa sau dezvoltarea disfuncţiei renale la pacientul cu IC" (17)
- "sindromul în care cordul sau rinichiul nu poate compensa disfuncţia celuilalt organ, ducând la un cerc vicios care determină decompensarea întregului sistem cardiocirculator" (18).

Toate definiţiile sunt considerate în prezent "prea simpliste pentru a fi exacte" deoarece reflectă prin enunţuri succinte modalitatea comună de utilizare a termenului, care pune în centrul problemei IC (19). La Congresul Mondial de Nefrologie din 2008, Ronco şi colab. propun o nouă definiţie a SCR. Autorii utilizează termenul de *sindroame cardiorenale* şi subliniază două aspecte: injuria primară poate fi cardiacă sau renală şi poate avea caracter acut sau cronic. Conform acestui concept, SCR este "dezordinea fiziopatologică a cordului şi rinichiului în care disfuncţia acută sau cronică a unui organ poate induce disfuncţia acută sau cronică a celuilalt organ" (20). Pornind de la o amplă analiză a secvenţei mecanismelor fiziopatologice implicate definiţia este completată cu o *clasificare* inovatoare şi foarte practică *în 5 tipuri* (21) (tab. I). Definiţia necesită câteva precizări (21):

- tipul 3 este incomplet caracterizat deoarece definirea uniformă a insuficienţei renale acute este introdusă recent în studii (criteriile RIFLE). În această categorie pot fi incluşi pacienţii cu stenoză bilaterală de arteră renală, cei din secţiile de cardio-chirurgie în perioada postoperatorie sau supuşi unei explorări angiografice cu substanţă de contrast.
- tipul 5, deşi nu se caracterizează prin afectare primară cardiacă sau renală, este inclus în SCR. Afecţiunile sistemice care conduc la injuria şi/sau disfuncţia simultană a celor două organe acţionează prin mecanisme comune în SCR şi anume stres oxidativ, inflamaţie, disfuncţie endotelială, activare neuroumorală.

TABELUL I

Clasificarea sindroamelor cardiorenale

Tip	Denumire	Mecanism	Condiţii clinice
1	Sdr. cardiorenal acut (27-40% în IC acută)	Agravarea bruscă a funcţiei cardiace determină insuficienţă renală acută	Şoc cardiogen, IC decompensată acut, sindrom coronarian acut
2	Sdr. cardiorenal cronic (63% în IC congestivă)	Insuficienţa cardiacă cronică determină afectare progresivă a funcţiei renale	IC cronică congestivă
3	Sdr. renocardiac acut	Agravarea bruscă a funcţiei renale determină insuficienţă cardiacă acută (frecvent edem pulmonar acut)	Insuficienţă renală acută prin ischemie renală acută, glomerulonefrite, stenoză bilaterală de arteră renală, nefropatie de contrast
4	Sdr. renocardiac cronic	Insuficienţa renală cronică contribuie la declinul funcţiei cardiace	Nefropatii glomerulare sau interstiţiale cronice
5	Sdr. cardiorenal secundar	Condiţie sistemică ce determină disfuncţie cardiacă şi renală concomitentă	Diabet zaharat, sepsis, lupus eritematos sistemic, amiloidoză, afecţiuni cronice inflamatorii

- sistemul de clasificare este considerat perfectibil deoarece mulţi pacienţi pot popula sau pot migra între diferite tipuri în cursul evoluţiei afecţiunii. Grupul de lucru ADQI opinează pentru îmbunătăţirea clasificării cu noi subtipuri ca situaţiile de disfuncţie reversibilă sau tranzitorie, afectarea rapid progresivă, boala stabilă etc.
- există deja opinia formulată de unii autori că tipurile 2 şi 4 ar trebui unificate, deoarece distincţia în practică nu este întotdeauna posibilă iar terapia nu este semnificativ diferită (22).

Indiferent de opinie, există consens că scopul final în definirea articulată a SCR rămâne facilitarea studiilor epidemiologice prospective cu design clar şi omogen, care să permită medicului practician identificarea populaţiilor ţintă pentru intervenţia terapeutică, diagnosticul după criterii standardizate, prevenţia şi tratamentul adecvat.

FIZIOPATOLOGIA: ÎNCĂ O NECUNOSCUTĂ

SCR este considerat mai mult decât o afectare simultană cardiacă şi renală. Factorii patogenici implicaţi (hipertensiunea arterială, diabetul zaharat, obezitatea, boala coronariană etc.) reprezintă precursori comuni ai IC şi BRC. Pacienţii cu SCR convieţuiesc cu un mediu fragil, dominat de modificări hemodinamice, neuro-umorale dar şi de mulţi alţi factori incomplet descifraţi şi clarificaţi (23).

Există totuşi câteva ipoteze cristalizate (24, 25):

1. ***teoria hemodinamică*** este explicată în mod tradiţional prin *mecanismul hipovolemic*. Declinul RFGe în IC reflectă perfuzia inadecvată renală secundară scăderii debitului cardiac, adică apare insuficienţa renală funcţională prin mecanism prerenal. Rezultă eliberare de renină şi activarea sistemului renină-angiotensină-aldosteron (SRRA) care determină retenţie hidro-salină, constricţia arteriolei aferente şi scăderea perfuziei glomerulare, stimulare neuro-umorală cu activitate proinflamatorie, proliferativă, profibrotică. Se discută şi eliberarea unui "factor depresor miocardic" care afectează suplimentar debitul cardiac (Blake şi colab. cit. de 26). Mecanismul este corect dar explică parţial evoluţia pacienţilor cu SCR. De asemenea nu s-a dovedit o relaţie evidentă şi constantă între evoluţia funcţiei cardiace şi renale pe termen scurt şi lung (24). La ora actuală este susţinută *teoria hipervolemică*, o reactualizare a modelului experimental descris de Watkins în 1931. Conceptul central este *congestia venoasă*, iar termenul propus în acest sens este de insuficienţă renală congestivă. Conform teoriei, creşterea presiunii venoase centrale se transmite retrograd şi determină:

a. creşterea presiunii în venele renale. Rezultă creşterea presiunii interstiţiale renale şi ischemia parenchimului renal. Concomitent, creşterea presiunii venoase într-un organ încapsulat cum este rinichiul, duce la distensia venulelor din jurul tubilor distali care pot fi obliteraţi. În ansamblu ischemia generează activare neuro-umorală cu efecte hemodinamice nocive.

b. creşterea presiunii intraabdominale ca parte a "sindromului de compartimentare abdominală". Mecanismul apare la peste 60% din cazurile avansate de IC. Rezultă un efect direct de compresiune renală dar şi de creştere a presiunii venoase centrale. Se închide un cerc vicios care compromite funcţia renală.

2. ***teoria conexiunii cardio-renale*** este o extensie a modelului Guytonian de control a volemiei şi tensiunii arteriale (TA) prin interacţiunea cord-rinichi, care iniţiază

răspunsul fiziologic numit reacţie hemodinamică defensivă (16, 27). La conceptul hemodinamic clasic, Bongartz adaugă rolul "*conectorilor cardio-renali*" reprezentaţi de balanţa improprie oxid nitric-radicali liberi de oxigen, activarea SRRA şi a sistemului nervos simpatic, inflamaţia. Când unul din organe devine insuficient conectorii interacţionează sinergic dar dezechilibrat şi întreţin deteriorarea funcţiei cardiace şi renale (19, 28).

3. ***ţintele renale potenţiale*** sunt reprezentate de factori/mecanisme clasice în IC: *sistemul arginin-vasopresină, sistemul peptidelor natriuretice*, mai nou *adenozina* cu efect negativ asupra feed-back-ului tubuloglomerular (29). Studiile clinice şi experimentale sugerează că aceste mecanisme ar produce în mod direct injurie tubulointerstiţială, independent de prezenţa sau absenţa IC. La aceste ţinte se adaugă rolul binecunoscut al deficitului de eritropoietină şi anemiei, frecvent întâlnite în SCR şi IC. Importanţa problemei este ilustrată de conceptul de *sindrom cardiorenal-anemie* sau "triada mortală" (24, 30, 31, 32) conform căruia anemia este actorul principal în inducerea inflamaţiei, stresului oxidativ şi apoptozei miocardice şi renale.

DIAGNOSTICUL ÎN PRACTICĂ: O PROBLEMĂ INCOMPLET REZOLVATĂ

Depistarea SCR presupune *identificarea factorilor de risc* pentru agravarea funcţiei renale. Profilul clinic este comun – pacient vârstnic, diabetic, hipertensiv, cu sindrom anemic, care poate avea istoric de infarct miocardic, IC sau BRC şi este sub tratament cronic cu diuretice, inhibitori ai enzimei de conversie a angiotensinei (IECA), sartani sau are tratament recent cu antiinflamatorii nesteroidiene. În mod particular prezenţa afectării renale intrinseci este unul din cei mai importanţi factori de risc pentru evoluţia spre SCR.

Pentru confirmarea diagnosticului grupul ADQI a propus o baterie largă de *biomarkeri cardiaci şi renali* utili în diferite subtipuri de SCR (21). Problema practică este specificitatea şi sensibilitatea pentru SCR, precum şi disponibilitatea, costul biomarkerilor. La ora actuală biomarkerii recomandaţi şi utilizaţi pe scară largă sunt *BNP (NT-proBNP) şi RFGe*. Alţi markeri ce necesită confirmare în studii viitoare sunt:

1. cardiaci – mieloperoxidaza, citokine (TNF-α, IL-6, IL-8, IL-18), procalcitonina; troponinele rămân utile în cazul sindroamelor coronariene acute.
2. renali:

a. de injurie renală: *NGAL* (gelatinaza neutrofilă asociată lipocalinei) – marker foarte precoce, înalt sensibil şi specific pentru afectarea ischemică sau nefrotoxică, KIM-1 (kidney injury molecule-1), N-acetil-ß-$_{(D)}$glucozaminidaza, IL-18, netrin-1, α şi γ-GST (glutation-S-transferaza), L-FABP (acizi graşi tipul L care leagă proteine) etc. Recent se discută şi rolul urotensinei II şi renalazei urinare (33, 34).

b. cystatin C (seric şi urinar) – marker cu nivel de predicţie superioară asupra funcţiei glomerulare respectiv tubulare, comparativ cu creatinina serică.

NGAL urinar şi plasmatic, cystatin C sunt biomarkerii cu şansele cele mai mari de a fi integraţi în practica clinică în viitorul apropiat.

Optimizarea supravegherii în SCR înseamnă monitorizarea:

1. funcţiei renale prin RFGe în mod obligatoriu. Scăderea cu 9-15 ml/min/1,73 m^2 este suficientă pentru a atrage atenţia asupra agravării funcţiei renale. Modificarea RFGe este precoce şi mult mai sensibilă

comparativ cu creatinina serică, utilizată ca surogat al funcţiei renale. Între cei doi parametri există o relaţie exponenţială, adică o modificare minoră a creatininei reflectă reduceri semnificative ale RFGe (35, 36). Pe de altă parte, în SCR, ureea serică este un marker înalt predictiv al mortalităţii în spital (trialul ADHERE) dar şi al evoluţiei pe termen scurt şi lung (37, 38). Hiponatremia refractară în ciuda tratamentului diuretic este un alt indicator al incapacităţii renale de a compensa hipervolemia independent de debitul cardiac (26). Markerul clinic cel mai sensibil pentru urmărire rămâne măsurarea greutăţii (39). În insuficienţa renală acută se impune pentru standardizare utilizarea criteriilor RIFLE (21).

2. cordului prin investigare seriată ecocardiografică, ce oferă posibilitatea evaluării neinvazive hemodinamice precise. Metoda rămâne standardul de aur, în timp ce monitorizarea invazivă a presiunii venoase centrale şi pulmonare capilare blocate rămâne controversată sau cel puţin limitată la anumite categorii de pacienţi (40). Grupul consens ADQI remarcă utilitatea unei metode deja disponibile – analiza vectorului de bioimpedanţă în monitorizarea şi managementul stării de hidratare (21).

TRATAMENTUL: O PROVOCARE MAJORĂ

Sarcina de a trata pacientul cu SCR este dificilă din mai multe motive: nu există dovezi epidemiologice consistente întrucât majoritatea trialurilor exclud disfuncţia renală, tratamentul este în general adresat IC în timp ce urmărirea funcţiei renale trece pe un plan secundar, nu există o terapie eficientă pentru toate categoriile de pacienţi. Terapia actuală este mai degrabă empirică decât bazată pe dovezi, iar decizia de tratament trebuie adaptată individual operând cu resursele disponibile (19). Există de *3 principii majore* ale conduitei terapeutice în SCR: evitarea agravării evoluţiei pe termen scurt cu terapia preexistentă, urmărirea activă a disfuncţiei cardio-renale, utilizarea medicaţiei protectoare renale (26).

Algoritmul practic de instituire a terapiei în SCR *trebuie să răspundă la 3 întrebări* (41):

1. care este statusul volemic? În caz de hipovolemie se constată agravarea funcţiei renale indusă de terapia IC. Se impune oprirea diureticului de ansă, reducerea dozelor/oprirea medicaţiei cu IECA, sartani, spironolactonă şi umplere volemică. Hipervolemia este sugerată de persistenţa dispneei şi a retenţiei hidrosaline, agravarea funcţiei renale, presiune venoasă centrală crescută. În acest caz problema devine manipularea adecvată a diureticului de ansă. Diureticele rămân piatra de temelie a tratamentului în IC, deşi dovezile epidemiologice sunt discordante vis-à-vis de efectele benefice (42, 43). Funcţia renală este vulnerabilă la terapia diuretică, deoarece există riscul de reducere a RFGe prin mecanisme multiple (42). În ciuda efectelor favorabile pe termen scurt, trialurile atrag atenţia că la 1 din 5 pacienţi edemele nu răspund favorabil la tratament (1), iar creşterea dozelor se asociază cu exces de mortalitate (Ahmed A. cit. de 43). În practica clinică a SCR se constată frecvent că retenţia hidro-salină persistă sau se accentuează în ciuda creşterii dozelor. Situaţia este numită *rezistenţă la diuretice* şi este consecinţa creşterii pragului renal de eliminare a sodiului şi a scăderii eficienţei diureticului. Tratamentul presupune recunoaşterea cauzelor: exces de sodiu în alimentaţie, utilizarea clandestină de antiinflamatorii nesteroidiene, absorbţie scăzută

a diureticului la nivel intestinal sau doze insuficiente de diuretic, hipoalbuminemie etc (44, 45). Al doilea pas este administrarea diureticului de ansă pe cale intravenoasă. Se preferă perfuzia cu doze mari de furosemid în 30-60 de minute şi nu bolusuri repetate, eventual utilizarea altor diuretice de ansă cu absorbţie eficientă pe cale orală (torsemid, bumetanid). O manevră utilă este asocierea dozelor mici de diuretic tiazidic pentru inhibarea reabsorbţiei distale de sodiu. În caz de hipoalbuminemie se recomandă asocierea albuminei umane desodate. În prezent se testează eficienţa dozelor mari de furosemid (500-1000 mg/zi) asociate cu soluţie salină hipertonă (150 ml cu concentraţie 1,4-4,6% în funcţie de sodiul seric) administrate în 30 de minute. Mecanismele posibile ar fi mobilizarea rapidă a fluidelor extravasculare în spaţiul intravascular, de unde pot fi mai uşor eliminate, modularea activării neuroumorale şi a funcţiei renale (46). Deşi există aceste alternative, se aşteaptă elaborarea unei decizii finale în urma publicării rezultatelor trialului DOSE-AHF (47).

O terapie la competiţie cu diureticele în SCR cu hipervolemie este *ultrafiltrarea*. Metoda are la bază principiul aquaferezei, adică eliminarea de lichide izotone cu prezervarea funcţiei renale. Studiată în cadrul trialului UNLOAD, ultrafiltrarea ameliorează semnificativ retenţia hidro-salină şi rata de respitalizare, dar nu şi-a dovedit eficienţa asupra ameliorării funcţiei renale şi supravieţuirii (48). În 2009 a fost iniţiat un nou trial CARESS-HF (CARdiorenal REScue Study in Acute Decompensated Heart Failure) actualmente în desfăşurare (49).

2. cum este perfuzia renală? Aspectul impune optimizarea parametrilor hemodinamici care condiţionează funcţia renală – TA şi debitul cardiac. Primul gest terapeutic este revizuirea medicaţiei vasodilatatoare şi diuretice utilizate (IECA, sartani, betablocante, spironolactonă) cu efecte benefice indiscutabile prin blocare neuroumorală, dar care poate avea impact negativ hemodinamic şi asupra funcţiei renale. În IC acută gestul terapeutic încetăţenit este administrarea agenţilor inotropi pozitivi sau a vasodilatatoarelor injectabile (nitroglicerină, nitroprusiat, nesiritide) după caz. Ca şi în cazul diureticelor există însă numeroase dovezi epidemiologice (trialurile OPTIME, SURVIVE, metaanalize referitoare la dopamină şi nesiritide) care sugerează efectul nefavorabil asupra funcţiei renale şi supravieţuirii pentru toate tipurile de medicaţie (50, 51, 52). În SCR atenţia se îndreaptă în continuare asupra dopaminei în doze mici de 2 γ/kg/min, strategie explorată în cadrul trialului ROSE-AHF (53). În ce priveşte nesiritide există câteva trialuri cu design comun care sprijină efectul benefic asupra funcţiei renale în cazul utilizării dozelor mici, fără efect asupra TA (54, Hebeler cit. de 26, Riter cit. 41). Verdictul va fi dat probabil de trialul ASCEND-HF la finele anului 2010 (55).

3. există afectare renală intrinsecă? În paralel cu asigurarea statusului de euvolemie şi optimizarea perfuziei renale, atenţia trebuie dirijată spre evaluarea cauzei afectării renale şi a factorilor precipitanţi. Afectarea renală intrinsecă trebuie suspectată dacă disfuncţia renală persistă după corecţie hemodinamică, situaţie întâlnită în mod tipic în diabetul zaharat, hipertensiunea arterială sau afectarea renovasculară.

Strategii de viitor în tratamentul SCR (23, 24, 26, 43, 50)

Interesul pentru managementul adecvat, centrat pe terapia protectoare renală, este susţinut de testarea noilor strategii cu clase

terapeutice deja cunoscute sau nestudiate până în prezent:

- *antagoniştii de vasopresină* (vaptani) sunt cunoscuţi din trialuri finalizate (ADVANCE, ACTIV, EVEREST etc.) ca medicaţie utilă în manipularea rezistenţei la diuretice fără a induce tulburări electrolitice, dar fără efect pe mortalitate sau de ameliorare a funcţiei renale. Rămâne de evaluat eficienţa şi superioritatea lor în comparaţie cu ultrafiltrarea.
- *antagoniştii de receptori de adenozină* A_1 sunt o ţintă terapeutică de studiu în IC acută şi cronică, pornindu-se de la rezultatele favorabile asupra diurezei, natriurezei şi funcţiei renale promovate de trialurile PROTECT-1 şi 2, TRIDENT, REACH-UP. Trialul PROTECT-III recent publicat nu confirmă efectele favorabile cardiace şi asupra funcţiei renale cu rolofylline (56). Rezultatele nu exclud această clasă terapeutică, un rol potenţial fiind prevenţia nefropatiei de contrast şi a rezistenţei la diuretice, sau în caz de hipotensiune (50).
- *peptidul natriuretic sintetic CD-NP* este o ţintă nouă investigată în trialul CONDITION-HF (57). CD-NP este un peptid chimeric inovator testat în studii experimentale, care are avantajul combinării efectelor favorabile ale peptidului C (antiproliferativ, venodilatator cu efect minim hipotensor) şi D (diuretic, natriuretic).
- *activatorii solubili de guanilat-cyclază* (BAY 58-2667 cinaciguat, HMR1766 atisciguat) au fost testaţi cu succes în studii experimentale. Mecanismul de acţiune este de inducere a vasodilataţiei via oxid nitric, iar la nivel renal creşterea excreţiei de sodiu cu prezervarea filtrării glomerulare.
- *darbepoetin alfa* este testat în trialul în desfăşurare RED-HF ca urmare a rezultatelor preliminare conform cărora utilizarea regulată de eritropoietină la pacienţii anemici cu disfuncţie renală ameliorează performanţa cardiacă şi întârzie progresia afectării renale. Nu este clar asupra căruia dintre conectorii cardio-renali acţionează predominant eritropoietina.
- *alte terapii de prezervare a funcţiei renale* în studiu sunt eliberarea dirijată intrarenală arterială pe cateter a medicaţiei sau extinderea indicaţiilor de utilizare a dispozitivelor de asistare mecanică ventriculară (INTERMACS Registry) (59).

CONCLUZIE

Mesajul cheie este că dezvoltarea sindromului cardiorenal este o etapă evolutivă dezastruoasă în istoria naturală a IC sau a BRC care trebuie depistată în mod activ şi precoce. În ciuda terapiilor inovatoare care se prefigurează, managementul adecvat este în impas, de aceea prevenţia şi optimizarea strategiilor existente rămân deocamdată cheia succesului.

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HEPATITA CRONICĂ CU VIRUS C, STEATOZA ŞI INSULINOREZISTENȚA – IMPLICAȚII CLINICE

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CHRONIC HEPATITIS C, STEATOSIS AND INSULIN RESISTANCE – CLINICAL IMPLICATIONS (Abstract): Chronic infection with hepatitis C virus is nowadays responsible for many cases of liver disease. According to recent research, it seems to be characterized by a great deal of metabolic abnormalities, most of them due to insulin resistance, which is present in the virus C-induced disease more often than in chronic hepatitis B or in the general population. As insulin resistance significantly impairs the natural course of the viral disease by reducing the efficacy of antiviral treatment and aggravating the development of fibrosis, it becomes more and more plausible that therapeutic intervention aiming to reduce it might contribute to a better course in many patients. Hepatic steatosis is also a frequent feature of chronic hepatitis C, being either induced by the infection with genotype 3 or by a predisposing metabolic background. The relation between steatosis and viral disease prognosis is controversial, probably due to the various factors inducing lipid accumulation. A better understanding of the metabolic substrate of chronic hepatitis C would help developing new approaches in its diagnosis and therapy, hopefully providing a better future to many patients. **Key words:** CHRONIC HEPATITIS C, INSULIN RESISTANCE, STEATOSIS, FIBROSIS

Medicina ultimilor ani conține variate exemple de descoperiri ştiințifice care îi induc un caracter interdisciplinar. Unul dintre aceste cazuri este conexiunea care a fost descoperită între diverse boli hepatice şi mecanismele de natură dismetabolică ce par a sta într-o mare măsură la baza acestora. Pe lângă steatohepatita nonalcoolică, o entitate clinică recent descrisă, dar care este deja considerată de mulți autori drept cea mai frecventă afecțiune hepatică în epoca contemporană, există numeroase dovezi că hepatita cronică cu virus C – o altă patologie de mare impact pe plan mondial la ora actuală – se caracterizează prin numeroase verigi fiziopatologice care o transformă măcar parțial într-o maladie metabolică, ce evoluează pe fondul unei rezistențe crescute la acțiunea insulinei şi a acumulării intrahepatocitare de lipide. Se speră chiar că în următorii ani, pe măsură ce aceste mecanisme patogenice vor fi înțelese, vor fi posibile adaptarea şi eventual completarea mijloacelor actuale de tratament antiviral în scopul creşterii eficienței terapeutice.

INSULINOREZISTENŢA ŞI HEPATITA CU VIRUS C

În ultimii douăzeci de ani s-au adunat treptat dovezi care leagă hepatita cronică cu virus C de un risc crescut de apariţie a insulinorezistenţei şi chiar a diabetului zaharat tip 2 (1).

Ciroza hepatică, indiferent de etiologie, se asociază cu perturbări ale metabolismului glucidic, printre care insulinorezistenţa, hiperinsulinismul şi uneori hiperglicemia (1). Prevalenţa diabetului zaharat este însă deosebit de ridicată, între 24 şi 50%, la pacienţii cu ciroză hepatică indusă de infecţia cu virus hepatitic C (2), adică de 3-5 ori mai mare decât la persoanele cu ciroză hepatică de altă etiologie, inclusiv cea datorată hepatitei cronice cu virus B (3).

Mai mult decât atât, diabetul zaharat este de 2-4 ori mai frecvent întâlnit la pacienţii non-cirotici cu infecţie virală C decât la persoanele cu hepatite cronice de altă etiologie, diverşi cercetători raportând o prevalenţă a diabetului zaharat situată între 7,6% şi 21% (3).

Pe de altă parte, comparativ cu populaţia generală, persoanele diabetice au un risc crescut de infecţie cu virusul hepatitic C, ceea ce se traduce prin existenţa markerilor serologici specifici acesteia la 4,2-10,5% dintre pacienţii cu diabet zaharat (3). Această constatare ar putea avea două explicaţii:

- susceptibilitate mai mare a diabeticilor la infecţia cu virus hepatitic C, datorată numărului crescut de spitalizări şi de manevre medicale invazive care le sunt necesare;
- inducerea hiperglicemiei de către virusul C.

Unele cercetări ştiinţifice par să dovedească fie că ARN-ul viral este prezent în ţesutul pancreatic, ceea ce ar putea determina un efect citopatic direct, fie că pacienţii cu hepatită cronică C manifestă un grad de disfuncţie β-celulară tradus prin alterarea răspunsului insulinosecretor la hiperglicemie. Ipoteza cea mai acceptată în ultimii ani de către lumea medicală este însă că efectul diabetogen al virusului hepatitic C se explică prin inducerea de către acesta a rezistenţei la insulină.

Pacienţii cu hepatită cronică C au un risc de 3 ori mai mare de apariţie a insulinorezistenţei şi a tulburărilor metabolismului glucidic. S-au raportat asocieri între severitatea rezistenţei la insulină şi mărimea încărcăturii virale (4). Insulinorezistenţa este adesea observată încă din stadii precoce ale leziunilor histologice hepatice (stadiile de fibroză 0 sau 1) (5) şi prezintă niveluri mai ridicate odată cu agravarea fibrozei şi independent de steatoză, ceea ce poate sugera că este unul dintre factorii determinanţi în progresia bolii hepatice (6). Se pare că hiperinsulinismul determinat de creşterea rezistenţei la insulină determină pe de o parte activarea directă a celulelor stelate, iar pe de altă parte induce – împreună cu eventuala hiperglicemie coexistentă – creşterea concentraţiei de CTGF (Connective Tissue Growth Factor), una din citokinele cu rol central în procesele de fibrogeneză. Interesant este faptul că unele publicaţii au sugerat că insulinorezistenţa asociată hepatitei cronice cu virus C are sediu predominant periferic (muscular) şi într-o mai mică măsură hepatic (7).

În unele studii s-a remarcat ameliorarea insulinorezistenţei la persoanele cu răspuns virusologic susţinut la tratamentul antiviral, respectiv lipsa acestor modificări la pacienţii *non-responderi* (8,9). Pe de altă parte, se pare că rezistenţa la insulină poate reduce de 2 până la 6,5 ori eficienţa tratamentului antiviral standard cu peginterferon-α şi ribavirină (10), începând să fie considerată un factor predictiv independent pentru obţine-

rea răspunsului virusologic precoce şi susţinut (10,11,12). Spre exemplu, atunci când s-a urmărit efectul terapeutic la persoanele obeze – ştiut fiind că excesul ponderal generează o rezistenţă crescută la insulină – s-a observat că pacienţii cu un IMC mai mare de 30 kg/m^2 au şanse de 4 ori mai mici de atingere a răspunsului virusologic susţinut. S-a propus chiar ca, în funcţie de nivelul insulinorezistenţei determinat prin HOMA-IR, să se opteze în viitor pentru regimuri diferite de tratament (13).

O serie de date din literatură atestă existenţa unor diferenţe în gradul de rezistenţă la insulină în funcţie de genotipul viral implicat: unii cercetători au identificat un nivel crescut al acesteia la pacienţii infectaţi cu genotipurile 1 şi 4 comparativ cu genotipul 3, iar alţii la genotipul 2a comparativ cu genotipul 1; există însă şi studii care nu au reuşit să demonstreze existenţa nici unei asocieri între insulinorezistenţă şi genotipul viral (5).

Sunt citate trei posibile mecanisme prin care virusul C induce insulinorezistenţă:

a. infecţia cu virus C determină încă din stadii precoce *creşterea nivelurilor serice ale interleukinei-6 şi TNF-α* (*Tumor Necrosis Factor-α*). Interleukina-6 inhibă autofosforilarea receptorilor insulinici hepatici şi fosforilarea IRS-1 şi IRS-2 (*Insulin Receptor Substrate-1/2*), cu un efect de scădere a sensibilităţii la insulină dovedit în unele studii experimentale. TNF-α creşte rezistenţa la insulină prin inhibarea receptorului insulinic, tirozinfosforilarea IRS-1 şi hipofosforilarea IRS-2, ceea ce alterează transmiterea semnalului postreceptor care determină mobilizarea GLUT-4 către suprafaţa membranei celulare, preluarea glucozei din mediul extracelular şi transportul transmembranar al acesteia (14);

b. virusul C determină *degradarea proteosomală a IRS-1* prin mecanisme moleculare genotip-specifice (15):

- genotipul 1 induce expresia SOCS-3 (*Suppressor of Cytokine Signaling-3*), iar genotipul 3 – expresia SOCS-7 (*Suppressor of Cytokine Signaling-7*); aceştia sunt inhibitori ai semnalului insulinic care acţionează prin ubiquitinarea IRS-1, ceea ce duce la preluarea lui intraprotosomală şi la distrugerea acestuia;
- genotipul 1b mai acţionează suplimentar şi prin activarea mTOR (*Mammalian Target of Rapamycin*), care determină serintreoninfosforilarea, redistribuţia intracelulară şi degradarea proteosomală a IRS-1;
- genotipul 3 induce şi inhibarea PPAR-γ (*Peroxisome Proliferator Activated Receptor-γ*), care implică de asemenea creşterea rezistenţei la insulină;

c. virusul C induce expresia *proteinfosfatazei 2A* printr-o cale care implică răspunsul stresor al reticulului endoplasmic; la rândul său, aceasta va defosforila o altă enzimă cheie din calea de transmitere intracelulară a semnalului insulinic, proteinkinaza B – PKB/Akt, transformând-o în varianta cu activitate kinazică scăzută (16); proteinfosfataza 2A modifică şi starea de fosforilare a AMP-kinazei, o altă enzimă cheie în homeostazia celulară energetică şi care este implicată în calea metabolică a gluconeogenezei (16).

STEATOZA HEPATICĂ ÎN INFECŢIA CU VIRUS C

Pacienţii cu hepatită cronică cu virus C prezintă steatoză hepatică în proporţie de 40-80%; cifrele variază în funcţie de coexistenţa altor factori cu potenţial de inducere a acesteia (consum de alcool, obezitate, diabet zaharat, medicaţii asociate etc.) (17). Chiar şi după excluderea influenţei exercitate de aceştia, prevalenţa

steatozei în infecţia cronică cu virus C rămâne de aproximativ 50%, mult mai mare decât cea citată în populaţia generală (circa 20%) sau la persoanele cu hepatită cronică virală B (18%) (18). La o treime dintre persoanele cu hepatită cronică C şi steatoză hepatică nu sunt prezente nici una dintre patologiile metabolice care favorizează în mod obişnuit acumularea lipidică intracelulară. Aceste constatări au determinat chiar pe unii autori să folosească – prin analogie cu NASH (*Nonalcoholic Steatohepatitis*) – termenul de VASH (*Virus-Associated Steatohepatitis*) pentru a denumi steatoza hepatică asociată infecţiei cu virus hepatitic C (1).

Efectul steatogen al virusului C este explicat de faptul că acesta utilizează mecanismele metabolismului lipidic intrahepatocitar pentru a se reproduce şi a intra în componenţa particulelor lipoproteice care se răspândesc apoi în circulaţia generală şi în întreg organismul (19), după cum se va vedea ulterior. Se pare că în membrana picăturilor lipidice care apar în citoplasma hepatocitelor secundar infectării cu virusul C se localizează întreg complexul de replicare virală, ca urmare a semnalului indus de proteina *core*, iar proteinele nonstructurale virale sunt şi ele localizate în imediata vecinătate a acestor incluzii lipidice (20).

Steatoza hepatică este mai frecventă la persoanele infectate cu genotipul viral 3a (74% faţă de 50% la alte genotipuri) (17). La acest grup de pacienţi gradul steatozei nu se corelează însă cu valoarea indicelui de masă corporală şi nici cu indicii de insulinorezistenţă, ceea ce ar putea sugera că secvenţele genotipului viral respectiv au acţiune steatogenă printr-un efect citopatic direct. Infecţia cu genotipul 3 induce dealtfel o încărcare grasă a hepatocitelor predominant în zona periportală şi nu în zona centrolobulară, ca în cazul steatozei induse de factorii metabolici. În plus, steatoza genotip 3-indusă se corelează cu replicarea şi încărcătura virală (17), nu se corelează cu progresia fibrozei (21), retrocedează în cazul succesului tratamentului antiviral şi reapare după o eventuală reinfecţie.

Potenţialul steatogen crescut al genotipului 3 se explică prin datele provenite din studii experimentale, care arată că picăturile lipidice intracitoplasmatice se formează ca urmare a unei acţiuni mai intense a proteinei sale *core*; aceasta a fost identificată chiar în membrana acestor vezicule lipidice (22). Se pare că prezenţa unui reziduu de fenilalanină în poziţia 64 a domeniului II al proteinei respective, spre deosebire de reziduul tirozinic care ocupă acest loc în cazul celorlalte genotipuri, induce o afinitate mai mare pentru lipide (23).

Se presupune că steatoza hepatică care se corelează cu nivelul rezistenţei la insulină ar putea fi în general determinată nu numai de efectul steatogen viral, ci şi de acţiunea altor factori de risc, precum obezitatea cu dispoziţie predominant abdominală, diabetul zaharat, dislipidemiile sau, eventual, consumul de alcool (24). La aceşti pacienţi este de obicei vorba despre infecţia cu genotipurile 1, 2 sau 4, steatoza pare să se asocieze cu leziuni mai avansate de fibroză şi cu o progresie accelerată a acesteia, iar încărcarea grasă nu retrocedează după încheierea tratamentului antiviral (25). Este posibil ca prezenţa încărcării lipidice intracelulare să sensibilizeze ţesutul hepatic la inflamaţie şi/sau moarte hepatocitară programată prin apoptoză, care favorizează activarea celulelor stelate şi extensia fibrozei.

Unii cercetători susţin chiar că, în cazul genotipurilor virale non-3, gradul steatozei

alterează şansa obţinerii unui răspuns viral susţinut (26), sugerând că pacienţii cu hepatită cronică C şi steatoză importantă ar trebui să înceapă cât mai precoce tratamentul antiviral, chiar dacă activitatea inflamatorie depistată histologic este doar uşoară (24). Câteva date experimentale sugerează că, în situaţii precum infecţia cu genotip 1b, insulinorezistenţa precede dezvoltarea steatozei hepatice, părând a fi cauza şi nu consecinţa acesteia.

Există trei mecanisme care explică apariţia steatozei hepatice legate de infecţia cu virus C:

a. *scăderea exportului de lipide din hepatocite*, prin formarea şi secreţia în cantităţi reduse de particule VLDL:

Proteina *core* a virusului C inhibă activitatea MTP (*Microsomal Triglyceride Transfer Protein*), o enzimă implicată în calea de asamblare a particulelor VLDL prin catalizarea transferului lipidic spre reticulul endoplasmatic, unde se produce asocierea acestora cu apoproteinele B în cadrul unor complexe stabile. Unii cercetători au identificat chiar o corelaţie inversă între gradul steatozei hepatice din infecţia cu virus C şi nivelul ARNm al MTP, independent de genotipul implicat (27). Reducerea activităţii MTP, cu consecinţele menţionate anterior asupra exportului hepatocitar de VLDL, explică de ce la pacienţii cu hepatită cronică C se constată niveluri reduse ale trigliceridelor serice (28), iar la cei care asociază şi steatoză severă sunt prezente şi niveluri plasmatice reduse de colesterol şi apoB. După alţi cercetători, proteinele non-structurale virale pot şi ele să reducă activitatea MTP, chiar în absenţa proteinei *core* (29).

Acţiunea inhibitorie a proteinei *core* asupra MTP, deşi prezentă la toate genotipurile, este mai puternică în cazul genotipului 3 (30). În cazul genotipurilor 1 şi 2, mecanismul de inhibare a MTP pare a fi o reducere a transcripţiei sub influenţa rezistenţei la insulină şi a hiperinsulinismului reactiv; acesta din urmă inhibă expresia enzimei pe o cale care implică o kinază denumită MAP-Kerk (*Mitogen-Activated Extracellular Signal-Regulated Protein Kinase*).

Conform altor date provenite din studii experimentale, reducerea sintezei şi secreţiei de particule VLDL se poate explica şi prin intervenţia fenomenelor de stress oxidativ. Acumularea proteinei *core* în interiorul mitocondriei duce la alterarea transportului de electroni din cadrul lanţului respirator mitocondrial, având drept efect producţia crescută de ROS (*Reactive Oxygen Species*). În plus, activarea răspunsului imun de către virusul C induce şi ea stress oxidativ prin eliberarea unor citokine şi activarea macrofagelor. Acumularea ROS provoacă peroxidarea lipidelor şi proteinelor structurale, alterând şi aparatul celular secretor de VLDL (31); în plus, ROS au un efect de stimulare a formării de citokine, inclusiv TNF-α, ceea ce duce la agravarea insulinorezistenţei şi prin aceasta a steatozei (19).

b. *reducerea consumului mitocondrial de acizi graşi*, prin inhibarea proceselor de oxidare lipidică:

Este posibil ca proteina *core* a virusului C să determine modificări structurale ale membranei mitocondriale, cu consecinţe negative asupra β-oxidării lipidelor, ceea ce poate explica acumularea citoplasmatică a lipidelor şi apariţia steatozei.

Tot proteina *core* pare a fi responsabilă de inhibarea expresiei PPAR-α, un receptor nuclear implicat în reglarea transcripţiei mai multor gene ale metabolismului lipidic; acţiunea inhibitorie a proteinei *core* este

mai intensă în cazul genotipului 3. Reducerea activităţii PPAR-α conduce la (32):

- scăderea expresiei acilcoenzimaA-oxidazei şi a CPT1A (*Carnitine Palmitoyl Acyl-CoA Transferase 1*) mitocondriale; ambele sunt enzime cheie în lanţul β-oxidării mitocondriale, aşa încât scăderea activităţii lor enzimatice se traduce prin reducerea oxidării acizilor graşi;
- inhibarea expresiei Mdr2, o proteină transportoare situată în membranele canaliculare care controlează secreţia fosfolipidică biliară, conducând implicit şi la excreţia redusă prin bilă a acizilor graşi.

c. *creşterea sintezei de novo a acizilor graşi*, prin activarea expresiei factorului de transcripţie SREBP-1c (*Sterol Regulatory Element Binding Protein Signaling Pathway-1c*) şi a receptorului nuclear RXR-α (*Retinoid X Receptor-α*); hiperactivarea acestor molecule determină supraexpresia unor enzime precum acid gras-sintetaza, acetilcoenzimaA-carboxilaza şi stearoil-coenzimaA-desaturaza, cu roluri cheie în lipogeneza *de novo*. Probabil tot proteina *core* este cea care cauzează şi activarea SREBP, întrucât transfecţia *in vitro* cu aceasta a unor linii celulare a dus la creşterea ARN_m al SREBP şi la sinteza intrahepatocitară crescută de acizi graşi (33). Există însă şi date care atestă activarea SREBP de către proteina non-structurală 2 (34).

Se pare că steatoza hepatică existentă la pacienţii cu hepatită cronică C poate favoriza, la rândul ei, replicarea virală. Virusul C se asociază cu lipoproteinele LDL în cadrul unor particule lipo-virale care vor fi captate din torentul sanguin de către receptorii hepatocitari pentru LDL, intrând pe această cale în celulă. Pe de altă parte, unii cercetători au descoperit existenţa unor anomalii în metabolismul VLDL şi LDL, cu un catabolism redus al VLDL (ceea ce accentuează transformarea particulelor VLDL în LDL în curentul circulator) şi un catabolism crescut al LDL, care duce la încărcarea hepatocitelor cu lipidele captate din sânge; deşi nu are încă o explicaţie la nivel molecular, această constatare îşi poate găsi motivaţia în eventuale conexiuni fizice stabilite între virusul C şi particulele lipoproteice respective şi/sau în modificarea de către acesta a compoziţiei lor moleculare (35). În felul acesta, alterarea raportului plasmatic între particulele VLDL şi LDL poate favoriza legarea virusului de hepatocite, astfel încât – odată cu reducerea secreţiei hepatocitare de VLDL – se închide un cerc vicios prin care virusul C îşi promovează propria intrare şi diseminare intracelulară (24).

CONSECINŢE TERAPEUTICE ALE ASOCIERII INSULINOREZISTENŢĂ – STEATOZĂ – HEPATITĂ CRONICĂ CU VIRUS C

După cum am menţionat anterior, rezistenţa la insulină şi steatoza hepatică pot avea de multe ori consecinţe negative asupra evoluţiei bolii şi a responsivităţii la terapia antivirală standard. Ghidurile actuale nu conţin însă recomandări specifice pentru diagnosticul sau tratamentul particularizat al insulinorezistenţei la pacienţii cu hepatită cronică C. Există şi autori care propun ca aceştia să fie monitorizaţi periodic prin determinarea indicilor de insulinorezistenţă (spre exemplu, HOMA-IR) şi depistarea din timp a unor elemente clinice ale sindromului metabolic (care are drept element fiziopatologic central rezistenţa la acţiunea insulinei), precum excesul ponderal, dislipidemia sau hipertensiunea arterială (1,11).

În cazul patologiilor metabolice, tratamentul uzual al insulinorezistenţei constă

în măsuri de optimizare a stilului de viaţă (implementarea activităţii fizice şi eventuale modificări dietetice, ce urmăresc reducerea excesului ponderal) şi eventual în medicaţie insulinosensibilizantă în unele cazuri (diabet zaharat tip 2, ovar polichistic etc.). Dintre aceste metode terapeutice, utilitatea mijloacelor nonfarmacologice de scădere ponderală şi a tratamentului cu thiazolidindione începe să fie testată şi la pacienţii cu infecţie cronică cu virus C.

Există dovezi că scăderea în greutate la pacienţii obezi are un efect benefic de reducere a leziunilor de steatoză şi fibroză din hepatita cronică C şi de favorizare a unui rezultat pozitiv al tratamentului antiviral. Dealtfel, consecinţele negative ale obezităţii asupra eficienţei terapiei standard din hepatita C se explică prin:

- legătura cauză-efect existentă între obezitate şi insulinorezistenţă;
- statusul proinflamator caracteristic obezităţii, cu niveluri crescute ale TNF-α şi ale leptinei şi niveluri scăzute de adiponectină.

TNF-α este o citokină proinflamatorie şi care induce rezistenţă la insulină, existând dovezi că nivelul său se corelează invers proporţional cu răspunsul la terapia antivirală. *Leptina* este o adipocitokină secretată proporţional cu capitalul de masă grasă al organismului; în cazul multor pacienţi se înregistrează de obicei un grad de rezistenţă la acţiunile sale de promovare a catabolismului care a condus – prin paralelism cu insulina – la denumirea de leptinorezistenţă. Întrucât leptina favorizează răspunsul imun celular mediat de limfocitele Th1, care par a avea un rol central în răspunsul susţinut la interferon, s-a formulat ipoteza că leptinorezistenţa ar putea avea efecte negative asupra eficienţei terapeutice. *Adiponectina* este o adipocitokină cu acţiune antiinflamatorie şi de antagonizare a TNF-α, care se pare că are niveluri scăzute atât la pacienţii obezi, cât şi la cei cu hepatită cronică cu virus C (36).

O absorbţie redusă a peginterferonului după administrarea subcutanată la pacienţii obezi, explicată prin drenajul limfatic defectuos prezent la aceştia şi care conduce la o biodisponibilitate scăzută a medicaţiei (37).

Referitor la tratamentul cu thiazolidindione, studiile de durată scurtă sau medie în care pioglitazona a fost folosită ca premedicaţie şi/sau *add-on* la tratamentul antiviral standard oferă informaţii contradictorii, oscilând între lipsa oricărui beneficiu terapeutic asupra infecţiei cu virus C (chiar dacă s-a reuşit ameliorarea sensibilităţii la insulină) şi obţinerea unui răspuns virusologic pe termen scurt sau lung (38-42). Sunt necesare cercetări desfăşurate pe loturi extinse de pacienţi şi pe intervale mai mari de timp pentru a putea concluziona dacă adăugarea terapiei cu thiazolidindione la tratamentul antiviral standard reprezintă o direcţie terapeutică de valoare pentru viitor.

Alte echipe de cercetare au obţinut rezultate favorabile *in vitro* utilizând lovastatin sau acizi graşi polinesaturaţi. Efectul favorabil al statinelor se explică prin acţiunea lor asupra 3-hidroxi-3-metilglutaril-Co-Areductazei, care determină inhibarea căii mevalonatului şi reducerea proceselor de prenilare a proteinelor. Se formează astfel mai puţine proteine asociate – necesare includerii în construcţia membranelor celulare, inclusiv în cea a picăturilor lipidice (43). Referitor la efectul acizilor graşi, se pare că cei saturaţi şi cei mononesaturaţi sunt necesari replicării virale, pe când acizii graşi polinesaturaţi o inhibă (44).

Trecerea în revistă a datelor obţinute de cercetările ultimilor ani ne permite să observăm că infecţia cronică cu virus hepati-

tic C se transformă într-o boală cu tot mai multe particularități metabolice. Deşi cunoştințele respective sunt încă incomplete şi de multe ori contradictorii, ele oferă motive de încurajare celor care caută un tratament cât mai eficient al acestei boli virale hepatice, întrucât se speră că – prin controlul mecanismelor fiziopatologice metabolice – va fi posibilă creşterea randamentului terapeutic.

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NOUTĂȚI

NOI DIRECȚII TERAPEUTICE ÎN NEFROPATIA INDUSĂ DE CYCLOSPORINA A

Activarea sistemului renină angiotensină aldosteron (SRAA) joacă un rol important în nefropatia indusă de cyclosporina A. Scopul acestui studiu este de a demonstra că administrarea de extract de ceai verde poate preveni apariția acestui tip de nefropatie. Pentru acest studiu au fost folositi şobolani care au fost împărțiți în patru grupuri (6 în fiecare grup) şi tratați 21 de zile: un grup de control(grupul 1) cărora li s:a administrat ser fiziologic, grupului 2 doar cyclosporină intaperitoneal 30 mg/kg/zi, grupului 3 cyclosporină şi extract de ceia verde 100 mg/kg/zi subcutanat iar grupului 4 doar extract de ceai verde. S-au observat nivele plasmatice crescute de uree şi creatinină în grupul 2 comparativ cu grupul 3 şi cu grupul 1. Analizele biochimine au arătat că activitatea plasmatică a reninei şi concentrația serică a aldosteronului au fost semnificativ crescute în grupul 2 față de grupul 1 şi semnificativ scăzute în grupul 3 față de grupul 2. Astfel, s-a demonstat că administrarea cyclosporinei creşte nivelele intrarenale ale reninei şi induce nefrotoxicitate. Efectele protective ale extractului de ceai verde în ceea ce priveşte nefropatia indusă de cyclosporină pot consta în blocarea SRAA. (Hyun HR, Hyun LK, Chung JH et al. Renoprotective effects of green tea extract on renin-angiotensin-aldosterone system in chronic cyclosporine-treated rats. *NephrolDial Transplant*. 2011; 26: 188-1193).

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PATOGENEZA COMPLICAŢIILOR TROMBOTICE ŞI HEMORAGICE ÎN SINDROAMELE MIELOPROLIFERATIVE ŞI MIELODISPLAZICE

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THROMBOTHIC COMPLICATION PATHOGENESIS IN CHRONIC MYELOPROLIFERATIVE DISORDERS AND MYELODISPLASTIC SYNDROMES (Abstract): Chronic myeloproliferative disorders (CMD) and Myelodisplastic Syndromes (MDS) represents a group of clonal pluripotent stem-cell pathologies. During their natural history, the clinical picture reveals both thrombosis and hemorrhage. The thrombosis could affect the microvessels, and also the large vessels, including even less usual territories (suprahepatic veins, porta vein, pulmonary vein). There are many factors contributing to thrombosis in myeloproliferative chronic disorders – the associated comorbidities, the numeric alterations of blood elements and also the disorders of the platelet's function. Thus, there were described quantitative and qualitative anomalies of platelet's receptors : GP Ib, GP IIb/IIIa, GP IV, GP VI, thrombopoietin receptor of the platelet cMPL, the increase of platelet activation; the increase of P selectin and thrombospondin and the increase on GP IIb/IIIa expression – they were all correlated with thrombosis. An important role has been attributed to JAK2 mutation, which affects the platelet receptor for thrombopoietin cMPL. Regarding the hemorrhage in chronic myeloproliferative syndrome, it is favored by many disorders in platelet's function, such as : the decrease of von Willebrand factor's receptor of the platelet, which leads to acquired Bernard Soulier syndrome; quantitative and qualitative disorders of dense granules of the platelet, decrease of the secretion and platelet aggregation after epinephrine, ADP and collagen stimulation. It was also described the acquired von Willebrand syndrome, most frequently type 2. **Key words**: MYELOPROLIFERATIVE CHRONIC SYNDROME, THROMBOSIS, HEMORRHAGE, PLATELET DYSFUNCTION

Sindroamele mieloproliferative cronice cuprind boli hematologice clonale care apar prin afectarea celulei hematopoietice pluripotente. Dintre acestea leucemia mieloidă cronică caracterizată prin prezenţa fuziunii BCR-ABL şi 3 boli mieloproliferative cronice Ph - policitemia vera, trombocitemia esenţială şi metaplazia mieloidă cu mielosclerozã. Conform noii clasificări OMS din acest grup mai fac parte mastocitoza sistemică, leucemia cronică cu eozinofile, leucemia cronică cu neutrofile, leucemia mielomonocitară cronică.

În evoluţie, tabloul clinic al acestor boli poate include şi manifestări trombotice sau hemoragipare. Manifestările trombotice sunt evidente mai ales la nivelul microcirculaţiei dar şi la nivelul vaselor mari – vene, artere (11). Acestea pot apare în evoluţie sau la debutul bolii, în faza preclinică, fiind necesare investigaţii pentru trombofilia dobândită, mai ales în cazul pacienţilor cu trom-

boze venoase situate pe vase mai rar afectate (vene suprahepatică, porta, pulmonară, sindromul Budd Chiari) (11).

La 32% dintre pacienţii cu SMPC s-au observat manifestări trombotice (arteriale, microvasculare) (21).

Manifestările hemoragipare sunt mai rare şi pot fi exprimate prin hemoragii mucoase şi cutanate minore care uneori pot preceda hemoragiile digestive superioare (11,12). Boala von Willebrand dobandită (cel mai adesea prin deficitul multimerilor de dimensiuni mari), o reducere a activităţii procoagulante a trombocitelor sau forme dobândite de sindrom Bernard Soulier au fost de asemenea raportate la pacienţii cu mieloproliferări cronice (16).

PATOGENEZA TROMBOZELOR

Cunoştiinţele despre patogeneza trombozelor în sindroamele mieloproliferative cronice sunt inferioare comparativ cu progresul cunoştinţelor din domeniul trombozelor. Cauzele care limitează cercetarea în acest domeniu sunt perioada lungă de urmărire a evoluţiei clinice, unii factori clinici asociaţi (impactul comorbidităţilor, terapia citoreductivă). Factori multipli contribuie la patogeneza trombozei – incluzând creşterea masei celulare în policitemia vera, creşterea posibilă a trombocitelor, activarea trombocitară şi leucocitară şi interacţiunea lor cu formarea de agregate leucotrombocitare în asociere cu factori protrombotici şi factori endoteliali (11).

În sindroamele mieloproliferative cronice apar numeroase **anomalii ale receptorilor trombocitari atât de natură cantitativă cât şi calitativă**. Aceştia sunt GPIb, GP IIb/IIIa, GPIV, GPVI şi receptorul pentru trombopoietină cMPL. Variabilitatea receptorilor trombocitari este de asemenea determinată genetic şi câteva studii au demonstrat că polimorfismul glicoproteinelor trombocitare poate contribui la tromboze (15,16). Prezenţa alelei PLA_2 a GPIIIa a fost asociată cu o creştere a incidenţei trombozelor arteriale dar această concluzie a studiului efectuat necesită confirmare în studii mai ample. Relevanţa polimorfismului GP1a, GP1bα şi integrinei α2β1 în special la pacienţii cu mieloproliferări cronice rămâne neclară (15,16). Într-un studiu s-a arătat că reducerea expresiei c-MPL este specifică la pacienţii cu policitemia vera şi nu este evidenţiată la pacienţii cu poliglobulie secundară (13).

Activarea trombocitară în mieloproliferările cronice a fost descrisă cu mult timp în urmă. Mai recent, creşterea expresiei P selectinei, trombospondinei şi a receptorului GPIIb/IIIa a fost corelată cu tromboza (16). Studiile efectuate au arătat că la pacienţii cu sindroame mieloproliferative cronice au fost evidenţiate o scădere a expresiei şi a capacităţii funcţionale a receptorului GPIIb/IIIa (CD41/CD61), receptorului pentru fibrinogen, fibronectina, vitronectina, trombospondina, factor Willebrand. De asemenea s-a asociat şi cu o scădere a fixării FvW pe trombocit (16).

Un studiu efectuat în 1989 în Japonia a urmărit **invesigarea anomaliilor influxului de calciu în trombocite la pacienţii cu boli mieloproliferative cronice**. Influxul calciului în trombocite a fost mai scăzut la pacienţii cu mieloproliferări cronice comparativ cu lotul de control. Concluzia autorilor a fost că anomaliile GP IIb/IIIa sunt implicate în scăderea influxului de calciu în trombocitele pacienţilor cu mieloproliferări cronice (17).

Patogeneza activării trombocitare în mieloproliferările cronice nu este cunoscută, deşi un număr mare de pacienţi au un deficit de lipooxigenază care poate creşte capacitatea endoperoxidazelor în producerea tromboxanului A2.

O explicatie alternativă a creşterii activării trombocitare include un efect al mutaţiei JAK2 (2). Datele recente sugerează că mutaţia JAK2 afectează receptorul cMPL--receptorul pentru trombopoietină de pe trombocit (fig. 1).

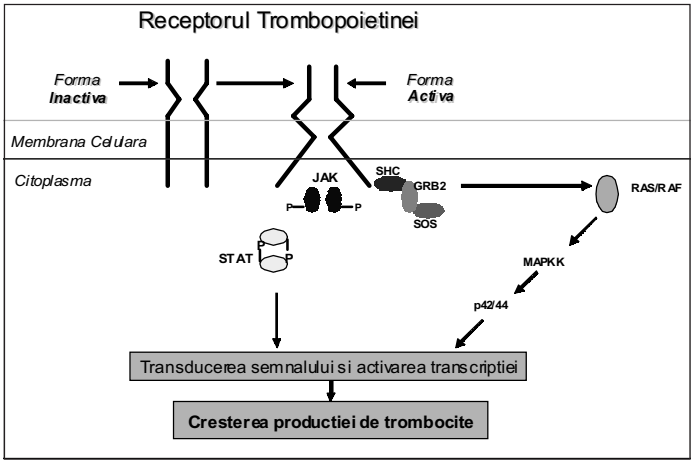

Fig. 1. Relaţia între mutaţia JAK2 şi receptorul cMPL-receptorul pentru trombopoietină

În mieloproliferările cronice mutaţia apare la nivelul celulei progenitoare multipotente (6). Progenitorii homozigoţi sunt mai frecvenţi la pacienţii cu policitemia vera şi s-a întâlnit rar la cei cu trombocitemie esenţială (9). Mutaţiile JAK2V617F apar la 90–95% din pacienţii cu PV, 50–70% din pacienţii cu TE, şi 40–50% din pacienţii cu MMM (14,15).

Această mutaţie poate apare în procente reduse şi în alte mieloproliferări acute sau cronice Ph(–) precum şi în mielodisplazii (1,3,7,14,15). Prezenţa mutaţiei JAK2 a fost evidenţiată la pacienţii cu leucemie acută mieloblastică, mai ales vârstnici, existând posibilitatea ca aceştia să prezinte sindroame mieloproliferative cronice anterior rămase nediagnosticate. Date recente au arătat că mutaţia JAK2 este prezentă într-o proporţie semnificativă la pacienţii cu leucemie acută mieloblastică care urmează evoluţiei sindroamelor mieloproliferative cronice. La pacienţii cu leucemie acută mieloblastică de novo incidenţa acestei mutaţii este redusă (10,12).

Domeniul intracitoplasmatic al acestui receptor este asociat cu JAK2, membru al familiei kinazelor Janus. Trombopoetina stimulează fosforilarea unor numeroase proteine printre care kinazele Janus JAK2 si TYK2 (17). Trombopoetina şi receptorul ei sunt cheile de control pentru reglarea numărului trombocitelor majoritatea pacienţilor cu policitemia vera şi metaplazie mieloidă cu mielofibroză au o reducere a nivelului receptorilor pentru trombopoietină şi o incompletă glicozilare a acestora. Acest defect nu a fost găsit la alte glicoproteine membranare trombocitare (GP IIb, multimerin). Defectul se corelează însă cu durata bolii şi hematopoieza extramedulară (8,16).

În sindroamele mieloproliferative cronice apar şi alte modificări ale receptorilor membranari trombocitari care pot favoriza manifestările trombotice-**GP IV (CD 36) receptor pentru trombospondina** eliberată din plachete în cursul secreţiei. Contribuie la consolidarea agregatelor trombocitare şi agregare ireversibilă. În bolile mieloproliferative cronice a fost observată o creştere a expresiei (25,26)**.** Prezenta formă proteolizată de 160Kda a trombospondinei (TSP) a fost evidenţiată la toţi pacienţii cu trombocitemie esenţială şi nu a fost găsită la pacienţii cu trombocitoză secundară (1). Într-un studiu s-a arătat că nivelul plasmatic al GP IV şi al celor de suprafaţă asociate cu TSP tind să se normalizeze în timpul tratamentului cu interferon pe când prezenţa formelor alterate ale trombospondinei persistă (18).

Trombocitele activate interacţionează cu alte componente ale sângelui şi au capacitatea de a provoca activare sau distrucţie a endoteliului.

Unele studii au demonstrat creşteri şi ale VEGF care demonstrează şi o **creştere a activării endoteliului**. Activarea leucocitelor (neutrofile şi monocite determină declanşarea coagulării prin eliberarea granulelor conţinute, formarea de agregate cu trombocitele, eliberarea unor citokine inflamatorii, a superoxidului de oxigen care produc afectarea endoteliului vascular (5). Interacţiunea între neutrofile şi trombocite apare după cascada adeziunii în care CD62P se leagă de P selectina de pe neutrofil. Fixarea adeziunii se face prin legarea CD11b a $ß_2$-integrinei în complex cu CD18 cu GP Ib trombocitar sau a fibrinogenului la GP IIb/IIIa trombocitară. În unele studii s-au arătat creşteri ale expresiei CD 62P, CD11b, CD 42b şi a complexelor CD11b/CD42b precum şi a complexelor CD62P/ CD11b la pacienţii cu policitemia vera şi trombocitemia esenţială (5). Studii recente au arătat o asociere între mutaţia JAK2 şi creşterea activării leucotrombocitare cu formare de agregate trombocitare (2).

Administrarea aspirinei poate fi eficientă prin impiedicarea formării acestor agregate. Hydroxiureea poate şi ea contribui la reducerea acestor agregate, posibil precum şi prin supraexpresia genei endotelinei 1 şi ICAM-1 ca şi creşterea nivelului oxidului nitric (1,4,5).

PATOGENEZA SINDROAMELOR HEMORAGIPARE

Receptorul trombocitar cel mai afectat care poate explica prezenta sindroamelor hemoragipare este GP Ib/IX (CD 42) receptor pentru factorul Willebrand. Capătul terminal este scindat de proteaze incluzând calpaina trombocitară rezultând un fragment solubil, glicocalicin, circulant în plasma cu valori serice normale 1-3μg/ml. Este proporţional cu producţia trombocitară. Acest receptor poate scădea cantitativ în sindroamele mieloproliferative cronice ducând la o variantă dobandită de sindrom Bernard Soulier (16).

La pacienţii cu leucemie acută, sindroame mielodisplazice şi mieloproliferative cronice au fost în literatură anomalii cantitative şi calitative ale granulelor dense trombocitare ale corpusculilor intracelulari (conţinând ATP, ADP, calciu, calcitonină şi pirofosfat) (28). Studii relativ recente au arătat o asociere între modificările granulelor dense trombocitare şi descoperirea leucemiei acute mieloblastice (27). Defectul apare ca şi consecinţă a anomaliilor de formare a acestor granule mai curând decât prezenţa unor vezicule goale sau o scădere a conţinutului acestora, consecinţă a activării trombocitare asociată secreţiei. Rezultatele sugerează că defectul granulelor dense trombocitare asociat cu leucemia acută sau sindromul mielodisplazic poate fi rezultatul alterării cromozomiale în linia celulară megacariocitară conducând la scăderea formării granulelor dense (27,28).

În bolile mieloproliferative cronice şi în mielodisplazii scade secreţia şi agregarea trombocitară după stimularea cu epinefrină, ADP, colagen. Defectul agregării la stimularea cu epinefrină se traduce prin absenţa primei unde de agregare. (28). Se mai poate observa în mieloproliferările cronice o scădere a multimerilor de dimensiuni mari (19). Un grup de cercetători din Argentina au luat în studiu un grup de 99 pacienţi cu boala von Willebrand dobândită (21). Aceştia au constatat că 75% dintre pacienţi asociau sindrom mieloproliferativ cronic în special policitemia vera sau trombocitemie esenţială (21).

Cel mai frecvent tip de boală Willebrand întâlnit a fost tip 2 (67,7%) Anomaliile timpului de sângerare, nivelul de factor VIII au fost observate la 83,3% din cazuri. Sângerările au fost prezente la 38,4% dintre pacienţi mai frecvent la pacienţii fără asociere de SMPC 58,3% vs pacienţi cu SMPC (32%) (p 0,022) cu o preponderenţă mai mare la femei (21).

Conform rezultatatelor publicate de Sanchez-Luceros et al. se conturează ideea că sin-

dromul von Willebrand dobândit la pacienţii cu SMPC este doar un diagnostic de laborator fără a fi evident clinic în multe cazuri deşi sângerările şi manifestările ischemice pot fi prezente. În schimb, la pacienţii fără SMPC Boala Von Willebrand dobândită se manifestă clinic mai evident prin episoade hemoragipare severe (21).

CONCLUZIE

Cunoaşterea aprofundată a mecanismelor care stau la baza diatezei trombotice şi hemoragipare în bolile celulei stem permite o abordare interdisciplinară şi complexă a problematicii, punând în discuţie aplicarea terapiilor cu viză patogenică, cu accentul pe terapiile cu ţintă moleculară.

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Rev. Med. Chir. Soc. Med. Nat., Iaşi – 2010 – vol. 114, nr. 4

PARTICULARITĂŢILE HIPERTENSIUNII ARTERIALE DIN SINDROMUL METABOLIC

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FEATURES OF ARTERIAL BLOOD PRESSURE IN METABOLIC SYNDROME (Abstract): The metabolic syndrome has high prevalence in hypertensive patients. Its presence leads to the early appearance of target organ damage, particularly of left ventricular hypertrophy. The 24-hour ambulatory monitoring of arterial blood pressure allows the estimation of the BP Load. The BP Load accomplishes the best correlation between the systolic blood pressure and left ventricular hypertrophy. The purpose of this study is to identify the prevalence of the metabolic syndrome and the particularities of hypertension in hypertensive patients suffering from metabolic syndrome, using 24 hour continuous blood pressure monitoring. **Material and method:** During a three years period (2007-2009), 303 hypertensive patients were assessed at the Internal medicine Section of the County Emergency Hospital in Bacau. **Results:** The metabolic syndrome occurred in 60.1% of hypertensive patients. The batch with metabolic syndrome contained 181 patients, a female/male ratio of 3,41 to 1, average age: 66.50 ± 10.28 years, 60.8% coming from urban environment. The monitorised systole-diastolic tension values studies indicate isolated systolic hypertension. No dipper and reverse dipper tensional profiles prevail. **Conclusions:** In patients with isolated systolic hypertension, the association of the metabolic syndrome is the main cardiovascular risk and is associated with increase of diastolic blood pressure, presence of left ventricular hypertrophy and increased hypertensive Load values. **Key words:** BLOOD PRESSURE, METABOLIC SYNDROME, BP LOAD

Sindromul metabolic recunoaşte inaltă prevalenţă în populaţia generală şi este întâlnit la circa o treime din pacienţii hipertensivi, mai ales la cei cu hipertensiune necontrolată.

Sub aspect morfofuncţional creşte stiffness-ul arterial cu reducerea distensibilităţii arteriale la pacienţii hipertensivi cu sindrom metabolic.

De asemenea, se raportează creşterea prevalenţei hipertrofiei ventriculare stângi, a disfuncţiei diastolice, aterosclerozei carotidiene precoce, reducerea distensibilităţii aortice, retinopatiei hipertensive şi microalbuminuriei (1). Apariţia precoce a leziunilor subclinice de organ poate constitui predictor independent de evenimente adverse vasculare şi poate explica parţial asocierea sindromului metabolic cu risc înalt cardiovascular şi renal.

Monitorizarea ambulatorie a presiunii arteriale se corelează mai bine cu leziunile de organ şi evenimentele cardiovasculare decât măsurarea manuală, constituind un instrument diagnostic şi prognostic deosebit de util la pacientul hipertensiv (2,3,4).

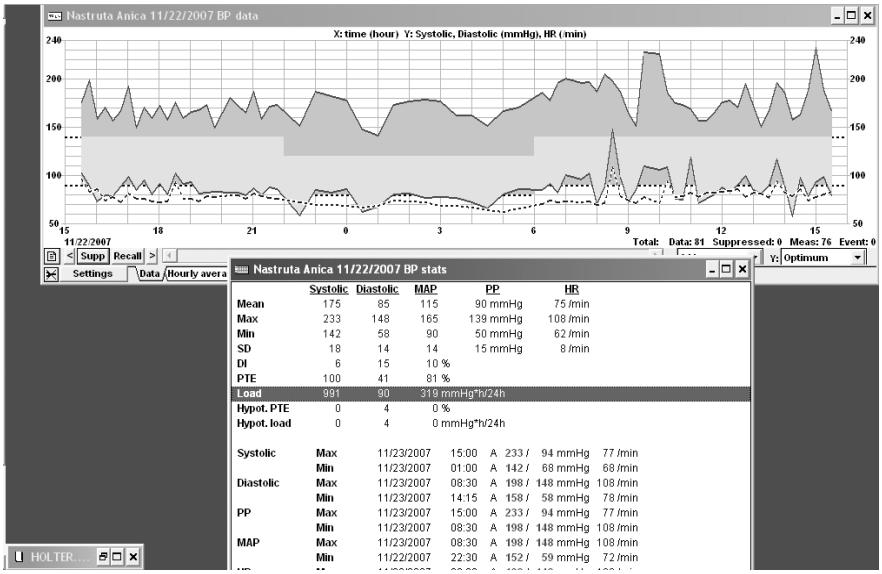

Fig. 1. Sarcina de presiune (LOAD)

Un parametru deosebit de util în practică, derivat din determinările tensiunii arteriale sistolice si diastolice, este denumit BP load (sarcina de presiune sau impactul hiperbaric), care reflectă procentul parametrilor presionali sistolici şi diastolici care depăşesc valorile normale. Practic este vorba de aria de sub curba tensională şi limita superioară a normalului. Unitatea de măsură este reprezentată de mmHg*h/24 ore, iar valoarea normală este de 60 mmHg*h/24 ore. Acesta poate diferenţia formele susţinute de formele episodice de hipertensiune arterială (5). Diversele studii au stabilit corelaţia acestui indicator cu leziune de organe ţintă, corectarea lui devenind obiectiv terapeutic (fig. 1).

Hipertensivul vârstnic se caracterizeaza prin labilitatea valorilor tensionale diurne, instalând peakuri hipertensive simptomatice chiar sub tratament corect condus, ceea ce defineşte HTA intermitentă. Deoarece mare parte dintre aceşti pacienţi prezintă afectare de organe ţintă, Verdecchia sugerează că încărcarea hipertensivă (sarcina de presiune, LOAD) poate fi mult mai strâns corelată cu riscul cardiovascular, când comparăm cu nivelurile medii ale TA înregistrate prin monitorizare continuă ambulatorie (6).

Estimarea sarcinii hipertensive (BP load) oferă relaţii mai precise despre expunerea sistemului cardiovascular la presiune pe parcursul a 24 ore (7). Numeroase evidenţe sugerează o relaţie directă şi semnificativă între sarcina hipertensivă şi leziuni de organe ţintă, acest parametru calculat numai prin monitorizare continuă are valoare predictivă superioară în relaţie cu morbiditatea şi mortalitatea cardiovasculară faţă de măsurarea izolată clinic a tensiunii arteriale. Mărimea sarcinii hipertensive realizează cea mai bună corelare cu HVS, în unele studii s-a demonstrat până la 90% corelarea HVS cu LOAD sistolic care depăşeşte 50% (8).

Scopul studiului este să identifice prevalenţa sindromului metabolic în rândul populaţiei hipertensive şi particularităţile hipertensiunii arteriale la pacienţii hipertensivi cu sindrom metabolic, utilizând monitorizarea continuă pe 24 ore a tensiunii arteriale.

MATERIAL ŞI METODĂ

Raportat la populaţia judeţului Bacău (n=725.005 locuitori), cu o eroare de eşan-

tionare de ±5,6% faţă de IC95% s-a stabilit dimensiunea eşantionului reprezentativ şi anume 303 pacienţi.

Medicină Internă a Spitalului Judeţean de Urgenţă Bacău. Selecţia pacienţilor a fost efectuată utilizând ca metodă selecţia sistemică, în sensul că în perioada de screening a fost examinat pentru studiu al cincilea pacient hipertensiv prezentat la consultaţie sau la internare şi care a prezentat hipertensiune arterială la prima vizită medicală. Diagnosticul de HTA s-a efectuat prin măsurarea TA la nivelul braţului bilateral, respectând recomandările Ghidului de hipertensiune din 2003 şi 2007. Pentru diagnostic şi stadializare s-a luat în considerare valoarea cea mai mare. La această primă vizită s-a solicitat semnarea consimţământului informat al pacientului în vederea introducerii în studiu. Pacienţii care au semnat formularul de consimţământ au fost invitaţi la alte două vizite medicale la intervale de o săptămână. Pacienţii care au prezentat valori definitorii pentru diagnosticul clinic de HTA la vizita din săptămâna a treia au fost randomizaţi şi incluşi în studiu în vederea monitorizării continue a tensiunii arteriale.

Au fost screenaţi un număr de 391 pacienţi la prima vizită medicală. Dintre aceştia 32 au prezentat tensiunea arterială normală la următoarele 2 vizite, 27 de pacienţi nu au mai revenit la una sau la ambele vizite din motive de necomplianţă, ceilalţi 332 pacienţi au intrat în studiu. După analizarea traseelor înregistrate prin monitorizarea continuă pe 24 ore a tensiunii arteriale, 11 pacienţi au avut trasee neinterpretabile (artefacte multiple, număr mic de măsurători corecte, decuplări ale manşetei) şi au fost excluşi. Alţi 18 pacienţi au înregistrat valori medii sistolodiastolice normale şi sarcina de presiune (load) absentă. Deoarece aceştia din urmă nu aveau antecedente hipertensive şi nu urmau tratament antihipertensiv, au fost consideraţi cu hipertensiune de halat alb şi au constituit un sublot analizat separat. Astfel au rămas în studiu 303 pacienţi hipertensivi, care au fost supuşi analizei statistice.

Monitorizarea continuă a tensiunii arteriale s-a efectuat cu sistemul de monitorizare care se numeşte ABPM-04, care corespunde cerinţelor protocoalelor de validare ale BHS (British Hypertension Society) şi al AAMI referitoare la tensiometrele automate.

REZULTATE

În studiul nostru, din 303 pacienţi hipertensivi, 181 de pacienţi (60,1%), cu raportul F/M = 3,41/1, au îndeplinit criterii de sindrom metabolic. Criteriile de diagnostic pentru sindromul metabolic au fost cele oferite de NCEP-ATPIII (minim 3 criterii), criteriul de bază fiind tensiunea arterială mai mare sau egal cu 135/85 mmHg. Vârsta medie a pacienţilor a fost de 66,50 ± 10,28 ani, variind între 18 şi 83 ani. De notat ponderea crescută a pacienţilor din mediul urban (60,8%).

Valorile tensiunii arteriale medii monitorizate pe 24 ore, în funcţie de sex, vârstă şi mediu de proveniență relevă valori ale tensiunii arteriale sistolice de 134,5 mmHg la pacienţii cu vârsta sub 65 ani şi 140,13 mmHg la grupa de vârstă peste 65 ani. Remarcăm creşterea valorilor presionale pe măsura înaintării în vârstă, evidenţiindu-se diferenţe semnificativ mai mari ale tensiunii sistolice la grupa de vârstă de peste 65 ani

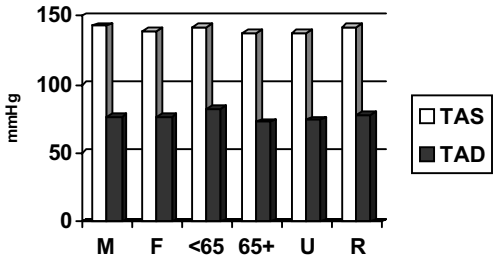

Fig. 2. Valori medii ale tensiunii arteriale monitorizate 24 ore pe sex, grupe de vârstă şi mediu de proveniență

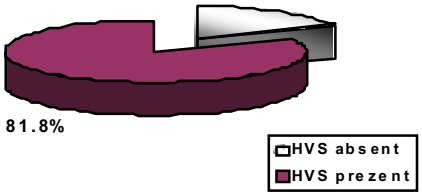

Fig. 3. Prezenţa HVS la lotul cu sindrom metabolic

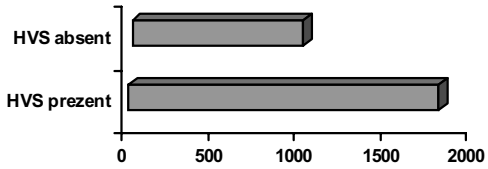

Fig. 4. Sarcina hipertensivă în funcţie de prezenţa HVS

($p < 0,05$). Valorile medii diastolice sunt 72 mmHg, respectiv 75 mmHg şi nu prezintă diferenţe semnificative între grupele de vârstă. În ansamblu tensiunea sistolică medie prezintă valori ce depăşesc valoarea de referinţă de 135 mmHg iar valori ale tensiunii arteriale diastolice nu ajung până la valoarea de referinţă de 85 mmHg, ceea ce sugerează că predomină hipertensiunea sistolică izolată la majoritatea pacienţilor (fig. 2).

Sub aspectul profilului circadian, dintre cei 181 de pacienţi hipertensivi cu sindrom metabolic un număr de 54 pacienţi (29,8%) au prezentat profil tensional non dipper, profil dipper fiziologic a fost înregistrat la 50 pacienţi (27,6%), reversedipperi au fost 49 cazuri (27,1%) şi 3 cazuri hiper dipper (1,7%).

Prelucrarea statistică a valorilor tensionale monitorizate, în funcţie de caracteristicile epidemiologice, relevă următoarele aspecte :

- la sexul masculin, atât valorile medii cât şi cele minime ale tensiunii sistolice sunt semnificativ mai mari comparativ cu cele monitorizate la sexul feminin ($p < 0,05$) ;
- indexul diurn relevă că lotul de pacienţi cu sindrom metabolic este preponderent non dipper, cu diferenţe semnificative între sexe ($p < 0,01$) ;
- valorile medii ale tensiunii diastolice şi cele minime sunt semnificativ mai mari la sexul masculin ($p < 0,01$), cu variaţii semnificative ale indexului diurn ($p < 0,05$) ;
- vârsta de 65 de ani a fost aleasă ca valoare de referinţă pentru compararea tensiunii arteriale deoarece vârsta medie a lotului cu sindrom metabolic a fost evaluată la 66,50 ani, evidenţiindu-se diferenţe semnificativ mai mari ale tensiunii sistolice la grupa de vârstă de peste 65 ani ($p < 0,05$) ; tensiunea diastolică nu prezintă diferenţe semnificative între grupele de vârstă ;
- pe medii de provenienţă mediile sistolo-diastolice nu evidenţiază diferenţe semnificative.

La pacienţii cu sindrom metabolic, în funcţie de prezenţa HVS pe electrocardiogramă şi valorile sarcinii hipertensive (load) pentru TA sistolică s-au evidenţiat următoarele aspecte :

- prezenţa HVS a fost identificată la 148 dintre pacienţii lotului cu sindrom metabolic (81,8%)(fig.3) ;
- valorile load au avut o variabilitate în intervalul 0 – 1858 mmHg*h/24h (fig.4) ;
- s-au înregistrat semnificativ mai multe cazuri cu load sub 500 la pacienţii cu HVS prezent ($p = 0,035$)(fig.5) ;
- valorile medii ale load au fost semnificativ mai crescute la pacienţii cu HVS prezent ($p < 0,01$)(fig.6).

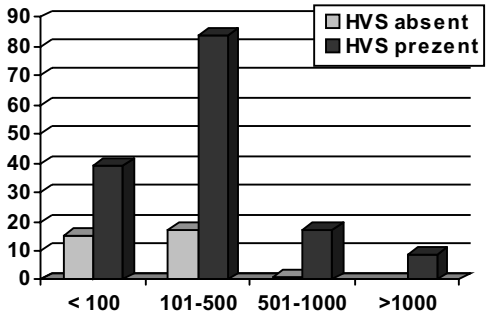

Fig. 5. Nivelul sarcinii hipertensive în funcţie de prezentţa HVS

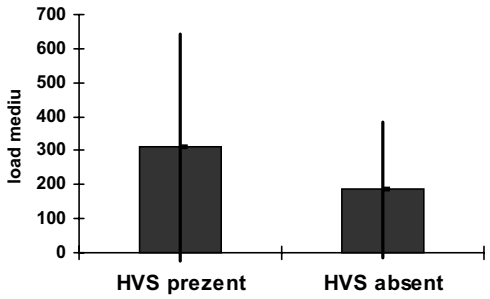

Fig. 6. Valorile medii ale sarcinii hipertensive în funcţie de HVS

DISCUŢII

Hipertensiunea arterială este adesea parte a unei constelaţii de anormalităţi clinice şi metabolice, realitate remarcată încă din 1920. Sindromul metabolic, în concepţia modernă poate fi trasat de la propoziţia lui Reaven din 1988, care a reunit sub denumirea de „sindrom X metabolic" asocierea hipertensiunii arteriale cu obezitatea abdominală (sau viscerală), dislipidemia aterogenă (HDL-colesterol scăzut, trigliceride crescute), intoleranţa la glucoză şi insulino-rezistenţa, acest ultim parametru fiind elementul central al sindromului (9).

Diagnosticul sindromului metabolic are importanţă deosebită la persoanele nediabetice deoarece identifică riscul crescut de dezvoltare a bolilor cardiovasculare, în concordanţă cu asocierea factorilor de risc implicaţi (10). În cazul identificării uneia dinre componente, este indicată căutarea sistematică a celorlalte elemente definitorii.

Definiţia originală şi cea revizuită a NCEP-ATPIII (National Cholesterol Education Program – Adult Treatment Pannel) este recomandată de Asociaţia Americană a Inimii pentru uzul clinic (11,12). Aceasta identifică 6 componente ale sindromului metabolic în relaţiile cu bolile cardiovasculare: obezitatea abdominală, dislipidemia aterogenă, creşterea presiunii sanguine, rezistenţa la insulină, intoleranţă la glucoză, statusul proinflamator şi protrombotic. Apariţia precoce a leziunilor subclinice de organ poate constitui predictor independent de evenimente adverse vasculare şi poate explica parţial asocierea sindromului metabolic cu risc înalt cardiovascular şi renal.

Date din studiul PROSPER (Prospective Study of Pravastatin in the Elderly at Risk) susţin că dintre componentele sindromului metabolic doar hipertensiunea arterială şi alterarea toleranţei la glucoză au valoare predictivă semnificativă pentru riscul cardiovascular (13). Un foarte recent studiu italian (14), studiul SardiNIA, demonstrează că elementele sindromului metabolic accelerează modificările morfologice şi funcţionale arteriale specifice vârstei.

Valorile tensiunii arteriale măsurate prin monitorizare 24 ore se corelează net superior cu riscul de leziuni a organelor ţintă. Primele evidenţe au fost legate de hipertrofia ventriculară stânga.

În studiul nostru 60,1% dintre pacienţii hipertensivi prezintă sindrom metabolic, cu pondere crescută a sexului feminin şi a populaţiei din mediul urban.

Vârsta medie a lotului fiind 66 ani, explică predominenţa hipertensiunii sistolice izolate la majoritatea pacienţilor.

Monitorizarea continuă pe 24 ore a tensiunii arteriale a evidenţiat creşterea progresivă a tensiunii sistolice odată cu înaintarea în vârstă dar şi creşterea nesemnificativă statistic a tensiunii diastolice, aspect datorat coexistenţei sindromului metabolic la vârstnici. Valorile tensiunii arteriale sunt semnificativ mai mari la sexul masculin, atât sistolice cât şi diastolice şi se însoţesc de alterarea profilului circadian.

Peste 80% dintre pacienţi au prezentat HVS electrocardiografică. Corelând HVS cu sarcina hipertensivă înregistrată prin monitorizare se remarcă predominenţa sarcinilor hipertensive uşoare şi medii (< 500 mmHg*h/24h) la majoritatea pacienţilor cu HVS însă valorile medii ale sarcinii presionale au fost semnificativ mai crescute la pacienţii cu HVS prezent.

CONCLUZII

Prevalenţa sindromului metabolic este foarte înaltă în rândul populaţiei hipertensive din aria geografică a judeţului Bacău şi creşte cu înaintarea în vârstă.

Predomină hipertensiunea sistolică izolată caracteristică populaţiei vârstnice, cu creşterea tensiunii arteriale sistolice odată cu înaintarea în vârstă dar şi creşterea tensiunii diastolice specifice sindromului metabolic.

Hipertensivii de sex masculin prezintă forme mai severe de hipertensiune, cu profil circadian patologic în proporţie semnificativ mai crescută.

Hipertrofia ventriculară stângă este întâlnită la 4 din 5 hipertensivi cu sindrom metabolic. Valorile medii ale sarcinii hipertensive sunt semnificativ mai crescute la hipertensivii cu HVS prezentă.

Monitorizarea continuă a tensiunii arteriale permite identificarea profilului tensional şi a sarcinii hipertensive. La hipertensivii cu sindrom metabolic predomină profilul nondipper şi reversedipper.

La pacienţii cu hipertensiune sistolică izolată, asocierea sindromului metabolic reprezintă principalul risc cardiovascular şi se asociază cu creşterea tensiunii diastolice, prezenţa hipertrofiei ventriculare stângi şi valori crescute ale sarcinii hipertensive.

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HELICOBACTER PYLORI – PRIETEN SAU DUŞMAN ? O CONVIEŢUIRE DE SECOLE

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HELYCOBACTER PYLORI- FRIEND OR FOE ? (Abstract) : *Helicobacter pylori* (*H. pylori*) is the bacterium that causes the most common infection in the world. Epidemiological studies show that over 50% of the population is infected, there are areas where virtually the entire population present the infection since childhood. Although the stomach seems to be a „hostile" environment for the development of microorganisms due to the acid pH not fit for life, it may be infected ; the first spiral organisms were described at this level as early as 1874. These microorganisms were isolated by Waren and Marshall in 1982 and named *Campylobacter pyloridis*. In 1989 Goodwin renamed the bacterium *Helicobacter pylori*. *Helicobacter pylori* is thus a bacterium which „manages" to survive in gastric acid environment producing an infection which, untreated, is likely to persist the entire life. This feature explains the high rate in the spread of the infection. The infection manifests itself in different ways, depending on the pathogenic strain and the host response, the majority of those infected being asimptomatic or paucysimptomatic. The great achievement of Warren and Marshall,who were rewarded with a Nobel prize in 2005, was to demonstrate that *H. pylori* can lead to gastric cancer, the bacteria being categorized as an oncogene agent. The anti *H. pylori* „campaign" was only a step away… and yet, the bacterium has been living in the stomach for centuries. We will try to list the pros and cons of *H. pylori* infection, when and how the infection should be treated, clearly indicating groups of patients in which eradication is absolutely mandatory. **Key words** : *HELICOBACTER PYLORI,* GASTRIC CANCER, GASTRITIS

Helicobacter pylori (*H. pylori*) este bacteria care determină cea mai răspândită infecţie de pe glob. Studiile epidemiologice arată că peste 50% din populaţie este infectată, existând zone în care practic toată populaţia prezintă infecţia încă din copilărie (1).

Deşi stomacul pare a fi un mediu „ostil" dezvoltării microorganismelor dat fiind pH-ul acid impropriu vieţii, acesta poate fi infectat, primele microorganisme spiralate fiind descrise la acest nivel încă din anii 1874 (2,3). Warren şi Marshall în 1982 izolează pentru prima dată o bacterie în stomac pe care o numesc *Campylobacter pyloridis* (4). În 1989 Goodwin redenumeşte această bacterie *Helicobacter pylori* (5).

Helicobacter pylori este o bacterie ce „reuşeşte" să supravieţuiască în mediu acid gastric producând o infecţie care, netratată, pare a persista toată viaţa. Această particularitate explică rata mare de răspândire a infecţiei. Mecanismul principal de transmitere a bacteriei este fecal-oral. Forma de manifestare a infecţiei este diferită, depinzând de tulpina patogenă şi de răspunsul gazdei. Majoritatea celor infectaţi sunt asimptomatici sau paucisimptomatici.

Marele merit a lui Warren şi Marshall, care au fost recompensaţi în 2005 cu premiul Nobel, a fost acela de a demonstra că *H. pylori* poate duce la apariţia cancerului

gastric, bacteria fiind trecută pe lista agenţilor oncogeni. De aici şi până la „campania" anti *H. pylori* nu a fost decât un pas... şi totuşi, bacteria convieţuieşte în stomac de secole.

Vom încerca să aducem argumente pro şi contra infecţiei cu *H. pylori*, cum şi când trebuie tratată infecţia, cu precizarea clară a grupelor de pacienţi la care eradicarea este absolut obligatorie.

EPIDEMIOLOGIE

H. pylori produce o infecţie cronică gastrică care survine după un episod acut, petrecut în copilărie în ţările cu un standard socio-economic scăzut şi la adult în cele dezvoltate. Prevalenţa infecţiei în ţările în curs de dezvoltare este la adult de 80-90% iar în ţările dezvoltate nu depăşeşte 60%. Incidenţa infecţiei este maximă în primii ani de viaţă, riscul dobândirii acesteia în timpul vieţii adulte scăzând exponenţial. Prevalenţa infecţiei creşte cu vârsta (6,7).

Gazda principală este reprezentată de specia umană. *H. pylori* are tropism tisular strict, reprezentat de celulele antrale gastrice. Poate fi identificat nu doar în stomac ci şi în zonele de metaplazie gastrică din duoden, esofag, fornix, diverticul Meckel, rect (8). Specii similare se pot întâlni şi la primate.

Teoretic toate persoanele sunt susceptibile la infecţie. Marea majoritate a studiilor indică un risc egal de infecţie cu *H. pylori* pentru ambele sexe. Incidenţa cea mai mare a infecţiei este în primii ani de viaţă şi în special în primul, fiind de 3,8% în ţările în curs de dezvolatare şi de 2,7% în ţările dezvoltate (9).

Factorii genetici (răspunsul imun al gazdei) sunt cei care, împreună cu factorii de virulenţă ai *H. pylori*, dictează tipul de infecţie cu *H. pylori* (gastrită, ulcer, cancer etc.) (10).

Consumul de alcool şi fumatul nu sunt factori de risc pentru infecţia cu *H. pylori*, existând unele păreri că alcoolismul ar avea un efect protector contra infecţiei prin alterarea mucusului gastric (8).

Calea principală de transmitere este cea fecal-orală, de aceea nivelul de dezvoltare economică şi educaţia populaţiei sunt principalii factori de risc pentru infecţia cu *H. pylori*.

PATOGENIE

Caracteristica *H. pylori* este tropismul strict pentru mucoasa gastrică datorită unor antigene de suprafaţă care se fixează de receptorii celulelor epiteliale şi de mucusul gastric, şi capacitatea de a „supravieţui" în mediul acid gastric, ostil altor microorganisme.

Cum reuşeşte *H. pylori* să „supravieţuiască" în mediu acid?

- bacteria secretă o serie de enzime, dintre care un rol major îl are ureaza. Aceasta scindează ureea în CO_2 şi NH_3 care fixează ionii de H^+ din sucul gastric, formând un sistem tampon în jurul *H. pylori*;
- în stadiul iniţial al infecţiei *H. pylori* produce o serie de substanţe care inhibă direct secreţia acidă gastrică (în primele luni de la infecţie pH-ul gastric creşte) urmând ca apoi, în faza cronică să aibă loc o stimulare constantă a secreţiei acide gastrice;
- bacteria se „poziţionează" sub stratul protector de mucus, deasupra celulelor epiteliale gastrice şi la distanţă de sistemul de apărare al mucoasei gastrice, care se găseşte sub lamina propria. Pentru a putea supravieţui însă bacteria are nevoie de nutrienţi care vor fi obţinuţi prin iniţierea unei reacţii inflamatorii, care afectează celulele epiteliale.

În concluzie, deşi infecţia cu *H. pylori* este predominant intraluminală este însoţită de o reacţie inflamatorie cronică, care în lipsa unui tratament va dura toată viaţa. Intensitatea acestui răspuns inflamator de-

pinde de virulenţa bacteriei şi de factorii genetici ai gazdei, ducând la manifestări diferite de boală (11,12).

Factorii de virulenţă

Unele tulpini de *H. pylori* sunt dotate cu o serie de factori de virulenţă, cei mai bine studiaţi fiind : insula de patogenitate cag, proteina vacA, cagA şi genele care codează preteinele membranare.

Insula de patogenitate cag reprezintă un fragment de genom de 40kb ce cuprinde aproximativ 30 de gene care codifică proteine ce asigură un aparat secretor de tip IV (cagE). Acest aparat secretor este folosit de *H. pylori* pentru a transloca în interiorul celulelor epiteliale diverse proteine dintre care cea mai cunoscută este proteina cagA. După translocare, cagA este fosforilată de kinazele Src cu inducerea unui proces de remodelare a celulei („hummingbird phenotype"). Sunt asfel modificate motilitatea şi lungimea celulelor epitaliale gastrice. S-a demonstrat de asemenea că tulpinile de *H. pylori* care conţin mutanţi ai insulei de patogenitate cagA (Hp0524, Hp0540, Hp0542, Hp0543) nu translocă proteina cagA dar induc o secreţie puternică a IL-8, care stimulează motilitatea celulară şi declanşează un răspuns inflamator sever. CagA interacţionează cu sistemele enzimatice celulare cum ar fi tirozin fosfataza SHP-2 (10,11,12).

A fost descris recent un alt *factor de virulenţă – dupA* (duodenal ulcer promoting gene) (jhp0917-jhp0918) – care creşte riscul pentru ulcer duodenal cu reducerea riscului pentru gastrita atrofică şi cancer gastric.

Citotoxina vacA este considerată un factor important de virulenţă în patogeneza ulcerului şi cancerului gastric. Această toxină poate induce multiple modificări celulare : vacuolizarea celulară, formarea de canale membranare, modificări în funcţionarea sistemului endosomal/lisosomal, apoptoză şi imunomodulare. Interacţionează, de asemenea, cu receptorii protein tirozin fosfatazei RPTPß – a căror activare induce vacuolizarea celulară şi fosforilare (Git1). Recent s-a demonstrat că vacA inhibă proliferarea celulelor T, cu blocarea secreţiei de IL-2.

Proteinele membranare sunt codate de o serie de gene ale *H. pylori*, cele descrise ca fiind factori de virulenţă sunt : BabA, SabA, AlpAB şi OipA, BabA, SabA, AlpAB.

SabA este implicată în activarea nonopsonică a neutrofilelor (13).

Modalităţile de răspuns la infecţia cu *H. pylori*

Răspunsul epitelial la infecţie este determinat de mai multe variabile : factorii bacterieni de virulenţă, modalităţile de semnalizare legate de receptorii specifici ce recunosc componente bacteriene şi mediul local reprezentat de hormoni, neurotransmiţători, citokine imune.

Răspunsurile celulelor epiteliale includ :

- modificări ale morfologiei celulare ;
- disrupţia joncţiunilor strânse intercelulare ;
- producerea de citokine ;
- creşterea proliferării celulare ;
- creşterea apoptozei ;
- activarea a numeroase gene asociate stresului inflamator.

În concluzie, infecţia cu *H. pylori* poate îmbrăca diferite aspecte. Cea mai mare parte a subiecţilor infectaţi prezintă doar o gastrită cronică superficială, cu evoluţie benignă, puţin simptomatică, 10% din cei infectaţi dezvoltă boală ulceroasă peptică, aproximativ 1% au o evoluţie spre adenocarcinom gastric şi sub 1% spre limfom MALT.

Funcţie de tipul gazdei (factorul genetic şi răspunsul imun) şi virulenţa bacteriei o parte din cei infectaţi cu *H. pylori* vor avea o evoluţie în ani spre adenocarcinom gastric, în secvenţa : gastrită cronică atrofică (pierderea ţesutului glandular), metaplazie intestinală (înlocuirea celulelor glandulare

cu celule de tip intestinal a căror marker este reprezentat de prezenţa vililor), displazie (apariţia de celule mari, cu atipii nucleare) şi neoplazie.

METODELE DE DIAGNOSTIC ALE INFECŢIEI CU *H. PYLORI*

- metode directe şi indirecte ;
- metode invazive şi non-invazive ;
- tehnici moleculare.

Scopul diagnosticului presupune pe lângă identificare infecţiei, verificare obligatorie a eradicării după tratament şi în cazuri speciale evidenţierea factorilor de virulenţă ai *H. pylori* şi tulpinilor rezistente la claritromicină.

Metodele indirecte – depistează prezenţa răspunsului organismului la infecţia. Cel mai frecvent se utilizează dozarea anticorpilor anti *H. pylori* în ser (metoda serologică). Rezultate fals negative se obţin în „fereastra" imunologică, la taraţi, imunosupresaţi iar cele fals pozitive după eradicarea infecţiei datorită persistenţei anticorpilor ani de zile. Această metodă este utilizată doar pentru diagnosticul infecţiei *H. pylori* şi nu pentru verificarea eardicării.

Cu ajutorul tehnicilor imunoblot se pot identifica şi factorii de virulenţă, respectiv Ac anti cagA, vacA. Prin dozarea gastrinei (G) şi a pepsinogenului (PSG) I şi II în ser se pot obţine informaţii preţioase cu privire la starea mucoasei gastrice (gastrită superficială sau atrofică). PSGI este secretat de celule oxintice din fundus şi corpul gastric, PSGII este secretat în întreg stomacul iar gastrina de celulele G din antrum.

În prezenţa gastritei supeficiale concentraţia serică de G, PSGI şi II creşte, pe când apariţia atrofiei gastrice duce la scăderea concentraţiei serice de G şi PSG, în special cea de PSGI. Raportul PSGI/II reprezintă un indicator important de urmărire a pacienţilor cu Ac anti *H. pylori* pentru depistarea apariţiei atrofiei gastrice (14).

Metodele directe invazive presupun evidenţierea directă a bacteriei sau produşilor de secreţie prin prelevarea de biopsii gastrice şi sunt reprezentate de :

- testul rapid al ureazei ;
- examenul direct al piesei de biopsie ;
- examenul histopatologic ;
- cultura ;
- tehnici PCR în biopsia gastrică.

Pentru evitarea rezultatelor fals negative trebuie luate unele precauţii :

- oprirea tratamentului cu inhibitori ai pompei de protoni (IPP) pentru cel puţin 14 zile ;
- întreruperea tratamentului cu antibiotice ;
- prelevarea biopsiei din regiunea antrală, la 1cm de pilor, de preferinţă 3-4 biopsii şi din corpul gastric la cei cu tratament cronic cu IPP ;
- spălarea sondei de biopsie de glutaraldehidă.

Examenul histologic are avantajul evaluării concomitente a leziunilor mucoasei gastrice, şi reprezintă gold-standardul diagnostic. Se folosesc coloraţiile hematoxilină-eozină, Giemsa modificată, Warthin-Starry. Coloraţia Warthin – Starry este cea mai fidelă, există studii care arată rezultate superioare tehnicilor PCR.

Cultura – este rezervată situaţiilor speciale, când există rezistenţă la tratament, pentru efectuarea antibiogramei. Necesită utilizarea a două medii de cultură, creşterea fiind lentă (7 zile).

Tehnici moleculare – prin tehnici PCR în piesa de biosie se pot depista markerii de virulenţă (vacA, cagA, dupA etc.) precum şi tulpinile rezistente la tratamentul cu macrolide. De asemenea este posibilă depistare *H. pylori* la cei cu limfom gastric MALT. Limita de detecţie a metodei este de 0,02pg Hp (aproximativ 10 organisme).

În concluzie, metodele directe invazive de depistare a *H. pylori* rămân "standardul de aur" al diagnosticului pentru că inventariază în acelaşi timp şi mucoasa gastrică şi permit izolarea bacteriei. Sunt indicate pentru diagnosticul infecţiei şi mai puţin pentru confirmarea eradicării (risc de re-

contaminare) (12,13,14).

Metodele directe neinvazive sunt indicate de elecţie pentru demonstrarea eradicării infecţiei. Prin clearence bacterian se defineşte dispariţia bacteriei după tratament, iar prin eradicare se înţelege dispariţia bacteriei la 4-6 săptămâni de la întreruperea tratamentului.

Există 2 metode directe neinvazive de diagnostic :

- depistarea Ag *H. pylori* fecal ;
- testul respirator cu uree marcată.

Rezultate fals negative se obţin în cazul consumului de IPP şi antibiotice precum şi în cazul gastritei atrofice.

Diagnosticul infecţiei cu *H. pylori* presupune efectuarea unui test indirect (serologic) şi a unuia direct. Infecţia este prezentă dacă cele două teste sunt pozitive şi este absentă dacă cele două teste sunt negative. Un test indirect negativ nu exclude infecţia, deoarece poate apare în infecţia acută şi la imunosupresaţi. Un test direct negativ nu exclude infecţia în prezenţa gastritei atrofice.

TRAMENTUL INFECŢIEI CU *HELICOBACTER PYLORI*

Are drept scop eradicarea infecţiei, ceea ce înseamnă absenţa *H. pylori* la 4-6 săptămâni după terminarea tratamentului, demonstrată, de preferat, printr-o metodă directă neinvazivă (endoscopia are un risc minor de reinfecţie) şi niciodată prin dozarea anticorpilor în ser (se menţin în ser ani, uneori toată viaţa).

Tratamentul standard constă în administrarea a 2 antibiotice şi un IPP cel puţin 7 zile. Metaanalizele recente arată o rată de eradicare sub acest tratament de sub 80%, de aceea, noile protocoale prevăd creşterea duratei de administrare a antibioticului la 10-14 zile, dublarea dozei de IPP, folosirea şi a altor antibiotice, tratamentul secvenţial (12,13).

În România s-a demonstrat o rezistenţă a *H. pylori* la metronidazol de peste 30%, de aceea acesta nu se utilizează în schemele de eradicare de primă intenţie.

Amoxicilina este antibioticul care nu dă rezistenţă, fiind inclusă în toate schemele de tratament.

Pentru a asigura un pH gastric constant ridicat, la care antibioticul să fie activ, se recomandă administrarea IPP în 2 prize. De asemenea, este de preferat ca antibioticul să fie dat în cel puţin în 2 prize (12).

Schema de tratament aplicată de primă intenţie în România constă în IPP (20 mg x 2/zi)+Amoxicilină (1 g x 2/zi)+Claritromicină (0,5 g x 2/zi), 7-14 zile.

Se mai pot utiliza următoarele scheme de tratament :

- IPP 40 mg/zi+ Denol (480 mg/zi) + Amoxicilină (2 g/zi) + Claritromicină (1 g/zi) ;
- IPP 40 mg/zi+ Denol (480 mg/zi) + Claritromicină (1 g/zi) + Tetraciclină (2 g/zi).

O strategie eficientă, relativ nouă, constă în tratamentul secvenţial de eradicare : 5 zile IPP 40 mg/zi + Amoxicilină 2 g/zi, urmate de 5 zile de IPP 40 mg/zi+ Metronidazol 500 mg x 2/zi + Claritromicină 500 mg x 2/zi, cu o rată de eradicare de peste 90%.

În cazurile speciale, cu *H. pylori* rezistent, dificil de eradicat, se recomandă introducerea unui nou antibiotic – Rifabutina 150 mg x 2/zi alături de IPP şi Amoxicilină, 10 zile (14).

Se mai poate recurge, ca tratament de rezervă, şi la combinaţia : IPP + Denol 120 mg x 4/zi +Metronidazol 250 mg x 4/zi+Tetraciclină 500 mg x 4/zi 14 zile, urmat de IPP încă 4 săptămâni.

Pe cine tratăm ?

Consensul de la Maastricht III indică următoarele grupe de pacienţi la care eradicarea infecţiei cu *H. pylori* este obligatorie :

- gastrita cronică atrofică cu metaplazie intestinală ;
- ulcerul duodenal şi gastric ;
- gastrita *H. pylori* pe bontul gastric după neoplasm gastric ;

- antecedentele heredecolaterale de cancer gastric ;
- limfomul MALT cu grad scăzut ;
- la cererea pacientului, după consultarea medicului curant.

Marea majoritate a pacienţilor infectaţi nu au indicaţii absolute de eradicare. Avantajul eradicărării ar fi scăderea rezervorului de bacterii şi împiedicarea evoluţiei spre gastrită cronică atrofică, stadiu în care eradicarea se face greu datorită scăderii numărului de colonii. Studiile recente arată că eradicarea infecţiei cu *H. pylori* în stadiile avansate de atrofie gastrică nu scade riscul de cancer gastric.

În practică se aplică eradicarea *H. pylori* la toţi pacienţii cu ulcer peptic (eradicarea „vindecă" ulcerul şi duce la dispariţia recidivei), la pacienţii cu infecţie pe bont gastric după chirurgia pentru neoplasm, la pacienţii cu limfom MALT şi la toţi pacienţii cu gastrită, simptomatici sau nu, dacă aceştia doresc.

Marele „dezavantaj" al eradicării „în masă" este reprezentat de selectarea tulpinilor de *H.pylori* rezistente. De altfel pentru România este demonstrată rezistenţa *H. pylori* la Metronidazol (utilizat foarte mult pentru tratamentul lambliazei şi în infecţiile genitale). S-a dovedit că la pacienţii cu boală de reflux gastro-esofagin întreruperea tratamentului cu IPP nu este urmată de rebound acid dacă există infecţie cu *H. pylori*.

CONCLUZII

H. pylori este bacteria care dă cea mai frecventă infecţie cronică la om din lume. Prin mecanisme specifice reuşeşte să „supravieţuiască" în mediu acid gastric. Tipul de inflamaţie depinde de factorul genetic şi de virulenţa bacteriei. Majoritatea celor infectaţi prezintă doar o gastrită cronică superficială, fără riscuri majore. Doar 1% din subiecţii infectaţi dezvoltă adenocarcinom gastric în secvenţa gastrită cronică atrofică-metaplazie intestinală- adenocarcinom. Tratamentul se face cu triplă terapie, scopul fiind eradicarea infecţiei. Se preconizează scăderea „rezervorului" uman de bacterii „preţul" fiind selectarea tulpinilor rezistente.

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CARDIOMIOPATIA – COMPLICAŢIE SUBEVALUATĂ ÎN CIROZA HEPATICĂ ?

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CARDIOMYOPATHY IN LIVER CIRRHOSIS – AN UNDIAGNOSED ENTITY ? (Abstract) : Cirrhotic cardiomyopathy is a condition recently known in liver cirrhosis consisting of systolic dysfunction to stress factors, diastolic dysfunction and electrophysiological abnormalities in the absence of cardiac disease. The prevalence of cirrhotic cardiomyopathy remains unknown until now. It can be diagnosed by using a combination of electrocardiograph, 2-dimensional echocardiography, and various serum markers (brain natriuretic factor - BNP, proBNP, TnI). Pathogenic mechanisms underlying cirrhotic cardiomyopathy development include abnormal signaling βadrenergic, cardiomyocites membrane fluidity changes, interstitial fibrosis, myocardial hypertrophy, altered transmembrane ion channels as intervention with negative inotropic effect of different substances whose concentration is increased in cirrhosis. Major stresses on the cardiovasculary system such as liver transplantations, infections, insertion of transjugular portosystemic stent-shunt (TIPSS) have been demonstrated to put in evidence the presence of cirrhotic cardiomyopathy. Heart failure is a significant cause of mortality after liver transplantation but the improvement of liver function determines cardiac abnormalities reversal. Current management recommendations include empirical, nonspecific and mainly supportive measures, no specific treatment can be recommended, and cardiac failure should be treated as in non-cirrhotic patients with sodium restriction, diuretics, and oxygen therapy when necessary. The exact prognosis remains unclear. The extent of cirrhotic cardiomyopathy generally correlates to the degree of liver insufficiency. Reversibility is possible (either pharmacological or after liver transplantation), but further studies are needed. **Key words** : CIRRHOSIS, CARDIOMYOPATHY, DIASTOLIC DYSFUNCTION, SYSTOLIC INCOMPETENCE, QT INTERVAL

EDITORIAL

KEEPING AN OPEN MIND ON LONG-TERM USE OF PROTON PUMP INHIBITORS

Since the release of omeprazole, the first proton pump inhibitor (PPI), in 1988 in Europe and, two years later, in the United States, six other PPIs have become available such as pantoprazole, esomeprazole, lansoprazole, dexlansoprazole, and rabeprazole. The discovery of omeprazole is the story of a huge therapeutic success, the result of over 20 years of hard work and meticulous research made by an extraordinary team of more than 150 researchers, who discovered and tested over 800 compounds, surmounting unimaginable obstacles.

Once introduced in the clinical practice, PPIs have revolutionized the management of acid-related diseases such as peptic gastric/duodenal ulcer (including bleeding peptic ulcer), gastroesophageal reflux disease, Helicobacter pylori (in combination with antibiotics), Zollinger-Ellison syndrome, being later recommended for the prophylaxis of upper gastrointestinal complications with nonsteroidal anti-inflammatory drugs therapy, in ICU prophylaxis for stress ulcer as well as in functional dyspepsia. Thus, PPIs have become some of the most widely prescribed agents throughout the world, with tens of billions of US dollars in global sales. Still, their use and sales are continuously increasing due to their efficacy and safety profile when administered according to the appropriate indications. Unfortunately, the use of PPIs outside their area of recommendation has increased alarmingly over the past decade, particularly in the more recent years, because them being available as over-the-counter medication in many countries, Romania included.

During almost three decades of clinical use, PPIs proved to be an efficient and safe group of drugs, with enormous benefits for millions and millions of people when used for well-established indications. Several studies found that the adverse effects occurring under PPI therapy are like those of placebo, while a study on the efficacy, tolerability and safety of up to 15 years' continuous maintenance treatment in severe acid-peptic disease reported no safety concerns (1).

Nevertheless, there is no pharmaceutical agent approved in clinical practice that does not carry a certain degree of risk. Even placebos, used in clinical trials for comparison with new drugs in terms of "safety", may have some adverse effects. Like in case of other drugs, PPIs are not always as safe as it has been thought, and concerns were raised about their potential association with several and serious adverse events, ranging from bone fractures (2) and community acquired pneumonia (3) to acute myocardial infarction (4) and inpatient death (5). It should be underlined that all studies regarding the association of PPI therapy with the risk for developing the above mentioned adverse events are supported mostly by observational research, and therefore, cannot confirm causality. The scientific strength of all observational studies is weak (odds ratio < 2), while most are retrospective and some are case reports

or case series. The quality of evidence regarding potential association with PPI therapy is consistently low to very low. Recently, in an excellent three review articles, experts critically analyzed the published literature about the potential adverse events associated with the long-term use of PPIs (6-8).

An association between PPI therapy and increased risk of bone fracture is based on several mechanisms such as reduced calcium absorption secondary to increased intragastrical pH levels induced by PPIs, but current evidence suggests a very weak connection (OR 1.24) (2). Moreover, the link between PPIs use and increased fracture risk has not been consistently demonstrated.

PPI therapy has also been associated with increased risk for community acquired pneumonia (3). The underlying mechanism of such an association remains unknown, although some researchers have suggested that PPI-induced low gastric acid secretion may lead to bacterial overgrowth in the stomach and from there, by an "upstream" effect, reach the oropharynx and then the upper airways. Overall, the association between PPI therapy and community-acquired pneumonia remains controversial, and clinicians should not refrain from prescribing PPIs, when indicated.

Cases of acute interstitial nephritis associated with PPIs use have been reported ever since the early 90s. Recently, one study reported that PPI users presented a significantly higher risk of chronic kidney disease probably due to recurrent episodes of acute nephritis (9), but such a connection is weak and remains uncertain.

Reduced gastric acidity with prolonged PPI therapy may interfere with the absorption of calcium, magnesium, iron, vitamin B12, and several cases of hypomagnesemia, hypocalcemia, iron deficiency anemia, and vitamin B12 deficiency have been reported (10). Nevertheless, data regarding an association between PPI therapy and micronutrient deficiencies are inconsistent and highly controversial.

Although several studies, systematic reviews, and meta-analyses (11, 12) reported an association between PPI use and the risk for *Clostridium difficile* infection (CDI) development, such a connection remains controversial as all the studies which support it are observational, with inherent accuracy limitations and significant heterogeneity as well as evidence of publication bias. In addition to this, most of the studies reporting associations between PPI use and the risk for CDI have a very low-quality evidence, not supporting a cause-effect relationship, while other studies failed to find such an association.

Observational studies have reported a link between PPI use and the risk for hepatic encephalopathy in a dose dependent manner (risk increases with dose) (13) or spontaneous bacterial peritonitis in cirrhotic patients (14), but the quality of evidence is low such connections are inconsistent and controversial.

More recently, several other associations between PPI therapy and serious adverse events such as dementia (15-17), stroke (18), acute myocardial infarction (4), and even inpatient death (5) have been reported. Thus, two clinical studies (15, 16) have shown that PPI use was associated with a 38% and 44%, respectively, increased risk for dementia. Such possible association caught great attention from the clinicians, considering the high prevalence (millions of patients) and economic impact (billions of US dollars) of this disease. There are two

suggested mechanisms linking PPIs to the development of dementia: vitamin B12 deficiency and enhanced brain beta-amyloid levels (17). However, the two-mentioned study (15, 16) present many limitations such as the lack of adjustments for some important risk factors for dementia (e.g., alcohol use, hypertension, family history of dementia). Thus, we do not have a clear evidence regarding an association between PPI use and the risk for dementia. Consequently, physicians should prescribe PPIs when the indication is well-established.

An increased risk of adverse cardiovascular events associated with PPI therapy have been reported by several studies. Recently, one study found that PPIs use is associated with the risk of first-time ischemic stroke in the general population (18). As with others retrospective observational studies, the strength of this association is low and a cause-effect relationship.

Several studies have suggested a potential association between PPIs use and the risk for myocardial infarction (4, 19). A leading hypothesis to explain such a risk is based on observational studies which showed that concomitant use of PPI and clopidogrel was associated with an increased risk of recurrent post-acute coronary syndrome. Ex vivo analyses suggested that PPIs competitively inhibit hepatic cytochrome P450 isoenzyme 2C19C (CYP2C19) necessary to metabolize clopidogrel in its active form and thus, increasing the possibility of clot formation and the risk of coronary thrombosis. Shah et al (4) reported a modest association between long-term PPI therapy and the risk of myocardial infarction in the general population. Nevertheless, all the studies regarding a potential association between PPI therapy and the risk of myocardial infarction are retrospective observational, and therefore cannot demonstrate a causal relationship.

PPI therapy has also been linked to a potential risk for gastrointestinal malignancies by causing hypergastrinemia which facilitates gastric colonization by *Helicobacter pylori* and stimulate hyperplasia of enterochromaffin-like cells leading to development of gastric neuroendocrine tumors (NETs). Nevertheless, large clinical studies have not confirmed an association between PPI therapy and gastric cancer or gastric NETs (20). Prolonged use of PPIs (more than 2 years) increased the risk of fundic gland polyps (21) which are not premalignant lesions.

Finally, a study just published reported excess risk of death among PPI users (5), among patients without gastrointestinal comorbidities; risk is also increased with prolonged duration of PPIs use. The mechanism of association between PPI use and risk of death remains unknown. Most probably, the occurrence of any of the adverse events associated with PPI long-term therapy mentioned previously (e.g., kidney disease) contributes to excess risk of mortality. This study is a retrospective, observational with known limitations, and therefore, further studies are needed to evaluate the association between PPI use and the risk of all-cause mortality.

All these studies mentioned above, reporting several adverse events of PPI use may create alarmism among physicians and spread fear among the informed patients, some of whom declining PPI therapy even when they have clear indications. The media also contributes to maintaining a state of panic among PPI users, with pessimist headlines such as “Heartburn pills hike stroke risk by 90%” (*Sun*, November 16,

2016). Despite such warnings, PPIs continue to be considered by most physicians as "safe" drugs, providing immense benefits when prescribed for appropriate indications. However, PPIs are widely used by millions of people for indications that were never approved.

As current evidence cannot confirm causality between PPI therapy and the risk for developing any of several above mentioned serious adverse events, a final estimation of the real risk is not possible. Yet, the existence of the risk for some adverse events of long-term PPIs therapy cannot be overlooked, and clinicians should be aware of them, and should inform their patients on such a risk, without, however, refraining from prescribing PPIs at the lowest effective dose and for shortest possible time only for clearly established indications.

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EDITORIAL

REFUSAL TO VACCINATE AND FALSE HYPOTHESES *VERSUS* NEW HYPOTHESES

Starting from February 2016, Romania faces an ample epidemic wave of measles, with 9760 cases and 35 deaths. Most of the cases affected children, mainly because of the refusal to vaccinate (1).

The expansion of the population's anti-vaccination attitude mostly occurred after 1998, when Andrew Wakefield published an article in The Lancet, suggesting as explanation for autism, the MMR (measles, mumps, rubella) vaccination which would inflammatory bowel disease, as a first stage that would determine the subsequent translocation of intestinal flora, followed by dissemination in blood, and brain lesion. Although the paper was based on the investigation of digestive manifestations associated with neurological disorders made on a study group of 12 children, of which 8 had been vaccinated against MMR and one had had measles, the conclusion, namely the association between autism and MMR vaccination, made the population refuse this vaccine (2). Later, multiple studies, starting with the one published in the same famous magazine in 1999 (3) brought strong scientific arguments to shake A. Wakefield's fake hypothesis. From then, even if The Lancet's editors retracted the quoted paper which generated among people the fear that MMR vaccine induces autism, the refusal to vaccinate phenomenon persisted, also comprising other types of vaccines. The data gathered to support the fight against Wakefield's hypothesis were synthesized by *Immunization Action Coalition* that listed 26 scientific papers (4). These rigorous studies argumentatively fight against erroneous assertions that explained the anti-vaccination trend but, obviously, they had no impact on the general population, at least in Romania, most probably caused by the lack of strategies of the Ministry of Health regarding the communication of scientific information, in a language that is accessible to general population. Obviously, the anti-vaccination group is more vocal, and its actions had a higher impact on the general population than the information and support campaigns meant to lead to vaccination acceptance supported by specialists.

If, initially, the relation between the MMR vaccine and autism was connected to the dysbiosis induced by the vaccine at the level of intestinal microbiome, the arguments that currently support the mechanisms that are responsible for the installation of autism spectrum disorders (ASD) are also correlated to microbiome, but with no implication of the vaccine as a trigger.

The new molecular biology methods used inclusively to characterize the human genetic luggage were refined, including for the study of microbiome, especially those at the intestine level, as it represents 70% of the 100 trillion microorganisms (bacteria, viruses, fungi, archaea) that constitute it. The evaluations in the human genome catalogue shows we have about 22,000 de genes, but as "non-redundant" genes - 3.3 million, in human intestinal microbiomes. The diversity among individuals' microbiomes is impressive: human is approximate-

ly 99.9% identical as regards their genome but, usually, as regards each of their microbiomes, the differences reach 80% to 90% (5).

Metagenomics and metatransriptomics studies allowed the description of 3 enterotypes at the level of the intestinal microbiome. An enterotype is a classification of living organisms based on its bacteriologic ecosystem, in intestinal microbiomes. The discovery of three human enterotypes was announced in 2011 by the research collective coordinated by Peer Bork. These enterotypes are not regulated by age, gender, weight or nationality. But there are indices showing that long term diet influences the enterotype. Type 1 is characterized by high levels of *Bacteroides*, type 2 by *Prevotella* and type 3 has high levels of *Ruminococcus* (6).

We do not know for sure which is the physiological role played by these enterotypes (eventually in inducing different conditions), but during the last years, different studies signaled the modification of the microbiome predominated by genuses and species associated to disease conditions (from metabolic diseases to celiac disease, Crohn's disease, ulcerative colitis, irritable bowel syndrome, obesity, non-alcoholic fatty liver, type 1 or 2 diabetes, to infectious diseases - *C. difficile* associated infections, translocation of *Enterococcus* resistant to vancomycin, to the chronic fatigue syndrome, thyroid dysfunctions, rheumatoid arthritis, ankylosing spondylitis, eczema, asthma and even ASD (as well as other neuropsychiatric disorders like depression) (7).

Some studies already bring arguments regarding the modifications of microbiome in different pathologies. For example, by sequence of some components of the intestine microbiota, (8) showed that in patients with acute diarrheal disease (ADD), the quantity of *E. coli* is higher, in comparison with healthy people, like after the ADD cure. The most frequent etiological agents mentioned in this study were the following: *Salmonella*, *E. coli* Shiga-like strains, *Campylobacter* and *Shigella* toxin producer, together with the quantitative increase of commensal *E. coli*. The study proposes to reconsider therapy, so that to control the quantity of *Escherichia* in cases of severe diarrhea and/or to promote the development of microbial populations, which are important for the good functioning of the healthy intestine. The results of the quoted study came from DNA sequencing from microbes at the intestine level in persons with diarrheic diseases caused by these four pathogen agents. Then, they compared the results with the microbes founded in healthy members of their family. As expected, the intestinal microbial communities differed in case of healthy and sick persons. On the other hand, the microbiota composition in patients with ADD was similar indifferently from the isolated etiological agent. Moreover, patients with similar specific profiles of the microbial community had a more severe disease and these modifications were similar, indifferently from age, gender or race. Future researches based on these discoveries should reconsider therapy strategies that involve probiotics or anti-microbial agents regarding the development of benefic microbes and fast decrease of proteobacteria populations, a major group of bacteria including several pathogen agents, like *Escherichia*. Such strategies could decrease the potential of intestinal infections and would facilitate the faster recoveries of those affected by ADD (8).

During the last years, studies based on

proving "intestinal-ADD microbiome" causality underlined the need to clarify pathogenic mechanisms as well as the concern about the explosive growth of the number of cases, namely from one case of 5000 children, to a case of 68 children (9). Per genders, boys are obviously more affected, about 1 case of 40 children for boys, and 1 case of 190 for girls, therefore a gender report of about four-five boys for each girl (10) Most probably, the signaled growth could be the consequence of specialists' concern on ASD, as well as on the new diagnosis criteria, not necessarily by a real growth of the number of cases, therefore an epidemic manifestation as initially thought. Of course, during the last years, the contribution of certain co-factors (including the environmental factors, antibiotics therapy and unbalanced diets etc.) also amplified, among which the most important role seems to be played by the constellation of intestinal microbiome (also modified because of the above-mentioned factors) (9).

The autism spectrum disorder (ASD) is defined as a set of neurologic development disorders, characterized by two types of main manifestations: social deficit and exacerbation of repetitive/stereotype behaviors. Together with the basic symptoms of ASD, different authors also noticed other comorbid conditions in some of the ASD patients, like gastric-intestinal complications (GI) and modifications of the intestinal microbiome (11, 12).

The relation between intestinal microbiome and ASD was suggested by the multitude of digestive symptomatology signaled in patients with ASD, namely, constipation or diarrhea or gastric acidity reflux. The brain - intestine - microbiome axis refers to interactions between the central nervous system, the gastrointestinal system and microorganisms from the gastrointestinal tract. The exploration of these interactions offers an explanation why gastrointestinal disorders are frequently met in children with ASD. The signs of brain-intestine affection are strongly related to functional disorders at the GI level that are frequently met in children with ASD. Studies on the microbiome in children with ASD suggest the intestinal microbiome modifications can be associated with ASD and gastrointestinal disorders in case of these children. But the data collected until present day are insufficient, so future studies are needed about the brain-intestine-microbiome axis, which could lead to new techniques meant to identify GI disorders in ASD children, as well as new therapies of the disorders accompanying this syndrome (13).

Emergent studies on the intestine-brain interaction indicate that the modifications of this axis can play a regulating role on the health condition, as well as in inducing certain conditions, including the ASD. Studies on real causes of the GI conditions in children are limited. A study that prospectively evaluated the possible causes of GI in children with ASD indicates that most of this symptomatology is not due to physiological or organic causes, but to functional causes (14).

Gorrindo et al., quoted by Luna et al. analyzed a group of 121 children, subsequently divided into 3 groups: ASD and GI disorders children, ASD children with no association of GI manifestations and children with GI manifestations and no ASD. The evaluation made of pediatrics gastroenterologists for the 2 groups of children with GI manifestations discovered that the functional gastrointestinal disorders (FGID) represent the most frequent cause

of GI accuses in both lots of patients. In the ASD group, 37 of the 40 children were diagnosed with FGID. Functional constipation was the most frequent GI disorder in this group. These findings correspond to the hypothesis according to which GID in ASD represent the pleiotropic expression of genetic risk factors, therefore medicines and diet do not influence the GI manifestations (13).

The practical recommendations and conclusions of other specialists in pediatrics continue to claim that most of the GI symptoms in children with ASD are due to functional disorders like those observed in children with neurotypical development and not to some physiological condition unique for ASD. The different studies that analyzed the influence of microbiome on ASD evolution concluded there was a definite relationship between the different types of intestinal microbiome and ASD, namely the association between the increase of the quantity of *Lactobacillus* and the decrease of the quantity of *Bifidobacterium* in feces (11). On the other hand, there are discordant results regarding the level of short chain fatty acids (SCFAs). According to some authors, this level is low, according to others, it is high. Thus, the low level of SCFAs (ex. acetate, propionate, valerate) was partially associated with children with ASD, with a high probiotics supply and, probably, partially due to a lower production (because of a lower sugar fermentation of benefic bacteria and/or low fiber supply) and/or higher absorption in the organism (due to a longer transit and/or due to a high intestinal permeability) (11).

Other authors (16) ascertains that the SCFAs elimination of feces is higher in children with ASD, but certain fatty acids (e.g. acetic, butyric, isobutyric) were high and caproic acid was high too. If the profile of colon microbiota would be identical in all children with ASD, we could identify those common markers present in most of the children with ASD, so that by finding it in feces and replacing the determination of the colon microbiota (implying sophisticated and expensive metagenomic and metaproteomic tests) highlighting these markers.

Unfortunately the intestinal microbiome profile differs from one individual to another with more than 50% (even 80-90% according to some authors), therefore it would be difficult for us to identify the most common profile of intestinal microbiome associated to ASD, so that prebiotics and/or probiotics adjuvant therapy would regulate the microbiome profile, encouraging benefic bacteria (e.g. *Bifidobacterium*) and discouraging the multiplication of dangerous bacteria (most probably associated to dysbiosis typically associated to ASD (e.g. *Clostridium*) (15).

In comparison with the intestinal microbiome in children without ASD, the intestinal microbiota in children with ASD is less diverse and it presents lower levels of *Bifidobacterium* and *Firmicutes* and higher quantities of *Lactobacillus, Clostridium, Bacteroidetes, Desulfovibrio, Caloramator* and *Sarcina* (11, 15). In children with autism presenting GI symptoms lower quantities of the following bacteria were found: *Prevotella, Coprococcus* and Veillonellaceae not classified, except that found in children with no GI symptoms. The fecal samples in children with ASD also have higher levels of the *Clostridium histolyticum* group (Clostridium, clusters II and I), in comparison with samples from healthy animals with no connection to children (12). On the other hand, in case of children brothers with GID, without au-

tism, they present an intermediary level of *Clostridium histolyticum* that do not significantly differ from the group of ASD children. *Clostridium* can produce neurotoxins and it could exercise systemic effects (12). The reduction of clostridia upon the administration of vancomycin has a benefic effect on the evolution of children with ASD (16), (17). Moreover, children with ASD present modifications of the levels of *Bifidobacterium*, *Prevotella* and *Sutterella*. As we already know, the intestinal microbiota in newborn differs according to the type of birth; in case of natural births we deal with *Lactobacillus*, while in case of C-section births, we deal with *Staphylococcus* and *Propionibacterium* and we noticed that C-section babies present a 20% higher risk to develop ASD (18). Still, the association did not persist when the control group used is that of the brothers of these children; this is the reason why we suppose genetic and/or environmental factors also interfere (18). Nowadays, it is unanimously accepted that both genetic and environmental factors are involved in the etiopathogenesis of the so-called non-syndromic ASD, which is not a side effect of a well-known genetic condition. For a long time, the percentage of the genetic element was essential but during the last years, considering the dramatic growth of ASD prevalence, the role of environmental factors gained a relatively greater importance. Beside these obviously epidemiologic considerations, as suggested by Wong et all, the hypothesis according to which exposure to the environmental factors can considerably contribute to ASD development is supported by the presence of different sub-sets of candidate genes, distinguished in each ASD subject and by a high phenotypic variability of these individuals. Despite this, there is still more to discover about the complex interaction between these causal factors. The relatively new concept of epigenetics can be very useful from this point of view at least for certain ASD cases. Epigenetics represents a fundamental regulation mechanism of the gene based on chemical modifications of the DNA proteins and histone, without changing the DNA sequence. It was proposed for the environmental factors like heavy metals and endocrine disrupting chemicals to be able to modify the epigenetic condition and genic expression, leading to ASD (19).

A recent synthesis tried to identify the environmental factors that could play a decisive role in ASD etiopathogenesis, counting air population, including perinatal and postnatal population, exposure to heavy metals (mercury, plumb, and arsenic), pesticides, nitric oxides, electromagnetic pollution or polyunsaturated fatty acids. Despite the critical analysis of numerous studies on this subject, authors could not reach to a clear conclusion on the role of environmental factors in inducing ASD, so they proposed to continue with these studies; future researches should investigate this relationship and bring solid proofs to certainly conclude on the significant difference in ASD prevalence in countries with high pollution levels *versus* those with low pollution levels (19, 20). We still have no information on the cause of digestive disorders in some of the children with autism. A co-factor that seems to influence the occurrence of ASD is the excessive use of oral antibiotics, which could also translate a deficit in immune defense in case of these children. The antibiotics therapies, especially those of broad spectrum, practically destroy normal flora, which is so useful in regulating intestinal functions,

including its motility and fluids contribution, breakdown of some vegetal polysaccharides, production of vitamins and competition against colon colonization with pathogen bacteria (11). The administration of certain antibiotics and probiotics (like fermented milk containing *Lactobacillus* strains, temporarily improves digestive symptoms, as well as symptoms associated to autism (21). The strong correlation between gastrointestinal symptoms and autism severity indicates that children with more severe types of autism present more severe gastrointestinal types of autism and *vice versa*. It is possible that autism symptoms would be amplified or even partially due to gastrointestinal issues. In a relatively recent study, in case of the autistic children, it was determined a low SCFAs level, partly associated with the use of probiotics, low level of sugar fermentation because of benefic bacteria that are practically absent or with the reduced contribution of soluble fibers, long transit and/or due to a high permeability at the intestine level.

CONCLUSIONS

Even if the ASD etiopathogenesis is not completely elucidated, it is evident from the studies conducted so far that this disorder is not induced by the MMR vaccine and that the intervention of several cofactors (genetic, environmental and especially of the intestinal microbiome) is responsible for ASD installation, which affect more and more children during the last years. Until the future studies are to be completed, the gathered data regarding this mechanism, especially about the intestine - brain axis and gut microbiome, we can claim that MMR vaccination, represents the most redoubtable weapon of the humanity, meant to prevent and fight against a dangerous disease.

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EDITORIAL

ANATOMY AT THE IASI FACULTY OF MEDICINE: SCIENCE, SUBJECT OF STUDY AND CONCEPT

Grigore T. Popa, in a memorable inaugural lecture entitled *What Does and What Doesn`t Anatomy Mean* (1928), summarizes a period of teaching and research, and traces the future of this subject of study and concept at the Faculty of Medicine of Iasi: `*No structure can be fully understood unless its reasons of occurrence, presence, and functional justification are obvious and able to explain its development and way to perfection. To list and describe the hand muscles is valueless unless the undergraduate can interpret the movements of the hand with which every day he washes his face*`. Our brief history has in view the long road to this concept of the Iasi school of Anatomy.

The first attempts at founding a medical institution of higher learning in the Romanian Principalities occurred in the first decades of the 19th century. Two such institutions are worth mentioning in this brief outline: N. Kretulescu's School of Elementary Surgery founded in Bucharest in 1842, and Neculai Negură's School of Surgery, started in Iasi in 1859. The former, although an institution of secondary education, played a key role in the foundation of the Faculty of Medicine in Bucharest in 1869. The latter was initiated by Neculai Negura, a graduate of the Faculty of Medicine from Berlin. Moreover, N. Negura`s school was the first academic medical school in Romania in which all courses were taught in Romanian. Unfortunately, its existence was ephemeral. The school had a brief existence as its initiator could not face the many difficulties encountered at the beginning. The same situation occurred when the University of Iasi was founded in 1860. De facto, the Faculty of Medicine was not among the four initial faculties of the oldest university in Romania. The Faculty of Medicine of Iasi was started only in 1879. On September 27, 1879, Nicolae Kretulescu, the then Minister of Public Education, signed the decree no. 11389 in which he approved the foundation of the Faculty of Medicine at the University of Iasi. Several days later, Prof. Petru Suciu, the Rector of the University of Iasi, to meet the demands of the minister`s decree, convened several meetings that took place on September 30, October 2and 5. Since 1879, the Faculty of Medicine of Iasi has been functioning without interruption. However, the existence of the new Faculty of Medicine depended on the quality of the teaching staff. In this respect, the Academic Council of the University recommended dr. Gh. Iuliano for the course of lectures in Descriptive Anatomy and Histology, and chemist Petru Poni for the Chemistry course. Both were recommended as substitute instructors. In his turn, dr. Telemac was recommended for the position of prosector. Despite this, the minister did not consider the local decision and appointed dr. Leon Scully for the course of lectures in Anatomy and Histology and pharmacist S. Konya for the course of lectures in Chemistry. Only the appointment of dr. Telemac as prosector was approved by the minister. With this initial teaching staff, the Iasi

Faculty of Medicine began its activity. At that stage, the Faculty enrolled only 14 students. The initial syllabus, received from Bucharest, was identical with the one used by the University of Bucharest. Two rooms in the University building were assigned for the courses of the Faculty of Medicine. The first Anatomy lecture was given by dr. Leon Scully. He also became the first dean of the Iasi Faculty of Medicine. He was appointed professor of anatomy in 1879 and two years later professor of surgery.

Dr. **Leon Scully** was born in 1853 in Piatra Neamt. He graduated from the University of Montpellier, where he obtained his degree in Medicine. He furthered his education at the Faculty of Medicine in Paris where he passed his PhD degree. At the time he gave his first lecture he was only 26 years old. In 1881, he was transferred to the position of Chair of the Clinical Surgery Department, a place he held until his death in 1912. Despite the various hardships and hostile attitudes, the Faculty of Medicine flourished. In the 1880-1881 academic year, the Faculty of Medicine was attended by undergraduates in the 1st and 2nd year of studies. The syllabus was enriched with new topics and new substitute instructors were appointed to the newly established departments. In the coming years, several new students were enrolled. The teaching programs were improved and the number of instructors increased. There were many signs at the time that the Faculty of Medicine had strengthened. More young doctors, graduates from Paris and Vienna, were employed during this time as substitute members of the teaching staff. Since 1884, following the graduation exam, the first series of doctors in Medicine and Surgery from Iasi left this institution of higher education. Starting from that year, the growing number of graduates reflected the stability and prestige of the institution. During that period, the Department of Descriptive Anatomy was provided with important teaching materials making it able to better train a substantial number of excellent specialists even from the first decade of activity of this faculty.

Nevertheless, the true founder of the School of Anatomy of Iasi was Professor **Aristide Peride**, who dedicated his entire activity to the organization of education in Anatomy at the Iasi Faculty of Medicine. Aristide Peride was appointed as a substitute professor in Anatomy in 1881. In 1889, he became a full professor. Professor Peride (1848 – 1906) linked his name with the construction and organization of the Institute of Anatomy, a remarkable structure which exceeded many similar institutes in this part of Europe. The newspapers of the time have long commented on this outstanding accomplishment. In this respect, the following "chronicle" in the "Hospital" (1890) gave interesting details: "*In a few days the building of the Institute of Anatomy, part of the Faculty of Medicine, will start in Iasi. The new building will be in the courtyard of the University building. When the work on the new building of the University will have been finished, it will serve as premises for the Faculty of Medicine. The architect of the new building is Prof. Stef. Emilian. We also learnt that the Ministry of Public Instruction commissioned the famous French sculptor W. Hegel, author of the beautiful statue of Miron Costin in Iasi and of other works, to create a large bas-relief of several meters, which will serve as a frontispiece to the Institute of Anatomy. The French sculptor will represent on the bas-*

relief the first lesson on the circulation of blood given by Harvey. The beauty of the work that will decorate one of the famous buildings of the University of Iasi is guaranteed by the name of the sculptor."

Provided with rooms for dissection and experimental surgery, an anatomical museum, annexes necessary to laboratory preparations, a library and an amphitheater, the Institute of Anatomy has ensured the training of many generations of students of the Faculty. Professor Peride himself contributed to the endowment of the museum collection of the institute. He identified himself, for a prolonged period (1881 and 1906), with the department he created. The department of Descriptive Anatomy, set up by Aristide Peride, who held the chair until 1906, was subsequently represented by two outstanding professors: **Ion Tanasescu** (1907-1913) and **Francisc Rainer** (1913-1919).

Ion Tanasescu was born in1875 in Fălticeni. He graduated from the Faculty of Medicine in Iasi in 1902 with the PhD thesis "General considerations on hydrocele and its treatment." While for a period of specialization in Paris, he worked for three years in the anatomy laboratory of Professor Poirier and three years in the surgery service run by Professor Hartmann. He returned to his native country in 1907, after graduating with honors in Anatomy, where he became Professor Leon Scully`s assistant. In 1908, he won the competitive exam for the chair of the Anatomy Department at the Faculty of Medicine in Iasi, where he became the 2nd professor, being promoted to a full professorship in 1913. In 1913, he was transferred to the Department of Topographic Anatomy and Surgery. Ion Tănăsescu was especially known as professor of Surgery. But Professor Tănăsescu was also an accomplished anatomist, combining erudition with exceptional surgical skills. He published important studies, especially on lymphatic vessels, cited in the classic European treatises. Prof I. Tănăsescu is the founder of modern surgery and functional anatomy at the Iasi University of Medicine. He was also the ``father`` of the Palace of Surgical Clinics, the most modern structure of this kind in South – Eastern Europe at the time.

Since 1900 the surgery school in Iasi has greatly developed and included the departments of External Pathology, Topographic Anatomy, Surgical Medicine, the surgical clinics and the specialized surgical clinics (Obstetrics and Gynecology, Genitourinary, Ophthalmology). The extension of the surgical school of Iasi was also due to the contribution of celebrated surgeons and great anatomists, such as Ernest Juvara and Ion Tănăsescu.

In 1898, Professor **Ernest Juvara** became a full professor at the Topographic Anatomy Department. In 1903, this department had to merge with the Surgery Clinics department, similarly to the counterpart department in Bucharest led by Prof. Toma Ionescu. **Ernest Juvara** (1870-1933) was born in Bârlad, in a wealthy family. In 1889, he started the medical studies at the Faculty of Medicine of Paris, where he worked in the chemistry laboratory of Professor Gauthier, then in the anatomy laboratory headed by Professor Poirier. In the laboratory of Profesor Poirier, Ernest Juvara wrote Poirier's lessons *"Quinze leçons d'anatomie pratique"* complemented by many original illustrations, a work published in five editions, also translated into German. Initially, he had the right as an undergraduate student to work in the hospitals of Paris, then he

headed the laboratory of Professor Poirier, and as instructor he worked in the pathological anatomy laboratory of Professor Duplay's surgical clinic. In 1895 he passed his PhD, at the age of 28. At this age Ernest Juvara passed an exam and was appointed as the 2nd professor in the Topographic Anatomy department of Iasi, where he was recognized as an anatomist of great learning and precision in the execution of drawings and anatomical preparations and as an accomplished surgeon. Some of the anatomical drawings made by Professor Juvara and some sections and anatomical parts he made are kept in the Museum of the Institute of Anatomy in Iasi, highlighting his skills. In 1912 Professor Juvara was transferred to Bucharest, where he headed the clinics at Filantropia and Brancovenesc hospitals. In 1906, he founded the 1st Clinic of Surgery at St. Spiridon Hospital of Iasi as a special surgical teaching unit.

Between 1913 and 1920, the Descriptive Anatomy Department was led by Francisc Rainer. **Francisc Rainer** (1874 – 1944), born in Bucovina (Rehozna) graduated from the secondary school and the Faculty of Medicine in Bucharest. He worked for a long period of time as head of the laboratory of Colţea Hospital. In 1913, he began his teaching career, as he became the Head of the Descriptive Anatomy Department in Iasi.

Professor Francisc Rainer's personality was many-sided. However, the greatest merit is the fact that he innovated the concept of Anatomy as a subject of study. He introduced, among the first in the world, the concept that Anatomy is the study of living, dynamic forms. The concept of functional biology opened men vistas to the interpretation of Anatomy overcoming the cumulative, strictly analytical study. Such an approach was a novelty for that period. Later, this concept was accepted by all specialists in the field. The transition of anatomy from a static, descriptive position to a biological interpretative position determined indirectly the new status of Anatomy among the other branches of Biology. Prof. Fr. Rainer was careful enough to place at the entrance to the dissection hall of the Institute of Anatomy the following words: *Anatomy, the science of living forms*. This statement is the essence of his scientific creed. When he left for Bucharest, prof. Rainer was sure that he had created a modern school of Anatomy at the University of Iasi. In 1919, Professor Rainer was transferred to Bucharest where he furthered his teaching and scientific activity. He is the founder the Institute of Anthropology in Bucharest. He died on the 4th of August 1944, a year after his election as an honorary member of the Romanian Academy. After prof. Rainer`s departure for Bucharest, for the next 9 years, two replacements for the Chair of Anatomy followed: Nicolae Hortolomei (1919-1925) and Gheorghe Zamfirescu (1925-1928).

In 1928 Professor **Grigore T. Popa** became the head of the Anatomy Department. His name was given to the University for Medicine and Pharmacy in Iasi. Grigore T. Popa was an outstanding personality of Romania. His discoveries in the field of physiology (the neuro-endocrine relations or the pituitary portal system) have won him world recognition. In 1963, Professor Harris of the University of Oxford, in an issue dedicated to these findings in the ``Triangle Magazine`` stressed the importance of Popa`s discoveries that became "a remarkable step in the history of world medicine".

Professor Grigore T. Popa was born in

1892 at Şurăneşti, Vaslui. In 1911, he graduated from the National College in Iasi. He received his title of MD in 1920, after graduating from the Faculty of Medicine in Iasi. Between 1914 and 1920 he worked as an honorary assistant at the Descriptive Anatomy Department. After obtaining his PhD he was transferred to the Department of Bucharest, where, after a few months, was promoted to the position of reader. He also became professor at the Anatomy and Embryology Department of the Physical Education Institute in Bucharest (1923-1925). Between 1925 and 1928 he worked in Chicago for the Rockefeller Foundation as Research Fellow. When he returned to Romania, Grigore T. Popa was appointed professor at the Department of Anatomy and Embryology, Faculty of Medicine in Iasi. Gr. T. Popa worked in Iasi until 1941 when he was transferred to Bucharest. In Iasi, he furthered the functional Anatomy school, gathering around him several young people who subsequently became distinguished professors or practitioners (Ion Iancu, Gheorghe Chipail, Nicolae Oblu and others). To his other physiological studies, he added his important research on the anatomy and function of the dura mater, pericardium mechanical structural characteristics, and his research on the vascular pituitary control system. The port microsystem is "a key for endocrine mechanisms" stated Harris. It was originally studied by Grigore T. Popa, then by Grigore T. Popa and Una Fielding of Cambridge. Nowadays the role of this system in neurosecretion is undeniable. Popa's discovery explains the interrelation system, a step towards the elucidation of endocrine physiological, and pathological or adaptive mechanisms like that stress type described by Hans Sellye. Grigore T. Popa`s discovery of the vascular pituitars control system is at the basis of modern endocrinology. A very refined Romanian, aware of the dangers of the period he lived in, Grigore T. Popa together with Mihail Sadoveanu and Mihai Codreanu, outstanding men of letters, founded the journal "Notes from Iaşi" – an important publication for the Romanian culture. In the journal, he contributed to the promotion of progressive social ideas. He also opposed fascism and communism.

In 1932, the head of the Topographic Anatomy Department was Professor **Ion Nubert** (1886-1975). Professor Ion Nubert was born in 1886 in Paşcani. He was a graduate from the National High School in Iasi (1906) and the Faculty of Medicine in Iasi (1914). Professor Nubert worked at the Institute of Anatomy until 1946. The results of his researches were cited in classical treatises. He educated a substantial number of undergraduates. His lectures were always illustrated with a rich iconography. Through his work Professor Nubert linked his name to the history of Anatomy in Iasi, a field in which he worked for more than three decades (beginning in 1915 when he was instructor, lecturer, and then reader - 1931 and full professor, 1932).

After World War II, the Department of Anatomy was led by Professor **Ion Iancu**, are outstanding personality of the medical school of Iasi. Professor I. Iancu was born in 1902 at Liești, Galați. After graduation, he worked as a urologist at "St. Spiridon" Hospital in Iasi after a period of specialization in Paris. Prof. Ion Iancu reorganized the Anatomy Department of the Faculty of Medicine in Iasi. He wrote more than 300 papers on the structure of the rear wall of the trunk, spine, psoas sheath, all his contributions remaining as reference material.

In recognition of his merits, the Institute of Anatomy of the Faculty of Medicine in Iasi was named after him. He was member of numerous Romanian and foreign scientific societies: the Balkan Medical Union, the Romanian Society of Surgery, the French Society of Urology. He furthered the achievements of his predecessors adhering to and promoting the principles of his forerunners, and making no concessions to the then prevailing communist ideology.

Another outstanding personality in the Department of Topographic Anatomy was Prof. Dr. **Nicolae Oblu** (1912-1995), founder of the first Hospital of Neurosurgery in the country (1972) and professors **Gheorghe Adomnicăi** (1923-2016) and **Gheorghe Frasin** (1922-1989) who enriched the studies on the functional anatomy of the abdominal wall.

This article is meant as a tribute to all those who have brought their outstanding contribution to the evolution of Anatomy, as a researcher topic and subject of study, which, regardless of any other opinion, will always remain the"fundamentum medicinae".

This brief presentation of the outstanding personalities of the Iasi school of anatomy aimed at following the main trends of research in a science that is rightly regarded as the key subject in any medical school that offers a sound basis for the medical education of Romanian physicians. Last but not least, the role of the Iasi school of anatomy in the development of the Bucharest school of anatomy and of the Romanian school in general cannot be denied or ignored.

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IN MEMORIAM

DOCTOR IULIAN STRATAN - THE PROFESSIONAL, THE MAN, THE FRIEND…

Born in 1938 in a christian family, his father being priest in parish Tibana, where he founded a new church, he was a meritous graduated of the boys' high school from Roman and in 1956 succeeded at the difficult entrance exam (13 candidates for a place) to the Faculty of Medicine in Iasi. In faculty, he belongs to one of the elite group of our series subjecting him to a terrible concurrence which has stimulated him permanent becoming one of the best students. He completed education in 1962 being scored with grade A (10) at the licence exam. According to repartition he opted for a job of trainee doctor at TB Sanatorium from Dobrita ("The Magic Mountain of Romania"), where he was affected to the surgical department. Having the support of an excellent physician Dr. Nicolae Rusdea, the young doctor was directed to the compartment of anesthesia, situated at the beginning in our country and he was precocious-forming in the new specialty.

Succeeding the first at the contest residency he chosen an appointment in Anesthesia-Intensive Care in the IIIrd Surgical Clinic, directed by renowned Professor Gheorghe Chipail who adopted, guided and formed him in the first years of career. He was also resident of Professor George Litarczek, in one of the first series of the newly established specialty and become successively specialist (1970) and senior physician (1978) and from 1980 to 2007 Head of Anesthesia and Intensive Care Clinic of "Sf. Spiridon" County Clinical Emergency Hospital from Iasi.

Devoted until to the sacrifice to his profession, he was continuously preoccupied by his theoretical and practical training and evolution by a solid documentation and frequent participation at refresher courses and national or international meetings getting in the last part of his career as a leading authority in the domain. Predicting perhaps his future professional consumption, he was married early during the college years, becoming a youthful father, grandfather and even great-grandfather. He has not neglected the family, but his time, energy and intelligence were confiscated by the medical activity.

We worked together for more than two decades, benefiting substantially by his competence and thoroughness solving as a team numerous difficult cases, especially in emergency but also in oncologic and endocrine surgery. We have collaborated directly and honestly without pride or preconceived ideas, but mostly conflicts. He did not flaunt his knowledge, but he argued loudest when justice was (almost always) by his side. He was unequaled in sensing of postoperative complications and predicting unfortunate evolutions.

We had no special relationship during the college, friendliness between us formed and perfected in the period of commune

activity, but also in the years we worked in different hospitals.

We both shared the passion for lecture and later for fine arts and classic music. He owned a vast library and was also a faithful auditor of the Philharmonic concerts in Iasi.

He has surprising wide knowledge in all these fields, where we had long colloquies in which I learned much. We often disagreed but without passion or preciousness. Considered to be a difficult man, he masked his shyness and sensibility by an apparent social misanthropy including in the professional environment of that total detached in private conversations where prove a wise, honest and generous man. He demonstrated all these qualities many years during some less happy events in both of our existence.

Finally, he supported with stoicism but conscient and especially with plenty dignity his prolonged sufferance during the last years, leaving us serene and reconciled.

Farewell Iulian….

Mihai Radu Diaconescu, M.D., Ph.D.
Professor of Surgery

EDITORIAL

MUCOSAL HEALING IN ULCERATIVE COLITIS – THE FOUNTAIN OF YOUTH?

Ulcerative colitis (UC) and Crohn's disease (CD) are two distinct pathologies commonly described as inflammatory bowel disease (IBD). UC is a chronic condition with frequent episodes of acute exacerbations and remissions. Its etiology and pathogenesis are still poorly characterized, although an aberrant immune response in individuals with a genetic predisposition is recognized (1).

Advances in the diagnosis and treatment of IBD have led to new perspectives on potential therapeutic targets. Recently, the therapeutic objectives have become more ambitious, going beyond clinical and biological remission, and aiming for mucosal healing, deep remission and even histological remission (2). In this way, clinicians aim for the modification of the natural history of the disease and an improvement in the quality of life.

Mucosal healing (MH) is not a new concept. A number of chronic pathologies, such as chronic duodenal ulcers, have MH as their therapeutic endpoint. Relatively recently Korelitz (3) used for the first time the term MH in the context of IBD.

The definition of MH is not universally accepted. In UC this can be either complete or partial (4):

- *Complete MH* involves the absence of friability, ulcerations and erosions in the rectum or colon (Mayo score 0).
- *Partial MH* involves an improvement in endoscopic appearance and the absence of ulcerations with a Mayo score of 1.

These definitions are however imperfect as they rely to a great extent on the endoscopist's experience and the methods used to improve endoscopic visibility (chromoendoscopy, high contrast imaging which amplifies mucosal details, etc.). Furthermore, there is no consensus regarding the optimal time interval from the commencement of therapy to the assessment of MH (8, 12 or 24 weeks), as well as the interval at which follow-up assessment takes place (5).

MH (the abscence of erosions, ulcerations and friability) is a separate concept from „*deep remision*", which is defined as the abscence of clinical, biological and imaging evidence of inflammation (6). *Histological remission* does not have a standardised definition; histological changes in remision include architectural changes of the crypts and a residual inflammatory infiltrates – a key diagnostic aspect being the abscence of neutrophil infiltration (6). A frequently asked question is whether MH can exist without a normal microbiota. Nevertheless, the answer to this requires further investigation and research.

In UC, MH is determined by colonoscopic means. The most frequently used endoscopic score is the Mayo score (score 0 = MH / inactive disease; score 1 = the presence of slight erosions or friability) and UCEIS (Ulcerative Colitis Endoscopic Activity Index or the Travis score). The latter has the advantage of a more detailed

assessment of the severity of endoscopic lesions, with more reproducible results (5). Although invasive, endoscopic assessment is relatively cheap and remains the principal method of evaluating and stratifying the activity of the disease, monitoring its evolution and assessing treatment response.

An alternative non-invasive method of assessing MH is the measurement of fecal calprotectin. This correlates not only with the extent of UC (higher values in pancolitis and left-sided colitis compared to proctitis) but also with the activity of the disease. Thus, calprotectin values under 50 μg/g suggest "deep remission"; an average value of 477 μg/g correlates with an acute exacerbation whilst two levels above 300 μg/g predict a future episode of acute flare (6). Many studies have demonstrated the role of calprotectin as a prognostic factor for MH (7,8). Roseth et al (7) demonstrated that a normalization in calprotectin levels correlates with endoscopic healing. Rikanek et al (8) demonstrated that the level of fecal calprotectin correlates not only with endoscopic, but also with histological healing.

The concept of MH as an ideal and final therapeutic target is extremely controversial. MH varies depending to the medical therapy used: infliximab 42-62%, adalimumab 25-47%, golimumab 42-45% and vedolizumab 52-56% (9). In some ways, MH has proven its benefits: a rise in quality of life, sustained clinical remission, a reduction in the use of steroids and immunosupressants, length of hospital stay, colectomy rates and risk of colorectal cancer down to similar rate to that in the general population (4,10). These would justify the adjustment in medical therapy to reach MH.

In contrast, an escalation in treatment exposes patients to side effects. According to the most comprehensive analysis published in 2016 (11), there is an increased risk (19%) for infections (not necessarily severe as patients are under surveillance) as well as a two-fold rise in the rate of opportunistic infections. This meta-analysis did not identify a rise in the risk of malignancy (possibly due to a short follow-up period) and did not identify significant differences between the different types of biological therapies concerning side-effects.

An additional controversial matter is whether the attainment of MH allows the cessation of medical therapy. The risk of recurrence at 1 year is 58% for patients in clinical remission, 36% in those with incomplete MH and 28% in those with complete MH (6). It is known that the episodic use of anti-TNF agents ('holiday drug administration') is associated with a raised risk of immunization, hypersensitivity and loss of effect (12). In contrast, the possibility of attaining an 'indefinite remission', the risk of side-effects and raised costs are justifications for cessation of biologic therapy in patients with MH. Recent studies (13) suggest that the treatment of a new flare episode with the same anti-TNF alpha agent that had been interrupted is efficient, leading to remission in 80-92% of cases. A possible explanation for this high remission rate could be the use of an anti-TNF agent in a selected group of patients that are responders to anti-TNF.

In conclusion, MH represents a therapeutic target with multiple benefits in clinical practice. There is insufficient data to make firm recommendations, with the decisions to increase (in order to achieve MH) or interrupt therapy (after complete MH) being case

specific, dependent on a risk-benefit analysis and taking in account the patient's choices. Complete MH does not however signify complete healing of the patient, mandating ongoing careful monitoring efforts. Despite all the recent advances, clinicians must have the wisdom to understand that they continue to treat IBD, not heal it.

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EDITORIAL

THE MEDICAL-SURGICAL JOURNAL IS 120 "VOLUMES" OLD

In his celebrated work, The Structure of Scientific Revolution (1962), Samuel Thomas Kuhn challenged the idea of cumulative unidirectional scientific progress. His theory of paradigms as sets of related concepts which compete for acceptance in times of rapid scientific change or revolution might be significant even for someone interested in understanding the development of Romanian science. The case of the Medical-Surgical Journal is a resourceful field in this respect.

In 1830, on 11 January, dr. Michael Zotta and dr. Iacob Czihac started *The Reading Circle (Cercul de Cetire)* with its 21 members. Three years later the Circle turned into *Societatea de Medici și Naturaliști (The Iasi Society of Physicians and Naturalists) (henceforth SMN*), the first modern scientific society in Eastern Europe, similar to institutions of the same kind in Western Europe. From the today perspective this society (SMN) may be considered the oldest scientific institution in Eastern Europe that introduced the scientific spirit of the age (1).

SMN was compared to a diamond in the crown of Moldavia that was later set into the great crown of modern Romania as its unionist members, assembled at its headquarters, in the very heart of the city of Iași, nominated and then elected Al. I. Cuza, prince of the united modern Romania, on Jannuary 5, 1859. The nomination and election of Al. I. Cuza in Wallachia too, on Jannuary 24, 1859 was a confirmation of the right decision taken by the Moldavian unionists, members of SMN: *"Nothing worthier of respect, admiration and imitation than the successful aspiration to our country's prosperity of those great men and scholars who planted the first tree to start an oasis of science in the wasteland of thought and culture that overwhelmed our beautiful Moldavia then, because of the difficult times it passed through. The foundation of SMN in Iasi was the first spark that illuminated the dark horizon of the Romanians' future prospects, who at the time enjoyed more freedom. From the deep and fertile furrow made by this scientific society, the Museum of Natural History, the Academy of Prince Michael Sturdza and finally the University of Iaşi were born "*(C. I. Istrati 1887) (cit 2).

Obviously a scientific society cannot long exist without a periodical that should aim at spreading the results of its members' research. Consequently, on the 1st of January 1844, C. Vârnav published an Adviser Journal for a Healthy and Economical life, a Periodical for the Romanian People, (Povățuitorul sănătății și economiei, foaie periodică pentru poporul românesc), similar to Franklin's almanac, that later on became the leaflet of the Society of Physicians and Naturalists from the Principality of Moldavia. As the scientific activity enhanced, in 1887 the leaflet was turned into the Bulletin of Society of the Physicians and Naturalists (Buletinul Societății de Medici și Naturaliști) (2). From 1924 on, the title of the periodical became The Medical-Surgical Journal (Revista Medico–Chirurgicală) (henceforth MSJ). The SMN members founded the Faculty of Medicine

(1879), *"which though recently initiated and in the process of development pioneered several research projects"* (2), making possible the emergence of one of the first genuine scientific publication in Romanian. The MSJ journal and the Faculty of Medicine have always lived in a happy symbiosis, symbolized by the image on the seal of SMN that is still in use: a snake and a cup, symbols of healing.

On January 1st 2016, 129 years since the publication of the first issue of MSJ was celebrated. In spite of this, because of the difficulties encountered during the two world wars, the 100th volume was issued only in 1996. It follows the MSJ appeared only 120 years out of 129. The 120 volumes are carefully preserved in the library of SMN, one of the oldest in Eastern Europe. Flipping through the 120 volumes of the Journal (MSJ) one lives a deep emotion that makes him/her aware that you are not only in front of a historical treasure in which you may identify the essential hallmarks in the development of medicine in Moldavia, via the research papers published here, but also an encounter with the outstanding personalities of the Iasi School of Medicine and of the other national medical schools (3).

Three main periods may be identified in the history of the MSJ: the first period, 1887-1923; the second period between 1924-1948; and the third: 1956 till our time which is also the most prolific period if we take into account the number of papers published in the issues of MSJ. A glance at the contents of the 120 volumes of MSJ allows us to assess that all the chief editors did their best to offer the MSJ readers highly original scientific papers. Moreover, "*this journal had from the inception a European level, and its publication was possible owing to the high scientific and cultural level reached by a multicultural Iasi* "(4).

The first issue of 1887 of MSJ was published in Romanian and French, the two versions being placed side by side on the same page. The editors stated in their editorial that *"As science enjoys the attribute of universality, those who work in this field should meet the requirements of this characteristic. It follows that MSJ could have been far from reaching its final goal if the papers published in MSJ had not been presented to the international readers in a form that could allow them to quickly inform themselves about the content of the papers they were interested in and easily guiding them to a correct assessment of their scientific value"* (3). In a period when the Romanian medical discourse was still in a process of formation and consolidation, MSJ played a major role in the development of the language of medicine in particular and the scientific discourse in Romanian in general. In this respect it is worth mentioning that the philologists SMN's may find the Journal a rich source for their scientific research. So far, some researchers wrote about the role of phonetic adaptability of neologisms from French in the formation of the language of medicine (4).

The first issue of SMN's journal, MSJ was published by an Editorial Board, that included dr. Aristide Peride, professor dr. Grigore Cobălcescu, Prof. Gustav Otremba, dr. Eugen Rizu, dr. Constantin Bottez, dr. Samoil Konya (although the first supporters of the idea were Anastasie Fătu and Ion Ciurea), who identified themselves with the SMN's board of directors. From 1888 on, the journal was published with an accompanying note*: "published under the direc-*

tion of dr. George Bogdan", the first outstanding personality from among the members of the editorial board. In this form the journal (MSJ) was published regularly until 1900. At the time, the journal (MSJ) was well known among European scholars as prof. George Bogdan took great care to provide*”a variety of printed materials that could interest both physicians and biologists”*. It is amazing for us, even today, that a society (SMN) made up of 49 ordinary members, in 1896, managed to support the publication of an important European journal. The proof of its popularity was the fact that 92 foreign scientific journals favoured exchanges with this outstanding scientific journal.

Between 1912 and 1916 MSJ was published exclusively in French under the title *”Bulletin et Memoires de la Societe des Medecins et Naturalistes de Iassy”.* The issues published in French strictly adhered to the high academic standards that had always characterized the Journal (MSJ). The contents of the issues published in the first period included medical studies, case studies, medical morbidity studies, reviews of the specialized journals of Europe, book and textbook reviews etc. (5).

At the beginning of the 20th century, until Romania became one of the belligerent countries, the Journal (MSJ) was coordinated by an editorial team that gradually became more and more dynamic. The editorial board included famous personalities of Iași school of Medicine. Among them E. Juvara, G. Socor, P. Bothezat, A. N. Brăescu, L. Russ Junior, C. Demetriade, P. Anghel, C .Botez, L. Buteanu, E. Pușcariu, I. Tănăsescu, S. Konya, C. Bacaloglu, P. Zosin, A. Tzaicu.

Other changes may be easily noticed during that period. If in the first years of MSJ’s existence, the number of articles signed by naturalists surpassed the number of medical studies, at the beginning of the 20th century the number of articles on medicine increased. (4).

Unfortunately, in the period 1916 - 1923 the publication of the journal (MSJ) was discontinued obviously because of the difficulties the editors encountered during the Great War when Iasi became the capital of unoccupied Romania. From 1924 on the bulletin was published with the title ”*The Medical- Surgical Journal”* (MSJ) , with a subtitle of *"transition"* which reminded readers of the old title *Bulletin of the Society of Physicians and Naturalists.* This time the journal became mainly medical in its content and aimed at concentrating Iasi medical creativity and spreading the new gains in the field at the international level.

In 1925, the MSJ was published in 6 issues. The editorial board consisted of outstanding members of Iasi school of medicine: C. Bacaloglu, N. Hortolomei, I. Mironescu, V. Răşcanu, A. Slătineanu, Elena Pușcariu, C. Ionescu Mihăești, M. Ciucă, I . Tanasescu, I. Enescu; the secretary of the editorial board was I. Alexa. During that period, the structure of the Journal (MSJ) was improved. The articles were grouped into: original papers, book reviews, review of reviews, aspects from the scientific life of SMN, in particular, and medical life, in general. Nobody can state that between the two world wars there were no other possibilities for publication, mainly abroad, but obviously the members of the teaching staff of the Faculty of Medicine preferred to publish the results of their research in MSJ aiming at the same time to achieve everybody’s dream: to offer the medical communities a journal that could rival other European publications

from the same field. No wonder that news about the latest discoveries in physics and chemistry that could have an impact on the medical science was always present (4).

During MSJ's second period, the most productive years were 1926-1931, as in other spheres of life, each volume had more than 600 pages. The topics of the papers published during that period reflected in fact the interests of the epoch mirrored in the main research projects. After 1940 celebrated professors from the Iasi School of Medicine were included in the editorial board. Unfortunately that glorious period in the history of SMN and MSJ came soon to an end. Among the new names of the editorial board it is worth mentioning the names of: L. Ballif, I. Nicolau, O. Franche, Gh. Tudoranu, J. Nițulescu, A. Moruzzi, Gh. Nastase, Gh Plăcinţeanu, N. Balan, L. Wasserman.

Although the Journal (MSJ) encountered countless difficulties during the Second World War, the enthusiastic editors succeeded in publishing the journal regularly until 1943: *"The printed materials for the last 1943 issues of MSJ were destroyed before their publication. The building of SMN was badly damaged by 11 bombs, 4 of which fully hit the building, and almost demolished it. It was no surprise to see that the medical collections gathered with so much hard work and displayed with so much care were lost for ever. In spite of this, after so much material loss, to say nothing of the spiritual and social waste, our predecessors found enough energy to gather all forces so that the publication of the MSJ could continue. Everybody dreamed of calmer and better times* ... "(vol. 57, 1946). (cit 4). But the hope was futile. In 1949 MSJ ceased to be published as the new communist authorities were obsessed of the idea of having an absolute control of everything that was published. All publications were supposed to be censured and distributed from Bucharest.

The policy of absolute control of all publications that could publish subversive material lasted until 1956 when the 60th volume was published. The editorial published in that issue was signed on behalf of the editorial board by Vasile Rășcanu, member of the Romanian Academy, who did his best to demonstrate to the communist authorities the importance of resuming the publication on of this outstanding medical journal. The title of the editorial was telling: *The Historical and Scientific Significance of MSJ of Iasi* (pp. 1-4) (5).

The publication of the 60th volume of RMC marked the third period, also a productive one. The members of the editorial board included V. Rășcanu, member of the Romanian Academy (editor in charge), professors Ș. Brăteanu, Vl. Butureanu (deputy editor), T. Economu, reader Dr. I. Kleinstein, reader G. Popovici and E. Ungureanu (secretaries of the editorial board.). Many consider that that was the gold age of RMC which culminated in the appointment professor Prof. Gregory Teodorovici as editorial secretary. Until 1966, all the published issues numbered almost 20 million pages. RMC had at the time 2,000 subscribers (3). Besides V. Rășcanu, member of the Romanian Academy, editor-in-chief between 1956– 1974 to whom we owe our gratitude for the persuasive plea in favour of resuming the publication of RMC, two other personalities are worth mentioning: Gregory Teodorovici (1975 – 1992) and Valeriu Rusu (1995 – 2014) who dedicated much of their lifetime to this journal. They employed all their competence and editorial talent to the prosperity

of MSJ. During that period the RMC was indexed in PUB MED and INDEX MEDICUS.

The scientific level of MSJ which celebrated the publication of its 120th volume was highly appreciated by similar journals and societies, among them the Society of Hygiene of the United Nations that ranked MSJ among the important medical publications, its history mirroring the ups and downs of Romanian medicine (6). In 2008, 1160 pages were published in the four issues. More than 1,000 medical specialists subscribed to this outstanding journal. Since 2012, MSJ has been published only in English. At the same time MSJ is currently being monitored to be included in the new database ISI Thompson , (03, 03, 201).

The late editor-in-chief who was extremely devoted to MSJ was Valeriu Rusu. He was aware of the steps that had to be taken in order to overcome to difficulties that MSJ encountered during the long period of transition that followed the 1989 events which more or less destroyed consciously or unconsciously some of the good achievements of the past: *"first of all we need a coherent editorial board that fully accepts the responsibility and obligations encountered by this position. Such o board of editors should be motivated to get rid of the sometimes exacerbated individualism if they were ready to offer their time and intelligence to this medical journal of Iasi that fully deserved that sacrifice. We should be aware that MSJ can "coexist" with similar publication that had been published under more favourable conditions if everybody accepts the idea that the journal (MSJ) means, after all, an important tradition that has to be defended and continued. This awareness should be developed in the collaborators who should be conscious of the exigencies of a journal indexed Index Medicus, PUB MED i.e. they should realize that their scientific contribution is signalled in the largest database in the world. Consequently, the editor should exercise a more rigorous assessment of all contributions, mainly by accepting the idea of the importance of external reviewers who could enhance the responsibility of the authors. Obviously the form of presentation is extremely important. The authors should strictly observe the presentation requirements of the journal"*(4).

A summary of the important contributions of MSJ is worth attempting. MSJ: encouraged the use of the Romanian language in science; contributed to the development of the medical discourse in Romanian; played a major role in foundation of the first institutions of high learning: Prince Michael s Academy (1835), the University of Iasi (1860), the Faculty of Medicine (1879); founded the first medical library as a result of exchanges with more than 150 periodical journals and of the book donations from the members of SMN; promoted scientific research, education and the spread of medical knowledge; made known the medical school of Iasi all over of the world; took part in the main national events always on the side of the progressive forces (e.g. the Union); mirrored the world scientific trends of the age; contributed to the promotion of the academic staff of the University of Medicine and Pharmacy of Iasi (a truth almost forgotten today by many members of the medical school); brought together scientists of various origins, a proof of the importance of positive multiculturalism; published outstanding original papers signed by many leading scientists from all over the world; promoted the movement towards education for

health, undergraduates' instruction and teaching staff's training.

This celebrated scientific society (SMN) and its journal (MSJ) passed through two world wars, the communist dictatorship, a long period of painful transition after 1989. In spite of all these difficulties they survived. It is once again a proof that *"the great works of humanity are achieved by people who succeed in overcoming the infirmities of an age."* (G. Scripcaru) (6). From the very beginning, MSJ had a distinct European personality, that aimed at strengthening and pursuing those values that embodied the scientific spirit of the age. This year we celebrate SMN's 186 years of existence and its long-life journal (MSJ).

This brief presentation of the key events in the development of Romanian medicine is also an encounter with the great personalities that gave life to the two institutions. All this has been done with a deep reverence given to the words on the 183 years old seal of SMN that is still made use of: *Semper Altius.*

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Note: *Those who are interested in furthering the study of the history of SMN and RMC should read Dana Baran's monograph, a formidable endeavour to cover thoroughly the ups and downs of this long – lived journal.*

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PHYSICIANS AND NATURALISTS SOCIETY, IASI-ROMANIA, SINCE 1833

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EDITORIAL

HUMAN PAPILLOMAVIRUS AND SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

Whole scientific world is talking about Human Papillomavirus (HPV) and his role in the oncogenesis, but after all who is this virus? .HPV is small (50-55 nm in diameter), non-enveloped, double-stranded DNA virus which primarily infect epithelial cells of the skin and mucous membranes. They are associated with a variety of clinical manifestations that range from benign lesions to cancer. The spectrum of diseases associated with HPV infections has accompanied humans throughout evolution. Papillomatous and verrucous skin and genital lesions have been described since ancient Greece and Rome. But until early twentieth century, research of HPV has not been started yet. Indication that now recognized HPV-associated diseases could be related to sexual transmitted infection dates from 1842 year when Italian physician Rigoni-Stern noted a high frequency of cervical cancer in married women, widows and prostitutes, but their rare occurrence in virgins and nuns. After visualization of papillomavirus particles in human warts by electron microscopy and characterization of HPV genome interest for HPV started to arise. Zur Hausen postulated and analyzed a possible role of HPV in cervical cancer development. The first HPV types (HPV16 and 18) were isolated from cancer biopsies of the cervix and were cloned in 1983 and 1984, respectively. Further epidemiological studies have confirmed that cervical infection by high-risk (HR) HPV types is the main risk factor for cervical cancer development. The role of HPV in the pathogenesis of squamous cell carcinoma of head and neck (SSCHNs) was first described in 1983, when histopathological features consistent with HPV infection were identified in oral cancers. Two years later, viral DNA from high-risk HPV-16 was detected in an oral carcinoma by Southern blot hybridization. By the early 1990s, discoveries of HPV in association with oropharyngeal carcinomas became consistent. In 2008, the Nobel Prize for Medicine was awarded to Dr. Zur Hausen for the discovery of the infection etiology of cervical cancer.

Currently, we have more molecular evidence that indicates a relationship between high-risk HPV and the pathogenesis of oropharyngeal cancers. Expression of HPV *e6/e7* oncogenes are the current criterion standard for determining a causal role of oncogenic HPV in human tumors. The *E6* and *E7*genes produce oncoproteins and are responsible for the degradation of p53 ,which are important events that lead to dysregulation of the cell cycle and thereby immortalization of the cells. The silencing of p16 by promoter methylation is commonly observed in SCCHNs. In contrast, HPV is readily detected in HPV-positive tumors and is highly correlated with p16 expression, which is most likely the result of transcriptionally active HPV infection. Therefore, there is a relationship between HPV the expression of p53 and p16 in oropharyngeal cancer and clinic-pathological variables. The diagnosis of HPV positivity should enter as a routine test for these patients. The most clinically

useful test, *in situ* hybridization, is available in only few centers, so that is unfortunate, even if p16 immunohistochemistry may serve as a reasonable surrogate and PCR – molecular detection of HPV DNA. It is widely accepted that the most common risk factors for SCCHNs are tobacco use, heavy alcohol consumption, betel quid chewing, and high-risk HPV infection. High-risk HPVs are currently well accepted as causative agents for most cervical cancers and have recently been associated with a subset of head and neck cancers. Oropharyngeal cancer has emerged as another mucosal cancer that is associated with HPV infection. HPV DNA is present in 20% of all SSCHNs and in nearly 60% of tonsillar cancers. Traditionally, most cases of oropharyngeal cancer were associated with chronic tobacco and alcohol use. This exposure resulted in the mutation loss of the genes p16 and p5. Decreased use of tobacco coupled with an increase in the HPV + of oropharyngeal cancer has shifted the majority of these types of cancers to now occur more commonly in non-smokers. An "epidemic " of HPV oropharyngeal related cancer has been identified and attributed to HPV + disease in many cases thought to result from oral genital contact.

Patients with HPV + cancer are less likely to smoke tobacco and less likely to use alcohol heavily, are heaving a better dentition, are heaving a higher incidence of oral sex practice, younger and higher socioeconomic status. Having in mind this two types of oropharyngeal cancers, HPV+ and HPV – and their molecular changes that concern the expression of p16 and p53 genes , we can conclude that patients with HPV + cancer have an higher expression of p16 and a lower expression of p53, responded well to treatment and showed good prognoses. The T stage in TNM (tumour, node, and metastasis) stage does not differ from HPV status, HPV + tumors are more likely than HPV –tumors to have a lower T stage, whereas advanced T stage is a significant risk factor for recurrence and death, independently of HPV status. Patients with p16 + oropharyngeal tumors are less likely to have persistent viable tumor in neck lymph nodes after (chemo) radiotherapy and hypothesized that a post treatment planned neck dissection may perhaps be avoided. Several authors indicate that the impact of HPV status must be evaluated in prospective clinical trials and included as a stratification factor to avoid potential confusion .The current American Joint Cancer Committee staging system for oropharyngeal cancer needs to be modified in order to be better reflect different prognoses for patients with HPV+ or HPV – tumors and those with vegetating or infiltrating tumors.

HPV-related SCCHN represents a new entity well- defined with a well- known characteristic that includes young age, good performance status, male gender, nonsmoking and nondrinking status, basaloid tumor histology and high-risk sexual behavior. The practicing oncologist needs to be aware of these new findings, and HPV testing with PCR or FISH, should be perform routinely. For now, results will have significant prognostic though not therapeutic implications. Still, changes come rapidly in this field and we expect that different treatment will be available to these patients in the near future.

In conclusion, what do we wish for these patients? We wish a modified TNM stage for HPV + tumors? We wish a genic and antiviral HPV specific – therapy? We wish a fuller utilization of HPV vaccine

and public education measures?

Personally, I am having great expectations within the scientific progress in this field so we could have some day the responses to those questions above, all for an early diagnosis, a specific treatment and a long term prognosis for the HPV positive related SCCHN s. Multidisciplinary dedicated clinical practice with integrated research, I consider to be an important aim.

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THE TRIBES AND TRIBULATIONS OF LARYNGOPHARYNGEAL REFLUX: A REVIEW OF RECENT STUDIES WITH IMPLICATIONS FOR INTERDISCIPLINARY COLLABORATIONS BETWEEN OTOLARYNGOLOGISTS AND GASTROENTEROLOGISTS

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THE TRIBES AND TRIBULATIONS OF LARYNGOPHARYNGEAL REFLUX: A REVIEW OF RECENT STUDIES WITH IMPLICATIONS FOR INTERDISCIPLINARY COLLABORATIONS BETWEEN OTOLARYNGOLOGY AND GASTROENTEROLOGY (Abstract) Laryngopharyngeal reflux has been recognized since 2006 (Montreal Consensus) as an extradigestive manifestation of gastroesophageal reflux disease. However, despite numerous research studies, the relationship between these two pathologies is yet to be fully understood. The aim of this paper is to review the literature of the last five years available via the PubMed database, looking at the controversies about the prevalence, pathophysiology and diagnosis of laryngopharyngeal reflux. **Keywords:** LARYNGOPHARYNGEAL REFLUX, GATROESOPHAGEAL REFLUX DISEASE.

Laryngopharyngeal reflux disease (LPR), also known as supraesophageal reflux disease, atypical gastroesophageal reflux disease (GERD) or reflux laryngitis, represents an extradigestive manifestation of GERD due to the reflux of gastric content through the esophageal/ pharyngeal/laryngeal axis (1). Although GERD and LPR are considered to be part of a spectrum within the same disease, they are different clinical entities produced through different pathophysiological mechanisms. Reflux laryngitis is still a challenge for both gastroenterologists and otolaryngologists. Even though the cause-effect relationship assertion between GERD and LPR has been strengthened by more recent evidence, the causality, diagnosis and treatment remain controversial. Since Koufman coined the term "LPR" in the 90's, reflux laryngitis has been most frequently recognized by Ear, Neck and Throat (ENT) surgeons and gastroenterologists, but also by other medical specialists (pneumologists, cardiologists etc), even to the point of being over-diagnosed (2). For example, in the USA, during a period of 11 years, the number of ENT consultations for clinical conditions related to GERD in-

creased by over 300% and the prescription of proton pump inhibitor (PPI) also multiplied about 14 times (2).

Epidemiology and pathophysiology. The mechanisms involved in the pathophysiology of LPR may be related to the direct injury of the esophageal and pharyngolaryngeal mucosa by the gastro-duodenal content, with or without micro-aspiration in the respiratory airways. The larynx is an organ about a 100 times more sensitive than the esophagus and is at high risk of injury because of its immediate proximity to the digestive system (3). Thus, only a small amount of gastric content is sufficient to cause lesions of the larynx. The second mechanism that is incriminated in the pathophysiology of LPR is the tracheal-bronchial reflex mediated by the vagal nerve, produced by the acidification of the distal esophagus, and which determines cough, throat clearing and laryngospasm (3). The vocal folds are covered by stratified squamous epithelium which creates a barrier against external and internal factors, including reflux. Erickson and Sivasankar conducted a study using transepithelial electrical resistance (TER) for assessing the permeability of the epithelium of the vocal folds. They found that the exposure of vocal folds to refluxate determines the decrease of TER, making them more susceptible to injury (4). It has also been demonstrated that carbonic anhydrase type III plays a protective role of the epithelium of the larynx, as it regulates cellular pH when the larynx comes in contact with acid by producing bicarbonate (5). Ford (1) showed that this enzyme was absent in 64% of patients with LPR. The peptic injury was also associated with depletion of other key protective proteins such as E-cadherin and the stress proteins (e.g. Sep 70) (5).

The exact actual prevalence of reflux laryngitis is hard to determine because of the different clinical presentations of LPR, added to the lack of consensus regarding LPR diagnosis and treatment. It is estimated that 4-10% of patients who seek otorhinolaryngologic services suffer from reflux disease. At the same time, LPR was identified in more than 50% of dysphonic patients (6). In last decades, the prevalence of GERD and LPR has increased dramatically. Using a statistical model in an analysis of 17 studies, El-Serag (7) showed that the average increase rate of reflux disease since 1976 has been 4% per year (P <0.0001). An interview-based study on 656 subjects revealed unexpectedly high percentages of people living with GERD - 40% (22% with typical GERD and 18% with LPR) across the USA. In addition, many patients were young – 21-30 years old (37%). Regarding causality, food additives were now considered to play a significant role, apart from the historically acknowledged obesity (8).

Clinical manifestations of LPR. Despite the association between GERD and LPR, the diagnosis of reflux laryngitis remains a difficult task. First, a complete and accurate anamnesis is needed in order to diagnose this pathology. According to the study published by Koufman two decades ago we should discriminate between GERD and LPR as distinct clinical entities (3). The symptoms of LPR are diverse and many physicians may not link these symptoms toLPR. The most common manifestations of LPR are dysphonia/ hoarseness, throat clearing or pain, chronic cough and globus (9, 10). But it is known that these symptoms are nonspecific to LPR and can also be found in other diseases such as postnasal drip syndrome, exposure to dif-

ferent allergens or environmental irritants like smoke or vocal abuse. Because symptoms are not exclusive to LPR, the clinicians should rely on a combination of manifestations and diagnostic tools such as laryngoscopy exam, impedance-pH monitoring and empiric trial of proton pump inhibitors (PPI) to establish an accurate diagnosis. It is important to emphasize that patients with LPR do not usually present typical GERD symptoms (heartburn and regurgitation), which can make proper diagnosis more difficult (1).

Diagnostic methods. ***Laryngoscopy.*** The diagnosis of reflux laryngitis is usually performed by ENT surgeons. Transnasal fiberoptic laryngoscopy is the first-line method to be used by otolaryngologists to diagnose LPR. Laryngoscopic exams are essential for excluding neoplasms, but their role in detecting GERD-associated laryngitis remains uncertain. There is a low inter-rater reliability among ENT surgeons evaluating the same laryngeal signs, and that is the reason why LPR diagnosis may be subjective and inaccurate. The specificity of the laryngeal signs of LPR is still debated in the literature. The most frequent laryngoscopic lesions that have been found to be related to GERD were edema and erythema, particularly in the posterior region (9). These were the main findings used by different researchers for the diagnosis of LPR, while ulcers and ventricular obliteration, nodules, polyps and leukoplakia were reported to be less useful. In another study, pseudosulcus was found to have 70% sensitivity and 77% specificity in patients with LPR (11). In 2007, Vavricka et al. (12) evaluated the prevalence of specific laryngeal lesions thought to be GERD-related, both in patients with documented reflux and normal subjects. They found that the prevalence of laryngeal lesions was the same in both groups. Only posterior pharyngeal wall findings showed a statistically significant higher prevalence in GERD patients as compared to the control group. Furthermore, the study of Hicks et al. revealed that the most signs seen in patients suspected of LPR were also found in healthy, asymptomatic subjects (13). To improve the diagnostic accuracy, Belafsky et al. developed the Reflux Finding Score (RFS) based on the presence and severity of the lesions found in laryngoscopy (14). This nine-item scoring system including the most common laryngoscopic lesions has great reproducibility and is useful to evaluate the effectiveness of therapy in patients with LPR. Thus, the laryngeal lesions have low specificity for LPR and that is the reason why patients initially diagnosed with reflux laryngitis do not respond to antireflux treatment. However, Park et al. showed a high specificity of the laryngeal findings for LPR (91%) (15). Rafii et al. demonstrated that LPR may be over diagnosed, all the 21 patients included in the study, had already been diagnosed with laryngopharyngeal reflux disease (16). In fact, all suffered from other laryngeal pathologies, different from LPR, as the cause for their chief complaint of dysphonia. The most common findings were vocal fold paresis (29%) and muscle tension dysphonia (14%). Therefore, the recommendation is for a diagnosis of reflux-induced laryngitis to not be made based on laryngoscopy findings alone.

Upper digestive endoscopy (UDE). UDE has excellent specificity for the diagnosis of GERD in the presence of an erosive esophagitis, but only one third of patients with GERD symptoms and even fewer after treatment with PPIs have ero-

sive esophagitis (17). However, erosive esophagitis could be generate also by other pathologies (e.g. Candida esophagitis, varices) that were found in 25% of LPR patients (18). It is important to notice that, although upper endoscopy is necessary to rule out esophageal cancer, it is not a specific diagnostic test for LPR. Indeed, it has been reported that up to 80% of LPR patients have normal UDE. Red flags that necessitate early endoscopy include dysphagia, bleeding, chest pain, weight loss or dyspnea.

Ambulatory pH monitoring/ impedance-pH monitoring. Ambulatory reflux monitoring is a diagnosis method which can demonstrate the presence of GERD by documenting an abnormal esophageal acid exposure. A 24-hour-double-probe (simultaneous pharyngeal and esophageal) pH monitoring has been the gold-standard for LPR diagnostic in last twenty-five years (19).

The sensitivity and specificity of ambulatory pH monitoring as a means for diagnosing GERD in patients with extraesophageal reflux symptoms have been challenged (6). Koufman showed a sensitivity of 24-h dual-probe pH monitoring ranging from 50% to 80% (8). In the presence of esophagitis, the sensitivity and specificity of this method is higher (up to 100%), but in patient without esophagitis, the sensitivity is dropped to about 70% (20). Also, it has been determined that pH monitoring cannot predict the severity of patient's reflux laryngitis symptoms or signs and only the heartburn correlates with LPR. There is great variability in the reported prevalence of abnormal pH monitoring in patients with laryngitis. A pathological reflux detected during a pH measurement does not confirm GERD as the cause of the laryngitis as well as a negative pH monitoring test can direct the diagnostic toward non-GERD etiologies. However, ambulatory reflux monitoring may be used to determine whether the patients' symptoms are related to reflux or not. The temporal association between reflux episodes and ENT symptoms may be evaluated by two methods: the symptom index (SI) and the symptom association probability (SAP) (21). For these methods to yield results, the patient has to keep a log and use a detection device accurately and timely.

The availability of multichannel intraluminal impedance and pH monitoring (MII-pH) seems to perform better in the diagnosis and management of extraesophageal manifestations of GERD than pH-testing alone (22). It is currently emerging as the new "gold standard" for clarifying differences in PPI refractory symptoms. Esophageal pH-impedance monitoring is a method that detects both the antegrade and retrograde movements of the refluxate and evaluates the physical (liquid, gaseous or mixed) and chemical (acid or non-acid) nature of the refluxate. According to the American College Guideline published in 2013, impedance-pH is used to establish the diagnosis of GERD when: the diagnosis is uncertain, or before consideration of endoscopic or surgical therapy in patients without esophagitis, or in the evaluation of patients refractory to PPI therapy.

A very recent study (23) comparing MII-pH and Bravo® capsule in patients with LPR symptoms found high prevalence of discordant esophageal pH results, most commonly a negative MII-pH testing, but positive Bravo testing. The researchers considered that the Bravo test results were more likely to be true, as the patients included in the study presented characteristics consistent with GERD (heartburn, defective LES, hia-

tal hernia). They concluded that MII-pH catheter study may be inadequate to diagnose GERD in the patients with both typical and atypical symptoms.

A new technology able to detect aerosols of acid and gaseous clouds of acid tends to be more used in patients with LPR. The Dx-pH measurement system® (Respiratory Technology Corp., San Diego, CA) is a highly sensitive and minimally invasive device for the detection of acid reflux in the posterior oropharynx. It uses a nasopharyngeal catheter with the pH probe placed in the oropharynx instead of hypopharynx and it measures the pH in either liquid or aerosol droplets (24). The advantages of this method are that it does not require manometry or endoscopy and the catheter is better tolerated as it does not traverse the upper esophageal sphincter. However, it is limited because it cannot confirm if a reflux event originates in the stomach, since it does not entail simultaneously probing the esophagus.

Empirical PPI treatment. The therapeutic test with PPIs is considered by many clinicians as a reasonable approach to the diagnosis of LPR, in which a favorable response is defined as diagnostic confirmation (18). Most published researchers consider a favorable response when there is a resolution of LPR symptoms after 3 months of double dose PPI trial. But the role of PPI trial is still controversial. A meta-analysis of randomized studies has demonstrated no benefit of PPIs vs. placebo in diagnosing GERD-related laryngitis (25). A placebo-controlled study including patients with LPR showed an improvement in 50% of patients when using placebo compared with 53% of patients who received PPIs (26**)** .Due to this seemingly strong placebo effect, patients who have some response to an empiric PPI trial may be incorrectly diagnosed with reflux disease. Nowadays, the current recommendation for the empirical trial is to include patients with concomitant typical symptoms of GERD (1). The advantage of this diagnostic tool is the cost-effectiveness, the ease of use and its sensitivity, but it lacks specificity.

Pepsin testing. The use of the pepsin test to diagnose GERD is justified, because pepsin is produced exclusively by the stomach. The pepsin test (Peptest) is a non-invasive, quick and inexpensive tool for the diagnosis of LPR. It consists of a lateral flow device that uses 2 monoclonal antibodies to human pepsin to detect the presence of pepsin in saliva. Its results can be read very easily in 5-15 minutes. Because of the benefits and ease of application, a positive salivary pepsin test in a patient suspected of having LPR can be a cost effective, accurate and alternative diagnostic method. Yuksel et al. (27) found sensitivity and specificity of 87%, a positive predictive value of 85% and a negative predictive value of 68% for the pepsin test in the diagnosis of LPR. In a very recent cross-sectional study, *in vitro* pepsin detection tests were compared to the 24-hour double probe pH monitoring results for patients with suspected LPR (28). The authors found a sensitivity and specificity of the pepsin detection test of 33% and 100%, respectively. A positive predictive value of 100% was recorded.

Current diagnostic tests for GERD and LPR are suboptimal and do not accurately and reliably measure chronicity of reflux. New methods to diagnose LPR are needed. Recently, a minimally invasive device has been developed to assess esophageal mucosal impedance (MI) as a marker of chronic reflux. Ates et al. conducted a prospective

longitudinal study using a prototype device to investigate MI patterns in patients with GERD and common non-reflux conditions, to assess MI patterns before and after treatment with PPI and to compare the performance of MI and wireless pH tests (29). They discovered that measurements of MI detect GERD with higher levels of specificity and positive predictive values than wireless pH monitoring. They are hopeful that this device may be employed in the future also in patients with LPR.

Another diagnostic method that offers an objective and quantitative alternative is computer-based analysis of laryngeal image color and texture features. The study of Witt et al. suggested that a combination of laryngeal hue and texture features could be used to diagnose LPR (30).

CONCLUSION

Laryngopharyngeal reflux is being diagnosed increasingly frequently in recent years, especially by ENT surgeons and gastroenterologists. It has become one of the most common reasons why patients see otolaryngologists. LPR is considered to be a different clinical entity than GERD as it features different symptoms, clinical manifestations and treatment objectives. The controversy about its pathophysiology and diagnosis continues still, despite the numerous studies that have been published in the last 5-10 years. We consider that upon initial consultation, the ENT specialist should be able to recognize reflux laryngitis signs and symptoms and refer the patient to a gastroenterologist, thus acknowledging the importance of interdisciplinary collaboration in such cases. Given that LPR diagnosis and treatment have not been standardized, we emphasize that further large randomized-controlled trials are needed for more accurate diagnosis.

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POINTS OF VIEW

THE METABOLIC SYNDROME: PROS AND CONS

The alarming increase in number of cardio-vascular diseases (CVD) in the 20th century has determined the onset of many epidemiological studies *(using the Framingham pilot-study model)* to identify the determinants, that led to the outlining of risk factors (RF); the prophylactic efforts focused on the changeable factors *(biological and behavioral)*, and the control over them proved to be efficient. Finding that the vascular accident risk increased by the number and quantity of such factors, created the possibility of assessing the global risk in each individual by a general risk score calculator, and the intensity of therapies was such shaped to achieve the therapeutic goals specific to the risk level. Finding that the combination of some major risk factors might have an additive or multiplying effect (1) has encouraged the development of holistic strategies, with arguments grounded on the outcomes of multiple therapeutic studies, according to the concept of evidence-based medicine.

The observance of more frequent combinations of risk factors led to the outline of the metabolic syndrome (MS) or cardiometabolic (initially proposed by Reaven under the name of Syndrome X) concept. MS appears as the result of this conceptual revolution in the management of CVD. The fact that, by adjusting the other major RFs (LDL-cholesterolemia included), the presence of MS would increase the mortality risk of CVD by approx. 3-4 times compared to the absence of MS *(Kuopio Study - posthoc analysis) (2)* enabled this concept to be put into practice. Finding that MS is often associated with a pro-inflammatory *(increasing PCR)* (3) and pro-thrombogenic (4**)** status, as well as the evidence-based association with the endothelial dysfunction and oxidative stress (5) supported the pathogenic relation to atherogenesis.

The existence of the syndrome has raised doubts and debates on its naming, components and pathogenesis from the very beginning. The dispute has even augmented to the attempt of approaching the MS as a disease with unique and generally accepted pathogenetic mechanism *(the* ***insulin-resistance*** *being, according to Reaven, the key mechanism)* (6) with a unitary curative and prophylactic strategy. The cardiologists had finally adopted the NCEP-ATP III guidelines for diagnosing (7) and quickly put it into practice, as it operated with easy to determine parameters *(abdominal circumference, BP, triglycerides, HDL-cholesterolemia and impaired fasting glycemia).*

By applying the ATP III criteria to a cross-sectional study, IIIrd NHANES determines a MS prevalence of 24% (1 of 4 persons) in USA, and therefore an extremely large proportion of population at high risk of diabetes and cardio- and cerebrovascular diseases, where the risk of individuals with MS to suffer a vascular accident *(stroke or AMI)* is 3 times higher than in those lacking the syndrome, the death risk carried by vascular accidents *(stroke or AMI)* is double *(8,9,10)*, and the risk to develop diabetes mellitus is 5 times higher (11) (or, 80% of diabetic persons die from cardio-vascular pathology); moreover, the

explosion in overweight in time has led to a MS prevalence increase from 23.1% (1988-1994) to 34 % (in 2010*)*, according to NHANES (12, 13).

The metabolic syndrome seems to be a major public health due to its

- major impact on the morbidity-mortality of obesity, atherosclerosis, diabetes mellitus and their complications;
- direct and indirect costs incurred *(medical care + economic loss consequent to temporary inactive periods, anticipated retirement and premature mortality);*
- behavioral changes incurred by an efficient prophylaxis *(nutrition, physical exercise);*
- need for involving governmental factors to ensure the logistic for these changes *(proper food, leisure parks, bike lanes etc);*
- need for involving the mass-media, school and family.

The huge stake of using the concept in setting preventive and therapeutic strategies has led to a great war in the medical media, with occasional polemic or sarcastic accents that have rarely been noticed in medical debates; we present below some eloquent headlines used by the opponents to this concept:

- Metabolic syndrome: clinical utility or futility; is this diagnosis necessary?- *(Reaven)* (14);
- Metabolic syndrome: a fata morgana?- *(Bakker et al)* (15);
- The myth of the metabolic syndrome- *(Gale)* (16);
- Metabolic syndrome . . . is dead - *(Jörgens)* (17);
- Should we dump the metabolic syndrome?: Yes . *(Gale)* (18);
- The concept of metabolic syndrome as a scientific instrument is a blunt sword *(Ritz)* (19);
- Metabolic syndrome - is it a syndrome? Does it matter? *(Kahn)* (20);
- The Metabolic Syndrome (Emperor) Wears No Clothes *(Kahn)* (21);
- The concept of the metabolic syndrome is it dead yet? *(Gupta et Poulter)* (22).

In reply, the metabolic syndrome supporters seem more balanced:

- Should we dump the metabolic syndrome? No. (*Alberti et Zimmet)* (23);
- Do not dump, but LEARN to recognize the metabolic syndrome *(Bense)* (24);
- The metabolic syndrome – revise the processes of care, don't dump the concept *(Holt)* (25);
- The metabolic syndrome still lives. *(Grundy)* (26);
- Metabolic Syndrome: older than usually assumed, but still too young to die *(Meisinger, Koletzko et Heinrich)* (27);
- The Metabolic Syndrome: perhaps an etiologic mystery but far from a myth (*Zimmet et Alberti)* (28).

We attempt to highlighting the main pros and cons:

Cons (21):

- Ambiguous or incomplete criteria and ambiguous reasoning of threshold values;
- The insulin-resistance as linking etiology is unclear;
- The insufficient prediction level of the syndrome, neither for CVD, nor for diabetes mellitus?
- The risk of association would not exceed either the sum of all component risks or even the risk of a single major component *(INTERHEART study)*;
- The lack of solid scientific evidence on clinical utility;

- The failure to establish a therapeutic strategy different from the approach of each component risk;
- The absence of a specific causal or pathogenetic treatment (the MS treatment is not different from the treatment of the sum of its components);
- Is it exaggerated to label over 25% of the population as ill (discriminatory? politically incorrect?).

It is also important that Gerald Reavan himself has reservations about on the practical utility of MS concept (14, 29)

To conclude, identifying each risk factor and a therapeutic approach for each of them would create a reasonable pragmatic conduct (30).

Pros

- The MS concept has already been proved as valid, solid and usefull in practice;
- MS should not be used as a risk quantification tool, but for the identification of high risk subjects who need an aggressive approach, both pharmacological and as a life style;
- The shared origin of MS components would require a holistic approach focused on behavioral changes (life style) at individual and population level, fact that allows the reduction of medication (31,32);
- The concept would also allow the medical approach of obesity, in addition to the purely cosmetic one *(Mc Guirre in 17)*.

To conclude, its usefulness would be sure in the awareness raising, education and motivation of high risk patients, targeting mainly the change in the life style.

Although (or because) the preventive power of using MS as a work tool equally targets DM and CVD, the diabetologists/endocrinologists (or, more precisely ADA and EASD) have alienate from this by publicly discouraging the use of MS concept *(33*), while most of the cardiologists and internists have enthusiastically adopted it, as AHA/NHLBI and ESC recommend it as a valid concept with real practical application. The strange polarization of the medical world, mainly by specializations (cardiologists versus diabetologists), made Scott Grundy consider the opposition of diabetology societies as the expression of a ***turf war*** subsequent the cardiologic societies entering a hunting ground the diabetologists consider it exclusively belong to them. Many people think about the media war around the metabolic syndrome as an exaggeration, or just a “a tempest in a teapot” or "a firestorm about nothing." (*Nathan in 17*). Finally, some contest MS as a scientific research tool, and others as a medical practice tool.

It is interesting that the Romanian endocrinologists and diabetologists accepted the use of the metabolic syndrome without aggressive disputes.

Although a truce has been sought for even from 2006 (accomplished only between IDF and AHA/ ESC), therefore a compromise solution that would not affect the aggressive and beneficient approach of risk factors, both disputants kept their ground, although there are many reasonable arguments among both diabetologists and cardiologists.

In 2006, in an attempt to harmonize the MS concepts, a consensus is reached between International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society and International Association for the Study of Obesity. They accept that the Metabolic Syndrome represents an

association (cluster) of risk factors for CVD and Type 2 DM that is more frequently met as the hazard may justify. These risk factors include: hypertension, dyslipidemia (hyper TG and low HDL-chol), increase in glycemia à jeun and central obesity. It has been accepted, in an attempt to unify the criteria, that the presence of 3 criteria out of 5 would allow the confirmation of the metabolic syndrome and, even if this should not be a compulsory criterion, the waist circumference is still a useful preliminary tool. It has been agreed the use of a unique set of cut-points, except for the waist circumference. To diagnose it, various criteria have been proposed for the definition of central obesity (compulsory component, with lower cut-points with ethnic specifications for IDF). It was also accepted that, in the interim, national or regional cut points for waist circumference can be used (31), *although certain studies state that* defining MS according to IDF criteria seems to be much better in CV risk predictors compared to that of NCEP-ATP III and NHLBI/AHA (35).

Although we agree with Scott Grundy that MS is still alive (26*)*, we have to accept that the researchers' interest in the metabolic syndrome has visibly reduced over the last years, and the topic seems to lose its importance. A good symptom for this is that ***The Journal of the CardioMetabolic Syndrome*** whose first edition seemed to be perfectly justified in 2006*(36)* ceased to exist. Another argument in this regard is the fading out of MS presence in the cardiovascular prevention guides; still, we have to note that ESC/EHS has relatively recently published a Guide dedicated to the metabolic syndrome and hypertension (37).

At the same time, we note the raise of surgeons' interest in obesity and MS, considering not only the impact of obesity and MS on the anesthetic and perioperative risks (materialized in heart, respiratory and renal complications, as well as in the increase in the hospitalization duration and cost) *(38)* but also the metabolic impact of surgical methods for treating obesity. Thus, the weight loss would be associated with reduced resistance to insulin, improved lipid profile and AT reduction *(39)*, meaning a reduced cardiovascular and diabetic risk, facts that justify the newer term of ***Metabolic Surgery***. The surgical therapy would be recommended for patients with diabetes mellitus or MS with BMI = 30-34.9 kg/m^2 (40).

The researchers' interest in the metabolic syndrome remains obvious in case of the researchers from developing countries usually confronted with tropical infectious diseases and malnutrition and where, implicitly, the metabolic syndrome represents a new challenge; this fact is visible both in the specialized Western publications *[Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy (USA) Metabolic Syndrome and Related Disorders (USA), etc.]* and in the medical publications in this area *[Journal of Endocrinology and Metabolism (Malaysia). Diabetes & Metabolic Syndrome: Clinical Research & Reviews (India), Journal of Diabetes and Metabolic Disorders (Iran), etc.]*.

Although some studies suggested the existence of a common genetic ground (41,42), the attempt to clearly demonstrate this has failed and thus strengthened the position of the concept's opponents (43). The failure of the attempt to consider the metabolic syndrome as a generally accepted entity is owed, beyond any vanity, to the lack of any undeniable evidence that proves the existence of a superior risk by the asso-

ciation of the constituent risk factors compared to the separated risk of each risk factor, as well as a clear and unifying pathogenetic mechanism or a clear common genetic cause that would allow the synthetization of a unique medicine (44, 45). Nevertheless, the efforts to identify unitary pathogenetic mechanisms and to identify a therapy target still go on, as the sirtuins have been recently proposed (46).

O reasonable, pragmatic and acceptable opinion we would like to note in order to conclude is that of Dr. Darren McGuire (17), who considered the "metabolic syndrome as a useful construct that helps in a broad population of patients to identify those at increased risk of developing either diabetes or cardiovascular disease at a time when lifestyle intervention can be very useful". Emphasizing that "the presence of metabolic syndrome is where weight worries stop being cosmetic and start being medical", he considered that the metabolic syndrome is particularly applicable in the primary-care, on the one hand, because the diagnostic is simple and cheap, and on the other hand, because it makes the patient more aware of the importance of lifestyle changes.

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0.000), egg white 19 cases (14.39%, p = 0.000), peanuts 10 cases (7.57%, p = 0,000), fish 8 cases (6%, p = 0.000), soy 6 cases (4.54%, p = 0.000), nuts 6 cases (4.54%, p = 0.000), wheat flour 5 cases (3.78%, p = 0.003), strawberries 2 cases (1.51%, p = 0.011) and rye flour 1 case (0.75%, p = 0.150) .

All allergens were statistically significant except for rye flour (p = 0.150) (fig. 2).

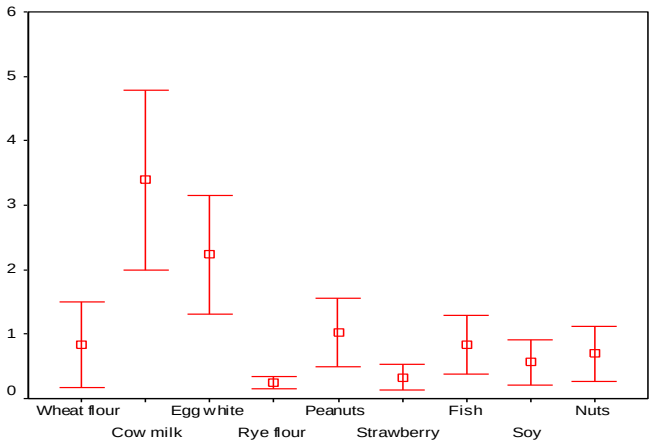

Fig. 2. The most common specific IgE allergens

In patients with low specific IgE levels but in which clinical manifestations and history were suggestive of allergy (47 cases - 35.60%), IgG4 antibodies against 20 food allergens were determined. Of these, 21 (15.9%) cases showed positive IgG4 levels against the following allergens: wheat flour (5 cases, p = 0.049), cow milk (3 cases, p = 0.072), egg white (2 cases, p = 0.061), peanuts (3 cases, p = 0.064), rye flour (2 cases, p = 0.040), strawberries (2 cases, p = 0.108), nuts (2 cases, p = 0.027), fish (1 case, p = 0.006) and soy (1 case, p = 0.087) (fig. 3). One patient presented mixed allergy to peanuts and nuts.

Of these allergens only wheat flour only (p = 0.049), rye flour (p = 0.040) and fish (p = 0.006) were statistically significant.

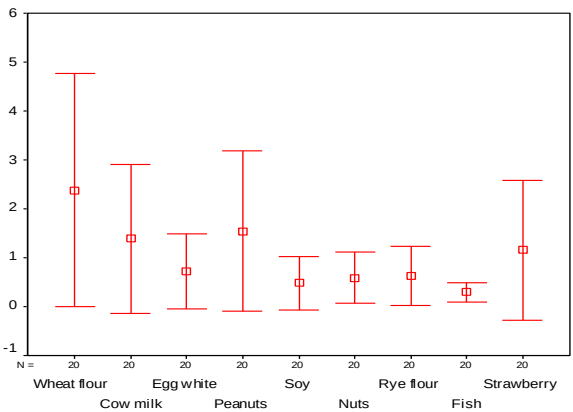

Fig. 3. The most common specific IgG4 allergens

In patients with positive IgG4 levels to different food allergens, 89% presented associated digestive and cutaneous symptoms, suggesting that the level of specific IgG4 antibodies is high in patients with gastrointestinal allergy, but has no predictive value.

Polysensitization to food and airborne allergens correlated with respiratory allergic manifestations (asthma and allergic rhinitis), occurring predominantly in the age group over 4 years, while atopic dermatitis was associated with food allergy in children under 4 years.

The reference values for specific IgG4 antibodies are listed in table I.

TABLE I
Interpretation of results for specific IgG4 antibodies

CLASS	REFERENCE VALUE	INTERPRETATION
0	<0.35 U/mL	**negative-** no IgG4 antibodies detected
1	0.35-0.7 U/mL	**borderline-** low level of IgG4 antibodies
2	0.7-3.5 U/mL	**positive-** moderate level of IgG4 antibodies
3	3.5-17.5 U/mL	**distinctly positive-** high level of antibodies
4	>17.5 U/mL	**Highly positive-** very high levels of IgG4 antibodies

DISCUSSION

Food allergy is defined as an immune response to food proteins affecting approximately 8% of children and 2% of adults (2), but it's the prevalence, as well as that of all allergic diseases is increasing.

In food allergy the mechanisms involved may be IgE or non-IgE mediated immune reactions. The immune mechanisms involved in non-IgE mediated reactions may be based on the participation of subtype 4 IgG antibodies. These types of IgG4 antibody mediated reactions are characterized by less severe and less specific symptoms, and have a late onset after the intake of the incriminated food (up to 72 hours).

There are several arguments supporting the involvement of IgG4 antibodies in non-IgE mediated food allergies: allergic reactions may occur independently of IgE specific antigen; subsequent decline of IgG antibody level when the incriminated food is removed from diet; specific IgG antibodies were identified in celiac disease, atopic eczema and in diseases associated with increased intestinal permeability (chronic inflammatory bowel disease) (3).

Besides hives and anaphylaxis triggered by IgE-mediated immune mechanisms, there are several cell-mediated diseases, such as food protein-induced eosinophilic esophagitis or enterocolitis. Non-IgE mediated food allergies that manifest with food protein-induced entercolitis syndrome or eosinophilic esophagitis are rare and have a favorable course on elimination diet (4).

Immune-mediated food allergies have to be distinguished from food intolerances caused by non-immune mechanism which are non-specific and their symptoms and those of irritable bowel syndrome often overlap (5). Allergic diseases by food sensitization are more common in the early years of life, their frequency decreasing with age, with the acquisition of tolerance

to food. Allergic manifestations with onset in the early years of life are the result of the interaction between genetic factors, environmental factors (artificial feeding, incorrect diversification, prolonged antibiotic therapy) and immaturity of the immune system in early childhood (6).

In our study group the diagnosis of food allergy was based on the criteria in international guidelines, suggestive clinical manifestations, thorough history, and positive levels of serum specific IgE and IgG4 against food allergens, positive skin tests, elimination diet, and oral loading test. The latter must be done under close medical supervision as it can cause such severe adverse reactions as anaphylaxis.

The analysis of specific IgE and IgG4 shows that 64.39% of the cases were IgE-mediated allergies and 15.9% IgG4-mediated allergies. The allergens most frequently involved were cow milk ($p = 0.000$), egg white ($p = 0.000$), peanuts ($p = 0.000$), wheat flour ($p = 0.003$) and fish ($p = 0.000$), in agreement with the data in literature (4, 7, 8). Correlation of skin test with specific IgE level showed a concordance between results. Skin tests have a sensitivity of 95% and a specificity of 50% (9). A negative skin test result has a high predictive value in children older than 1 year, while in children less than 1 year its negative predictive value is lower due to the particularities of cutaneous mast cells (small number and low reactivity). A positive skin test has a positive predictive value of only 50%. If history is not strongly suggestive of an allergy, the diagnosis should be confirmed by a challenge test (10).

With regard to egg allergy, recent studies estimate that it affects approximately 0.5-2.5% of children (11, 12), but there are studies reporting a higher percentage; thus, an Australian study suggests that 8.9% of children have allergic reactions to egg (13). In our study egg allergy is the second most common food allergy. Egg allergy is associated with a high risk of allergy to peanuts or other food allergens, atopic dermatitis, occurrence of respiratory allergy and asthma (14, 15, 16). The rate of resolution of egg allergy is slow; most studies show that tolerance to egg develops at age 3 (17), but there are studies suggesting that about half of children develop tolerance at age 12 (18). The latter study demonstrated persistent egg allergy in 42% of children in their teens, suggesting that the number of adults with egg allergy can increase with time, although current estimate of egg allergy among adults is 0.2% (19).

Factors predictive of the resolution of egg allergy are: initial reaction characteristics (isolated hives/angioedema versus other events), specific IgE level, intensity of skin prick test reaction, severity of atopic dermatitis, IgG4 level, response of interleukin 4 (IL-4). Of these, the first two are the most important predictors for resolution (11).

CONCLUSIONS

This study shows that IgE-mediated food allergies are more common, the highest percentage being recorded in children less than 4 years of age due to the interaction of genetic and environmental factors and immaturity of immune system in the early years.

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IN MEMORIAM

PROFESSOR GEORGETA DATCU, M.D., Ph.D.

(27 August 1939-10 August 2015)

The academic community of "Grigore T. Popa" University of Medicine and Pharmacy Iasi expressed by the voice of 1962 promotion its deep regret for unexpected loss of our distinguee colleague Professor Doctor Georgeta Datcu, teacher and head of the Ist Medical Clinic for more than four decades.

Originating in Bacau in a family of intellectuals, his father dr. Demostene Teja being a renowned radiologist and her mother being pharmacist, she naturally oriented toward medical career. High school graduating with honors she benefited a merit bachelor being admitted without examination and between 1956-1962 pursued the General Medical faculty courses distinguishing herself for intellectual qualities and passion of perfection in medical profession. As colleague and chief of our group she proved modesty and diplomacy, never wanting social roles, her politics being only school performance.

Due to its endowment but also to the great generation of masters we have benefited: I Iancu, I. Nițulescu, Gh. Chipail, I Enescu, Vl. Butureanu, Maria and O. Franke, Th. Economu i.e., she graduated with one of the highest accolades.

She became by competition apprentice (1960) and *house officer* (1962) carrying out her training in prestigious clinics mainly in internal medicine services.

In 1962 she sustained the licence thesis intitled *"Pseudartrosis of the rachisyntesis graft in Pott's disease in children"* conducted in Pediatric Surgery Clinic and appreciated with maximum qualifier.

In the Ist Medical Clinic she was spotted and guided by Professor Constantin Negoiță, known for the rigor of its staff selection who was her mentor and role model for entire career, dr. Georgeta Teja being the first in a series of valuable graduates, four of which becoming later heads of clinics. Therefore in 1965 she became jr assistant of this clinic where will activate for 44 years being successively promoted assistant (1968), lecturer (1983), associate professor (1990) and professor (1994). She became also registrar (1968) and consultant in internal medicine (1978) and cardiology (1983).

She led the clinic for 15 years until her retirement with professionalism, strictness and balance maintaining its excellence and reputation.

As practician outside her encyclopedic knowledge and holistic approach of the suffering, Professor Datcu had particular clinical feel destined only to "true doctors" always surprising by the finesse and accuracy of diagnosis and optimal therapeutic solution

Although her priority was competence in cardiology, like her illustrious predecessors Professors Bacaloglu, Niculescu, Tudoranu and Negoiță, Professor Datcu

demonstrated broad knowledge in other specialties of internal medicine.

She gave equal importance to teaching activity through clinical internships and demonstrations filled of attainments, dedication and patience stored in the memory of students but also of young colleagues, residents or physicians in training, all of them decisive benefiting in their education and perfection.

Her lectures have excelled by sober style without pedantry, logic and systematized, oriented to practice, all dressed in an elegant, worship language, always appropriate to the audience. Like her mentor, Professor Datcu had an impressing scientific activity not by the number but by the value of its works. Most of publications realized together with Professor Negoiță and collaborators from the clinic include clinical trials or casuistry published in specialty literature. As a tragic coincidence our last common work treating cardiothyreosis was printed quite in her dying day.

In 1984 she sustained her doctoral thesis having as theme the *"Study of capacitant vessels in systemic arterial hypertension"* headed by Professor Negoiță completing an original research spread over several years enjoying unanimous appreciations. In 1996 Professor Datcu became Ph.D. superviser in internal medicine and cardiology directing with her proverbial competence and exigency a number of theses.

Its main medical writings still remain chapters included in the treaty of Medical Clinic under the editorship of Professor Negoiță in 1995 (bronchiectasis, bronchial asthma, bronchopulmonary carcinoma) and those under editorship of her colleague and husband Professor Mihai Dan Datcu *"Clinical confrontations - commented cases"* 2003 and *"Controversies and diagnostic errors in cardiology"* 2009, all of great use and practical interest for students, residents but also specialized doctors.

She also participated actively and consistently with papers, reports or in quality of chairman at numerous scientific meetings with topics of internal medicine and cardiology, either autochthonous - some organized in Iasi – either abroad, established itself as one of the most prominent contemporary personalities of specialty Colleague and friend for almost sixty years I could appreciate the intelligence and culture not only professional of Professor Georgeta Datcu, her great character full of frankness, honesty, loyalty and altruism, all wrapped in a mantle of humor and Moldavian kindness.

Her disappearance - the first female professor of internal medicine in our country - is a heavy loss both for the Romanian medical world and for collaborators, colleagues and its friends.

God rest her soul….

Dr. Mihai Radu Diaconescu,
Professor of Surgery

LAUDATIO

PROFESSOR ALLA VÂȚĂ, M.D., Ph.D.

A CAREER OF SUCCESS

Prof. Alla Vâță, PhD is one of the most important Romanian specialists in the field of infectious diseases. A genuine and dedicated scholar, she is endowed with a rare intelligence. She has always enjoyed the respect and admiration of the whole medical community. She is admired for her excellent professional training that became obvious whenever she presented her opinions about a diagnosis or whenever she taught her undergraduate and graduate students. Her lectures captivated the audience. She is a modest lady and an exact and patient scientist. Prof. Alla Vâță led with a firm hand the Iasi Infectious Diseases Hospital. She was also a remarkable researcher in her field.

She was born in 1920 in Kishinew, where she completed her secondary studies. In the period 1940-1945 she attended the Iaşi Faculty of Medicine**.** Her professors were among others**:** Al. Țupa, C. C. Dumitriu, I. Enescu, I. Tănăsescu, D. Cornelson, all of them outstanding scientists in their field.

In 1945, after a competition she was accepted in the externship program at the Hospital of Infectious Diseases, led by prof. Maria Franche, PhD, who became Mrs Vâță"s model throughout her life. She started her academic career in 1946 at the same clinic. A brilliant career followed: junior teaching assistant, assistant lecturer, lecturer (1952), associate professor (1961), professor (1970). In 1963 she defended her PhD in medical sciences, complemented in 1972 by the highest Romanian academic degree that was awarded after defending the second doctoral dissertation. Prof. Dr. Alla Vâță has been an honorary Member of the Academy of Medical Sciences since 1996.

In 1981 she became consultant on the staff of the Hospital of Infectious Diseases. During her teaching career she supervised 16 doctoral dissertations at the Iasi ″Gr. T. Popa″ University of Medicine and Pharmacy. During this prodigious career she was driven by the desire for continuous improvement despite the isolation of Romania from the western world.

Prof. Dr. Alla Vâță enjoyed a remarkable teaching career**.** She used to teach the 6th year medical students and postgraduates, being admired and respected by all her students and colleagues.

She taught a course of lectures on infectious diseases. Moreover, she managed to create a school of highly trained specialists in infectious diseases, many of them becoming in their turn professors in this field (St. Dumitriu, E. Brauner, V. Luca), senior consultants (dr. E. Beşleagă, dr.C. Scurtu), and heads of department from of hospitals all over Romania. All of them were able to solve the most difficult problems this specialty was faced with at that time.

She was a remarkable manager, leading

IMPLICATIONS OF GHRELIN AXIS IN BREAST CANCER - REVIEW

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IMPLICATIONS OF GHRELIN AXIS IN BREAST CANCER - REVIEW (Abstract): Breast cancer is, by far, the most frequent cancer among women and many factors influence the physiological and pathological growth and development of the mammary gland. There is developing evidence that the hormone ghrelin, known for the growth hormone releasing effect and food intake modulator, could also play a role in the pathogenesis of breast cancer and may represent a new diagnostic marker and a potential therapeutic target. We performed a PubMed Database search of relevant studies and ten papers were included in our systematic review. Ghrelin axis seems to be definitely involved in the pathogenesis of breast cancer, although a precise role has not been yet established. In order to verify the precise role of ghrelin axis in breast cancer further studies with larger populations are necessary that should include the analysis of metabolic, genetic and environmental factors which are expected to influence the results. **Keywords:** GHRELIN, BREAST CANCER, NOVEL THERAPEUTIC TARGETS

Breast cancer is, by far, the most frequent cancer among women with an estimation of 1.67 million new cases diagnosed in 2012 (25% of all cancers). Also it is the most frequent cause of death in women in less developed regions, while in more developed regions it is now the second cause of death (after lung cancer), ranking as the fifth cause of death from cancer overall (522,000 deaths) (1).

There are many factors that influence physiological and pathological growth and development of the mammary gland. Endogenous hormones (sex hormones, growth hormone axis), reproductive factors (lower parity, older age at first giving birth, younger age at menarche, older age at menopause), oral contraceptives and hormone replacement therapy, family history and genetic factors (BRCA1 and BRCA2 mutations), alcohol and smoking, diet, overweight and obesity are known to be risk factors in breast cancer (2).

There is developing evidence that ghrelin, known for the growth hormone (GH) releasing effect and food intake modulator (3) could also play a role in the pathogenesis of breast cancer and may represent a new diagnostic marker and a potential therapeutic target.

THE GHRELIN AXIS

From its discovery in 1999 (3), further studies revealed that ghrelin axis is not simple, but a complex, diverse system, regulated at several stages, which determines diverse transcripts and proteins with multiple functions, many of them still likely to be discovered.

Ghrelin is a unique 28-aminoacid (AA) peptide containing an n-octanoyl group on the serine in position 3 and it is the natural ligand for the growth hormone secretagogue (GHS) receptor (GHS-R) (3, 4, 5). Circulating ghrelin consists of more than 90% of des-acyl ghrelin and less than 10% acyl ghrelin (6).

The human ghrelin gene is localized on the chromosome 3p25-26 (7). Fig. 1 summarizes the steps which lead to the production of the different ghrelin gene products. Preproghrelin mRNA is a transcription isoform of the ghrelin gene that leads to the translation of preproghrelin (117 AA) (3). Preproghrelin contains an N-terminal signal peptide (23 AA) that is cleaved from the polypeptide to form proghrelin (94 AA). Proghrelin is further processed (mainly by prohormone convertases - PC1/3) to produce the ghrelin peptide (28 AA) (8,9).

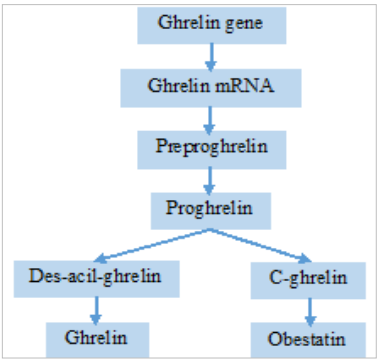

Fig. 1. Sequential steps of the production of the major ghrelin gene-derived products

The cleavage of the ghrelin molecule from proghrelin produces C-ghrelin (66 AA), which is then further cleaved to form obestatin (23 AA). Both C-ghrelin and obestatin circulate in human plasma (10).

Proghrelin can be posttranslationally modified with the addition of an octanoyl group to the serine 3 residue of ghrelin, through an acyl-bond, by the enzyme ghrelin O-acyltransferase (GOAT) (11,12). Not all circulating ghrelin is octanoylated and the majority of plasma ghrelin is actually the non-acylated form termed des-acyl ghrelin (27 AA). Acyl-protein thioesterase 1 (APT1) has been described to convert ghrelin to des-acyl ghrelin in the plasma (13).

Ghrelin can be also acylated with groups other than octanoic acid, depending on dietary modifications, such as decanoic acid resulting in a decanoylated form termed D-ghrelin (14).

The ghrelin receptor belongs to G-protein coupled receptors (GPCR) superfamily. The *GHSR* gene is located on chromosome 3q26.2 (15). There are documented two splice receptor variants, type 1a (GHSR1a) and type 1b (GHSR1b). GHSR1a is known as the functional form of the receptor that mediates many of the ghrelin effects. GHSR1b is a truncated form and has been considered as a non-functional receptor because ghrelin does not bind or activate this receptor (16).

A range of alternative splice variants arise from the ghrelin gene, many of which may encode novel peptides. Although their receptors have not yet been identified, they have already proven to be active, having intriguingly subtle but opposite physiological actions to ghrelin (17, 18, 19, 20).

PHYSIOPATHOLOGICAL ROLES OF GHRELIN WITH IMPLICATIONS IN BREAST CANCER PATHOGENESIS

Ghrelin and ghrelin-receptors are expressed in a wide range of normal and tumor tissues suggesting it has many physiological functions and pathological implications. Ghrelin can exert its effects through endocrine or autocrine / paracrine actions.

The major role of ghrelin is to stimulate GH secretion, via its binding to GHSR1a on the pituitary somatotropic cells, acting in synergy with GH-releasing hormone (GHRH), thus activating GH/IGF-1 axis with major proliferative effect on both normal and tumor cell types (21, 22). Ghrelin is also able to stimulate the pituitary secretion prolactin (PRL) and adrenocorticotropic hormone (ACTH) inducing further cortisol release (23).

Ghrelin is also a major food intake modulator stimulating appetite by central and peripheral pathways and *via* the vagus nerve. Ghrelin is secreted in a pulsatile manner increasing before the onset of meal, during fasting, and decreasing after feeding (24). The clinical application of these effects have been investigated in both eating disorders and muscle wasting conditions, including obesity, cachexia and sarcopenia, conditions that are also encountered in the tumoral genesis and evolution of breast cancer (25).

Besides these two major effects, ghrelin has a multiplicity of other physiological roles affecting energy and glucose homeostasis, gastrointestinal, cardiovascular, pulmonary systems, immune function and bone physiology (25).

Ghrelin is involved in long-term weight regulation fluctuating in a compensatory manner to body weight modifications, thus decreasing with weight gain (overfeeding, high fat diet) and conversely increasing with weight loss (food restriction, cachectic conditions like in advanced cancers) (25).

In connection with body weight and body fat weight it was shown that ghrelin and des-acyl ghrelin may inhibit aromatase expression and activity in human adipose cells. This is a very important effect because aromatase converts androgens into estrogens and its expression within adipose stromal cells is believed to be the major driver of estrogen-dependent cancers in older women (26).

Concerning glucose homeostasis (with implications in insulin/IGF-1 axis), ghrelin was shown to decrease insulin sensitivity depending on the acyl ghrelin/des-acyl ghrelin ratio as acyl ghrelin determines insulin resistance, while des-acyl ghrelin neutralizes this effect (27).

Ghrelin also controls several aspects of female reproductive system inhibiting hypothalamic gonadotropin-releasing hormone (GnRH) and of both LH and FSH secretion (28, 29, 30).

More direct effects in cancer pathogenesis were studied and showed that ghrelin is involved in a number of processes associated with cancer progression, including cell proliferation (probably *via* MAPK / ERK1/2 pathway), cell migration and invasion, the inhibition of apoptosis and ghrelin could play a role in angiogenesis and cancer-related inflammation (31).

GHRELIN AXIS AND BREAST CANCER

Although it is known that many tumors express members of the ghrelin axis, their role in cancer has not been extensively studied. Searching PubMed database on the implications of ghrelin axis in breast cancer

(excluding articles on orexigenic effect of ghrelin), ten original articles were found concerning this subject. A similar search was performed in 2012 by Stefanaki et al (32) and our systematic review is updated with the latest studies results.

The first study to show that ghrelin has a potential clinical application in breast cancer was performed by Cassoni *et al* in 2001 (33). The study demonstrated that there are specific receptors present in human breast carcinomas, in both estrogen-dependent and estrogen-independent breast cancer cell lines, which bind natural and synthetic GH secretagogues, thus proving they may have other biological activities, independent of their GH releasing effect. The same study concluded that these receptors were also having an inhibitory action on breast cancer cell proliferation *in vitro*, probably *via* activation of binding sites, different from GSHR1. Additionally, the entity of the binding was independent from the histological type of the tumor, stage, estrogen receptor status, proliferative index and whether patients where in pre- or post-menopause. However, it was positively correlated to the grade of tumor differentiation, in a way that well-differentiated carcinomas showed a higher GHS binding than moderately and poorly differentiated carcinomas (33).

The following study was performed by Jeffery *et al* in 2005 (34). The aim of this study was to investigate ghrelin expression in breast cancer tissues and cell lines and to examine the effect ghrelin has on breast cancer cells proliferation *in vitro*. According to their results, ghrelin plays a role in breast cancer by stimulating cell proliferation in both estrogen-dependent and estrogen-independent tumors. They reported that the truncated isoform GHSR1b is highly expressed in breast cancer tissue with negligible expression in normal breast specimens. Taking into account that GHSR1 is expressed in both normal and cancer tissues and cell lines, this may suggest that GHSR1b could represent a new marker for breast cancer. In their study, Jeffery *et al* also described the expression of exon 3-deleted preproghrelin (a human proghrelin isoform), which encodes mature ghrelin and a novel C-terminal peptide. This peptide was found to be highly expressed in the estrogen-independent cell lines, but also in the glandular epithelium of high-grade cancer tissue, exhibiting the highest immunoreactivity and thus indicating it may play a functional role in breast pathogenesis (34).

In 2006, Wagner *et al* (35) investigated the effect of a total 12 single-nucleotide polymorphisms and 3 dinucleotide repeats in the genes encoding *GHRH*, *GHRL* and somatostatin (*SST*) and their receptors (*GHRHR*, *GHSR*, *SSTR2*) on the risk of breast cancer. The results demonstrated that the *GHRL*, *GHRHR*, *SST* and *SSTR2* gene polymorphisms may contribute to a decreased breast cancer risk and this would be consistent with a decreased activity of the IGF-1 pathway (35).

Another study was performed by Dossus *et a*l in 2008 (36), which investigated the association of 15 genetic variants in the *GHSR* and *GHRL* genes with breast cancer risk, circulating IGF-1 and IGFBP-3 levels, and anthropometric measures. In their study, GHRL SNP rs171407 was found to be associated with an increased breast cancer risk (20%), with a greater height (+0.4%) and with a lower BMI (-1.9 %). Similarly, GHSR SNP rs572169 was also associated with increased breast cancer risk (20%) and greater height (+0.4%), but not

with BMI. Another GHRL SNP - r3755777 - was associated with higher circulating IGF-1 levels. The findings of Dossus *et al* study might reflect an effect of these polymorphisms in *GHSR* and *GHRL* genes on breast cancer through the GH axis, independently of BMI or obesity and consequently independently of the ghrelin action on appetite and food intake (36).

In the same year, Feigelson *et al* (37) conducted a case-control study among postmenopausal women to establish whether genes associated with obesity increase the risk for breast cancer. Among these genes was also *GHRL* gene and the study found no association between the gene and breast cancer risk (37), contrary to previous study of Dossus *et al* (36).

Also in 2008, Ordway *et al* (38) conducted a study on DNA methylation alterations in breast cancer that identified a single locus within the promoter region of *GHSR* gene that is hypermethylated, which is capable of distinguishing infiltrating ductal breast carcinoma from cancer-free breast tissue with a sensitivity and specificity of 90% and respectively 96% (38).

Later, in 2011, Gahete *et al* (39) showed than In1-ghrelin - a variant transcript processed from the *GHRL* gene - was expressed about 8 times higher in breast cancer samples compared to normal mammary tissue. The levels of In1-ghrelin expression was strongly correlated in breast tumors with GOAT, GHSR-1b, cyclin D3 (a proliferation marker) and Ki67, but not with native ghrelin or GHSR-1a, thus suggesting a possible relevant role in the pathology of breast cancer. Further, the In1-ghrelin over expression in estrogen-dependent breast cancer cell lines caused increased proliferation rate suggests that the blockade of In1-ghrelin variant may have some therapeutic benefit (39).

In the same year, Grönberg *et al* (40) conducted a study to evaluate the potential biological and prognostic role of ghrelin and obestatin expression in invasive breast cancer. The results showed that ghrelin and obestatin were positively correlated to ER expression and negatively to proliferation rate, tumor size and to the Nottingham histologic grade (NHG) status. Also ghrelin expression was associated with a prolonged survival, which is consistent with the negative correlation to tumor grade, size and proliferation rate. Further, the results showed that tumors expressing ghrelin had 2.5-3 times lower risk for recurrence or breast cancer-specific death than those lacking ghrelin expression, proving a potential role of ghrelin as a prognostic marker in breast cancer. Even though ghrelin and obestatin were significantly correlated to each other, obestatin did not provide any prognostic information (40).

Botla *et al* (41) performed in 2012 a study aimed to identify methylation variation that could differentiate between breast cancers and other breast tissue. They have established a signature associated with two genes - *GHSR* and *SFRP2* (associated with poor patient survival) - which exhibited significant hypermethylation in cancers suggesting an epigenetic defect. Concerning diagnosis, the *GHSR* pattern demonstrated a very high sensitivity and specificity of 89.3% and 100%, respectively. To date, this represents the highest combined sensitivity and specificity for a DNA-based biomarker in breast cancer reported (41).

CONCLUSIONS

In this systematic review we tried to identify the implications of ghrelin axis in breast cancer. The ghrelin axis seems to be

definitely involved in the pathogenesis of breast cancer, but a precise role is not yet established. Studies performed up to date are not sufficient to support that ghrelin itself should be used in current management strategies, but they show much promise concerning early detection, prognostic and therapy in breast cancer.

Further studies with larger populations are necessary that should include the analysis of factors (metabolic, genetic and environmental) which are expected to influence the results, in order to verify the precise role of ghrelin axis in breast cancer.

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NEWS

SUDDEN DEATH OF AN INFANT WITH CORONARY INVOLVEMENT DUE TO TAKAYASU ARTERITIS

Takayasu arteritis is an inflammatory disease that involves large vessels, predominantly the aorta and its main branches. This disease is much more common in females than in males, and the age of onset usually ranges between 10 and 30 years old, but it has been reported in children as young as 2 years of age. Coronary artery lesions are occasionally found in adult patients with Takayasu arteritis and rarely in younger children. Myocardial infarction or heart failure due to coronary involvement can be one of the potentially fatal complications in this disease. Usually, the involved arteries, aorta and its main branches, were thickened and stenotic. In the reported case, the ascending aorta was thickened and showed ostial stenosis in the coronary arteries bilaterally. The proximal segment of the left and right coronary arteries showed approximately 60% and over 90% occlusion, respectively. Microscopically, the intima was thickened due to an increase of intimal cells and fibers, and the adventitia showed thickening with fibrosis. In addition, remarkable infiltration of inflammatory cells, including multinuclear giant cells phagocytosing fragmented elastic fibers, and destruction of elastic fibers were observed in the media. However, Takayasu arteritis has not been recognized as a cause of sudden coronary death in infants. This report presents a case of sudden infant death due to severe stenosis of the left and right coronary arteries caused by Takayasu arteritis, which had not been diagnosed before death. In conclusion, Takayasu arteritis can be a life-threatening condition even in infants. There may be other similar cases that were unrecognized at autopsy in children with sudden unexpected death that are needed to be diagnosed (Wang EL, Sato Y, Kitamura O, et al. Sudden death of an infant with coronary involvement due to Takayasu arteritis. Cardiovascular Pathology 2013, 22: 109–111).

Doina Butcovan

CURRENT CONCEPTS IN MINIMAL HEPATIC ENCEPHALOPATHY

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CURRENT CONCEPTS IN MINIMAL HEPATIC ENCEPHALOPATHY (Abstract): Minimal hepatic encephalopathy (MHE) defines the presence of neurocognitive impairments in patients with cirrhosis or portal-systemic shunting that show a normal neurologic and psychiatric status on clinical examination. Although ammonia has the central role in MHE pathogenesis, factors such as infection, oxidative stress, manganese or intestinal bacterial overgrowth contribute to the development of the neurocognitive deficits associated with this disease. Many methods have proven useful in identifying MHE but because of the major drawbacks (standardization requirements, high price, sophisticated equipment, and limited access) a gold-standard test is still missing. Although beneficial, the treatment of MHE is not routinely recommended and should be taken into consideration in patients at risk for accidents and in those with cognitive complaints or decline in work performance. **Keywords**: MINIMAL HEPATIC ENCEPHALOPATHY, LIVER CIRRHOSIS

Hepatic encephalopathy (HE) is a brain dysfunction caused by liver failure and/or portosystemic shunts (PSS). The clinical picture of HE includes neuropsychiatric abnormalities, which may vary from subclinical manifestations to coma (1). Factors such as multifactorial etiology, polymorphism of clinical manifestations, oscillating evolution, and the wide variety of precipitating factors constituted prerequisites for developing a guideline for classification, diagnosis and treatment of this pathology. The American Association for the Study of Liver Diseases and the European Association for the Study of the Liver established a working group that put forward guidelines regarding the nomenclature used for defining and systematizing HE based on clinical manifestations (fig 1).

The current standardization individualizes Minimal Hepatic Encephalopathy (MHE) as a separate entity together with the old West Heaven criteria. According to the nomenclature used by the International Society for the Study of Hepatic Encephalopathy and Nitrogen Metabolism (ISHEN), MHE and grade I HE are included into covert HE (1, 2). MHE is defined by neurologic and psychiatric status within normal limits during clinical examination, but with neurocognitive deficits detectable by psychometric or neuropsychological testing (3). Neurocognitive abnormalities include impaired attention, deficits in fine motor skills, disorders of the visual-spatial perception and altered executive functions (4, 5).

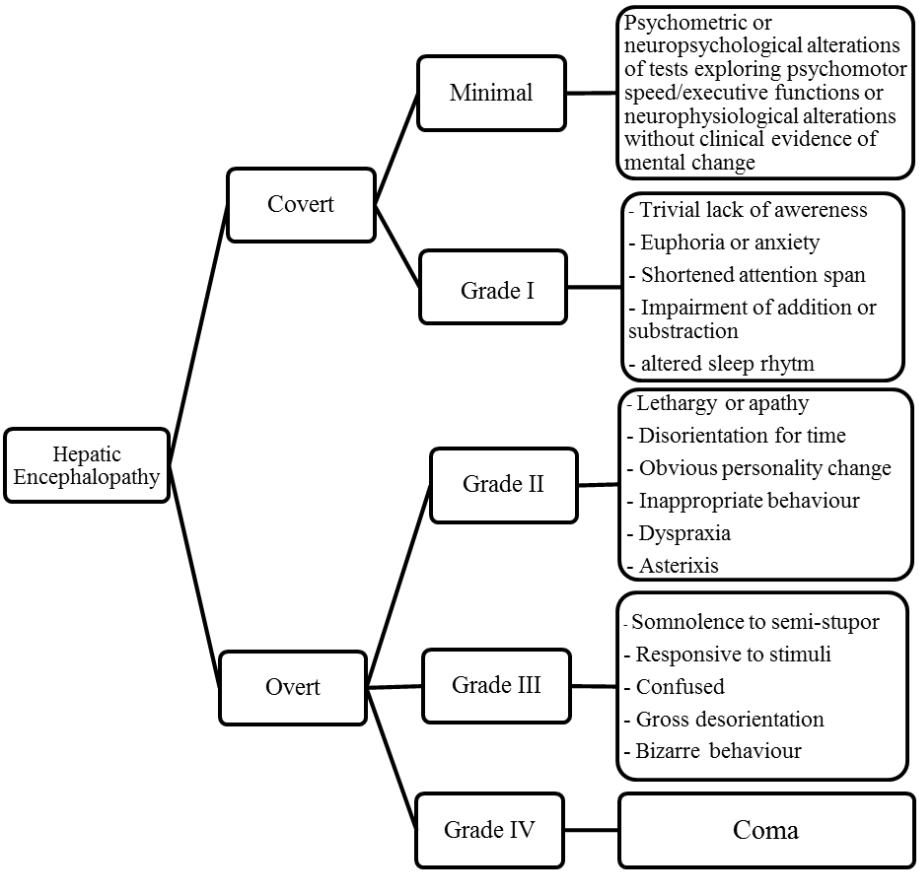

Fig. 1. Classification of Hepatic Encephalopathy adapted from Vilstrup et al. - Practice Guideline by The European Association for the Study of the Liver and The American Association for the Study of Liver Diseases (1)

PATHOGENIC THEORIES

The mechanisms involved in MHE pathogenesis are not fully understood, but even in 2014 ammonia continues to play the central role. Recent studies have pointed to the importance of additional factors, such as inflammation, oxidative stress and intestinal bacterial overgrowth, in generating neuropsychiatric abnormalities in this category of patients (6).

Ammonia has a decisive role in the pathogenesis of MHE. Physiologically, ammonia is converted to urea in the liver. This metabolite is water-soluble and it is excreted from the body by the kidneys through urine. In patients with chronic liver diseases or portosystemic shunts, this metabolic pathway is compromised and ammonia degradation occurs primarily in muscle cells, and in the cells of the central nervous system. The extrahepatic catabolic process makes use of the amino-glutamic acid to transform ammonia into glutamine (7, 8). MHE is a form of gliopathy caused by type II astrocytes, the only cells at brain level containing glutamine-synthetase that can

metabolize ammonia. The intracellular glutamine concentration within astrocytes increases proportionally with the level of ammonia in the blood. An active osmolyte, glutamine draws water inside the atrocytes, determining their increase in volume, while causing the secondary development of brain edema and intracranial hypertension (9, 10). This theory is supported in a study by Tanigami et al. performed on animal models, which revealed the lack of astrocyte volume expansion following the administration of methionine-sulfoximine (glutamine-synthetase inhibitor) (11).

Secondary to prolonged exposure to high ammonia concentrations, astrocytes undergo morphological and functional changes. During this stage, brain cells undergo a transformation process turning into type II Alzheimer atrocytes, characterized by large nuclei, prominent nucleoli and chromatin marginal arrangement (12). In order to compensate for the increase in volume, the astrocytes release osmotic-active molecules, such as myo-inositol and taurine (13, 14). According to a study by Shawcross et al. (15), the low levels of intracellular myo-inositol are associated with increased risk of neurological impairment. Glutamate transporters situated along the astrocyte membrane are inactivated and the activity of post-synaptic receptors for glutamate is inhibited. These functional transformations determine alterations of the neuronal function, reducing the conduction of nervous impulses (16, 17). Ammonia alters the function of the hematoencephalic membrane and determines the decrease of small and medium chain amino-acids and an increase in the inflow of neutral long-chain and aromatic amino-acids. Secondarily, the intracerebral synthesis of catecholamines (serotonin and dopamine) is reduced, with inhibitory effects on neurotransmission (18, 19).

Infection and inflammation. Although ammonia is an important factor in MHE pathogenesis, 10% of HE patients have normal levels of ammonia in the blood (20). Except for ammonia, there are other neurotoxic compounds derived from the bacterial metabolism showing high serum levels in patients with chronic liver diseases: phenols and mercaptans. Produced by bacteria in the bowel, these substances are not catabolized in the liver due to the impaired liver function and they are transported to the brain, where they exert their neurotoxic effect (21). A recent study by Bajaj et al. emphasizes the important role of acute bacterial infections and associated inflammation in the development of neuropsychiatric anomalies in patients with HE. The authors identified imbalances in the intestinal microbiome of cirrhotic patients. The presence of certain bacterial families (Alcaligeneceae, Porphyromonadaceae, Enterobacteriaceae) in the stool of patients with overt hepatic encephalopathy (OHE) was associated with systemic inflammation and the presence of cognitive deficits (22). Moreover, inflammation and inflammatory mediators enhance the negative effects that ammonia has on the central nervous system (23). Studies on astrocytes by Alvarez et al. and Rama Rao et al. demonstrated an increase in mitochondrial permeability under the influence of ammonia, TNF-α, IL-6, IL-1β and IFN-γ. High levels of IL-6 and TNF-α proinflammatory cytokines were found in the blood of patients with MHE (24, 25).

Oxidative stress. Molecular studies on rats revealed a rise in the synthesis of reactive oxygen species (ROS) and reactive nitrogen species (RNS) by the astrocytes

following exposure to ammonia, pro-inflammatory cytokines or benzodiazepines (26, 27, 28). On the other hand, Reinehr et al. revealed an increase in astrocyte volume when exposed to ROS and RNS (29). According to the hypothesis of Albrecht and Norenberg, glutamine determines an enhancement of the oxidation processes in the astrocytes through intra-mitochondrial release of ammonia. It later favors the creation of ROS and RNS through calcium-dependent reactions. Moreover, ROS mediates tyrosine nitration reactions. Tyrosine nitrate alters the permeability of the hematoencephalic membrane, favoring the expansion of the astrocyte volume and cerebral edema (30).

Manganese is a neurotoxin that accumulates in the central nervous system (CNS) preferentially in the basal ganglia and may be pointed out by magnetic resonance imaging (MRI) (31). In a MRI study on cirrhotic patients with portacaval anastomoses or transjugular intrahepatic portosystemic shunt (TIPS), Spahr et al. revealed a hypersignal at basal ganglia level in 88% of the patients. Additionally, 89% of the patients presented extrapyramidal symptoms and 67% of patients had increased levels of manganese in the blood. These findings are reversible after the normalization of liver function (32,33). Manganese homeostasis alterations may contribute to the development of cognitive deficits present in the clinical picture of patients with liver cirrhosis (31, 32, 33).

Neuromodulators. Ammonia and manganese determine increase in the density of peripheral type benzodiazepine receptors (PBR) in the brain. These receptors regulate the synthesis of neurosteroid hormones like tetrahydro-progesterone (THP) or tetra-hydroxycorticosterorne (THDOC) by the astrocytes and are present in high numbers in the CNS of patients with MHE. THP and THDOC potentiate the activity of gamma-aminobutyric acid (GABA) receptors, with inhibitory effect on the nerve conduction in the CNS (34, 35). This theory might explain the impairment of fine motor skills and coordination or the psychomotor disorders seen in patients with MHE (36, 37).

*Intestinal bacterial overgrowth (IBO)***.** A study on patients with MHE by Liu et al. revealed changes in the intestinal microbiome with an increase in the number of urease producing bacteria. Therapeutic intervention (synbiotic treatment) was associated with improvement of bowel flora imbalances, as well as better psychometric tests results. (38). In another study, Gupta et al. revealed a high prevalence of IBO in patients with MHE (38.6%) (39).

CLINICAL AND SOCIOECONOMIC IMPLICATIONS

MHE has a negative effect on the quality of life and determines alterations of the psychomotor skills and the ability to process information (40, 41). Prasad et al. revealed the impairment of daily activities such as social interaction, emotional behavior, reading, alertness and recreation periods in patients with MHE (42). Moreover, sleep disorders constitute a frequently met feature associated with chronic daytime fatigue and circadian rhythm disorders (43, 44). Weissenborn et al. outlined the negative impact that MHE exerts over short-term memory. Memory disorders have also been associated with attention and visual perception deficits (45).

Particular aspects of cognitive deficits in MHE determine differences in the ability to work in various professions. Cirrhotic patients involved in professions that require

constant vigilance and coordination (e.g. operators of industrial machinery or drivers) are more severely affected by MHE compared with the patients dealing with predominantly intellectual or verbal tasks (e.g. administrative or executive functions) (46). MHE may adversely impact the socioeconomic status of patients by reducing workplace performance. Approximately half the patients diagnosed with MHE do not have a job (47). The decrease in workplace performance and the diminution or the absence of a salary involves substantial moral and material losses for the individual (46). MHE affects both the individual and the society, generating high costs through repeated hospitalizations and treatment, in the evolution towards OHE development (48). Besides affecting the quality of life, MHE patients have an increased risk of progression to OHE. The patients constitute a potential danger both to themselves and to the wider community (49). Attention deficits, memory alterations and persistent fatigue associated with MHE contribute to impairment of driving abilities and an increased number of driving errors (exceeding the permissible speed, illegal turns, or accidents) (50, 51, 52).

Although MHE represents the initial stage of the neurocognitive impairment spectrum associated to cirrhosis, some studies correlate it with increased mortality (53). In a study by Dhiman et al., MHE patients showed faster progress towards grade I HE or OHE, and an increased rate of progression to liver failure. In addition, the study showed higher mortality rates in patients with MHE as compared to those without MHE (39.1% vs. 22.9%). (54)

DIAGNOSIS

MHE diagnosis requires the use of neuropsychological and/or neurophysiological tests in order to document neurocognitive alterations occurring in patients with chronic liver diseases or PSS. Standard clinical examination does not reveal any neurological anomalies or mental disorders. In what follows, we will point out MHE diagnosis criteria adapted from Simon-Talero et al.: diagnosis of a disease that can cause MHE - cirrhosis, PSS or portal vein thrombosis; normal neuropsychiatric status on clinical examination; absence of grade I HE or OHE; documentation of neurological alterations by formal neuropsychological assessment, neuropsychological tests, computerized tests or neurophysiological tests; the exclusion of other factors or diseases that can cause neurological impairment - metabolic, cerebrovascular, neurodegenerative diseases, alcoholism, vision impairment etc. (55).

The tests used for MHE diagnosis assess multiple cognitive areas including: working memory, inhibitory control, psychomotor speed, reaction speed and visual-motor coordination. We list below the techniques used for MHE diagnosis adapted from Dhiman et al.: Neuropsychological assessment using series of tests with proven clinical applicability, although time-consuming. Computerized tests (Critical Flicker Frequency, Inhibitory Control Test, Continuous Reaction Times) are rapid, easily applicable tests but with limited data regarding diagnostic significance and requiring standardization prior to use. Neurophysiological tests (Electroencephalography, Spectral Electroencephalography, Evoked Potentials P300) are objective, may be repeated but require sophisticated equipment in order to be applied. Short neuropsychological tests (PHES) have high sensitivity, demonstrated clinical applica-

bility with quick results but also have limited access and requirements of standardization prior to use. CDR computerized assessment battery are sensitive with rapid results but the limited access, high price and the need for training courses prior to use constitute major drawbacks for widespread usage (56). ISHEN recommends psychometric tests such as PHES, as a first-line option for the diagnosis of MHE. In the absence of prior PHES standardization, previously studied alternative tests will be used, for which normative data is available (57).

At present there is no universal test for the diagnosis of MHE, and there are controversies in the literature regarding the benefits resulting from the treatment of this pathology. Screening all cirrhotic patients for MHE is not recommended. The neurocognitive assessment should be individualized and taken into consideration especially in patients with an increased risk of accidents (labor or machine) and in those with cognitive complaints or associating alteration of the work capacity (55).

TREATMENT

Given the central role ammonia has in MHE pathogenesis, literature studies focused on investigating pharmacological agents that decrease ammonia levels. The therapies are similar to those used for the treatment of OHE: non-absorbable disaccharides (lactulose), antibiotics (rifaximin), probiotics/synbiotics and branched aminoacids (58). In clinical setting, non-absorbable disaccharides are most commonly used for the treatment of MHE (58). However, a meta-analysis of randomized trials suggests that antibiotics such as rifaximin may prove more useful (59). Recent studies have shown the benefits of lactulose and rifaximin in improving the cognitive function and the quality of life in cirrhotic patients with MHE. (60, 61, 62, 63) Given their modulating properties of the intestinal microbiome, probiotics, synbiotics and yoghurts may stand as effective, reliable and long-term solutions for the treatment of MHE. Studies to date are inconclusive because they were conducted over short periods of time and on a relatively small number of patients (64, 65).

MHE medical treatment should be individualized and requires a dynamic evaluation following objective indicators for an accurate assessment of the therapy response. Treatment is mainly targeted at patients with cognitive complaints, those associating alteration of the work capacity and drivers. (55)

CONCLUSIONS

MHE is the initial stage of the spectrum of neurocognitive impairment associated with liver cirrhosis. Despite the term "minimal", this entity is associated with reduced quality of life, socioeconomic status deterioration, reduced driving ability and faster progression to OHE.

Ammonia has a central role in MHE pathogenesis. Complementary factors such as inflammation, oxidative stress, manganese and IBO require further studies in order to determine the exact extent of their involvement in the development of MHE. Although not routinely recommended, early diagnosis and treatment in patients with MHE leads to an improvement of the cognitive deficits and increased quality of life.

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EDITORIAL

THE LINK BETWEEN DIET AND CANCER

Cancer has gradually become one of the most important pathologies associated with both significant physical or psychological suffering and important financial costs that put a strain on economy. Identifying cancer risk factors is a constant goal of many epidemiological studies and recent data indicates that lifestyle is a major contributor to the general increase in cancer incidence. Currently, diet, obesity and the lack of physical exercise are believed to be responsible for at least 35% of all human cancers. In under-developed countries, a diet that lacks certain nutrients and vitamins, together with improper conditions for food storage and other discrepancies between food requirements and daily intake are the main mechanisms responsible for diet-related cancer. In developed countries, high-fat, sugary diet, food additives and obesity are believed to increase cancer risk (1, 2).

The relationship between diet and cancer risk is complex. Diet can (1) represent a source of procarcinogens or carcinogens, (2) increase carcinogen formation through transforming nitrates and nitrites (abundantly found in some types of food and/or in water) in nitrosamines, (3) modulate carcinogenic effects or (4) modify gene expression through epigenetic regulative processes.

Thermal food processing can result in carcinogenic byproducts. For instance, frying or smoking meat can lead to the formation of polycyclic aromatic hydrocarbons such as benzopyrene. Smoked meat and smoked fish consumption have been linked to gastric cancer in countries with high intake, such as Japan. Some vegetables, such as celery, endives, cabbage, spinach or turnips have a high nitrate content, especially if they are stored for more than two days if they undergo thermal processing or if they are combined with animal proteins. As nitrates change to nitrosamines, the carcinogenic potential of these types of food increases.

Most of the processes through which food is conserved can increase the risk of cancer. Both salt and salting as a conservation technique have been linked to gastric cancer. Some additives or food preservers, currently used for adding color, taste, aroma or consistency have mutagenic potential, which is why some have been withdrawn from the food processing industry. Nitrates, sometimes used as food preservers for meat, are transformed in the gastrointestinal tract into nitrites if a *Helicobacter Pylori* infection is present. Nitrites then combine with secondary amines to form nitrosamines, which is why any type of food that contains nitrates should be consumed in moderation. Also, a high saccharine intake has been linked to bladder cancer in mice. However, currently there is no evidence to link saccharine consumption and cancer risk in humans.

Diet can also act as a protection against cancer. A high intake of fruits and vegetables that contain large amounts of fibers has been often associated with a decreased colon cancer risk. Similar associations have been noted for gastric cancer and for other epithelial malignancies such as lung, phar-

ynx, larynx, esophageal and breast cancer. Consuming large amounts of food that contain phytoestrogens (selective estrogen modulators) may contribute to primary and secondary prophylaxis of endocrine-dependent cancers.

Among xenobiotics, the following associations are currently considered scientifically valid:

- a diet rich in saturated fats is associated with an increase in colon, breast, prostate and endometrial cancer;
- deep-fried meat contains polycyclic amines, linked with gastric, colon, rectal, pancreatic and breast cancers;
- diets which exceed the daily recommended caloric intake increase the risk of biliary, breast, endometrial, colon and prostate cancer;
- an excess of animal proteins, particularly from red meat, are associated with breast, colon and endometrial cancer risk;
- excessive alcohol consumption is linked with oral, pharynx, larynx, liver and esophageal cancers, especially in combination with smoking;
- a high-salt diet and smoked meat consumption is associated with esophageal and gastric cancer;
- food preservers and food additives are associated with intestinal cancer (3, 4).

Red meat intake

As previously mentioned, both red meat (beef, pork, lamb, horse) and processed meat (salami, pastrami, ham, or other types of meat preserved by means of salting, smoking, deep frying or adding food preservers) increase the risk for pancreatic, colon, rectal, breast, kidney and prostate cancer. Eating red meat more than one time per week increases colon and rectal cancer risk by approximately 40%, whereas each additional 50g/day of processed meat increases cancer risk by 20%.

One of the most popular theories that explains the relationship between red meat intake and cancer risk is that red meat has a high hemoglobin content which irritates the colon and rectal mucosa and leads to an electrophilic aggression that initiates carcinogenesis. One other mechanism is the transformation of red meat amino acids in aggressive intermediate compounds that react with nitrates and nitrites in the bowel, a chemical reaction catalyzed by the hem from hemoglobin. The recommended amount of red meat intake is 500 g/week (a maximum of 5 meat servings/week). Also, current healthy eating guidelines advice against eating meat prepared by means of deep frying.

Vegetable products

Most types of food believed to protect against cancer have vegetable origin. Starch-free vegetables protect against upper gastro-intestinal cancers: oral cancers, pharynx, larynx, epidermoid esophageal cancers, gastric adenocarcinomas, colon and rectal cancers, pancreatic, breast and bladder cancer. A high fruit intake protects against oral and pharynx cancers, esophageal, lung, colon and rectal cancers. There is some evidence suggesting that garlic intake protects against colon and rectal cancers and a high tomato intake protects against prostate cancer (most likely due to the high amount of lycopene found in tomatoes).

The protective effect against cancer found for both fruits and vegetables is most likely due to a high content of vegetable

fibers, specific vitamins and certain bioactive compounds such as carotene (found in "red vegetables"), folates (found in "green vegetables"), glycosylates (brassica) and alkyl sulfates (garlic).

Current guidelines recommend a daily vegetable intake of 600-800 g/day and eating at least two fruits to decrease breast, colon, rectal and pancreatic cancer risk. Of these, at least 400 g/day (preferably divided into five portions) should consist in starch-free vegetables, fruits or unprocessed seeds (6). Some additional information regarding the choice of fruits and vegetables:

- the highest number of antioxidants can be found in strawberries, raspberries, blackberries and wild berries ("small red fruits");
- vitamins C, E and A, together with Q coenzyme, lycopene, melatonin, quercetin, Selenium and Zinc represent natural antioxidants;
- vitamin C has a strong antioxidant and anticancer effect and can be found in several types of fruits such as: blackberries, cranberries, broccoli or red cabbage. Because most of the vitamins are photosensitive, fruits should be stored in a dark dry place;
- vitamin A is found as β-carotene in: carrots, spinach, broccoli and green cabbage. Heavy smokers (more than one pack of cigarettes/day) should avoid vitamin A supplements because they have been linked with an increase in lung cancer incidence for this population group;
- vitamin E can be found together with unsaturated fatty acids in eggs, meat, fish, cereals, seeds, nuts and soy. Some studies have linked a high vitamin E intake with a protective effect against prostate cancer;
- starch-free vegetables have a low caloric count and a diet rich in these vegetables also protects against gain weight.

Other types of food and cancer risk

Phosphoric calcium may reduce colon cancer risk through its ability to bind biliary acids and free fatty acids, thus blocking their effects on the gastro-intestinal tube.

A high milk intake could increase prostate cancer risk through increasing blood Calcium levels and insulin-growth factor 1 (IGF-1) (5, 6). High Calcium levels inhibit 1,25-dihydroxyvitamin D formation, which could in turn stimulate prostate cell proliferation.

Omega-3 unsaturated fatty acids that are found in high concentrations in fish oil protect against several types of cancer and exhibit cardiovascular protective effects. However, there is one study that linked a high fatty acid intake to prostate cancer.

Obesity

After reviewing all epidemiological studies performed in the past thirty years, the *International Agency for Research on Cancer* (IARC) has recently determined that obesity is an important risk factor for several types of cancer. Obesity is responsible for the death of one in seven males and one in five females in the United States and accounts for 4% of male cancers and 7% of female cancers in the European Union. The link between cancer and obesity is quite complex (1, 3). Each five-kilogram weight gain is associated with a 1.08 increase in breast cancer in menopausal women. Each one-unit increase in the Body Mass Index increases breast cancer risk by 3%. In the United States, obesity is responsible for 20% of all breast cancers in men-

opausal women and 50% of all breast cancer-related deaths in this population group.

Being overweight is one of the most important risk factors for several types of cancers such as: colon cancer, breast cancer (in menopausal women), endometrium cancer, esophageal adeno-carcinoma, pancreatic and kidney cancer and, to a certain extent, biliary cancer (4).

Some more information regarding obesity-related cancers:

a) endometrial cancer risk in women with a more than twenty-kilogram weight gain after the age of eighteen is five times higher than in the normal population;
b) colon cancer incidence is significantly higher in both men and women that are obese;
c) obesity increases likelihood of kidney cancer especially in women, although the exact mechanism is not yet understood;
d) esophageal cancer: obese individuals have a higher incidence of gastro-esophageal reflux disease (GERD), which is associated with Barret's esophagus, a precursor metaplastic lesion. People with Barret's esophagus have a higher risk for esophageal adenocarcinoma. However, some studies indicate that the link between esophageal cancer and obesity is independent of GERD;
e) other cancers for which the link with obesity is not yet fully certified: pancreatic cancer (risk is increased 2x for obese individuals), hepatic caner, cancer of the cardia (most likely due to Barret metaplasia), ovarian and cervical cancer (limited data) and lymphoma. Additional epidemiological studies are required to firmly link these cancers to obesity.

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METABOLIC SYNDROME A WIDESPREAD THREATENING CONDITION; RISK FACTORS, DIAGNOSTIC CRITERIA, THERAPEUTIC OPTIONS, PREVENTION AND CONTROVERSIES: AN OVERVIEW

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METABOLIC SYNDROME A WIDESPREAD THREATENING CONDITION; RISK FACTORS, DIAGNOSTIC CRITERIA, THERAPEUTIC OPTIONS, PREVENTION AND CONTROVERSIES: AN OVERVIEW (Abstract): Metabolic syndrome diagnostic criteria include at least three of five of the following medical conditions: abdominal obesity, high blood pressure, abnormal high fasting plasma glucose, elevated serum triglycerides and low HDL (high – density cholesterol) level. Sedentary, overweight and obesity are characteristically associated with the syndrome, nevertheless there are new studies which indicate that chronic stress, through deregulation of the hypothalamic-pituitary-adrenal (HPA) axis is also involved in the development of the syndrome. Metabolic syndrome represents a significant risk for the development of the cardiovascular diseases. New studies in the USA have estimated its prevalence at approximately 34 % of the adult population and its prevalence is increasing with age. Diagnosis guidelines are establishing the metabolic syndrome diagnostic when are met three of the following five conditions: fasting glucose ≥ 100 mg/dL or type 2 diabetes receiving drug therapy for hyperglycemia; high blood pressure ≥ 130/85 mm Hg or high blood pressure receiving drug therapy; triglycerides ≥ 150 mg/dL or treatment for plasma hyper-triglyceride value; HDL-C < 40 mg/dL in men and < 50 mg/dL in women or if under therapy for reduced HDL-C levels and waist circumference of ≥ 102 cm in men and ≥ 88 cm in women. Besides important change of lifestyle, often drug therapy is needed (diuretics and ACE inhibitors, cholesterol drugs and weight loss medications). The value of physical activity and diet in prevention and treatment of the syndrome is supported by numerous studies. Association of metabolic syndrome and cardiovascular risk is still a matter of controversy, in what concerns the lack of significant cardiovascular risk in the case of obesity itself without metabolic syndrome. **Keywords**: METABOLIC SYNDROME, OBESITY, HPA (HYPOTHALAMIC-PITUITARY-ADRENAL) AXIS, HDL CHOLESTEROL, LDL CHOLESTEROL

Metabolic syndrome is a disorder affecting the energy use and storage. Criteria needed for diagnosis are including at least five of the following medical conditions: abdominal obesity, high blood pressure, abnormal high fasting plasma glucose, elevated serum triglycerides and low HDL (high density cholesterol) level. Metabolic syndrome represents a significant risk for the development of cardiovascular diseases and diabetes. Different studies have estimated the prevalence of the syndrome in the USA at approximately 34 % of the adult population (1, 2).

Metabolic syndrome is also known as: insulin resistance syndrome, syndrome x, metabolic syndrome x, cardio metabolic syndrome. The term of metabolic syndrome was first used in the late 1950 s and became commonly used at the end of 1970 s, first to describe association of risk factors for diabetes (3). In 1947 it was first noticed that the upper body obesity was predisposing to diabetes, atherosclerosis and gout. In 1977 was used the term of metabolic syndrome to describe the association between obesity, diabetes mellitus, hyper-lipoproteinemia, hyperuricemia and hepatic steatosis and thus to contour the risk factors for atherosclerosis (2, 4).

RISK FACTORS

New researches are indicating that chronic stress is involved in the development of the metabolic syndrome, by disruption of the hormonal balance of the hypothalamic-pituitary-adrenal axis (HPA-axis). Disruption of HPA-axis induces increased circulatory cortisol levels with consequently high glucose and insulin levels, finally leading to insulin-triggered effects on adipose tissue and resulting into visceral adiposity, insulin resistance, dyslipidemia, high blood pressure and effects on bone metabolism, with the development of "low turnover" osteoporosis. Disruption in the functioning of HPA-axis is explaining the involvement of the abdominal obesity in the development of the cardiovascular disease, type 2 -diabetes and stroke (5).

Overweight and obesity is the main characteristic of the syndrome, reflected in the strong link between its prevalence and waist circumference, associated with increasing adipose mass. Nevertheless, there are cases which show normal weight but do have the syndrome (6). Sedentary behavior is one of the main risk factors for cardiovascular diseases and mortality. Most of the metabolic syndrome's features are associated with sedentarism: increased abdominal adipose tissue, low HDL cholesterol level, high blood pressure, high glucose levels (7).

Metabolic syndrome affects 44 % of the US population over 50 years of age. The syndrome is more frequent in female sex and its prevalence is increasing with age, all over the world. Metabolic syndrome is increasing five folds the risk of type 2 diabetes mellitus, which is seen like a complication of the syndrome. It is believed that pre diabetes and metabolic syndrome are representing in fact the same disorder, but defined by different sets of biological markers. Metabolic syndrome is associated with a higher risk for cardiovascular disease, than the cases with type 2 diabetes mellitus without associated metabolic syndrome. Hypoadiponectinemia is increasing insulin resistance and it represents also a risk factor for the development of the metabolic syndrome.

Patients with schizophrenia and other psychiatric diseases are at high risk for metabolic syndrome, a risk which is increased in such cases by sedentary behavior, noxious dietary habits and antipsychotic drug – induced adverse effects. 32 to 51 % of individuals with psychiatric disorders have metabolic syndrome, with a higher prevalence in women than in men (2, 8). Common triggers, such as excessive sugar intake and generally overabundant food environment are incriminated in the concomitant development of multiple metabolic abnormalities at the same time, pointing once more the dysfunction of energy use and storage paths in the metabolic syndrome (8, 9, 10).

Markers of systemic inflammation, such as C-reactive protein, fibrinogen, interleukin 6, tumor necrosis factor alpha (TNF-α) are increased in the metabolic syndrome. TNF-α is causing the production of inflammatory cytokines and is inducing cell signaling through interaction with TNF-α receptor, which may lead to insulin resistance. Increase of adipose tissue is increasing the number of immune cells inside it, cells which are involved in the chronic inflammatory processes; chronic inflammation being a risk factor for the development of arterial hypertension, atherosclerosis and diabetes (11).

DIAGNOSTIC CRITERIA

Main symptom of metabolic syndrome is the central obesity (also-known as visceral, male-pattern or apple-shaped adiposity) – an overweight with accumulation of adipose tissue mainly around the waist and trunk (6). Signs of metabolic syndrome include: high blood pressure, decreased fasting serum HDL cholesterol, elevated fasting serum triglyceride level (VLDL triglyceride), impaired fasting-glucose level and insulin resistance. Metabolic syndrome is usually associated with hyperuricemia, hepatic steatosis, polycystic ovarian syndrome in women and erectile dysfunction in men, and acanthosis nigricans, xanthomas or xanthelasmas, in patients with severe dyslipidemia (12).

The diagnosis guidelines from National Heart, Lung and Blood Institute (NHLBI) and the American Heart Association (AHA) are stipulating that the diagnosis of metabolic syndrome is established, when are met three of the following five conditions (13):

- fasting glucose ≥ 100 mg/dL or type 2 diabetes which is receiving drug therapy for hyperglycemia;
- blood pressure ≥ 130/85 mmHg or high blood pressure which is under drug therapy;
- triglycerides ≥ 150 mg/dL or if treated for hypertriglyceridemia;
- HDL-cholesterol < 40 mg/dL in men and < 50 mg/dL in women, or if under therapy for reduced HDL-cholesterol;
- waist circumference ≥ 102 cm in men, or ≥ 88 cm in women.

Complaints of chest pain, dyspnea or claudication are frequent symptoms of potential complications. Obstructive sleep apnea seems to be a novel risk factor for metabolic syndrome (14).

A matter of controversy is represented by the fact that adipose tissue and resulted obesity are generally explaining most of the cardiovascular risk. Nevertheless, obesity itself, without metabolic syndrome is not associated with a significant cardiovascular risk, while metabolic syndrome without obesity is associated with a significant risk of cardiovascular disease and diabetes.

Generalized lipodistrophy characterized by an almost complete absence of adipose tissue develops signs of metabolic syndrome in the absence of adipose tissue, resulting in type 2 diabetes. Type 2 diabetes is cured if in transgenic mice with lipodistrophy, adipose tissue is transplanted (15, 16).

THERAPEUTIC OPTIONS AND PREVENTION

Change of lifestyle is the first choice treatment of the metabolic syndrome. If the efforts of reversing the risk factors are not giving expected results in 3 to 6 months, then drug therapy is needed. Diuretics and ACE inhibitors are used to treat arterial hypertension. Cholesterol drugs are used to

reduce LDL cholesterol and triglycerides elevated levels and to increase HDL low levels. Sometimes are used weight loss medications, which may result in weight loss.

As obesity is generally considered the main feature of the syndrome which is leading to the development of the other symptoms, weight loss as well as lifestyle changes, including diet and physical activity, may reduce the need for medications. Many studies are supporting the value of physical activity and diet in the therapy of the metabolic syndrome. Restriction of the total dietary carbohydrate intake shows most efficiency in decreasing common manifestations of the metabolic syndrome. Unfortunately, high-dose statins therapy has been resulted in rapid progression to diabetes (17).

Different strategies are used to prevent the development of the metabolic syndrome, like increased physical activity (for example walking 30 minutes daily) and low caloric diet. International Obesity Task force is supporting the idea of a need for sociopolitical measures to decrease the rate of metabolic syndrome development in population. Caerphilly Heart Disease Study which followed 2375 male subjects over 20 years suggested that daily intake of approximately 568 ml of milk or correspondent dairy derivatives reduced with more than 50 % the risk of metabolic syndrome. There are studies which support these findings, while others are questioning them (7, 18, 19).

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NEWS

ONCOPLASTIC MAMMOPLASTY ASSOCIATED WITH POSTMASTECTOMY RADIATION THERAPY: THE PLAN FOR REDUCING RECONSTRUCTIVE COMPLICATIONS

It is widely accepted that breast cancer patients who require radiation therapy after mastectomy and immediate reconstruction treatment, suffer high complication rates. A group of researchers from University of California showed that combining neo-adjuvant chemotherapy with oncoplastic mammoplasty is one of the best solutions for high aesthetics results with low local recurrence rates for patients needing radiotherapy treatment after surgery. 64 patients mastectomized with immediate reconstruction were compared with 37 who underwent the new procedure. After using the Fisher's exact test, the results showed rates of complications for infection 16.2% *versus* 35.9% ($p < 0.04$), for skin flap necrosis 10.8% versus 29.7% ($p < 0.05$) and for unplanned intervention 2.7% versus 37.5% ($p < 0.001$), much higher for the mastectomy group (excepting seroma – 7.8% versus 5.4%, $p = 0.6$). The authors show that oncoplastic mammoplasty is highly recommended to patients with local tumor in advanced stages, in order to avoid complications of multiple long lasting surgical procedures, improving quality of our patients' life. (Peled AW, Sbitany H, Foster RD, Esserman LJ. Oncoplastic mammoplasty as a strategy for reducing reconstructive complications associated with postmastectomy radiation therapy. *Breast J* 2014; 20(3):302-7)

Ioannis Gardikiotis

NO COLORECTAL CANCER SCREENING PROGRAM IN ROMANIA! THUS, START WITH OPPORTUNISTIC SCREENING

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NO COLORECTAL CANCER SCREENING PROGRAM IN ROMANIA! THUS, START WITH OPPORTUNISTIC SCREENING (Abstract). Colorectal cancer is a major health problem in the world. It can be detected in asymptomatic early stages so screening programs have been implemented in many countries for many years. Unfortunately, in Romania, the screening program for the detection of colonic pathologies is still not working. This paper suggests that an opportunistic colorectal cancer screening may be a solution in our country. This means that when a physician is contacted by a subject older than 50 years, a colorectal screening procedure – fecal occult blood testing or colonoscopy should be suggested to him/her. Thus, by collaborating between physicians from many specialties (gastroenterologists, general practitioners, internal medicine specialists, and surgeons) we hope to decrease the rate of advanced colorectal cancers found in daily practice. **Key words:** COLORECTAL CANCER SCREENING, COLONOSCOPY, FECAL OCCULT BLOOD TESTING.

Colorectal cancer is an important oncologic problem both in Europe and in the world. Even if it is in the top three human cancers, it has the advantage that it can be detected in asymptomatic early stages, by screening programs. On the other hand, progression from adenomatous polyps to cancer is a known and documented fact. Thus, by performing polypectomies, we can decrease the rate of colon cancer.

This is why screening programs for the detection of asymptomatic colorectal cancers and of colonic polyps were implemented in many countries, years ago.

Several studies proved that colorectal cancer screening programs decreased the mortality by colonic cancers (1-3), regardless of the screening strategy (based on fecal occult blood testing – such as FOBT or FIT - or on colonoscopy).

Many years ago, in Romania it was decided that colorectal cancer screening programs are valuable and a pilot study was started. Unfortunately, even after 10 years, the screening program for the detection of colonic pathologies is still not working.

A legislative program for cancer screening in three organs was issued some years ago: for breast, cervix and colon cancer. But no financial support was provided for the colorectal cancer screening during the last years!

Efforts were made by the National Society of Gastroenterology and Hepatology (SRGH) to obtain funding for this program, but nothing happened!

Thus, what to do? There is not time to wait since in every day practice, the endoscopists find too many advanced colorectal cancers (sometimes with metastases).

Of course, it is a priority to continue the pressure on politicians to find financial support for such a program, but this is not enough!

Can we find other available methods?

We believe that we must start with an opportunistic colorectal cancer screening.

What does this mean?

It means that when a physician is contacted by a subject older than 50 years, a colorectal screening procedure should be suggested to him/her.

These colorectal screening procedures can be performed according to the financial means of patients or according to the physician's preference (fecal occult blood tests are very cheap and can be preferred by general practitioners - GP, while colonoscopy may be preferred by gastroenterologists, internal medicine specialists or surgeons).

In a recent study (4) performed in Romania in a large cohort of asymptomatic subjects (2433 patients), using colonoscopy as screening method for colonic pre neoplasic and neoplasic lesions, we found in polyps in 32.1% of cases (in 7.2% cases polyps larger than 1 cm) and carcinomas in 2% of cases. What does this mean? By adding cancers and large polyps, in 9.2% of cases we found an advanced disease (1/11 asymptomatic subjects have pre neoplastic or neoplasic lesions).

Thus, using this modality of screening that can be offered to all patients over 50 that contact a physician, we can discover a lot of pathological cases in early stages.

In addition we can decrease the risk for further cancers by performing polypectomy for all detected polyps.

Gastroenterologists play a major role in the awareness of colorectal cancer risk. We must train the other specialists regarding this risk and we should work together for the opportunistic screening (at least until a population based screening program will properly funded).

The GPs must know the real value of fecal occult blood tests (and which is the difference between FOBT and FIT), how often to propose these tests to the patients (annually or biennial), or if they should propose a colonoscopic evaluation (with the advantage that a normal colonoscopy must be repeated only after 10 years).

On the other hand, all must be informed regarding the importance of colonoscopy's quality (cecal intubation in more than 90-95% of cases); of the preparation for colonoscopy; regarding which is the risk for complications and on other issues on this topic.

Of course, we can talk about the funding of a screening program. Usually this is covered by the National Health Authority!

But since this is still not happening in Romania, we must do something. And the only thing that we can do (excepting political pressure) is to work in the patients' interest, with the opportunistic screening.

Thus, by collaborating with physicians from other specialties: GPs, internal medicine specialists, surgeons and maybe others, we can decrease the rate of advanced colorectal cancers found in daily practice.

Thus, don't worry..... begin the opportunistic screening stating from tomorrow, so we can fulfill our task of decreasing the number of advanced cases.

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NEWS

BETA-BLOCKER MEDICATIONS AND RISK OF PSORIASIS

Psoriasis is an immune-mediated skin disease which can affect internal organ such as cardiovascular system. Besides, cardiovascular diseases have been linked to psoriasis development. In a study published in *JAMA Dermatology*, the authors evaluated the association on hypertension and beta (β)-blocker medication with risk of psoriasis. The study group included 77 728 normotensive and hypertensive women, with or without antihypertensive medications. The results showed that the hypertensive women with long-term status (6 years or more) have had a higher risk of developing psoriasis compared with normotensive women. Also, the highest risk of psoriasis was documented among hypertensive women who used β-blockers for 6 years or more. These results sustain the role of hypertension and medications in psoriasis development. (Shaowei Wu, Jiali Han, Wen-Qing Li et al. Hypertension, antihypertensive medication use, and risk of psoriasis. *JAMA Dermatology*, 2014; DOI: 10.1001/jamadermatol.2013.9957).

Cătălina Luncă

A NOVEL TRANSUNGUAL PERMEATION ENHANCER FOR TOPICAL DRUG DELIVERY SYSTEM IN ONYCHOMYCOSIS

The treatment of onychomycosis is difficult because of the barrier properties of the nail plate that impede the passage of antifungal drugs. In a study by Chouhan et al., hydroxypropyl-β-cyclodextrin (HP-β-CD) was tested as a transungual drug permeation enhancer for poorly water soluble antifungal drugs such as terbinafine hydrochloride. According to Chouhan et al., HP-β-CD improves hydration of nail plates and increases solubility of terbinafine hydrochloride. Using HP-β-CD as a permeation enhancer, the researchers designed a nail lacquer formulation (containing 10%w/v of HP-β-CD) to deliver the drug in an effective concentration across nail plates, proving *in vitro* higher flux ($4.586 \pm 0.08 \mu g/mL/cm^2$) than the control formulation ($0.868 \pm 0.06 \mu g/mL/cm^2$). The study concluded that HP-β-CD is an effective permeation enhancer for transungual delivery of terbinafine hydrochloride and other poorly water soluble drugs (Chouhan P, Saini TR. Hydroxypropyl- β -cyclodextrin: a novel transungual permeation enhancer for development of topical drug delivery system for onychomycosis. *J Drug Deliv*. 2014;2014:950358. doi: 10.1155/2014/950358).

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CARDIAC TUMORS

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CARDIAC TUMORS (Abstract): Cardiac tumors are a group of rare disorders with a frequency that varies in population studies between 0.0017% and 0.33%. There are primary cardiac tumors with an incidence of 5% of all cardiac tumors and secondary tumors (metastases of the heart) in 95% of cases. Symptoms are nonspecific and can mimic many other heart diseases. This fact makes the diagnosis of cardiac tumors very difficult. Approximately 75% of the primary cardiac tumors are benign; the most frequent histological type encountered is myxoma in 50 % of cases, followed by cardiac fibromas, lipomas, rhabdomyomas, hemangiomas, teratomas, papillary fibroelastomas, pericardial cysts or cystic tumor of the atrioventricular node region. Secondary cardiac tumors (metastases) are 20 times more common than primary cardiac tumors. Paraclinical methods (especially imaging evaluation) are always necessary for the complete diagnosis: transthoracic and transesophageal echocardiograms are the gold standard investigations in the diagnosis of the cardiac tumors; CT scans together with MRIs are complementary diagnostic methods that are useful when the diagnosis is uncertain. In the majority of cases surgical treatment is recommended. **Keywords:** CARDIAC TUMOR, MYXOMA, ECHOCARDIOGRAPHY.

Cardiac tumors are a group of rare disorders with a frequency that varies in population studies between 0.0017% and 0.33% (1). There are primary cardiac tumors with an incidence of 5% of all cardiac tumors and secondary tumors (metastases of the heart) in 95% of cases (2). The diagnosis of primary cardiac tumors is frequently a challenge. Symptoms associated with most of them are nonspecific and can mimic many other heart diseases. Therefore, the diagnosis of cardiac tumors is difficult to be made when the first signs appear.

Classification

Approximately 75% of the primary cardiac tumors are benign; the most frequent histological type encountered is myxoma, in 50 % of cases. There can also be cardiac fibromas, lipomas, rhabdomyomas, hemangiomas, teratomas, papillary fibroelastomas, pericardial cysts or cystic tumors of the atrioventricular node region (2).

The remaining 25% are malignant tumors most frequently represented by sarcomas (angiosarcomas, fibrosarcomas, liposarcomas, leiomyosarcomas, osteosarcomas), lymphomas and mesotheliomas (2, 3).

Paragangliomas and pheochromocytomas can also be, depending on their anatomopathological characteristics, benign or malignant cardiac tumors (4).

Secondary cardiac tumors (metastases) are 20 times more common than primary cardiac tumors. In various necropsy studies in patients with malignant diseases, the heart is affected in 10% of cases (5). Cardiac metastases can occur by: direct invasion: lung cancer, breast cancer; hematogenous: melanoma, sarcoma; lymphatic extension: epithelial carcinoma.

Pathophysiology

Pathophysiological changes caused by cardiac tumors depend on: nature of the tumor (benign *vs.* malignant), localization, histological type, embolic potential and secretor or non-secretor character of the tumor.

Primary cardiac tumors in adults most commonly develop in the subendocardial tissue, rarely at the pericardial or myocardial level. Cardiac metastases frequently locate in the pericardium (epithelial neoplasms). Tumors that develop from the big blood vessels can metastasize in the endocardium (4, 5).

There are some preferential localizations of the cardiac tumors (5):

- Left atrium: myxoma and myxomatous hypertrophy of the interatrial septum;
- Right atrium: angiosarcomas;
- Left ventricle: fibromas, rhabdomyomas;
- Ventricular septum: angiomas;
- Valves of the heart: papillary fibroelastomas.

Clinical signs

Signs and symptoms related to cardiac Tumors can be very different; therefore the diagnosis might be considerably delayed. They depend primarily on the localization of the tumor and the anatomopathological characteristics.

Patients can be completely asymptomatic or may present:

- Signs of right or left heart failure: dyspnoea, oedema of the legs, fatigue; they can also present acute pulmonary oedema and low cardiac output syndrome;
- Supraventricular (atrial fibrillation or flutter) and ventricular arrhythmia;
- Atrioventricular blocks;
- Signs of valvular disease;
- Pericardial effusion;
- Pulmonary or systemic thromboembolism;
- Signs and symptoms that most frequently occur in malignant Tumors: fever, weight loss, impaired general condition, immunological signs: arthralgia and Raynaud's phenomenon (4).

Cardiac tumors can mimic a variety of other cardiac and non-cardiac pathology. Right atrial tumors can mimic Ebstein disease, constrictive pericarditis or atrial septal defect; left atrium tumors may be confused with mitral valvular heart disease or endocarditis; the right ventricle Tumors with pulmonary stenosis or pulmonary embolism and the left ventricle Tumors with aortic valvular disease or obstructive hypertrophic cardiomyopathy (4).

Paraclinical signs

Paraclinical, hematological and biochemical changes and the imaging evaluations are essential for assessing the surgical risk and patients' follow-up.

Hematological and biochemical changes include: signs of inflammation (increase in erythrocytes sedimentation rate, C reactive

protein and fibrinogen), presence of anaemia or polyglobulia, thrombocytosis, thrombocytopenia or leukocytosis. Protein alterations may also occur through the increase in gamma globulins (4).

The electrocardiogram is nonspecific most of the times. The patient usually has a sinus rhythm. The existence of a large left atrial myxoma can lead to atrial fibrillation. Atrioventricular blocks can also occur. Ischemic changes or necrosis can occur when tumor fragments embolise at the level of the coronary arteries.

Thoracic radiography can be normal. Cardiomegaly, cardiac calcifications or pericarditis can indicate the existence of a cardiac tumor formation.

Transthoracic and transesophageal echocardiograms are the gold standard investigations in the diagnosis of the cardiac tumors. They have a sensitivity of 93%, 97% respectively (4, 5). They are a non-invasive diagnosis method that offers a multitude of morphological and functional information about the tumor. They can identify the tumor localization and size, the existence of vascularisation, as well as the impact it has on the heart function. The transesophageal echocardiogram offers a better imaging of the atria and the interatrial septum. It provides a higher resolution imaging of the tumor and it can detect such growths that are not visible in a transthoracic echocardiogram. It provides a better visualization of the right atrium Tumors (2).

CT scans together with MRIs are complementary diagnostic methods that are useful when the diagnosis is uncertain. The CT scan is a useful investigation for assessing the degree of intramural, pericardium and extracardiac damage. The MRI provides good accuracy in the 3D scans of the tumors and in their differentiation from other intracardiac formations (5).

Cardiac catheterization and angiography are not indicated as routine investigations due to the high risk of peripheral embolisation through the dislocation of a tumor fragment or an associated thrombus. They are reserved for the cases where the visualization of the tumor is difficult using the non-invasive diagnosis methods and in the case of other associated cardiac diseases. The situation is different in the case of vascularised tumors where the cardiac catheterization and coronarography can be used to identify the coronary artery that provides the vascularisation of the tumor (4).

Treatment

Cardiac tumors treatment is influenced by the histological type of the tumor. In the case of benign cardiac tumors, the treatment primarily includes surgery for tumor excision. This depends on its type and on patient's symptomatology. In the case of the symptomatic patients the complete excision of the tumor leads to their healing.

In the case of cardiac myxomas, surgery is recommended to be done as soon as possible, even if the patient is symptomatic or not, due to the high risk of embolism through the breaking of small tumor fragments. Surgery involves the complete excision of the tumor together with the pedicle or/and the insertion area (*fossa ovalis*) plus 5 mm of surrounding normal endomyocardial tissue in order to avoid recurrences (5). The relapse risk is also high in the case of cardiac fibromas, hemangiomas and lymphomas.

Similarly, the decision for surgery has to be prompt in the case of a mobile papillary fibroelastoma of over one cm.

On the contrary, in patients diagnosed with cardiac lipomas or lipomatous hyper-

trophy of the atrial septum the surgery should be indicated only for those who show significant hemodynamic disorders.

The treatment for malignant tumors is similar to the cases in which these anatomopathological subtypes are located in other places in the body. Although surgery offers the chance of a curative treatment, this is in many cases a difficult target to hit due to early metastases.

Most treatments are palliative and are done in order to remove the cardiac obstruction, improving the heart functions for a limited period of time. Chemotherapy can help in certain cases by reducing the mass of the tumor and facilitating the excision.

Sarcomas benefit from surgical treatment with curative effect in the case of small tumors without secondary dissemination. Cardiac angiosarcomas require a multidisciplinary approach that includes surgery, chemo radiotherapy and immunotherapy (6).

The inoperable cardiac sarcomas or the ones with metastasis at the moment of diagnosis mainly benefit from chemotherapy.

Despite the use of these multiple therapies, the average survival of the patients diagnosed with cardiac sarcoma is of one year at the most (6).

Cardiac lymphomas are generally sensitive to chemotherapy. Prompt chemotherapy with or without adjuvant radiotherapy became the elective treatment of these tumors. Surgical excision remains the second principle of treatment that can be approached (6, 7).

Usually, the treatment of secondary cardiac tumors is palliative and symptomatic. Surgery procedures, chemo and radiotherapy can be used.

CONCLUSIONS

Cardiac tumors are a group of rare disorders that, due to the physiopathological changes, lead to the increase of mortality in the absence of an efficient treatment. The diagnosis and treatment of primary cardiac tumors will always be a challenge for doctors and surgeons due to their rare occurrence as pathology and the lack of a predefined guide for their management. However, taking cardiac tumors into account within the differential diagnosis together with a high suspicion index are essential in making a correct diagnosis.

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A REVIEW ON THE CAUSE-EFFECT RELATIONSHIP BETWEEN OXIDATIVE STRESS AND TOXIC PROTEINS IN THE PATHOGENESIS OF NEURODEGENERATIVE DISEASES

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A REVIEW ON THE CAUSE-EFFECT RELATIONSHIP BETWEEN OXIDATIVE STRESS AND TOXIC PROTEINS IN THE PATHOGENESIS OF NEURODEGENERATIVE DISEASES (Abstract): Protein aggregates are the defining pathological feature of human neurodegenerative diseases. Studies have revealed that mutant huntingtin, polyglutamine-expanded ataxin-1 and ataxin-3 can cause elevated levels of reactive oxygen species in neuronal cells. It has also been indicated that the normal host prion protein behaves as an antioxidant, while the neurotoxic peptide based on the sequence of the scrapie isoform increases hydrogen peroxide toxicity in neuronal cultures. Additionally, not only can oxidative stress contribute to the aggregation of β-amyloid and α-synuclein, but both β-amyloid and α-synuclein can induce oxidative damage. Furthermore, oxidative stressors have been shown to play a critical role in neurofibrillary pathology leading to tau hyperphosphorylation. In conclusion, the present review supports a cause-effect relationship between oxidative stress and toxic proteins in the pathogenesis of neurodegenerative disorders. Keywords: NEURODEGENERATIVE DISEASES, PROTEIN MISFOLDING, OXIDATIVE STRESS

Neurodegenerative disorders as diverse as Parkinson's disease, prion diseases, polyglutamine diseases, Alzheimer's disease and related tauopathies share a common feature – aggregation and deposition of abnormal protein. Expression of mutant proteins in transgenic animal models recapitulates features of these diseases. Neurons are particularly vulnerable to the toxic effects of mutant or misfolded proteins. When they accumulate in sufficient quantity, misfolded proteins are prone to aggregation. Failure to detect and eliminate misfolded proteins contributes to the pathogenesis of neurodegenerative disease (1).

As previously mentioned, the common mechanistic theme of these diseases is the formation of aggregates containing misfolded proteins with a concomitant gain of function which eventually leads to neuronal death. A gain of function, rather than a loss of function, is sustained by the observation that the aggregates are toxic *per se*. Several studies have addressed the following question: what makes the aggregates toxic? In this regard, a widely invoked cause of membrane damage has been free radical oxidative stress (2). The oxidative damage induced by the redox activity of a target protein, which interacts with free radicals and metal ions, has been found as a typical hallmark in the majority of neurodegenerative

disorders. Thus, protein aggregation and oxidative stress have been demonstrated to be the major factors involved in the neurodegenerative process (3).

The objective of the current review would be to examine a possible cause-effect relationship between oxidative stress and the toxic proteins in the pathogenesis of neurodegenerative diseases.

POLYGLUTAMINE DISEASES

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by an expansion of cytosine-adenine-guanine (CAG) repeats located in the coding region of the IT15 – interesting transcript 15 – gene on chromosome 4, whose product is named huntingtin (htt) (4). In addition, intranuclear inclusion bodies have been found in the striatal neurons of transgenic mice expressing the CAG repeat containing exon 1 of the htt gene (5). The expanded polyglutamine (polyQ) tract contained by the encoded protein htt may be toxic directly and/or through interactions with other cellular proteins (6).

The interaction between the mutated N-terminal part of htt and the glycolysis enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) supports a role for oxidative damage in the disease process, as it impairs the glycolitic activity of this enzyme. The use of spectrophotometric assays in postmortem brain tissue has revealed impaired oxidative phosphorylation enzyme activity restricted to the basal ganglia in HD brain (7). GADPH interaction with htt has been further increased by the presence of the expanded polyQ stretch (8).

It has been observed that mutant htt (mhtt) causes increased levels of reactive oxygen species (ROS) in neuronal SK-N-SH (human neuroblastoma) cells and non-neuronal COS-7 (monkey kidney) cells. Thus, it has been postulated that endogenous heat shock protein 27 (HSP27) in its unphosphorylated form suppresses polyQ-mediated cell death by protecting against ROS induced by mhtt. It has been shown that HSP27 mutants showing protection in the polyQ model increase survival of HeLa cells exposed to hydrogen peroxide (H_2O_2). Oxidative stress is likely to be relevant to polyQ-induced death, since both N-acetyl-L-cysteine and the reduced form of glutathione (GSH) have inhibited polyQ-mediated cell death. Furthermore, cells expressing mhtt have presented decreased levels of GSH, which have been increased by co-expression of HSP27 (9).

A consequence of the defect in energy metabolism caused by mhtt may be an increase in oxidative injury, a regionally specific mechanism of neuronal death in HD. A well-established marker of oxidative damage to deoxyribonucleic acid **(**DNA) is 8-hydroxy-2-deoxyguanosine (OH8dG), and a significant increase in OH8dG in nuclear DNA has been found in the caudate nucleus in HD post-mortem tissue (7). A significant increase in OH8dG has also been found in mitochondrial DNA of parietal cortex from HD patients (10).

In addition, lipid peroxidation (LP), induced by the formation of free radicals, has been determined in the striatum, cortex and cerebellum of R6/1 transgenic mice that express a human mutated htt exon 1, with approximately 116 CAG repeats, and wild-type (WT) control mice at 11, 19, 24 and 35 weeks of age. A tendency of elevated levels of LP in the R6/1 mice has started by 19 weeks and has become significant by 24 weeks; at 35 weeks, LP in transgenic striatum was 274% higher than in non-transgenic mice. As a result, mutated htt specifically

induces progressive oxidative damage to the striatum of transgenic mice (11).

Spinocerebellar ataxia 1 (SCA1) is an autosomal dominant neurodegenerative disorder caused by the expansion of CAG trinucleotide repeats encoding the polyQ tract in ataxin-1, the SCA1 gene product. PolyQ expansion leads to the aggregation of ataxin-1 proteins in the nucleus. It has been shown that the large aggregates formed by polyQ-expanded ataxin-1 recruit the Cu/Zn-binding isoform of superoxide dismutase, SOD1, in the nucleus of HeLa cells. In the case of mutant ataxin-1-expressing cells, most of SOD1 has been detected in nuclear aggregates; in the case of normal ataxin-1-expressing cells, some Cu/Zn-SOD has been found co-localized in ataxin-1 aggregates and some diffused in the cytosol. In addition, it has been revealed that the oxidation of intracellular proteins occurs with a higher frequency in the presence of mutant ataxin-1 relative to normal ataxin-1. When treated with both BSO (an inhibitor of γ-glutamylcysteinyl synthetase, the first enzyme in the GSH biosynthetic pathway) and t-butylhydroperoxide (TBH), mutant ataxin-1-expressing cells have proved to be more prone to mitochondrial dysfunction than normal ataxin-1-expressing cells. These results suggest that polyQ-expanded ataxin-1-expressing cells increase the levels of ROS in HeLa cells (12).

Spinocerebellar Ataxia 3 (SCA3)/ Machado-Joseph Disease. Human SK-N-SH neuroblastoma cells stably transfected with full-length mutant Machado-Joseph disease (MJD) with 78 CAG repeats have been established to analyze the role of mutant ataxin-3 and mimic an early disease stage, as less than 1% of the mutant cells contain nuclear aggregates under basal conditions. To test the hypothesis that oxidative stress might contribute to the progression of the disease, TBH has been used to assess the oxidative tolerance of cells. It has been noticed that SK-N-SH-MJD78 cells, stably expressing expanded ataxin-3, are more susceptible to low concentrations of TBH (1 and 3 μM) up to 24h treatment than the parental SK-N-SH cells. Additionally, Western blot analysis has indicated that the protein expression of HSP27 has dramatically decreased in both neuronal and non-neuronal cells with expanded ataxin-3, which is possible to result in an increase of ROS in the cellular model. The same significant reduction of HSP27 has been further confirmed in the lymphoblastoid cell lines from two MJD patients, compared with that from a normal individual. Semiquantitative reverse transcription-polymerase chain reaction and microarray analysis have ruled out the possibility that the reduced HSP27 expression could be due to transcriptional repression of HSP27 gene, further supporting the evidence that expanded ataxin-3 increases the vulnerability to oxidative stress (13).

PRION DISEASES

According to "prion hypothesis" formulated by Prusiner in 1982, transmissible spongiform encephalopathies are caused by "novel proteinaceous infectious particles" corresponding to the scrapie isoform (PrPsc) of the normal host prion protein (14).

It has been suggested that the conformational conversion of normal, protease-sensitive, primarily α-helical form of prion protein (the cellular isoform, PrPc) into a pathogenic, protease-resistant, β-sheet-rich form of the protein (PrPsc) may be the result of imbalances in the level of copper. The changes in copper concentration have

altered prion protein (PrP) ability to act as an antioxidant by modifying its conformation. Truncated PrP could be a result of self-proteolysis by PrP when Cu^{2+} physiological concentration is too high (15). Moreover, it has been reported that PrPc undergoes a site-specific cleavage, both Cu^{2+}- and ph-dependent, of the octapeptide-repeat region on exposure to ROS such as H_2O_2 and superoxide ions (O_2^-). Such ROS cleavage might be involved in the formation of the scrapie isoform, as abnormal cleavage of PrPsc occurs into the octarepeat region (16).

The flexible amino-terminus of PrPc contains the octapeptide PHGGGWGQ, which is repeated four times and is among the best-preserved regions of mammalian PrPc. Proton nuclear magnetic resonance (NMR) spectroscopy has indicated that the histidine residues in each octarepeat are coordinated to the copper ion. It has been concluded that host PrPc gains a Cu,Zn-SOD-like antioxidant activity, dependent on copper incorporation (17). Immunoprecipitated PrPc from WT mouse brain has also mimicked SOD1 activity, which confirms that native PrP behaves as an antioxidant. Additionally, PrPc dependent SOD1 activity has been abolished by the precise deletion of the octapeptide-repeat region (18). Moreover, native PrPc, affinity-purified from mouse brain, has exhibited the same SOD1-like antioxidant activity, as determined by spectrophotometric assay. In conjunction, incubation of purified PrPc with PrP106-126, the neurotoxic peptide based on the sequence of PrPsc, has significantly inactivated the SOD1-like activity (19).

It has also been demonstrated that SOD1 activity varies with the level of PrPc expression. Thus, prion protein-deficient cells proved to be more sensitive to oxidative stress due to reduced SOD1 activity (20). In addition, SOD1 activity has proved to be significantly higher in cerebellar cells overexpressing PrP than for WT extracts. Moreover, the expression of PrPc may regulate Cu, Zn-SOD activity by influencing copper incorporation into the SOD1 molecule (21).

Furthermore, a study on 8 week-old prion protein knock-out mice (Prnp-/-) has emphasized a role for PrP in modulating oxidative homeostasis in vivo. In this regard, higher levels of oxidative damage to proteins and lipids have been found in the brain lysates of Prnp-/- as compared to WT mice of the same genetic background (22). PrP-/- neurons also proved to be significantly more susceptible to H_2O_2 toxicity than WT neurons after a 6 and 24 hour exposure. This result might be related to the significant decrease in glutathione reductase (GR), but not glutathione peroxidase (GPx), activity detected in PrP-/- neurons both in vitro and in vivo. These findings have been supported by PrP106-126 that has significantly inhibited GR activity and increased H_2O_2 toxicity in neuronal cultures (23).

ALZHEIMER'S DISEASE & PICK'S DISEASE

The presence of intracellular neurofibrillary tangles, containing hyperphosphorylated tau protein and apolipoprotein E, and of extracellular senile (neuritic) plaques, including non-soluble β-amyloid, ubiquitin, apoE, presenilins and alpha-antichymotrypsin, are considered hallmarks of Alzheimer's disease (AD) (24). AD is associated with an increased cerebral accumulation of amyloid beta-protein (Aβ), a 39-42 amino acid peptide. Soluble Aβ monomers aggregate to form antiparallel β-

pleated sheets as proved by circular dichroism studies (25).

As Aβ is considered to have a causal role in AD, the effect of oxidative stress on its metabolism within the cell has been studied. It has been reported that oxidative stress induced by H_2O_2 promotes intracellular accumulation of Aβ in human neuroblastoma cells through enhancing the amyloidogenic pathway (26). A possible role for LP in the pathogenesis of AD has also been suggested. It has thus been determined that membranes containing oxidatively damaged phospholipids promote beta-sheet formation in a 42-residue Aβ and accumulate Aβ significantly faster than membranes containing normal saturated phospholipids (27).

It has also been shown that H_2O_2 mediates Aβ-induced neurotoxicity, which explains why catalase, that degrades H_2O_2, protects the cells from β-amyloid toxicity. In addition, Aβ has induced the activity of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappa B), a transcription factor thought to be regulated by oxidative stress (28). It has been suggested that amyloid peptides including Aβ may be toxic via a common oxidative mechanism and that their amphiphilic form, rather than the structure in β layers, may be the cause (29, 30).

Moreover, it has been emphasized that, under some conditions, toxic Abeta peptides Aβ1-40 and Aβ25-35 enhance transition metal-catalyzed oxidation of hydroxylamine derivatives (31). Several studies have also concluded that cortical or hippocampal synaptosomes isolated from AD brain or from rodent brain or rodent cultured hippocampal neurons incubated with Aβ1-42, Aβ1-40 or Aβ25-35 have increased protein oxidation; non-toxic reverse peptides have not caused protein oxidation (32).

It can thus be proposed the amyloid β-peptide-associated free radical oxidative stress model for neurotoxicity in AD: Aβ-initiated ROS react rapidly with several moieties in the plasma membrane and cause protein oxidation and LP; toxic products of LP, such as 4-hydroxy-2-nonenal and acrolein (2-propenal), having longer half-lives than free radicals, migrate to different parts of the neurons causing multiple alterations of cellular function such as sharp increases in intracellular Ca^{2+} and ultimately leading to neuronal death. Moreover, it has been suggested that methionine-35 (Met-35) is a key amino acid involved in Aβ radicalization, aggregation and neurotoxicity, since it is the most susceptible Aβ residue to oxidation in vivo, especially under oxidative stress conditions. Methionine oxidation to the sulfoxide leads to predominantly β-sheet conformation adopted by toxic Aβ. Examination of senile plaque-resident Aβ1-40 has detected a high proportion of methionine sulfoxide (33, 34).

However, it has been indicated that, rather than being a source of ROS, β-amyloid may be a modulator of ROS production (35).

Furthermore, the hyperphosphorylation of the microtubule-associated protein tau (τ) is considered a hallmark of tauopathies such as AD and Pick's disease. It has been hypothesized that oxidative stressors play a critical role in neurofibrillary pathology by activating stress-activated protein kinases such as p38 pathway that leads to τ phosphorylation in vitro and co-localizes with phosphorylated τ in vivo (36). It has been found that acrolein, a LP product from arachidonic acid, increases τ phosphorylation due to p38 stress-activated kinase (37).

PARKINSON'S DISEASE & DEMENTIA WITH LEWY BODIES

The synucleins (α, β and γ-synuclein) are a small family of proteins highly expressed in nervous tissue. An obvious link with Parkinson's disease (PD) has been established by showing that two missense mutations in the α-synuclein gene (A53T and A30P) are responsible for rare familial forms of the disease (38). In both PD and dementia with Lewy bodies α-synuclein accumulates in the form of fibrillar aggregates inside the Lewy bodies, cytoplasmic inclusions deposited within dopaminergic neurons.

Alpha-synuclein appears to increase ROS levels in dopaminergic neurons via more than one process. First, α-synuclein interacts with the dopamine (DA) transporter and facilitates its clustering at the plasma membrane. Consequently, DA uptake is accelerated leading to increased susceptibility to DA-induced apoptosis. Therefore, the re-uptake of more DA intracellularly can be a source of increased ROS due to DA metabolism (39). It has been indicated that over-expression of α-synuclein and especially its mutant forms exaggerates the vulnerability of neurons to DA-induced cell death through excess intracellular ROS generation (40). Additionally, transfection of cell lines with mutant forms of α-synuclein have increased the baseline levels of oxidative damage, reflected by decreased levels of GSH and enhanced levels of 8-hydroxyguanosine (8-OHG), protein carbonyls and LP (41).

It has also been shown that both full-length α-synuclein and its synthetic peptide fragment, when incubated in solution, have the ability to generate hydroxyl radicals upon the addition of ferrous ion (42). In conjunction, it has been revealed that oxidative stress can induce the aggregation of human α-synuclein (43). This bi-directional molecular pathway could create a vicious cycle of α-synuclein changes and excessive oxidative stress.

Moreover, it has been shown that parkin, an ubiquitin-protein ligase, interacts with α-synuclein; double staining of PD brains has illustrated that parkin and α-synuclein co-localize to the same pathological structures (both Lewy bodies and axonal spheroids) (44). In at least one patient, mutations in parkin have led to Lewy body formation as seen in sporadic PD. It has been investigated how overexpression of WT and mutant parkin proteins modulates proteasomal activity, ubiquitinated proteins accumulation, oxidative stress indices or antioxidant defenses. Increasing expression of WT parkin by gene transfection in two cell lines has led to increased proteasomal activity, decreased levels of protein carbonyls and a trend to a reduction in ubiquitinated protein levels. Transfection of the two cell lines with DNA encoding three mutant parkins associated with autosomal recessive juvenile parkinsonism have not altered antioxidant enzyme activities, namely SOD1, SOD2, catalase, GPx and GR, but have increased oxidative stress, as indicated by decreased levels of GSH, smaller increases in proteasomal activity and elevated levels of protein carbonyls and LP (45). Impairment of proteasomal function leads to free radical generation and oxidative stress (46). Therefore, altered activity of the ubiquitin-proteasome system, which degrades oxidatively damaged proteins, may play a key role in PD pathogenesis.

CONCLUSIONS

The present review provides strong evidence for a cause-effect relationship be-

tween oxidative stress and toxic proteins in the pathogenesis of neurodegenerative diseases.

In this regard, it has been suggested that a toxic gain of function of mutant htt arising from altered interactions with other proteins may lead to oxidative damage. In addition, transgenic mice over-expressing the mutated amino terminus of human htt have been shown to suffer the effects of increased oxidative damage. Moreover, studies have indicated that both polyglutamine-expanded ataxin-1 and ataxin-3 increase the vulnerability to oxidative stress.

It has also been emphasized that the loss of resistance to oxidative stress due to inactivation of the function of the normal form of prion protein, either due to conversion or through interaction with the pathogenic form of the protein (the scrapie isoform), may be important to the pathogenesis of prion diseases.

Furthermore, several studies have demonstrated not only that the oxidative processes can contribute to the aggregation of β-amyloid, but that β-amyloid itself can be considered a source of free radicals, thus supporting amyloid β-peptide-associated free radical oxidative stress model for neurotoxicity in AD.

Finally, this review points out that α-synuclein increases ROS levels in dopaminergic neurons and oxidative stress can induce the aggregation of α-synuclein.

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NEWS

THE INFLUENCE OF BAROREFLEX ACTIVATION THERAPY ON RENAL RESPONSES IN PATIENT WITH RESISTANT HYPERTENSION

It is widely accepted that renal system play an essential role in terms of long-term blood pressure regulation. Recently, a non-pharmacological theraphy represented by baroreflex activation has provided to be a promising alternative in order to reduce arterial pressure, particularly in patient with drug-resistant hypertension. A multicentre, double blind Rheos Pivotal Trial was designed to investigate the renal response and efficiency to prolonged baroreflex activation theraphy in patient with drug resistant hypertension. 322 patients aged 21 to 80 years, who had blood pressure ≥ 160/80 mmHg despite maximal tolerated therapy with ≥ 3 antihypertensive medication including a diuretic, were divided in two groups. Group 1 consisted of 236 patients who started baroreflex activation therapy 1 month after device implantation, whereas in the 86 patients from group 2 the device was activated 6 months later (deferred baroreflex activation theraphy). Serum creatinine, urine albumin/ creatinine ratio and estimated glomerular filtration rate were determined to asses renal function. The **results** showed that in group 1, serum creatinine increased from 78 to 84 μmol/L, and glomerular filtration rate decreased from 92 to 87 mL/min per 1.73m^2 at month 6, whereas albumin/ creatinine ratio did not change. Group 2 demonstrated similar renal responses at month 6 and month 12 as group 1. In **conclusion,** chronic baroreflex activation does not result in further decrease in renal function, suggesting that this non-pharmacological approach is safe, effective and promising. (Alnima T, De Leeuw PW, Tan F, Kroon A. Renal Responses to Long-Term Carotid Baroreflex Activation Therapy in Patients With Drug-Resistant Hypertension. *Hypertension* 2013; 61(6):1334-9).*Ionut Tudorancea*

CORRELATIONS BETWEEN THE PRESENCE OF SIGMOID INTERVENTRICULAR SEPTUM AND INCREASED RELAPSE RISK OF STROKE IN HYPERTENSIVE PATIENTS

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CORRELATIONS BETWEEN THE PRESENCE OF SIGMOID INTERVENTRICULAR SEPTUM AND INCREASED RELAPSE RISK OF STROKE IN HYPERTENSIVE PATIENTS (Abstract) **Objectives:** Comparison between cerebrovascular accident (CVA) relapse in hypertensive patients with sigmoid interventricular septum (SIS) and CVA relapse in hypertensive patients without SIS. **Materials and methods**: We selected 36 hypertensive patients, all of them complicated with previous CVA; 18 patients had SIS (Group A) and 18 patients didn't have SIS (Group B). The utilized methods were the following: clinical examination, electrocardiogram, transthoracic echocardiogram, biochemical findings and complete blood count, performed every 3 months, during 2 years of observation. Another method, addressed for CVA relapse patients, were cerebral computed tomography or magnetic resonance imaging .The recommended treatment was administered according to the European therapeutic guidelines. **Results**: 6 (out of 18) patients with SIS presented a CVA relapse and only 1 (out of 18) patient without SIS revealed a CVA relapse**. Conclusions**: Our results support recent conclusions from another publications: patients with SIS (an apparently benign condition, which is a part of the cardiac modifications usually discovered in elderly people) have a higher cardiovascular risk for acute events, than the patients without SIS.
Keywords: SEPTUM, HYPERTENSIVE, CEREBROVASCULAR

The physiological aging of the heart can reveal different aspects: myocardium atrophy, isolated amiloid deposits, mitral annulus calcification or the angulations of the interventricular septum to the ascending aorta (1). This last mentioned modification is known (in echocardiography) as sigmoid interventricular septum (SIS) and has been described in the medical literature since 1969 (2). Initially, SIS has been just a physiological variant of the heart aging. Afterwards, its occurrence has been explained as due to the ascending aorta dilation (in elderly people) and to the tilting of the aorta towards the interventricular septum. The basal portion of the interventricular septum is bulging into the left ventricular outflow tract (LVOT) and is becoming sigmoid-shaped, without significant clinical consequences (3). The same authors, Chan and Veinot, suggested the fact that the benign aspect of the SIS could have

hemodynamic consequences (the obstruction of LVOT) if the patient had left ventricular hypertrophy associated to SIS. A physiopathology impact could occur in some cases of heart aging. Several Japanese authors mentioned the age-related morphological changes of the inter-ventricular septum; a part of them had proven the fact that the thickening of basal portion of the interventricular septum was more significant in hypertensive patients than in normotensives, during follow-up spanning 10 years (4); other authors (5) remarked the basal portion of the interventricular septum hypertrophy (age-related) even in normotensives and no hypertrophy of the mid-ventricular portion. All these studies allowed a better understanding of the so-called "sigmoid interventricular septum" modification. A new concept about SIS developed during the last 10 years: a part of these patients could have dynamic obstruction into the left ventricular outflow tract, followed by hemodynamic consequences: a decreased coronary and/or cerebral perfusion pressure and an increased telediastolic left ventricular pressure. The clinical consequences generated by these hemodynamic changes consisted of: angina pectoris, lipothymia / syncope and dyspneea on effort. The treatment of the patients with SIS and dynamic obstruction in LVOT, using a medication which decreased the pressure gradient, became absolutely required. Clinical studies have proven the beneficial effect (decrease of the gradient and improvement of the symptoms) of the association between negative inotropic agents: betablockers and cibenzoline (6, 7) or betablockers and dysopiramide (8).

MATERIAL AND METHODS

The study included 36 patients (men: 60%; women 40%), aged between 65-79 years.

The inclusion criteria were: arterial hypertension (with an evolution of at least 10 years); ischemic stroke (past medical history); adherence to medical recommendations.

The exclusion criteria were: smoking or alcohol intake, at the beginning of the study; diabetes mellitus; association of severe medical diseases (neoplasia, severe heart/ renal/ respiratory/ liver failure).

The patients were monitored through the following methods (every 3 months, during 2 years): clinical examination, biochemical findings (serum creatinine, transaminases, glycemia, and lipid profile), complete blood count, electrocardiogram, transthoracic echocardiogram (we used a Fukuda Denshi UF-850 XTD echograph machine).Cerebral computed tomography or magnetic resonance imaging were performed only for the CVA relapse patients. We had 2 groups of patients, after echocardiography: group A- 18 patients with SIS and group B-18 patients. We selected (through echocardiography) these 18 patients with SIS, CVA and arterial hypertension, from 107 patients with arterial hypertension and previous CVA.

Clinical examination revealed the following: 1. angina pectoris (4 classes' classification, according to the Cardiology Canadian Society); 2.dyspneea (4 classes classification, according to the New York Heart Association); 3.vertigo; 4.sensibility and/ or motility disorders (confirmed by neurological examination); 5.blood pressure values (clinostatic and orthostatic positions). The CVA relapse has been clinically suspected; neurological consultation and cerebral computed tomography/magnetic resonance imaging were required for CVA confirmation.

Medical treatment (for group A,with

SIS) consisted of betablockers (Metoprolol succinate 50-200 mg/day), antiplatelet agents (Clopidogrel 75 mg/day), statins (Atorvastatin 40-80 mg/day or Rosuvastatin 20-40 mg/day), diuretic „thiazide-like": Indapamide 0.625-1.5mg/day, single or associated (with angiotensin converting enzyme inhibitors), into a fixed combination- Perindopril / Indapamide 2.5/ 0.625 mg or 5/1.5 mg, daily. Those patients with SIS and obstruction in LVOT received smaller doses of diuretics or fixed combination (for blood pressure control); this caution was necessary for the prevention of gradient increasing in LVOT, followed by redoubtable clinical consequences. We selected calcium channel blockers (Amlodipine 2.5-10 mg) and diuretics (Indapamide 0.625-1.5 mg/day) for group B- the hypertensive patients without SIS, according to European therapeutic guidelines in elderly hypertensive with CVA. We preferred beta blockers (Nebivolol 2.5-10 mg/day or Carvedilol 6.25-12.5 mg, twice daily) instead of calcium channel blockers, for those hypertensive with associated ischemic heart disease.

The patients without SIS and elevated blood pressure values, despite treatment with calcium channel blockers and diuretics, also received angiotensin converting enzyme inhibitors (Perindopril 2.5-10 mg/day) or sartans (Telmisartan 20-80 mg/day). All the patients without SIS (group B) received Clopidogrel 75 mg/day and statins (Atorvastatin 40-80 mg/day or Rosuvastatin 20-40 mg/day), considering previous CVA.

RESULTS

I. Group A. This group was formed by the following: group A_1 -7(out of 18) patients with SIS, LVOT obstruction and cardiovascular symptoms; group A_2- 4(out of 18) patients with SIS, symptoms and without LVOT obstruction; group A_3 - 7 (out of 18) patients with SIS and without symptoms or LVOT obstruction. All those 7 patients of group A_1 had cardiovascular symptoms: IIIrd class angina pectoris (angina on minor efforts) and IIIrd class dyspneea (dyspneea on minor efforts). Group A_2 had the following symptoms (fig.1): IIIrd class angina pectoris-2 patients; IInd classdyspneea-1 patient and IIIrd class angina pectoris plus dyspneea-1 patient. The patients of group A_3 had no cardiovascular symptoms, but they have SIS. All the 7 patients of group A_1 improved after 3 months treatment their effort tolerance (IInd class instead of IIIrd class). The patients of groups $A_2$and A_3 remained in the same situation concerning effort capacity.

II. Group B. This group was formed by the following: group B_1 (fig.2) -10 (out of 18) patients without SIS, had the following symptoms: IIIrd class angina pectoris-4 patients; IInd class angina pectoris - 2 patients; IIIrd class dyspneea-1 patient; IIIrd class dyspneea plus angina pectoris- 3 patients; group B_2-8(out of 18)patients without SIS and without cardiovascular symptoms. Five (out of 7) patients from group B_1 had a favorable evolution of cardiovascular symptoms (they passed from IIIrd class to IInd class). Concerning the 8(out of 18) patients of group B_2 we noticed the same clinical condition throughout the 2 years study.

About CVA relapse, we revealed the fact that 6 out of 18 patients (33%) with SIS (from group A) had a CVA relapse, during 2 years study. All these 6 patients had LVOT obstruction, suggesting a strong correlation between the presence of hemodynamic significant gradient and CVA

relapse risk. The CVA relapse rate was significantly reduced - 5% - (1 out of 18 patients) among the patients without SIS (group B).

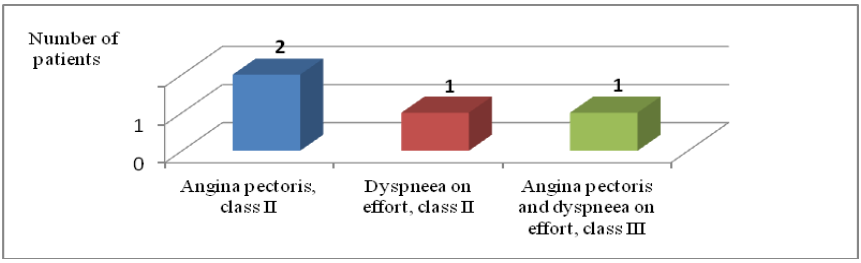

Fig.1. The symptoms of the patients with SIS and without obstruction in LVOT

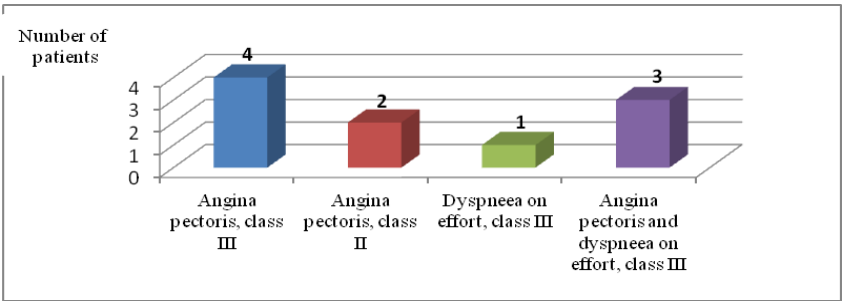

Fig. 2. The symptoms of the patients without SIS

Echocardiography revealed obstruction in LVOT (mild / moderate obstruction: LVOT gradient was between 25-52 mmHg), in all 7 patients of group A_1. All these 7 patients recorded a qualitative echocardiographic improvement after the treatment: the highest LVOT gradient was34 mmHg (after 3 months), comparing with the highest gradient-52 mmHg (at the beginning of the study).

The biochemical findings revealed the following aspects: 1) 2 patients from the group A with SIS maintained (at 3 months revaluation) a lipid profile associated with high cardiovascular risk (total cholesterol≥ 230 mg/dl). These 2 patients required the addition of Fenofibrate 160 mg to the previous statin and they had a favorable evolution of cholesterol values (at 6 months revaluation).

They were part of the 6 patients group with the relapse of the CVA; 2) at the 3 months revaluations of the serum creatinine, renal dysfunction has been precociously revealed in 3 (out of 18) patients from group B without SIS (treated with Perindopril 10 mg). We stopped Perindopril administration for these patients; replacing that medication with betablockers (the patients also continued the diuretics and calcium channel blockers).

We haven't discovered right or left renal artery stenosis at these patients (there was the possibility they had atherosclerotic stenosis of the small renal arteries, undetectable with echography).

Cerebral computed tomography and magnetic resonance imaging proved the CVA relapse in all 7 patients clinically suspected: 6 (out of 18)-33% from group A

and 1 (out of 18)-5% from group B.

DISCUSSION

The clinical studies have proven the fact that an apparent benign aspect of heart aging, like sigmoid interventricular septum, could precipitate severe obstruction in LVOT, under special circumstances. The hemodynamic worsening (vasoplegic syndrome determined significant gradient in LVOT) after lumbar anesthesia, in patients with SIS, was an example for this situation (9). Another clinical situation which could develop the aforementioned gradient was anemia associated with SIS; anemia could induce hyperkinesias (with powerful ventricular contractility) and could decrease blood flow volume, both of them leading to sudden increase of the LVOT gradient (10). Our study has related results with other publications (11, 12): an apparent physiological heart aging situation – SIS - could increase the risk for ischemic cardiovascular events (cerebral or myocardial) and medical intervention could decrease this risk.

CONCLUSIONS

Our study noticed that increasing LVOT gradient in SIS patients with LVOT obstruction lead to a higher relapse of CVA (33%- 6 from 18 patients) than in patients without SIS (5%-1 from 18 patients). Despite medical treatment which had benefices towards LVOT gradient, those 6 patients with LVOT obstruction unfortunately developed CVA relapse.

That means they had done some mistakes according life style and behavior: they had a sudden increasing in LVOT gradient, due to heavy efforts or psychological distress. Those patients who performed heavy efforts developed a sudden more powerful left ventricle contraction, so an increasing LVOT gradient and decreasing cerebral blood flow; therefore they made an ischemic CVA under these circumstances (medical treatment could not protect the patient because the effort was too strong).

Those patients who had a major psychological distress had a sudden cathecolamines releasing, which provoked a more powerful contraction of left ventricle, followed by the same consequences as aforementioned heavy efforts.

After a detailed anamnesis, we discovered one of this etiology at all our patients with CVA relapse. Future studies, including more patients, are necessary to complete these observations.

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NEWS

INTRAVASCULAR LARGE B-CELL LYMPHOMA

Intravascular lymphoma was first described in 1959 by Pfleger and Tappeiner in Germany. It was described as a neoplasm of the vascular endothelium, due to intravascular growth of malignant cells, hance the initial name of the disease as "angioendotheliomatosis proliferans systemisata". In 1986 Sheibani et al proved, via immunohistochemical investigation, that the cells of intravascular lymphoma were of lymphoid origin and described the disease as angiotropic (intravascular) large-cell lymphoma. In 2008, the World Health Organization defined intravascular large B-cell lymphoma (IVLBCL) as a type of extranodal large B-cell lymphoma where growth is restricted to the lumina of vessels, particularly capillaries. Intravascular large B-cell lymphoma is rare, occurring at an estimated frequency of less than 1 person per million. Two forms have been described: classical, also known as Western form, with a cutaneous variant; and an Asian form occurring more frequently in the Far East. Intravascular lymphoma is most frequently a disease of B lymphocytes, although rare cases of thymus-cell (T-cell) and natural killer–cell disease have been reported; the World Health Organization considers these cases to be a separate entity. Intravascular large B-cell lymphoma is aggressive, and has historically been a rapidly fatal malignancy when diagnosis and treatment is delayed. Intravascular large B-cell lymphoma is a rare type of extranodal large B-cell lymphoma. It tends to occur in elderly patients, occurs with only slight predilection for male sex, and is without known risk factors. Accurate diagnosis is elusive, requires strong clinical suspicion, and tissue sampling and microscopy of at least 1 of what is typically several disseminated lesions. Microscopy will demonstrate large B cells sequestered within the intravascular spaces; these cells inconsistently express several of the typical B-cell antigens, with CD79a, CD20, MUM1/IRF4, and CD19 being the most commonly expressed. With the exception of a cutaneous variant, IVLBCL is aggressive and carries a grim prognosis, although the prognosis has improved with recent introduction of rituximab to combination chemotherapy regimens. These anthracycline-based or CHOP-like regimens plus rituximab have resulted in improvement of clinical course, and prolonged remission and survival (Dennis E. Orwat,; Nicholas I. Batalis, Intravascular Large B-Cell Lymphoma, *Arch Pathol Lab Med*. 2012;136:333–338).

Doina Butcovan

GASTROESOPHAGEAL REFLUX AND METABOLIC SYNDROME

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GASTROESOPHAGEAL REFLUX AND METABOLIC SYNDROME *(*Abstract) The **aim** of our study was to evaluate gastric, duodenal and gallbladder motility disorders in patients with gastroesophageal reflux disease (GERD) and metabolic syndrome. **Material and methods**: We studied 128 patients with GERD divided into two groups: first group with metabolic syndrome and the second without metabolic syndrome. By abdominal ultrasound we monitored our patients for the gastric emptying rate, the duodenal motility and the nonlithiasic pathology of gallbladder (cholesterolosis). **Results**: We found that patients with metabolic syndrome had three kind of abnormal motility disorders including stomach, duodenum, and gallbladder. The patients without metabolic syndrome we found only two abnormal motility disorders: of the duodenum and gallbladder. Hyperglycemia and high serum cholesterol level in the first group were correlated with stomach, duodenum and gallbladder abnormal motilities. In our opinion metabolic syndrome can aggravate gastroesophageal reflux disease due to these metabolic abnormalities. **Conclusions**: We consider that treatment of reflux disease in these particular cases must also involve measures to correct metabolic disorders. **Keywords**: METABOLIC SYNDROME; CHOLESTEROLOSIS GALLBLADDER GASTROESOPHAGEAL REFLUX.

Metabolic syndrome is a cluster of metabolic disorders defined as the presence of an increased waist circumference and two of the following components: high blood pressure, hypertriglyceridemia, low levels of high density lipoprotein (HDL)-cholesterol, or diabetes mellitus (DM)/ hyperglycemia (1). This syndrome is associated with high risk for cardiovascular diseases and has become one of the major health problems worldwide (1).

Gastroesophageal reflux disease is characterized by the presence of esophageal mucosal injury or reflux symptoms caused by the abnormal reflux of gastric content into the esophagus (2).

Several studies have shown the relationship between obesity, erosive esophagites and GERD symptoms (3). Also other studies demonstrated that metabolic syndrome was associated with GERD (4) through the precise mechanisms have not been fully determined. However, literature on whether metabolic syndrome influences the gastric, duodenal and gallbladder motility in patients with GERD is scant.

The aim of this study was to determine whether metabolic syndrome influences the gastric, duodenal and gallbladder motility and aggravates the symptoms in patients with GERD.

MATERIAL AND METHODS

Patients

We conducted a prospective case-control study, on patients admitted in a tertiary hospital, from January 2010-December 2012, diagnosed with GERD.

We excluded patients with prior gastric surgery, gastric cancer and peptic ulcer.

After a medical history was taken, all patients had physical examination, hematological and biochemical blood tests, an abdominal ultrasound and completed a questionnaire.

Patients were diagnosed with metabolic syndrome if they had an increased waist circumference and two of the following component: high blood pressure (≥130 mmHg systolic or ≥85 mmHg diastolic), hypertriglyceridemia (≥150 mg/dl), low levels of HDL-c (≤40 mg/dl in males or ≤50 mg/dl in females), or DM/hyperglycemia (1).

All participants completed a validated questionnaire that identified reflux symptoms, such as irritating heartburn and/or regurgitation experience during the preceding year (5). Values between 0-7 points were associated with less probable GERD, a score between 8-10 points revealed GERD with discomfort and unpleasant symptoms and a score of 11-18 points characterized GERD with discomfort and severe symptoms.

Measurements

The sonographic examination was performed using a Philips XD11XE ultrasound machine, using a C5-2 transducer.

The gastric motility was assessed by the method proposed by Dareiche and Osman (6). The method considers that gastric volume is directly proportional with antral area (Aa). Gastric emptying rate (GER) represents the percentage of Aa reduction measured 15 minutes and 90 minutes after a standard meal. Normal values for Aa are 489 mm^2 (377-745 mm^2) for 15 minutes and 166 mm^2 (124-279 mm^2) for 90 minutes. The average GER value is 63% (58-69%) (6).The longitudinal diameter (D1) and anteroposterior diameter (D2) of the antral section were obtained placing the traducer in a longitudinal position at the level of the abdominal aorta and the left hepatic lobe. The formula for Aa is: Aa= 3,14 x D1xD2/4 and it was applied for Aa measured at 15 minutes and at 90 minutes. GER =(Aa90'/Aa15'-1) x 100 (7). We used a standard meal which contained 50g carbohydrates, 15g proteins and 12g lipids.

The duodenal motility was assessed by sonographic observing of peristaltic and antiperistaltic waves. This type of movements could be observed during the physiological phase of duodenal digestion and if they are enhanced it could have pathological consequences.

Gallbladder colesterolosis was defined using ultrasound criteria described by Sporea (7).We calculated the gallbladder volume before and after Boyden meal and then we calculated the ejection ratio (ER) of gallbladder by the formula: E.R= V1-V2/V1x 100, where V1 is gallbladder volume before Boyden meal , V2 is gallbladder volume after Boyden meal. The formula for gallbladder volume is V=0,5x Lxl x g, L is the length of gallbladder, l is the width of gallbladder, g is the thickness of gallbladder wall. Normal values are 50-60%.

Statistical analysis

Statistical analysis was performed using the χ^2 test for comparison of discrete variables and the *t*-test for comparison of continuous variables. The continuous variables measured in this study were expressed as the mean±SD. Correlations were performed

using Spearman correlation test. Linear and logistic regression were used to calculate odds ratio (OR) and 95% confidence interval (CI). A two-tailed p value of<0.05 was considered statistically significant.

This study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.

RESULTS

In this study we included 128 patients diagnosed with GERD. Fifty-nine patients had metabolic syndrome and 69 patients represented the control group, without metabolic syndrome (tab. I).

TABLE I

Baseline characteristics of the study groups

	GERD with metabolic syndrome (n=59)	GERD without metabolic syndrome (n=69)	*p*
Age (years)	41±2.3	40±2.9	0.553
Sex (M/F)	35/24	40/29	0.486
BMI (kg/m^2)	34.77±5.2	29±4.4	0.036
Smoker (%)	58	55	0.125
Triglyceride (mg/dl)	255±45.2	159±32.9	<0.0001
LDL-col (mg/dl)	176±21.4	112±20.3	<0.0001
Cholesterol (mg/dl)	290±43.6	236±35.3	<0.0001
HTA(%)	78	51	<0.0001
DM (%)	39	16	<0.0001
Hiatal hernia (%)	75	68	0.058
Esofagitis (%)	24	19	0.244
GERDQ questionnaire (points)	14±2.4	9±1.2	<0.0001

Abbreviations: BMI: body mass index, LDL: low density lipoproteins, HTA: arterial hypertension, DM: diabetes mellitus, GERDQ: gastroesophageal reflux questionnaire.

The mean age was 40.5 years and 58.5% of the subjects were men.

We found a significant increased in the mean BMI, systolic blood pressure, glucose, cholesterol in patients with metabolic syndrome compared to the controls.

Patients with GERD and metabolic syndrome had a higher GERD questionnaire score than controls (p=0.033), corresponding with more severe reflux disease.

The presence of metabolic syndrome determined a high rate of cholesterolosis, an increased duodenal motility and ER of gallbladder, and decreased gastric emptying in cases than controls (tab. II). None of the controls had low values of GER.

In group with metabolic syndrome we found three kind of abnormal motility disorders: low GER values (100%); enhanced duodenal motility (40%) and high values of gallbladder ER (42%). In the control group we found only two kinds of motility disorders: enhanced duodenal motility 16% and high values of gallbladder ER. We diag-

nosed gallbladder cholesterolosis in cases 45% than controls in 10%.

TABEL II

Odds ratio for metabolic syndrome and GERD by multiple logistic regression

Metabolic syndrome and GERD		
	OR	CI 95%
Decreased GER	2.32	1.36-5.48
Increased duodenal motility	1.58	0.97-5.63
Colesterolosis	1.21	0.83-7.45
Gallbladder Er>50-60%	0.98	0.53-2.77

Abbreviations: GERD: gastroesophageal reflux disease, GER: gastric emptying rate, ER: emptying rate.

In patients with GERD and metabolic syndrome we found an inverse correlation between serum glucose and GER value, and a direct correlation between cholesterol values and duodenal motility.

DISCUSSION

GERD is caused by the abnormal reflux of gastric contents into the esophagus (4). Its occurrence is related to multiple factors, such as gastric acid secretion, hiatal hernia, lower esophageal sphincter (LES) function, esophageal motility, and esophageal perception (2). In addition, dietary habits, such as alcohol intake and eating foods with high components of fat or in large portions, may cause not only visceral fat accumulation, but also frequent gastroesophageal reflux due to a reduction in LES pressure and an increase in the number of transient LES relaxations (TLESR) (2).

In the present study we investigated the relation between metabolic syndrome and gastric, duodenal and gallbladder motility disorders, and our results clearly demonstrated that the presence of metabolic syndrome was associated with decreased gastric emptying, and increased duodenal motility. In addition, the presence of metabolic syndrome was suggested to worsen reflux symptoms (3) fact confirmed by our study.

All cases with cholesterolosis had high values of gallbladder ER and associated enhanced duodenal motility. This fact means that the bile which is rich in cholesterolosis reaches the duodenal lumen quickly and can induce the enhanced duodenal movements which sometimes opens the pylorus and allows the reflux of duodenal juice containing bile and pancreatic juice in the stomach. If the gastric motility is low (low GER values) the gastroesophageal reflux is getting worse.

In the first group patients with three motility disorders had high values of GERDQ (11-18 points) meaning that the reflux was severe. Controls, with only two abnormal motilities disorders, had lower values of GERDQ (8-10 points) which means that the gastroesophageal reflux was not severe as in the first group.

Previously, Chung et al. (8) demonstrated that the presence of metabolic syndrome and visceral obesity in a Korean population were significant risk factors for the occurrence of reflux esophagitis. The results of our study appear to fit well with those findings. An augmented effect of dyslipidemia on the prevalence of reflux esophagitis was also demonstrated in the study of Chung et al. (8), though the precise mechanism of the dyslipidemia-induced high prevalence of GERD and reflux esophagitis remains unclear. We demonstrated that high cholesterol levels imply high excretion into biliary tract followed by development of gallbladder cholesterolosis. The bile rich in cholesterol reaches the duodenum and induces enhanced

motility. Therefore, the intake of meals with a high level of fat might induce not only dyslipidemia, but might also lead to aggravate GERD symptoms.

In the present study, low values of GER were statistically related with high values of blood glucose. It is known that GERD prevalence in patients with DM, is higher in comparison with that in healthy individuals, and is reported to be caused by a decline in salivary secretion, delayed gastric emptying-induced increases in TLESR, and an increase in gastroesophageal reflux (3, 4). In our study, we observed that low values of GER in the first group were similar to those from diabetic patients (7) in which the vegetative vagal neuropathy induced delayed gastric emptying.

CONCLUSIONS

Our results indicate that metabolic syndrome enhanced GERD symptoms by gastroduodenal and gallbladders motility disorders. These results allow us to assert that in all cases with gastroesophageal reflux which associates metabolic syndrome the treatment must be addressed also to this complex entity.

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EFFECTIVENESS OF THE IMMUNOCHEMICAL FECAL TEST (FIT) FOR DETECTION OF ADVANCED ADENOMAS IN COLORECTAL CARCINOMA SCREENING IN AN ASYMPTOMATIC POPULATION

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EFFECTIVENESS OF THE IMMUNOCHEMICAL FECAL TEST (FIT) FOR DETECTION OF ADVANCED ADENOMAS IN COLORECTAL CARCINOMA SCREENING IN AN ASYMPTOMATIC POPULATION (Abstract): **Aim:** The aim of the study was to evaluate the benefit of the immunochemical fecal blood test (FIT) for detection of advanced adenomas. **Material and methods:** We have conducted a screening study on a average-risk cohort of subjects, aged 50 –74 years. We used a rapid quantitative FIT to perform the screening. Colonoscopy was recommended in all participants with positive FIT. We assessed the participation rate and analyzed advanced adenomas detected in the screening examinations. **Results:** Our study enrolled 1111 asymptomatic participants. Immunochemical test results were positive in 72 subjects (6.5%). Colonoscopy was performed by 50 /72 (69.4%) subjects. The detection rate for advanced adenomas was 2.2%. A total number of 28 advanced adenomas were detected in 24 subjects, meaning 1.2 advanced adenoma/subject. Analyzing the 3 characteristics of advanced adenomas, we observed in our study that 25/28 adenomas (89.3%) had sizes ≥ 10 mm, 11/28 adenomas (39.3%) were tubulovillous/villous and 19/28 adenomas (67.9%) had high dysplasia. The majority of advanced adenomas were found in the left colon as compared to the right colon: 71.4% vs. 28.6%, p=0.0008. **Conclusions:** In our study was obtained a 2.2% detection rate for advanced adenomas, most of them (71.4%) being located in the left colon. The dominant feature of advanced adenomas detected was the size ≥ 10 mm (89.3% of cases). **Keywords:** IMMUNOCHEMICAL FECAL TEST, ADENOMAS, COLORECTAL CARCINOMA, SCREENING.

Colorectal cancer (CCR) is a major cause of mortality in the developed countries, with a 6% risk to develop the disease during life, with an incidence of more than one million new cases per year and with a long term survival of 50% (1).

Detecting the early stages of CCR and adenomas in an apparently healthy population using the screening tests reduces incidence and mortality due to colorectal car-

cinoma. Noninvasive testing as fecal occult blood tests (FOBT) is now widely accepted as a screening tool for detecting CCR (2). At the beginning the guaiac fecal occult blood tests were used for screening but due to their low sensitivity and specificity (they are not specific for human hemoglobin and they need dietary restrictions) they are less used nowadays (3).

The fecal immunochemical test (FIT) is considered a superior screening test, because of its better reproducibility and acceptance. Detection rates for advanced adenomas and cancer are higher compared to guaiac fecal occult blood test (gFOBT) (4). Therefore the American Cancer Society has replaced the guaiac based test with immunochemical tests as a screening tool for CCR (5). A better sensitivity in detecting CCR in early phases when the disease is asymptomatic has been demonstrated by fecal immunochemical test than gFOBT (1). Data from Saito demonstrated a sensitivity of 67-89%% in detecting CCR using immunochemical test compared with 33-37% using Hemoccult. A reduction in CCR mortality with 60% was demonstrated with annual screening (6).

The aim of the study was to evaluate the benefit of the FIT for detection of advanced adenomas knowing their malignant potential.

MATERIAL AND METHODS

Our study included a cohort of average risk persons, meaning asymptomatic subjects aged between 50 and 74 years. To limit the percentage of FIT false positives results the following subjects were excluded subjects with adenoma or colonic carcinoma or with family history of adenoma or colonic carcinoma (first – degree relatives), subjects with inflammatory bowel diseases, people that took part in a screening program and performed one of the screening methods accepted by the international guidelines in the last years (2 years for FOBT, 5 years for virtual colonoscopy/barium enema/flexible sigmoidoscopy and 10 years for colonoscopy). Subjects with a short life expectancy (less than 10 years) were also excluded.

All subjects included in our study signed an informed consent; the study was approved by the local Ethics Committee and was in accordance with the Helsinki Declaration of 1975.

The stool sample was collected in a clean dry screw-top container according to the instructions that they have received. The stool sample was handed to the laboratory on the same day of the collection or within the first 24 hours. The test format of HEM CHECK-1 (VedaLab, France), used in our study, employs the standard antibody/antigen lines of capture, typically with a control line to interpret a positive and negative result. Test results were sent to the participant, physician and medical screening program coordinator.

Patients with positive FIT were sent to the Gastroenterology Department of Arad County Hospital to set an appointment for the colonoscopy. The Gastroenterologist explained to the subjects the benefits and the risks of performing a colonoscopy and if needed, a polypectomy. Preparation for colonoscopy was done with polyethylene glycol. All colonoscopies were performed under sedo-analgesia. Anesthesia, colonoscopy and polypectomy were performed only after obtaining the written consent from the patient. Administration of anticoagulant therapy, non-steroidal anti-inflammatory drugs or clopidogrel was stopped in the last 5-7 days prior to the

endoscopic procedure. The colonoscopy result includes the number of polyps, their size (≤ 5mm, from 5-9 mm, and ≥ 10 mm) and their location. The histopathology result of the biopsy mentions besides the size (≥ 10 mm), the tubulovillous or villous structure and the high grade mucosal dysplasia of the adenoma to confirm the diagnosis of advanced adenoma.

We used the term advanced adenomas for adenomas with at least one of these characteristics: size ≥ 10 mm and / or high-grade mucosal dysplasia and / or with a villous structure of ≥ 20% (tubulovillous, villous).

Statistical analysis was performed using the MedCalc Software, version 12.4.0 (MedCalc, Belgium). The distribution of the numerical variables was first tested by the Kolmogrov-Smirnov test. In case of numerical variables with normal distribution mean value and standard deviation were calculated, while in case of non-normal distribution median values and range interval were presented. Qualitative variables were presented as numbers and percentages. The Fisher's exact test was used for the comparison of two proportions expressed as a percentage. 95% confidence intervals were calculated for each predictive test. A *p*-value less than 0.05 were regarded as significant for each statistical test.

RESULTS

Our study enrolled 1111 asymptomatic participants with mean age 61.4 ± 12.8 years, 461 (41.5%) men and 650 (58.5 %) women. FIT results were positive in 72 (6.5%) persons with mean age 62.1±11.9 years, 34 (47.2%) men and 38 (52.8%) women. The mean age of women with positive FIT was similar with the mean age of men with positive FIT: 62.3±12.1 years vs. 61.9±12.1years, p=0.76.

Among the 72 participants with positive FIT, 50 (69.4.%) accepted to perform a colonoscopy. The others refused colonoscopy or delayed this investigation. Advanced adenomas were detected in 24 subjects (2.2%), 16/24 subjects (66.7%) were men and 8/24 subjects (33.3%) were women. The mean age of men with advanced adenomas was similar with that of women detected with advanced adenoma: 63.5±11.4 years vs. 62.6±12.1 years, p=0.85. A total of 28 advanced adenomas were detected in these subjects, meaning 1.2 advanced adenoma/ subject. Association of advanced adenomas with colorectal cancer was met in 5 patients (20.8%) (tab. I).

TABLE I

The distribution of advanced adenomas according to the age

Age group (years)	Total investigations		Advanced adenomas		Number of advanced adenomas	Number of advanced adenomas /subject
	number of subjects	%	number of subjects	%		
50-54	225	20.3	3	1.3	3	1
55-59	256	23.0	3	1.2	4	1,3
60-64	254	22.9	6	2.4	7	1.2
65-69	178	16	6	3.4	7	1.2
70-74	198	17.8	6	3.0	7	1.2
Total	1111	100	24	2.2	28	1.2

Analyzing the 3 characteristics of advanced adenomas, we observed that 25/28 adenomas (89.3%) had sizes ≥ 10 mm, 11/28 adenomas (39.3%) were tubulevillous/villous and 19/28 adenomas (67.9%) had high dysplasia.

In our study 7/28 (25%) advanced adenomas showed a single characteristic: 5 adenomas had size ≥ 10 mm , one adenoma had tubulovillous/villous structure and one adenoma was with high grade dysplasia. Two characteristics were found in 15/28 (53.6%) adenomas: 11 adenomas had size ≥ 10 mm and high grade dysplasia, 3 adenomas had size ≥ 10 mm and had tubulovillous/villous structure and one adenoma had tubulovillous/villous structure and high grade dysplasia. All 3 characteristics were found in 6/28 (21.4%) adenomas.

Analyzing advanced adenomas by its location we observed that most of them were found in the left colon as compared to the right colon: 71.4% vs. 28.6%, p=0.0008 (tab. II).

TABLE II

Distribution of advanced adenomas at colonoscopy

Location	Adenomas	
	Number	%
Rectum	6	21.4
Sigmoid colon	12	42.9
Descending colon	2	7.1
Transvers colon	5	17.9
Ascending colon	3	10.7

DISCUSSION

The purpose of screening for CCR is to reduce the morbidity and mortality because the prolonged evolution of the adenoma to carcinoma (10-15 years) is well known (7). Colonoscopy is the first choice screening option recommended by the international guidelines because of its high sensitivity/specificity and therapeutic utility for advanced adenomas, but it is costly and it requires specialized personnel and corroborated with the risks that are involved (like perforation) (8) make this method a difficult one to be implemented in a national screening program in our country.

The aim of the present paper was to evaluate the effectiveness of FIT in detecting advanced adenomas as the removal of these lesions could be considered a warrant of invasive cancer prevention. We chose the FIT because among the other non invasive test, such as the guaiac based fecal test, it proved better sensitivity, specificity and a higher compliance (9). We studied an average-risk population cohort, aged 50-74 years, using a rapid qualitative FIT, Hem Check 1, produced in France by VedaLab.

In our study the detection rate of advanced adenomas was 2.2%, similar values being obtained in other studies (9,11) and remained considerably higher compared to the guaiac-based fecal test detection rate (10,11).

In this study we detected 28 advanced adenomas. Dominant characteristics of advanced adenomas were size ≥ 10 mm (89.3%) and high-grade dysplasia (67.9%). 39.3% of advanced adenomas contained tubulovillous/villous. Large size, high-grade dysplasia and villous pattern are pathological characteristics that are known to increase the probability of malignant

transformation (12). It is known that polyp's ≥ 10 mm have a 1% malignancy rate per year and they turn into cancer in 24% of cases. Adenomas less than 10 mm may develop invasive cancer in less than 5 years extremely rare (13). Some studies (14, 15) proved that greater size of the adenomas determined higher rate for detection of advanced adenomas or cancer in follow–up colonoscopies and a villous pattern of more than 20% is not a constant risk factor for detecting advanced adenoma during follow –up colonoscopy (1). We obtained similar values compared to the data published by Yang et al evaluating the effectiveness of the FIT in a 5919 cohort study in 2011 when they analyzed the characteristics of the advanced adenomas (16).

Our data analysis supports the hypothesis that FIT is more efficient for detecting left-sided advanced adenomas than right-sided advanced adenomas The location of advanced adenomas in our study was mainly in the left colon 71.4% vs 28.6% the right colon ($p<0.0008$). The differences in detecting advanced adenomas in left and right colon are similar to the findings reported by Morikawa et al (2005) regarding immunochemical FOBT (17) and is also supported by other studies, one of them conducted by Haug et al. in 2011 using FIT (18), and another one reporting a test performance of a guaiac-based FOBT (19). Most studies proved a better efficiency in detecting left sided lesions (rectum, sigmoid, descending colon, splin flexure) than in the right colon (transverse, ascending colon,caecum) probably because of the stool consistency and because the blood is more homogeneous distributed in the right sided colon and is more superficial in the left colon (18).

Authors of the "European guidelines for quality assurance in colorectal cancer screening and diagnosis guidelines" (1) gathered information from several studies stating that older age comes with increased risk of detecting advanced adenomas (15,20). Our study cohort showed a greater rate of advanced adenoma in the group age ≥ 60 years old, but should be specified that a relatively small number of FIT positive subjects were included in each group. Although the majority of the studies (1) analyzing the detection rate of advanced adenomas in older age showed the same conclusions, advanced age is not an indication for more intense surveillance because of the slow progression of advanced adenomas to cancer (1). In our study we detected a greater number of advanced adenomas in men; similar values were obtained in some studies (15) but not in all (20).

CONCLUSIONS

In this study we obtained a 2.2% detection rate for advanced adenomas, most of them (71.4%) being located in the left colon. The dominant feature of advanced adenomas detected was the size ≥ 10 mm (89.3% of cases). Knowing that, positive FOBT and, more useful – FIT combined with cut – off hemoglobin levels, are recommended as methods which are correlated with colonic diseases – especially polyps and cancer.

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CLINICAL, BIOLOGICAL AND EPIDEMIOLOGICAL ASPECTS OF INFLAMMATORY BOWEL DISEASES IN NORTH-EAST ROMANIA

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CLINICAL, BIOLOGICAL AND EPIDEMIOLOGICAL ASPECTS OF INFLAMMATORY BOWEL DISEASES IN NORTH-EAST ROMANIA (Abstract): **Aim:** Inflammatory bowel diseases (IBD) are chronic conditions of unknown etiology with increasing incidence in South-East Europe. The epidemiological characteristics, clinical, biological and endoscopic profiles are very different worldwide, with little data existing from Moldova region. This study aims to characterize IBD in North-East Romania from the mentioned points of view. **Material and methods:** All patients with Crohn's disease or ulcerative colitis (UC) admitted to a tertiary hospital during a three year interval were retrospectively analyzed in terms of epidemiological characteristics. All IBD patients hospitalized during 2010 were complexly analyzed by noting the type and form of disease, the biological picture, the clinical and endoscopic scores. **Results:** UC represents 77% of all cases (one of the highest values of the recent studies). Age at onset is higher in the studied region, with the average over 40 years. In UC the forms of severity and extension are uniformly distributed, but in Crohn's disease there is a predominance of colonic forms and the number of complicated cases is high - over 50%. The parameters with the best correlation with endoscopic scores were the clinical Mayo score for UC, serum C-reactive protein and fibrinogen. **Conclusions:** There is a definite predominance of UC in North-East Romania, but Crohn's disease tends to present an increased number of complications. The age of onset of the two diseases is high. There is a good correlation between the clinical and biological scores with endoscopic activity, but without this assessment inflammation may be underestimated. Systematic exploration of the small bowel may increase detection of ileal manifestations. **Keywords:** INFLAMMATORY BOWEL DISEASES, ULCERATIVE COLITIS, CROHN'S DISEASE, EPIDEMIOLOGY, MONTREAL CLASIFICATION.

Inflammatory bowel diseases (IBD) are chronic conditions of uncertain cause comprised of two distinct entities- ulcerative colitis (UC) and Crohn's disease (CD). If UC only affects the colon and rectum by continuous inflammation limited to the mucosa, CD may involve any segment of the digestive tract (most commonly the ileum and the colon), inflammation being discontinuous and extended in depth (1). With an incidence of tens to hundreds per 10^5 people and a prevalence of a few hun-

dreds, IBD represent an important cause of morbidity and impaired quality of life especially in young and middle-aged patients, by the chronic relapsing nature and lack of curative treatment (2). There is important variation worldwide in the incidence, prevalence, clinical and evolving features according to geographic region, ethnicity and race (3). It is estimated that the incidence in South-Eastern Europe is slightly increasing, especially for CD, compared to Northern and Western Europe, where it is stabilized (4,5).

The few published studies for Romania have shown a lower incidence compared to the neighboring regions, a higher mean age and the prevalence of mild to moderate forms of disease. However, the data are slightly contradictory and there are methodological difficulties (6, 7).

This study aims to evaluate from epidemiological, clinical, biological and endoscopic perspective all cases of inflammatory bowel disease submitted to a tertiary medical center within three years.

MATERIAL AND METHODS

This study represents a retrospective analysis of all cases diagnosed with inflammatory bowel disease hospitalized in the Center of Gastroenterology and Hepatology from the "St. Spiridon" University Hospital between 1st January 2008 and 31st December 2010. Epidemiological data as disease type, number of cases, age and gender of patients were recorded. Cases classified as indeterminate colitis were interpreted as UC or CD considering the dominant clinical, endoscopic and histological characteristics. A sample composed of all patients hospitalized during the year 2010 was complexly analyzed by studying data recorded in the medical observation documents and analyzing the colonoscopy registers and video recordings, noting the following parameters: type of disease and its duration, main symptoms, personal and family history (including possible extra-intestinal manifestations), classical inflammation tests values (ESR, fibrinogen, C-reactive protein - CRP), the extent and severity of UC and Crohn's disease location and behavior according to the Montreal criteria (8).We also calculated the Mayo Clinical score (for UC) (9) and Harvey-Bradshaw index (for Crohn's disease) (10) and endoscopic Mayo score and respectively simple endoscopic score for Crohn's disease (SES-CD) (11). Finally we assessed the degree of correlation of endoscopic scores with clinical scores and biological parameters of inflammation. Data analysis was made using *Microsoft Excel* and *Minitab* software.

RESULTS

The total number of patients (excluding multiple hospitalizations within one year) and the proportion of the two types of disease are illustrated below (fig. 1).

There is a clear predominance of UC, this represents 77% of all cases. Most affected age groups were 25-35 and 35-45 years, totalizing 46.8% of all patients, the prevalence decreasing progressively with age. The mean age of patients was 45.7 ± 15 years, being lower for CD (44.09 ±14.9 years) than UC (46.02 ±15.1 years).

There were no statistically significant gender differences, with a slight predominance of CD in men (87 vs. 80) and UC in women (285 *vs.* 270), the total number being fairly equal (365 women and 375 men).

The number of patients diagnosed with IBD hospitalized more than one day in 2010, which was further taken into account

was 125.

In what regards the disease duration, as shown in the most hospitalized cases are newly diagnosed and those under 2 years from the onset, totalizing 47.5% of all cases (fig. 2).

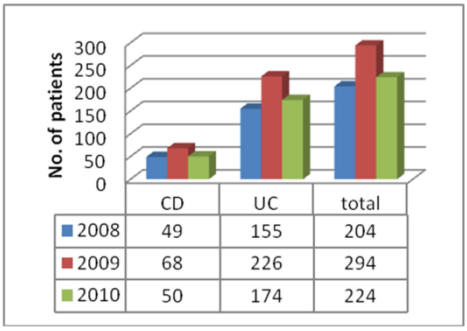

Fig. 1. Number of patients by year and type of disease.

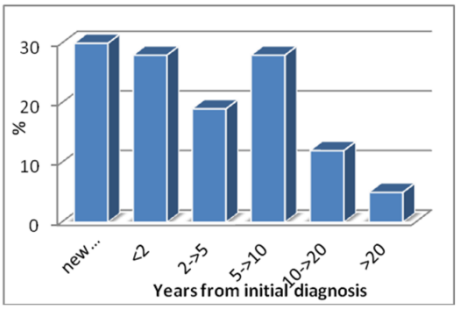

Fig. 2. Duration of the disease at evaluation.

Calculating the age at diagnosis it can be noticed that the maximal incidence in the analyzed cohort was in the age group 35-45 years (35%), followed by age group 25-35 years (27%). The mean age at onset among people with CD was 37.7 ± 13 years and in those with UC 42.8 ± 14 years.

Family history of IBD was reported in only one case (1.19%), whereas colon cancer was found in first-degree relatives in a higher proportion than in the general population (5.95%). Most subjects are non-smokers (57%) or former smokers (20%), the proportion of active smokers being of 23%.

Ulcerative colitis is most commonly (in almost half of cases) presented as left side colitis inflammation up to the splenic flexure) - 46.3%, followed by pancolitis (29.3%) and isolated rectal injury (24.4%). In Crohn's disease colonic involvement accounts for more than half of all cases, isolated ileal disease affecting just 14.3% of subjects. Almost 10% had lesions of the upper digestive tract and almost a quarter had perianal manifestations during evolution (fig. 3).

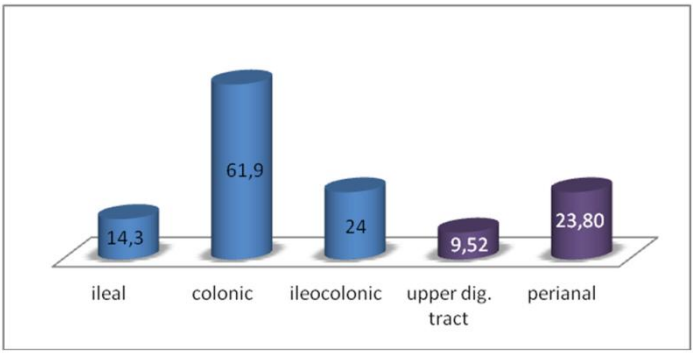

Fig. 3. Crohn's disease classification depending of localisation (%) .

Most cases of Crohn's disease were non-stricturing and non-penetrating (42%), followed by stricturing and penetrating forms (29%); 16.1% are stricturing and 12.9% are penetrating forms.

Mayo clinical UC score shows a balanced distribution of the three forms of severity (37% score 0-3, 33% score 4-6 and 27% score over 7). The calculation of Harvey-Bradshaw index highlights the prevalence of mild and moderate disease forms as follows: 21.4% score<5, 17.8% score 5-7, 50% score 8-16 and only 10.7% score over 16.

The most common extraintestinal manifestations were axial arthritis (ankylosing spondylitis and seronegative spondylarthropathies) in 13.6% of patients, followed by cholestatic syndrome evidenced by increased alkaline phosphatase and ocular manifestations - uveitis, scleritis and episcleritis (fig. 4). Overall, 35.25% of patients had at least one extraintestinal manifestation. Aphthous stomatitis has not been evaluated.

Colonoscopic evaluation was performed in 72.8% of patients. Over half of the subjects had a UC Mayo score of 2 (52.38%) and another quarter score 3 (26.19%). The analysis of simple endoscopic score of Crohn's disease shows mild to moderate forms predominance, with less than 10% being severe endoscopic forms: 28.5% score<3, 23.8% score 4-10, 38.09% score 11-19 and 9.52% score>20.

The correlation expressed by Pearson coefficient (r) of endoscopic Mayo score and SES-CD index with clinical scores and biological parameters of inflammation and P value is presented below (tab. I).

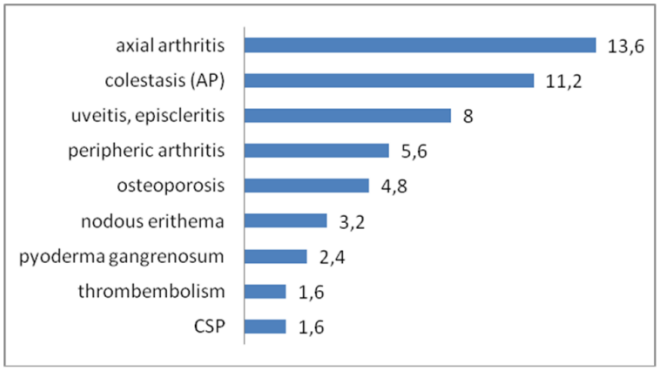

Fig. 4. Extraintestinal manifestations (%).

TABLE I

The correlation coefficient *r* of endoscopic Mayo score and SES-CD with various clinical and biological parameters and the corresponding *p* value

	Mayo endoscopic	P	SES-CD	P
Clinical score	0,727851	0	0,486445	0,109
WBC	0,522412	0,003	0,548403	0,101
ESR	0,562575	0,001	0,483462	0,125
Fibrinogen	0,695638	0,003	0,709565	0,049
CRP	0,711294	0,014	0,684553	0,134

We can notice that Mayo clinical score is a good indicator of the colorectal inflammation (strong correlation) compared to HBI score of Crohn's disease where the correlation with endoscopic activity is moderate. In both diseases, the correlation of the parameters of inflammation with endoscopic scores revealed that CRP and fibrinogen were superior to white blood cells and ESR.

DISCUSSION

One first aspect that stands out in all the 3 years studied is the small proportion of patients with Crohn's disease compared to UC cases (about 23%). This can be considered a particular aspect of the NE region of Romania, the prevalence ratio of CD for the neighboring regions being at least 30% in most cases (4,12). A possible explanation may be that Crohn's disease is under diagnosed, especially the forms located to the small bowel, given the technical and material difficulties of exploring this territory.

The age group distribution of our patients is similar to that reported for Europe, with the predominance of disease in young adults (up to 45 years). Most of the addressed cases are newly or recently diagnosed, probably because the optimal treatment for maintaining remission is reached after few years. Age of onset was higher among patients with UC, which is found in most epidemiological studies. Its average in the studied population is higher than in areas with a high incidence from northern Europe, being around 40 years (13). The second peak of incidence observed in older studies is not present in our cohort. The high age at onset may be a characteristic of the studied region.

Although there are no significant differences in prevalence according to patients' gender, we noticed a slight predominance of CD in men and UC in women, opposite to the trends from other regions (14).

Considering the limits of a retrospective study, we can conclude that extraintestinal manifestations are still in considerable proportion (about one third of the subjects) and in accordance with the literature (15). Osteoarticular manifestations are by far the most frequently encountered, and among them we mention the seronegative spondyloarthropathies and ankylosing spondylitis.

On a review of disease types, if for UC the relatively balanced distribution of the three forms with the predominance of left-side colitis is most often seen, in Crohn's disease we noticed an unusual predominance of colonic and ileo-colonic forms, the number of limited ileal forms being extremely small (14%). We believe that this may represent a feature of CD in this region, although insufficient exploration of the small bowel may contribute to the low proportion of pure ileal forms.

Even though the incidence and prevalence of CD are smaller than in neighboring regions (6), however the disease tends to be more aggressive, 48% of cases presenting an endoscopic score of moderate or severe injury, and the proportion of the stricturing and/or penetrating cases is more than a half (58%).

Another interesting aspect especially seen in UC by comparing the clinical and endoscopic scores is that although more than 70% of cases can be clinically classified as mild or moderate; in endoscopic examination 78% of subjects have Mayo endoscopic score of 2 or 3. Hence it can be concluded that UC is often undervalued when taking into account only the clinical picture, many less symptomatic patients

presenting at least moderate inflammation of the colonic mucosa.

From the biological parameters correlated with intestinal inflammation, C-reactive protein is superior to ESR and white blood cells in the evaluation of inflammation. Serum fibrinogen had surprisingly similar correlation numbers to CRP and thus significantly better than the ESR and WBC. Therefore, this often neglected and abandoned parameter in assessing systemic inflammation remains extremely valuable, especially where CRP determination is not available.

CONCLUSIONS

The main epidemiological features of inflammatory bowel diseases in NE region of Romania are the clear predominance of UC compared to Crohn's disease, older age at onset for both entities, higher tendency of CD to develop intestinal complications (stenosis, fistulas, perianal manifestations) and underestimation of intestinal inflammation by using only clinical and biological parameters. We propose a more careful and systematic exploration of the small bowel (videocapsule endoscopy, ileoscopy during colonoscopy, enteroscopy, CT, enterography) to diagnose Crohn's disease in case of subtle symptoms and in those already diagnosed with colonic forms and also the evaluation whenever possible of inflammation by endoscopic examination with biopsy or at least by the new non-invasive markers (calprotectin, lactoferrin).

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NEWS

TREATMENT OF TB-HIV CO-INFECTION - A BIG CHALLENGE

Tuberculosis (TB) is a major public health problem worldwide. The association of TB with HIV infection induces a higher rate mortality caused by TB complications or by evolution towards AIDS. The treatment of patients TB-HIV co-infected is a big challenge. Even though the deferment in the initiation of antiretroviral treatment (ART) increased mortality, frequently the start of ART is delayed after completing TB therapy as a result of drug interactions between rifampicin and antiretroviral drugs, summation of side effects and low compliance to treatment with many drugs. By analyzing the several clinical trials, Naidoo and his collaborators showed that initiating ART earlier (within 2–4 weeks) after TB treatment initiation has induced reduction in mortality for the TB–HIV co-infected patients with advanced immunosuppression (CD4+ T-cell count <50 cells/μl). Mortality rate for the patients with baseline CD4+ T-cell counts of 50 cells/μl or higher was not influenced by the time of initiating ART in the course of TB treatment. The same analysis showed that an earlier ART initiation in immunosuppressed patients was associated with a higher risk for Immune Reconstitution Inflammatory Syndrome (IRIS). The same researchers showed that early ART initiation in the course of TB treatment for immunosuppressed patients will be based on compromise between increased survival and increased risk of IRIS (Naidoo K, Baxter C, Abdool Karim SS. When to start antiretroviral therapy during tuberculosis treatment? *Curr Opin Infect Dis*. 2013; 26: 35-42).

Cătălina Luncă

THE PLACE OF ULTRASOUND ASSISTED MEGALIPOSUCTION IN THE ROMANIAN OBESITY THERAPY DEDICATED PROGRAMS

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THE PLACE OF ULTRASOUND ASSISTED MEGALIPOSUCTION IN THE ROMANIAN OBESITY THERAPY DEDICATED PROGRAMS (Abstract): Obesity global epidemic and increasing number of super obese people requires a firm stand on the need for medical education of the general population and the need for aggressive therapeutic, but effective, methods, as its consequence is growth rate of comorbidities. The treatment of obesity is a combined treatment worldwide: lifestyle changes, medication and surgery. **The aim** of this article was 1) to highlight the obesity therapy in the world and the Romanian experience regarding bariatric surgery and the UAM (Ultrasound Assisted Megaliposuction) technique, 2) to underline the necessity of implementation of all new methods of obesity treatment in Romania and the possibilities to do that and 3) to determine the knowledge and the compliance of the Romanian obese patients regarding the new surgical obesity therapy technique. **Material and methods:** We conducted a personal single center survey on 30 obese persons in order to demonstrate the necessity of adjusting the obesity treatment in Romania to the European standards and the complete Romanian patient compliance regarding the new surgical obesity therapy technique. Responses to two questionnaires were evaluated. **Results**: Interpretation of the scores of the first questionnaire revealed a significant impairment of life quality. Evaluation of the second questionnaire results revealed that 60% of people studied had morbid obesity. These people may theoretically benefit from bariatric surgery techniques and UAM. **Conclusions**: The implementation of all new methods of obesity treatment in Romania, as an UE member state, is not an illusory idea, but a reality and it should be a matter of national interest. **Keywords**: OBESITY, ULTRASOUND ASSISTED LIPOSUCTION, MEGALIPOPLASTY.

Obesity is often neglected but is a very severe illness by morbid complications associated with it. Obesity global epidemic (GLOBesity) and increasing number of super obese people (BMI> 50 kg/m^2) requires a firm stand on the need for medical education of the general population and the need for aggressive therapeutic methods, but effective, as its consequence is the growth rate of comorbidities: cardiovascular (hypertension, cardiac overload, tachycardia), respiratory (breathing muscle wasting due to increased work needed for ventilation, sleep apnea syndrome), metabolic (glucose intolerance, diabetes, dyslipidemia), mobility (joint strain), endocrine (hormonal disorders) (1, 2) and premature death (3, 4) in this population.

Worldwide, 1 billion adults are overweight and 475 million are obese, leading

to more than 2.5 million deaths per year. In Europe, 260 million adults and 12 million children are overweight or obese. In Romania, 20% of men and 30% of women are obese. Over time were proposed several ways to treat obesity.

The aim of this study was: 1) to highlight the obesity therapy in the world and the Romanian experience regarding bariatric surgery and the UAM technique; 2) to underline the necessity of implementation of all new methods of obesity treatment in Romania and the possibilities to do that both technically, economically and in terms of housing and 3) to determine the knowledge and the compliance of the obese Romanian patients regarding the new surgical obesity therapy technique.

MATERIAL AND METHODS

We conducted a single survey center in Iaşi University Center, included 30 persons, 10 men and 20 women, aged between 16 and 60 years old (36.7+/-13.9) and a BMI between 30.1 and 55, 1 kg/m2 (38.6+/-6.3). The aim of this survey was to underline the necessity of implementation of all new methods of obesity treatment in Romania and to determine the patient compliance regarding the new surgical obesity therapy technique. Responses to two questionnaires were evaluated. This survey was approved by the Ethical Committee of the University of Medicine and Pharmacy "Grigore T. Popa" - Iasi and every person who participated in the survey has signed an informed consent.

The first questionnaire is a "quality of life" questionnaire, proposed by George Parkerson et al. in 1990 (5). The second questionnaire is a personal questionnaire that included 20 questions and try to emphasize knowledge of the studied persons about treatment methods of obesity and their compliance on UAM technique. The questionnaire was accompanied by an information sheet on bariatric surgery techniques and UAM.

For the rest of the data presented, the Internet was the source of information.

RESULTS AND DISCUSSION

Worldwide, the treatment of obesity is a combined treatment. Basically there are three major therapeutic principles: lifestyle changes, medication and surgery (bariatric and plastic surgery).

Surgery is the most effective way to combat morbid obesity after the U.S. National Institutes of Health (6). Surgery is dedicated to only selected cases, when other treatment methods have been exhausted: diet, sports, psychotherapy, anorexogenics, lipase inhibitor, behavioral therapy, therapy for possible endocrine disorders.

After bariatric surgery, the patient may benefit from a series of plastic surgery procedure to reshape the body: liposuction, dermolipectomy, brahioplasty, abdominoplasty.

UAM is a liposuction technique based on the use of ultrasonic energy, which allows the selective destruction or fat emulsification, which is then aspirated. With this method the blood loss are minimal (7,8) and the amount of lipoaspirate that can be eliminated in a single session can reach up to 20 liters. For this reason UAM can be used in the treatment of obesity. Unlike traditional liposuction, UAM selectively destroys only target adiposity and spares support structures of the skin and dermis, such as vessels, nerves, collagen matrix and elastic fibers, thereby preserving the elasticity of treated areas (9).

It has been hypothesized that liposuction should not only have a remodeling role but also a functional one, removal of large

quantities of fat lead to increased lipase activity, leading through a feedback mechanism, to a continuous reduction in body weight, linked to inhibition of hunger center (10, 11, 12). For this reason, liposuction is performed not only for aesthetic reasons but also for therapeutic purposes to improve the quality of life of obese patients.

UAM can be practiced in combination with bariatric surgery in the treatment of morbid obesity, but it may represent a reliable alternative to this.

In Romania, there are about 300,000 obese patients who should benefit from such interventions. Of these, only 4,500 were operated in the last 20 years, over 2,500 by Dr. Catalin Copaescu and his team, at BariXL Center, the only center of excellence in the treatment of obesity and metabolic diseases in Romania, center accredited by specialized forums in Europe and the United States (IFSO- EC).

In Romania, laparoscopic gastric bypass, gastric banding and gastric sleeve procedures are performed in Bucharest, Timisoara, Cluj Napoca, Târgu Mures. In Romania, the first laparoscopic gastric bypass surgery was performed in 2002 (13, 14). Since then, 170 operations of this type were performed. This experience is a priority in Romania. The experience of Romanian surgeons in the laparoscopic treatment of obesity approaches 1,000 patients, thus constituting one of the most important experiences in the Balkans. However, it cannot be said the same about their experience in terms of UAM technique. There are no publications concerning the application of this method in Romania.

Technically and in terms of housing, UAM can be introduced in the Romanian obesity therapy dedicated programs in centers where there is an interdisciplinary group involved in the study and treatment of obesity (table I) and only after surgeons were familiarized and trained in performing this surgical techniques, in order to minimize intraoperative and postoperative complications. The learning curve of this technique is long (15,16), it involves a minimum of 30 patients and it requires careful attention to detail in terms of volume of tumescent solution infiltrated and ultrasound aspiration time (17).

TABLE I

Interdisciplinary group involved in the study and treatment of obesity

NUTRITION AND METABOLIC DISEASES	GENERAL SURGERY	PLASTIC AND RECONSTRUCTIVE SURGERY	PSYCHIATRY PSYCHOLOGY
Bariatric Medicine	Bariatric Surgery	Body reshaping surgical techniques	Treatment of eating disorders
Control of Diabetes		U.A.M.	

From an economic perspective, in the year 2000 it was published a comparative evaluation of systemic effects, efficacy and cost for SAL, PAL and UAM (16). Cost analysis (table II) showed that UAM is the most expensive technique. The choice of the ideal technique depends always on the surgeon. Thus, UAM is the technique preferred by surgeons for patients with morbid obesity, being the only method that allows liposuction of a large amount of adipose tissue in one surgical session, with minimal blood loss (7,8). UAM equipment is indispensable for the complex treatment of obesity and it should be found in all specialized centers.

Nowadays, the public health sector in Romania is going through a difficult period. Not the same can be said about private medical services, which are more present than ever.

The number of private hospitals, increased from 12 in 2006 to 84 in 2012, are able to raise to the necessary UAM application requirements in the treatment of obesity. Thus, the implementation of all new methods of obesity treatment in Romania, as an UE member state, is not an illusory idea, but a reality and it should be a matter of national interest and a priority of the Ministry of Health.

TABLE II

Total cost of a single procedure (1 unit = 500 USD) (adapted from [16])

	Cost (units)		
	SAL	**PAL**	**UAL**
Hospitalization	1	1	1
Surgery room	1	0.75	1.25
Equipment	0.06	1	6
Total	2.06	2.75	8.25

Our survey was approved by the Ethical Committee of the University of Medicine and Pharmacy "Grigore T. Popa" - Iasi and every person who participated in the survey has signed an informed consent.

Interpretation of the scores of the 10 scales of the *Duke Health Profile* questionnaire revealed a significant impairment of life quality both physically (43.3+/-24.4), mentally (55.3+/-40.1) and from the socio-professional point of view (51.6+/-30.5). Most people have a real perception (45+/-37.9) of their general poor health condition (50.06+/-25.6). For this reason we notice a decrease in the self-esteem score (53.3+/-35.7) and depression-anxiety installation (Duke-Ad Score 44.4+/-32.05). Duke Health Profile questionnaire assessment results emphasized once again that obesity is a disease often overlooked due to the lack of pain (Pain Score 38.3+/-33.9) but very severe and associated with morbid complications (Disability Score 60+/-33.2). Resolution of co-morbidities and regaining of self-esteem would improve dramatically the quality of life.

By evaluating the responses to the *20 questions of the personal questionnaire*, we reached the following results: 30% of people had a I^{st} grade obesity (BMI 30.1 -33.8 kg/m2); 30% of people had a IInd grade obesity (BMI 35.6 -38.5 kg/m^2) of which 20% of people show associated comorbidities and 40% of people had IIIrd grade obesity (BMI> 40.1 to 55.1 kg/m^2). In conclusion 60% of people had morbid obesity.

In our study, *40% of people, 30% with I^{st} grade obesity and 10% with IInd grade obesity but without associated comorbidities*, can benefit from medical treatment and only for inefficiency of this therapy after 1 year, we can discuss a UAM technique, not having bariatric surgery indications in these cases. Only half of them (1 man and 5 women) had medical therapy instituted: 1 man and 1 woman had diet associated with sports and drug therapy and 4 women had diet associated with sports and psychotherapy. Although all 5 young people (2 men and 3 women) aged between 16 and 19 years old had a form of depression, only 2 women of 16 and 18 years old have received medical psychotherapy. The medical treatment was of minimum one yearin 4 cases and of 6 months in 2 cases. Its efficiency could be observed only in 3 cases of the 4 who benefited from therapy more than one year. The patient who received this therapy for one year and to whom it proved ineffective, can benefit from UAM. The other two cases should continue therapy for at least one year and

for the 6 cases who remained untreated, it can be established a complex medical therapy for at least 1 year. In case of its inefficiency after one year (30% of people), UAM may be considered for patients without contraindications.

From all the patients, *60% of people with morbid obesity, 20% with IInd grade obesity and associated comorbidities and 40% with IIIrd grade obesity*, can theoretical benefit of bariatric surgery techniques and UAM as conservative therapeutic methods (diet, exercise, medications) are effective in patients who do not have severe forms of obesity (BMI < 35 kg/m^2). In the case of morbid obesity results are poor, but conservative treatment methods must be applied for at least one year before surgical techniques. Obesity surgery is considered today the most effective treatment for morbid obesity. Only 30% of people with morbid obesity had indications for bariatric surgery and 16.6% for UAM. Contraindications were represented by depression (23.3%), varices (10%), inguinal hernia (6.6%), and weight > 140 kg without weight loss diet (6.6%), effective gastric banding (3.3%), diagnosis of less than 5 years (3.3%).

In conclusion, assessment of the second questionnaire results revealed that in the moment of the study only 30% of people with morbid obesity had indications for bariatric surgery and 16.6% for UAM. In case of inefficiency of the one year medical therapy in the case of the 30% people with no morbid obesity, 46.6% had indications for UAM.

With regard to information held by 30 people, only 36.6% (age 16-29) had summary information about bariatric surgery and only 13.3% (age 25-36) about UAM. The source of information was represented by the physician in 16.6% cases and by Internet in 33.3% cases. Given this situation, people compliance on this technique was very low 16.6%. The people compliance on this technique has increased 3.6 times after reading the information sheet attached to the questionnaire concerned.

All patients have shown interest in being included in a therapeutic program for obese patients, including: diet, sports, psychotherapy, bariatric surgery, UAM, body shaping and other techniques.

It should be noted that our survey, being accomplished in a single center, can be only a mere guide, thus it is necessary a multicenter study to define the result.

CONCLUSIONS

Obesity is a serious medical condition. Co-morbidities related to obesity are responsible for decreased life expectancy by nine years to women and by 12 years to men. In Romania, one of two people is overweight and one of four is obese.

Treatment of obesity should be a combined treatment: diet, sports, psychotherapy, drug therapy, bariatric and plastic surgery. Surgery is the most appropriate therapeutic option for patients with morbid obesity.

UAM has metabolic effects and significant morphological effects, improving quality of life of patients who maintained a constant body weight (12). UAM can be practiced both in combination with bariatric surgery in the treatment of morbid obesity, but it may represent a reliable alternative to this, when patients refuse it. In addition, UAM represents an attractive alternative for patients with BMI 30-40 kg/m^2 and surgical recommendation, who are not candidates for bariatric surgery and are unresponsive to other forms of therapy. UAM is also a solution in the case of juvenile obesity (young people up to 18 years old, not having a bariatric surgery indica-

tion, for which other treatment methods have failed).

The experience of Romanian surgeons in the laparoscopic treatment of obesity is one of the most important experiences in the Balkans, but there are no publications concerning the application of UAM method in Romania, although it has become in Europe and the United States one of the basic techniques of body reshaping methods arsenal since 2001.

For the UAM technique to become a reality also for obese patients in Romania and for obese patients to be included in a specific therapeutic program, several conditions must be taken in consideration:

- creation of centers adequately equipped in the existing private hospitals, with an interdisciplinary group involved in the study and treatment of obesity (nutritionist, general surgeon, plastic surgeon, anesthesiologist, psychologist);

- organization of UAM learning technique trainings for surgeons;

- creation of a grant program for patients with indications for operation, given the precarious pecuniary situation of the population;

- organization of information programs both for population and for family physicians (brochures, radio, TV, internet, conferences, symposia, etc.).

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STATISTICAL ANALYSIS OF SUICIDE CHARACTERISTICS IN IASI COUNTY

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STATISTICAL ANALYSIS OF SUICIDE CHARACTERISTICS IN IASI COUNTY (Abstract): A prospective study intended for statistic analysis of suicide events occurring in 2004-2009 period, in Iasi County, was performed. Specific data emerged from the conventional investigation, focusing on the sex, age, seasonality, marital condition, occupation status, blood alcohol concentration, religion adherence, and previous suicide attempts of the persons who committed the lethal self-aggression. The results showed a males: females (M:F) ratio of 4.13:1, central tendency to suicide towards the 46 years, a mean age of the self-murderers series of 45 years, while the most frequent age was 49 years. The interquartile range expanded from 33 to 56 years. The rural: urban (R: U) ratio was 1.38:1, whereas a statistically-significant seasonal variation was found in villages. Suicide events occurred more frequently around the Easter and Christmas, whereas the orthodox Christian believers seemed to suicide more than Catholics. Additionally, a correlated analysis, based essentially on data provided by the local Institute of Legal Medicine and Psychiatry Hospital, offered a comprehensive understanding of the mental state of the self-murderers and their psychiatric profile. **Key words**: SUICIDE, SEASONALITY, ALCOHOL, MENTAL DISEASES, RELIGION

The human history knows several forms of deliberate killing, the main types focusing on the well-known human-animal linkage, the relation of a person with his fellow(s), or the relationship of a person with himself. Not exhaustively, the first was named food-motivated act, defending or entertainment, the following murder or self-defense, while the third as suicide or martyrdom. However, the above inventory can modify according to the social, moral, ethical, religious and legal values or standards taken into account.

All the above exposed killing types are important and challenging from the scientific standpoint, being continuously investigated to make clear their causes, level of significance, frequency, and consequences. However, of all killing forms, suicide is the most investigated phenomenon. The rationale sustaining the high received interest comes from the fact that it is a complex act, with numerous influences, including personal (biological, psychological, psychiatric), social, economic, political and cultural components (1-3).

Consequently, suicide-related up-to-date studies are needed to periodically be performed to investigate as much as possible the previous "inner state" of a population to make correct decisions on the appropriate prophylactic means to be consid-

ered for suicide prevention.

In this work, we focus on a deliberate killing involving suicide-related phenomena. The statistic analysis over a local population was performed in order to evaluate a series of variables such as sex, age, marital status, occupation category, seasonality, religious adherence etc. in order to well establish their influence on the suicidal act and to be further used to make correct decisions on the suitable prophylactic methods. Apart from the above described analyzed variables, the study sought also for a comparison between rational and pathologic suicide to underline possible correlations between the mental healthiness, inner psychological state and the lethal act.

The analysis is a prospective one and is focused on the suicidal events performed in Iasi County, a representative region of the North-East part of Romania, comprising a population of 816 910 persons (according to the 2002 census) and a relatively homogenous religion-related distribution (4).

MATERIAL AND METHODS

This work has taken into account a six-year period, i.e. 2004-2009, and comprised 679 suicidal events that were issues of the local Institute of Legal Medicine, Clinical Hospital of Psychiatry "Socola", and Register Office of Iasi County, Romania. A correlated analysis was needed because despite the Romanian legislation imposes forensic autopsy of the self-murderers by which important medical information focused on the death's causes are obtained, the requirements to clearly establish the psychically integrity of the self-murder in the moment he acted as such remain unfulfilled.

The statistical analysis was based on descriptive parameters like median, mean, mode values, and standard deviation interquartile range. The frequencies were analyzed by using the Pearson's chi-square test of "goodness to fit", with an imposed 0.05 limit of probability.

RESULTS

During the six years studied, 17 154 suicides were committed in Romania, among them 679 cases (3.96 %) occurring in Iasi County, in a population that represents 3.77 % from the Romanians. The annual average suicide rate, calculated for the six years, was 13.85 per 100,000. Suicide distribution, on the sex, rural and urban media, and age categories, is displayed below (tab. I).

TABLE I

Annual average suicide rate on the sex, area, and age category

Sex / Area / Age category	Number of cases (six years)	Total inhabitants (Iasi County)	Annual suicide rate (to 100,000 inhabitants)
Male	540	401,516	22.41
Female	139	415,394	5.58
Rural	410	429,124	15.92
Urban	269	387,786	11.56
< 20 years	50	235,179	3.54
20 – 39 years	200	261,744	12.73
40 - 59 years	301	183,794	27.29
> 59 years	128	136,193	15.66

As table I points out, the most encountered suicide cases were found to males (M:F = 4.13:1), and emerged more from the rural area (R:U suicide rate ≈ 1.38:1), while the most affected population segment was 40 - 59 years.

Absolute number of the self-murderers has increased yearly, in spite of apparent Gaussian distribution of the urban suicide events (fig. 1).

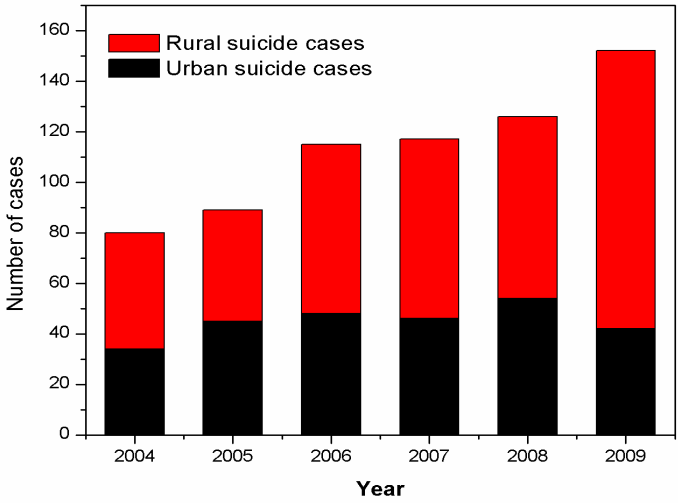

Fig. 1. Year-dependent distribution of suicide events

According to Table II, the central tendency to suicide was towards the 46 years (median parameter), mean age of the self-murderers series was 45 years, while the most frequent age was 49 years (mode parameter). The middle fifty, i.e. interquartile range, expanded from 33 to 56 years. As it can also be observed, men suicide slightly earlier during their life as compared to women, whereas the age difference was of only 1-2 years.

The number of season-distributed self-murderers and statistical significance related to urban and rural media are summarized in Table III. Season variation is significant only in rural medium where the highest value is attained in autumn period (29 %), whereas the occurrence of such events is less observed in winter (17 %). However, for the total cases, the highest value of suicide events moved to summer (28.57 %).

TABLE II

Age descriptive parameters

Age parameter	Global (years)	Male (years)	Female (years)
Median	46	46	48
Mean	45	45	46
Mode	49	48	49
Interquartile range (Q_3-Q_1)	56-33	55-34	60-31

TABLE III
Seasonality of suicide events and statistical significance

Season	Total			Urban			Rural	
	Observed	Expected		Observed	Expected		Observed	Expected
Spring	178	170		70	68		108	102
Summer	194	170		81	68		113	102
Autumn	182	170		63	68		119	102
Winter	125	170		55	68		70	102
p-value	$p < 0.001$			$p = 0.14$			$p < 0.01$	

As regarding the family status of the persons who committed suicide, from the 679 cases investigated, marital status was found for only 385. Among them, 200 persons were married, 139 un-married, 22 widowers, whilst 11 cases were noted as concubinage.

We also analyzed some data related to alcohol intake at the moment the self-murderers committed the lethal act. Accordingly, from the 679 suicide cases, 288 persons consumed alcohol (42.42 %), 267 being men and 21 women. Therefore, taking into account all cases, i.e. 540 men and 139 women, 49.44 % out of men and 15.11 % out of women consumed alcohol before suicide. As regarding the blood alcohol level, 78 % self-murderers from the 288 cases had a blood alcohol concentration higher than 1 g ‰.

Due to the underreporting of suicidal deaths in the registry system, the socio-economic status was available just for 358 persons, 23.46 % being employed, 32.69 % retired, 32.4 % unemployed or without occupation, and 11.45 % pupils or students.

From the education standpoint, data for 365 cases were available. The analysis showed 9.04 % persons with university education, 66.57 % with high school education, 23.83 with primary education, and 0.55 % without school instruction. However, these data were not complete and therefore could not be compared with their corresponding items in the population of Iasi County to draw out relevant conclusions.

Referred to all studied cases, a medical record of mental illness was found for 189 self-murderers (27.83 %), 80 persons being registered once, 32 twice, and 14 were found with 3 records summing 18.55 % from all suicide cases. The most involved mental diseases were depression (35.45 %), alcoholism (31.74 %), schizophrenia (6.88 %) and dementia (5.29 %).

On the other hand, we found 92 cases (13.55 %) of previous suicide attempts, but the exact number of attempts was generally found to be registered only for less than 3 attempts, the others being noted as "multiple". Referred to their counterparts, i.e. 540 men and 139 women, 12.22 % (66 cases) occurred for men, whereas women represented 18.7 % (26 cases). In addition, from all the 92 cases, 46 self-murderers (50 %) had a medical record of mental illness at "Socola" Hospital.

We investigated as well the religious adherence of the self-murderers in a Romanian region where orthodox Christians are majorities (92.55 %) whereas the second important religious minority is Catholic (4.94 %). From the 354 cases whose religious confession was found, 341 (96.32 %) belonged to Orthodox Church, 11 (3.1 %) to Catholic Church and 2 (0.56 %) were non-religious. Mirrored in their corre-

sponding items in the total population of Iasi County, the obtained data showed a slightly increased suicide rate of the Orthodox Church's believers.

We observed that close to the main Christian holydays, i.e. Easter and Christmas, the suicide incidence increased. Thus, taking for analysis one month (2 weeks around Easter day plus 2 weeks around Christmas day) per year, 69 self-murderers were recorded for the entire analyzed period. Therefore, for the cumulated six months, the monthly suicide rate was 11.5. Comparatively, for all the six years, monthly suicide rate was 9.43.

DISCUSSION

For our target population, the suicide rates are higher in villages, with a peak in autumn and a gap in winter. It is somehow a contradictory result if a prophylactic role of the work is considered. Since the work activities in rural areas are highly increased in autumn and at low level in winter, in order to comprehend the results of the study we should give credit to other explanations such as season-related physiological alterations. Indeed, it is reported that sunlight, for instance, with higher activity in spring, summer and autumn, through interactions with brain serotonin, is able to trigger increased impulsivity and suicidal behaviors (5-8). In other words, winter acts as a prophylactic factor by providing a sort of "hibernation" state leading to the amelioration of the inner psychological pain by "restarting" the person's emotional sphere towards a new possible successful beginning in the next year.

Alcohol intake is a frequent precursor to suicide, and alcoholics are at a high risk. Generally, studies on suicide showed that alcoholics account for 20 % - 40 % of all suicide events (9). Therefore, the alcohol-related data emerged from our study fitted fairly well with specific literature data, taking into account a value of 42.42 % obtained for both alcoholics and non-alcoholics self-murderers that consumed alcohol just before suicide event. To alcoholics, the alcohol, by itself, is not the suicide's cause, but the social and familial tensions, disruptions, isolation, and associated depression, emerging due to alcohol long-term misuse, are the real causes of the alcohol-influenced suicide. Also, the relatively high blood alcohol levels (> 1 g/l) can be correlated with their need to fight the inner fear and maybe to stop conscience accusing them against consecutive consequences of this act on, for examples, family, friends etc.

Only 23.46 % of investigated cases were represented by the employed people, pointing out the protective role of the work. In this context, it is possible that an indirect cause for the pupils/students suicide to emerge also from family work-related economical problems.

We also observed that women are more susceptible to suicide attempts as compared to men, the women-to-men suicide attempts ratio being 1.5: 1. This result follows the profile of the general trend of the globally encountered behavior of suicide attempts. However, 50 % of persons with suicide attempts were observed to have at least one psychiatric recording, emphasizing how important is the proper mental state in limit conditions.

Focusing on the appropriate medical recordings of the local psychiatric hospital, an increased percentage (about 30%) of self-murderers had psychic deficiencies. For these patients, psychiatric healthcare seemed to be insufficient to solve only by

itself the most profound problems. Moreover, the psychiatry-suicide relation takes out collateral questions. For instance, what if another important part of the self-murderers suffered psychically and no psychiatric recording was made throughout their life? Or, equivalently, how many self-murderers, viewed mentally as healthy people, had been suffered actually by hidden minor-to-medium mental problems or, at least, had been combined a minor mental illness with a medium-to-high psychological problem ? Furthermore, could the suicide thoughts be truly encountered also to important percentages of normal people? Is there an equilibrium barrier between life and death desire so fragile that can break down for many normal people? We assume there are few normal people able to recognize the presence of such thoughts because of the increased risk to degrade their social, professional, and familial image.

Finally, the increased suicide rates around important Christian holidays can be explained by considering an exacerbation of certain feelings triggered by the meaning of these special days where sole people, predisposed generally to depression, become more vulnerable to suicide. Also, the connection of the suicide date with central Christian holidays can be intended for the amplification of an accusing message against those considered guilty for their death.

CONCLUSIONS

Applied to Iasi County, our study showed an average suicide rate of 13.85/100,000. Also, the statistical analysis pointed out that males suicide much more than females (M:F = 4.13:1), whereas cases emerged from rural regions are more frequent as compared with urban areas (R:U suicide rate ≈ 1.38:1).

The central tendency to suicide was towards the 46 years, mean age of the self-murderers was 45 years, while the most frequent age was 49 years.

The study underlined that two groups present high suicide risk: men and middle-aged population.

Alcohol, season, period of day, and main Christian holydays were shown to trigger the suicide behavior. Moreover, the analyzed suicide events were shown to comprise important percentages of psychiatry-related cases, mental problems playing a decisive role to view suicide as an escaping way, possible from the "prison" of psychic torture.

The suicide prophylaxis should be more focused on the higher-risk population groups and should supplementary address to the risk factors affecting all groups of self-murderers. Probably, cooperation with local Churches to prevent this hardly predictable phenomenon and, further on, to assist the affected families would be highly helpful.

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NEWS

NEW TOOLS FOR FIGHT THE FLU

A new research was developing for construction of computer-designed proteins to fight the flu. The researchers have suggested that these proteins found in nature, but that do not normally bind the flu, can be constructed via computer modelling to act as broad-spectrum antiviral agents against a variety of flu virus strains.Hemagglutinin enables the virus to attach to the surface of cells lining the airways. After virus penetration into cells, under the influence of acid intracellular pH, the hemagglutinin change shape and thus allows initiating viral RNA replication. By binding the hemagglutinin in a very specific orientation, like a key into a lock, the computer-designed proteins block his shape change and viral invasion of cells. These inhibitor proteins bind to all types of the Group I Hemagglutinin, a group that includes H1, the pandemic H2 and avian H5 strains.The scientists could use protein interface design to generate a variety of proteins that would block pathogen-host cell interaction.

1. Timothy A Whitehead, Aaron Chevalier, Yifan Songrille Dreyfus et al. Optimization of affinity, specificity and function of designed influenza inhibitors using deep sequencing. *Nature Biotechnology*, 2012; DOI: 10.1038/nbt.2214.
2. University of Washington (2012, June 1). Computer-designed proteins programmed to disarm variety of flu viruses. *ScienceDaily*. Retrieved June 16, 2012, from http://www.sciencedaily.com- /releases/2012/06/120601231756.htm.

Cătălina Luncă

FUNCTIONAL STATUS OF CHRONIC RENAL REPLACEMENT THERAPY IN ELDERLY PATIENTS – COMPARISON BETWEEN HEMODIALYSIS AND PERITONEAL DIALYSIS

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FUNCTIONAL STATUS OF CHRONIC RENAL REPLACEMENT THERAPY IN ELDERLY PATIENTS – COMPARISON BETWEEN HEMODIALYSIS AND PERITONEAL DIALYSIS (Abstract): Nowadays, nephrologists are confronted with an increasing number of elderly patients diagnosed with end-stage renal disease (ESRD) in need of dialysis. The benefits of renal replacement therapy are uncertain in this group of patients. Most studies show that the quality of life and survival of elderly dialyzed patients are worse than in younger patients because of multiple comorbidities. Functional status is an important aspect of the quality of life, a strong predictor of survival and a determinant of the health care systems costs. **Method:** In the present research, we compare the change in the functional status – appreciated with the MDS-ADL score – in a cohort of hemodialyzed versus peritoneal dialyzed elderly patients (>65 years) during a period of 3 years after starting dialysis treatment. **Results:** At the time of initiating dialysis, the median minimum data set of activities of daily living (MDS-ADL) score in hemodialysis (HD) elderly patients was 4.04 and in continuous ambulatory peritoneal dialysis (CAPD) group was 6.27 (the median MDS-ADL score at the moment of starting dialysis was statistically significant higher in peritoneal group than in hemodialysis elderly group). **Conclusions:** The results conclude that elderly treated with peritoneal dialysis have a better evolution of functional status than hemodialyzed elderly patients do. **Key words:** END-STAGE RENAL DISEASE, ELDERLY, HEMODIALYSIS, PERITONEAL DIALYSIS, FUNCTIONAL STATUS, MDS-ADL SCORE.

In the last two decades, an increasing number of elderly patients diagnosed with end-stage renal disease (ESRD) have started dialysis (1,2). In most of developed countries, more than 40% of the patients initiating dialysis are aged over 65 years old (3). In 1995, in Europe, 52% of the patients who begun dialysis were more than 60 years old (4).

In 2008, data from USRDS (United States Renal Data System) shows that in the USA – the country with the highest incidence of CKD (chronic kidney disease) – the median age of dialysed patients was

64.4 years (5). Comparative to 1998 (when elderly represented 47% from all dialysed patients (5)), the raise of ESRD incidence in persons >65 years was of 11%, comparative with 2.4% in 20 – 44 years age-group and 6.1% in 45 – 64 years age-group (5).

The benefits of dialysis in elderly adults are uncertain (6,7,8). Mortality in the first year after the initiation of dialysis exceeds 35% among patients older than 70 years and exceeds 50% among patients older than 80 years (8). Clinical trials comparing survival of elderly uremic patients treated with dialysis versus conservative management show that the prognostic depends less of the patient's age, but more of the number of comorbidities and early referral to a nephrologist (1,6,7,9). Most of these studies concluded that, in the presence of multiple comorbidities, survival is identical in both types of treatment (1,9).

The optimal modality of dialysis in elderly is a matter of debate, as well (7). Most studies comparing hemodialysis (HD) and peritoneal dialysis (PD) in elderly – in terms of quality of life or survival – failed to demonstrate the superiority of one therapy versus the other (3,8).

Functional status – the ability to perform activities such as walking, bathing, dressing, getting out of bed, and using the toilet – is an important aspect of the quality of life in elderly patients, a strong predictor of survival and a determinant of the health care system costs (6,8,9,10). In our clinical research, we compare the change in functional status – appreciated with the MDS-ADL score – in a cohort of hemodialysed versus peritoneal dialysed elderly patients (>65 years) during a period of 3 years after starting renal replacement therapy (RRT). Limited data regarding this comparison is present in the literature, most studies being performed in nursing homes. As in Romania, nursing homes are practically absent and the management of elderly patient needs family collaboration with the nephrologist, we consider useful to evaluate the change in functional status in both types of dialysis therapy in order to appreciate the optimal modality of treatment.

MATERIALS AND METHOD

During January 2006 – December 2007, nondiabetic patients diagnosed with ESRD who initiated dialysis and were > 65 years old at the time of initiation were included in the study. The follow-up period was for 3 years from the moment of the dialysis initiation and the study ended in December 2010. The patients were classified as HD or PD treated patients only after 3 month of stable dialysis therapy.

Functional status of the patients have been measured at 6-months interval using **MDS-ADL** score (**M**inimum **D**ata **S**et of **A**ctivities of **D**aily **L**iving) (tab. I). Originally developed by *Sidney Katz* in 1963, the Index of Independence of Activities of Daily Living, or Index of ADL, is one of the oldest and most widely used health measures (11). Over the years, *Katz*'s system has been modified and expanded (2).

Each ADL item has six possible categories of response representing observations made by a trained clinical professional over an assessment period of seven days. Assessments based on observed rather than self-reported physical function were considered more reliable.

The categories of response for the MDS-ADL self-performance scores were ranged from '0' (total independence) to '4' (total dependence). The MDS Long Form ADL scale is a sum of the responses to all the seven individual ADL items and has a

range from '0' to '28' (higher scores indicating a greater dependence). The score modality in MDS-ADL questionnaire is: **0** – total independence or no or little help with an activity; **1** – supervision provided 3 or more times during last 7 days; **2** – limited assistance by another person (medical staff, family member) with the patient highly involved in activity; **3** – extensive assistance by another person (medical staff, family member) with the patient performing part of the activity; **4** – total dependence and full participation of another person (medical staff, family member) in activity during the entire 7 days. Evaluation of MDS-ADL was performed at the moment of dialysis initiation and then at 12-months intervals. Statistic validation of data was performed using rank *Mann – Whitney, Z and T tests*.

TABLE I
The items included in the MDS-ADL score

ITEM	DESCRIPTION
Eating	How the patient eats and drinks, including other means of intake of nourishments (tube feeding, gastrostomy).
Dressing	How the patient puts on, fastens, takes off all items of street clothing.
Locomotion	This includes how the patients move between locations in their room and corridor outside their room.
Personal hygiene	This relates to how personal hygiene is maintained, including combing hair, brushing teeth, washing and drying face and hands; baths and showers are excluded from this item.
Bed mobility	This includes how a patient moves and turns his body position while in bed.
Transfer	This includes how a patient moves between surfaces such as a bed and chair.
Toilet use	How a patient uses a toilet, commode, bedpan or urinal and transfer on and off the toilet.

RESULTS

Between January 2006-December 2007, 65 elderly ESRD patients have begun dialysis therapy: 18 on CAPD and 47 on HD. From all new patients who started CAPD in the mentioned period, elderly represented 47.36%. The median age was 73.16 years. Ischemic nephropathy was the main primary renal diseases (66.7%); in 6 patients it was associated with other cause of renal failure. From all new hemodialyzed patients, elderly represented 19.18%. The median age was 70.53 years. The main causes of renal failure were ischemic nephropathy (29.78%) and tubule-interstitial chronic nephropathy (29.78%). ESRD etiology was mixed in 6 patients. At the time of initiating dialysis, the median MDS-ADL score in HD elderly patients was 4.04 (fig.1): 10 patients (21.27%) had a MDS-ADL score ≥ 7, needing extensive assistance from a family member in order to perform usual daily living activities; 20 patients (42.55%) had a MDS-ADL score between 1-7; 17 patients (36.17%) had a MDS-ADL score 0, being completely able

to perform daily activities with no help or minimal help from another person. In CAPD (continuous ambulatory peritoneal dialysis) group, at the time of initiating dialysis, the median MDS-ADL score was 6.27 (fig. 1): 7 patients (38.88%) had MDS-ADL score ≥ 7; 7 patients (38.88%) had MDS-ADL score between 1-7; 4 patients (22.22%) had MDS-ADL score 0.

After starting dialysis, evaluation of MDS-ADL score was performed at 6 months intervals. Obvious, as the time passed, the number of patients in which the MDS-ADL score was evaluated changed because of death reported in both groups (fig.2, fig.3).

In the HD group, the median MDS-ADL score sharply increased in the first 6 months. After that, we recorded a stability of the score until the end of the first year. In the second year and in the third year, the MDS-ADL score increased progressively until the end of the study (tab. II). The raised of the score was with 2.22 points. As the evaluation of MDS-ADL score was performed only in patients who remained alive and presuming that the patients with the highest scores died until the end of the study, the 2.22 points increase of the score seems important.

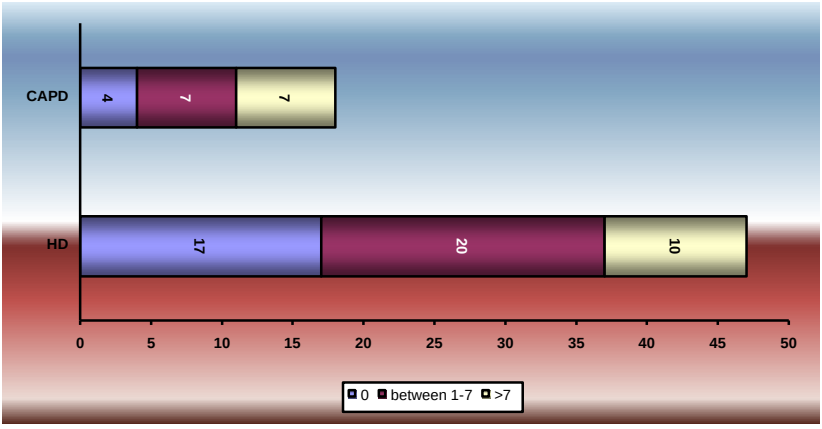


Fig. 1. MDS-ADL score at the time of dialysis initiation in the two groups of elderly patients

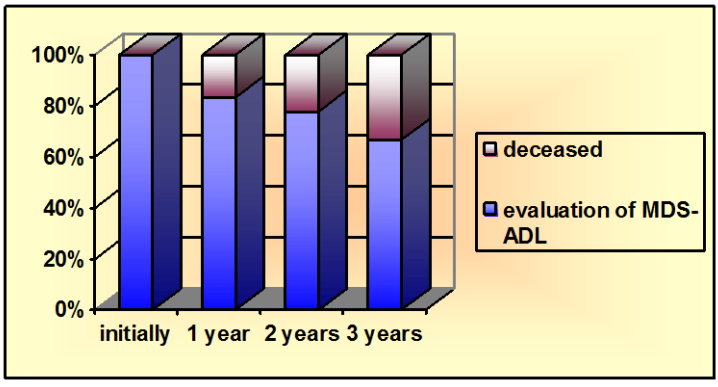


Fig. 2. Number of CAPD patients in which the MDS-ADL questionnaire was performed

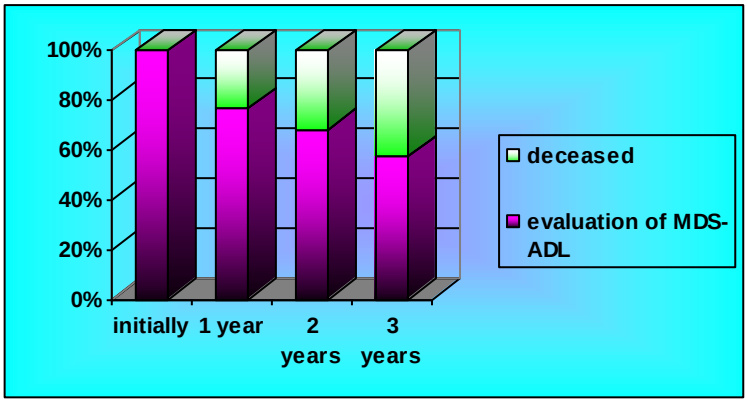


Fig. 3. Number of HD patients in which the MDS-ADL questionnaire was performed

TABLE II

Evolution of MDS-ADL score in hemodialyzed elderly group

ADL score	0	6 months	12 months	18 months	24 months	30 months	36 months
0	17	12	12	10	9	9	9
1-7	20	14	16	15	15	13	11
> 7	10	11	8	9	8	8	7
Deaths	0	10	11	13	15	17	20
Median ADL score	**4.04**	**4.40**	**4.27**	**5.32**	**4.97**	**6.16**	**6.26**

In peritoneal dialysis group, the median MDS-ADL score decrease significantly in the first 18 months from the dialysis initiation, proving a substantial improvement of the functional status. After that, the median MDS-ADL score increased, but more slowly than in hemodialysis elderly group (tab. III).

TABLE III

Evolution of MDS-ADL score in peritoneal dialysis elderly group

ADL score	0	6 months	12 months	18 months	24 months	30 months	36 months
0	4	5	5	5	5	5	5
1-7	7	6	6	6	5	4	3
> 7	7	5	4	3	4	4	4
Deaths	0	2	3	4	4	5	6
Median ADL score	**6.27**	**3.75**	**3.40**	**3**	**3.14**	**3.46**	**3.83**

DISCUSSION

When dialysis was started, the proportion of elderly patients completely independent (score 0) or partially dependent on help from another person (score 1-7) was significantly lower in peritoneal dialysis

group (fig.4) – *T-test*.

The median MDS-ADL score at the moment of starting dialysis was statistically significant higher in peritoneal group than in hemodialysis elderly group (*Mann-Whitney rank test*) (fig. 5). These results concluded that elderly patients with debilitating comorbidities that reduce the ability to perform daily minimum activities were preferentially oriented to peritoneal dialysis. Peritoneal dialysis, a procedure that is considered more "physiological" than hemodialysis was chosen to protect the patients from the potential risks of hemodialysis. As in Romania, homedialysis or nursing homes (where a specialized person performs dialysis) are not available as in developed countries. The choice of hemodialysis in patients with severe comorbidities becomes an important problem even if we see it only from the point of view of patient transportation to and from the hemodialysis center.

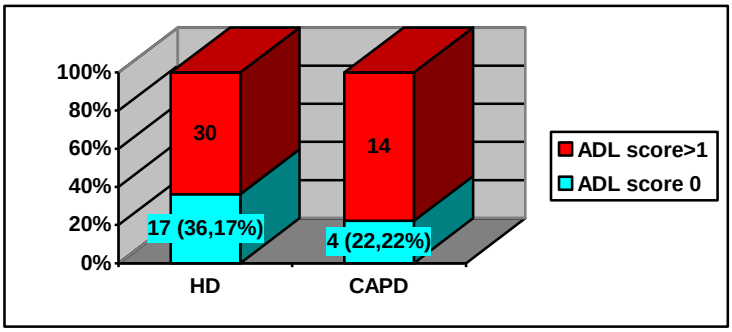
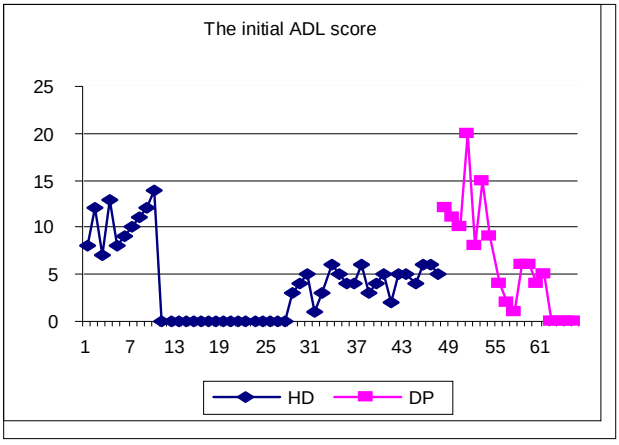


Fig. 4. Percent of the patients with preserved independence in performing daily activities (MDS-ADL score zero)



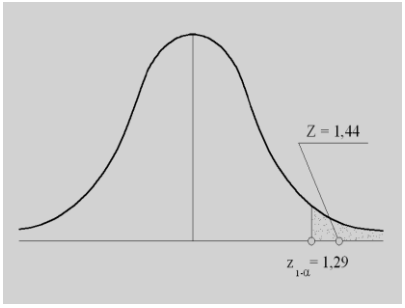


Fig. 5. The median MDS-ADL score was statistically significant higher in CAPD group than in HD group, when dialysis was started

Statistic analysis of the evolution of MDS-ADL score during the study revealed that, in both groups, the MDS-ADL score increased in the third year of the

study. This increment was statistically slower in PD group than in HD group (*Mann-Whitney rank test*) (fig. 6). The success of this attitude (to include in peritoneal dialysis elderly patients with severe initial functional dysfunction) requires patient's family support. In our CAPD elderly group, 12 patients (66.66%) had full assistance from a family member and 6 patients (33.33%) had partial help (one member visiting the patients at least once daily).

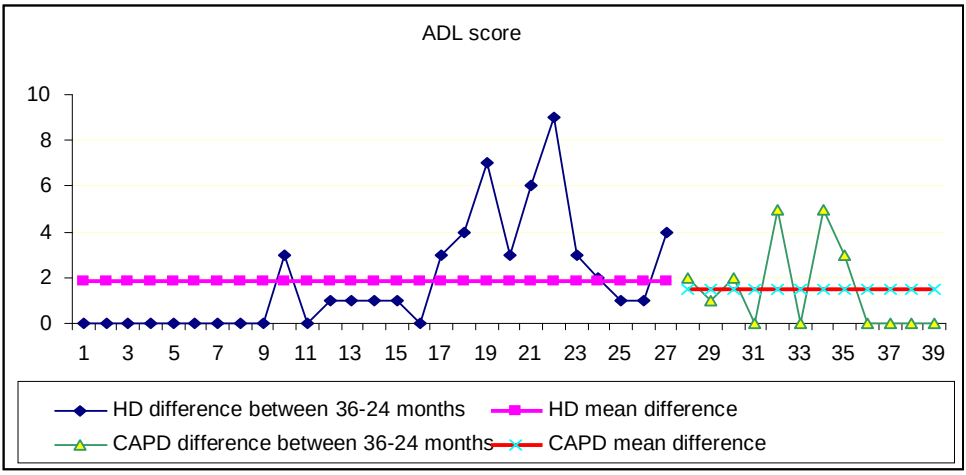


Fig.6. In the third year of the study the MDS-ADL score increased more rapidly in the HD group

CONCLUSIONS

This study – part of a larger research on comparison of peritoneal dialysis versus hemodialysis in elderly – is observing the evolution of functional status for 3 years after starting dialysis. The results conclude that elderly treated with peritoneal dialysis have a better evolution of functional status than hemodialyzed elderly patients do. Protecting vascular stability, lower incidence of severe and/or acute complications, dialysis access and dialysis dose easier to obtain, all of these are possible explanations of the advantages of peritoneal dialysis versus hemodialysis in elderly and of the results obtained in the above study.

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NOUTĂȚI

NOILE VALORI ALE PUNCTELOR DE RUPTURĂ STABILITE DE CLSI ŞI SENSIBILITATEA TULPINILOR DE *ENTEROBACTERIACEAE* PRODUCĂTOARE DE BLSE

În 2010, Clinical and Laboratory Standards Institute (CLSI) a redus valorile punctelor de ruptură funcție de care se stabileşte sensibilitatea la unele cefalosporine şi aztreonam pentru speciile de *Enterobacteriaceae* şi a eliminat necesitatea de a efectua screening-ul pentru tulpinile care produc beta-lactamaze cu spectru extins (BLSE) şi testele de confirmare.Wang et al. au efectuat un studiu prin care au incercat să evalueze cât de multe dintre tulpinile producătoare de BLSE ale trei cele mai comune specii de *Enterobacteriaceae* (*Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis*) sunt declarate sensibile la antibiotice beta-lactamice conform noilor valori ale punctelor de ruptură. Sensibilitatea la antibiotice au testat-o prin metoda diluțiilor în mediu solid şi interpretarea au realizat-o funcție de noile valori ale punctelor de ruptură, iar genele care determină producerea de BLSE au fost amplificate prin PCR şi detectate prin secvențiere. Rezultatele au arătat că toate tulpinile de *Enterobacteriaceae* producătoare de BLSE au fost rezistente la cefazolin, cefotaxim, ceftriaxonă, dar un număr substanțial de tulpini producătoare de ESBL aparținând speciilor de *Escherichia coli* şi *Proteus mirabilis* ar putea fi raportate ca fiind sensibile la ceftazidim, cefepim şi aztreonam în conformitate cu noile valori CLSI. Wang et al au explicat rezultatele prin prezența unei prevalențe înalte a tipului CTX-M a BLSE. (Wang P, Hu F, Xiong Z. Susceptibility of extended-spectrum-beta-lactamase-producing *Enterobacteriaceae* according to the new CLSI breakpoints. *J Clin Microbiol* 2011; 49(9): 3127-31).

Cătălina Luncă

COMPARATIVE EVOLUTION OF HEMODIALYSIS *vs.* PERITONEAL DIALYSIS IN ELDERLY

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COMPARATIVE EVOLUTION OF HEMODIALYSIS VERSUS PERITONEAL DIALYSIS IN ELDERLY (Abstract): The prevalence of treated patients with end-stage renal disease (ESRD) has considerably increased in the last decade mainly in the elderly age groups. The type of optimal mode of dialysis therapy in elderly is difficult to establish and most studies comparing hemodialysis and peritoneal dialysis in elderly failed to demonstrate the superiority of one therapy versus the other. The present article uses Charlson Co morbidity Index to compare evolution of an elderly population in HD versus PD. **Material and methods**: Nondiabetic patients (> 65 years) who initiated dialysis therapy for ESRD during January 2006-December 2007. **Results**: In HD group, the median CCI calculated 6 month before initiating dialysis therapy was 5.87, with limits between 3 and 11. At the time of initiating dialysis, the median CCI was 6.55 (limits 3-11). In peritoneal dialysis group, CCI evaluated 6 months before initiating dialysis had a median value of 7.38, with limits between 3 and 11. At the time of initiating dialysis procedure, the median CCI was 7.72. **Conclusions**: This study demonstrates that PD is, in the majority of situations, a better option for dialysis in elderly. **Key words**: END-STAGE RENAL DISEASE, ELDERLY, HEMODIALYSIS, PERITONEAL DIALYSIS, CHARLSON COMORBIDITY INDEX.

Incidence of chronic kidney disease has considerably increased in elderly (> 65 years) as a result of population ageing, the age-related increase in the incidence of hypertension and diabetes mellitus (the main causes of CKD) and the worldwide increase of accessibility to renal replacement therapies.

Data from USRDS (United States Renal Data System) show that in 2008, in the USA – the country with the highest incidence of CKD – the median age of dialyzed patients was 64,4 years (1). Comparative to 1998 (when elderly represented 47% from all dialyzed patients (2)), the raise of ESRD incidence in persons >65 years was of 11%, comparative with 2,4% in 20-44 years age-group and 6,1% in 45-

64 years age-group. In Great Britain, more than 50% of the patients entering dialysis therapy are aged more than 65 years (3). In other words, ESRD have actually become a predominantly geriatric disease; therefore, nephrologists should accumulate increasing knowledge in this field and collaborate with gerontologists as well (4).

The type of optimal mode of dialysis therapy in elderly is difficult to establish and most studies comparing hemodialysis and peritoneal dialysis in elderly failed to demonstrate the superiority of one therapy versus the other (5).

Incidence of co morbidities in elderly with ESRD is extremely high and clinical outcome, as well as the survival, are worse then in younger adults (6). In order to determine the best choice for renal replacement therapy for elderly people, it is necessary to consider the advantages and the disadvantages of each method in this population (7) (table I).

The present article compares the evolution of HD versus DP in an elderly dialyzed population using Charlson co morbidity index (8).

TABLE I

Advantages and disadvantages of peritoneal dialysis and hemodialysis in ESRD patients>65 years

	Peritoneal dialysis	**Hemodialysis**
Advantages	No need of vascular access Longer preservation of residual renal function Patient independence of hospital Can be performed by a family member	Suitable for the majority Independent of patient's ability
Disadvantages	Difficult in anurics or patients with history of abdominal surgical interventions Difficulty in learning the procedure	Risk of intradialytic hypotension Frequent vascular access problems, frequent changes for central catheters, augmented risk for infection

MATERIAL AND METHODS

Nondiabetic patients who initiated dialysis therapy for ESRD during January 2006-December 2007 and who were > 65 years old at the time of initiation were included in the study. The follow-up period was for 3 years from the moment of initiation of therapy, the study ended in December 2010. The patients were classified as HD or PD treated patients only after 3 month of stable dialysis therapy.

In all patients, at the beginning of the study it was recorded the following data: age 65-75 years or > 76 years; gender; GFR at the time of initiation of dialysis calculated with MDRD formula; primary cause of renal failure. Patients' comorbidities were classified using Charlson Co morbidity Index (CCI) (8) calculated at the time of initiation of dialysis and, then, at 6 month interval (table II). Comparisons between HD and DP elderly patients were made. Statistic validation of data was performed using *t* and *z* tests.

TABLE II
Charlson Co morbidity Index Scoring System*

Co morbidity	Score
Myocardial infarction (history or ECG changes)	**1**
Congestive heart failure	**2**
Peripheral vascular diseases (includes aortic aneurysm>6cm)	**1**
Cerebrovascular disease (CVA with mild or no residual or TIA)	**1**
Dementia	**3**
Chronic pulmonary disease	**2**
Connective tissue disease	**1**
Peptic ulcer disease	**1**
Mild/moderate liver disease	**2**
Diabetes without end-organ damage (excludes diet-controlled only)	**1**
Hemiplegia	**1**
Moderate/severe renal disease	**3**
Diabetes with end-organ damage (retinopathy, neuropathy, nephropathy)	**2**
Tumor without metastasis	**2**
Leukemia (acute or chronic)	**2**
Lymphoma	**2**
Severe liver diseases (cirrhosis, hepatic failure)	**4**
Metastatic solid tumors	**6**
AIDS	**4**

* For each decade>40 years, a score of 1 is added.

RESULTS

65 years old elderly ESRD patients have begun dialysis therapy between January 2006-December 2007: 18 on CAPD and 47 on HD.

From all new patients who started CAPD in the mentioned period, elderly represented 47.36%. There were 9 men and 9 women. The patients ≤ 75 years predominated (55.56 % ≤ 75 years, 44.44 % ≥ 76 years); the median age was 73,16 years (limits between 65-82 years). Ischemic nephropathy was the main primary renal diseases (66.7%); in 6 patients it was associated with other cause of renal failure. GFR at the time of initiating dialysis therapy had a median value of 6.68 ml/min, with limits between 4.4 and 8 ml/min.

Elderly who begun HD represented 19.18% from all new hemodialysed patients. The median age was 70.53 years, with limits between 66 and 78 years; 44 patients had ≤ 75 years and 3 ≥ 76 years. There were 29 man and 18 women. The main causes of renal failure were ischemic nephropathy (29.78%) and tubulointerstitial chronic nephropathy (29.78%); etiology of ESRD was mixed in 6 patients. GFR at the time of initiating dialysis therapy had a median value of 6.60 ml/min, with limits between 4.1 and 9,3ml/min.

In peritoneal dialysis group, CCI evaluated 6 month before initiating dialysis had a median value of 7,38, with limits between 3 and 11. At the time of initiating dialysis procedure, the median CCI was 7.72 (limits 4-11).

In HD group, the median CCI calculated 6 month before initiating dialysis therapy

was 5,87, with limits between 3 and 11. At the time of initiating dialysis, the median CCI was 6.55 (limits 3-11).

DISCUSSION

The proportion of elderly treated with CAPD was greater than those treated with HD: 47% in CAPD versus 19% in HD (fig.1). In the CAPD elderly group, there were treated significantly more patients >76 years than in HD elderly group: 44.44% versus 6% (fig.2) – *t* test.

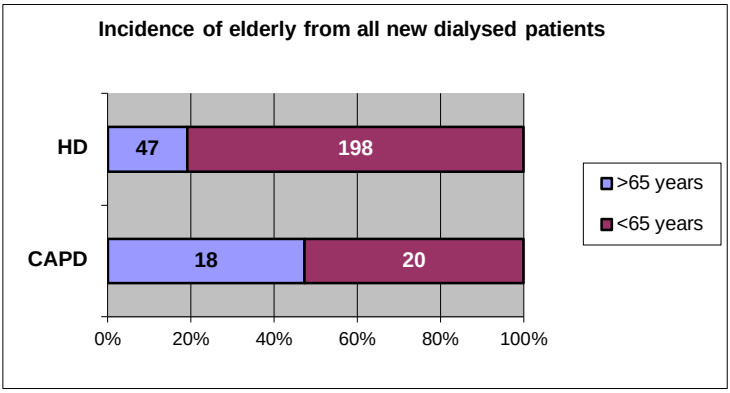

Fig. 1. Incidence of elderly from all dialyzed patients in CAPD versus HD

	CAPD	HD
Median age (years)	73.17	70.53
SD	5.43	3.46
Number of patients (n)	18	47

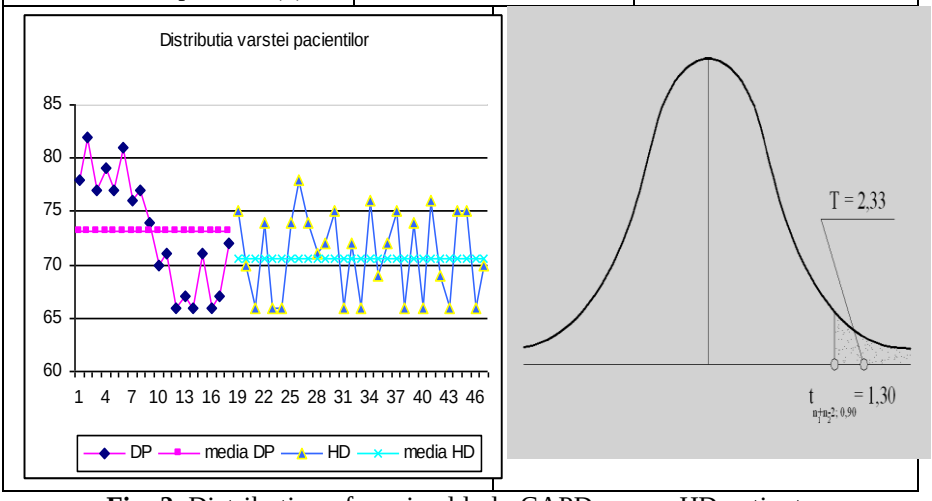

Fig. 2. Distribution of age in elderly CAPD versus HD patients

These data might be explained by the modality of selection of dialysis therapy in elderly in clinical practice. PD is preferred in those situations in which HD would be

associated with multiple risks for patients, meaning: elderly with multiple co morbidities, those with difficulty of transportation to the hospital, those cu hemodynamic instability or those with impossibility to obtain a permanent vascular access.

This observation is supported by the fact that residual GFR at the time of beginning dialysis therapy was comparable between the two studied group (6,68ml/min in PD group and 660. ml/min in HD group), which demonstrates that, in the present study, the selection of elderly for PD was not planned (as theoretically would be expected). Except few cases with severe complications of uremia (pulmonary edema, hyperkalemia – 4 patients in our study) who first needed several HD sessions, in the vast majority of elderly, PD was preferred as first choice of therapy due to severe co morbidities (malnutrition, dementia or other sequel of CVA, angina, hemodynamic instability) with high risk of aggravating in HD therapy.

The fact that, in clinical practice, DP is considered in elderly like an "escape valve" for severe cases is further supported by other two results in our study:

1. CCI at the time of initiating dialysis was more severe in elderly treated with PD (median 7.72) versus those treated by HD (median value 6.55) – *T* test (fig.3). In PD elderly patients there was a predominance of complications of systemic atherosclerosis – especially cerebrovascular and coronary diseases, while in HD elderly patients there was a predominance of congestive heart failure.

2. The primary renal disease (PRD) (fig.4):

- ischemic nephropathies was the PRD in 66% of elderly PD patients versus 30% in elderly HD group (*Z* test);
- several kidney diseases with oligoanuria and the necessity of high ultrafiltration (oliguric glomerulopathies, nephropathies associated with congestive heart failure) or obstructive nephropathies with chronic kidney infections and the necessity of periodic urologic interventions were predominant in HD elderly group

	Charlson index at the time of initiating dialysis therapy		
	Media	**SD**	**N**
CAPD	7.72	1.90	18
HD	6.55	2.19	47

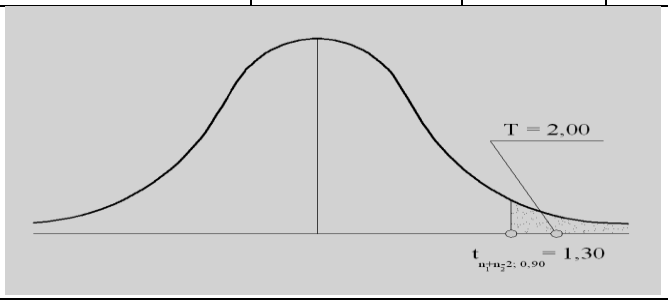

Fig. 3. Charlson Co morbidity Index at the time of initiating dialysis is more severe in CAPD elderly patients

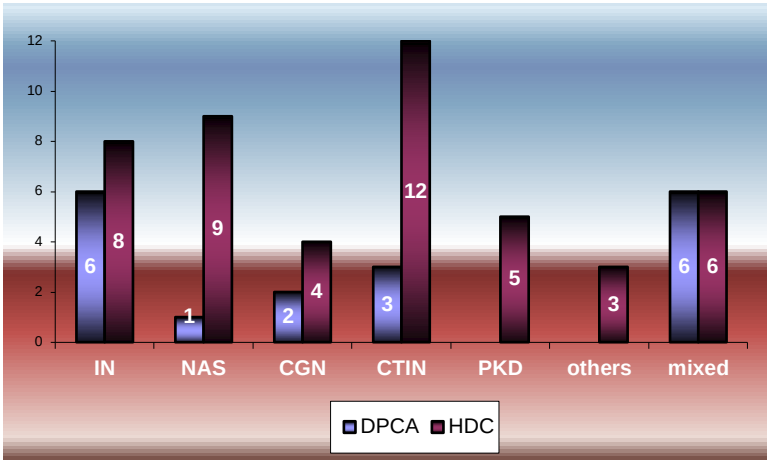

Fig. 4. Comparative distribution of primary renal diseases in CAPD versus HD elderly group

The most important finding in the present study is the comparative evolution of CCI during the 3 years of observation (fig.5). In elderly PD group, it was noted a stability of CCI value for a period of approximately 1,5 years, followed by an increase as a result of several co morbidities aroused in the patients' evolution:

- Cerebrovascular accidents (CVA) were recorded in 3 patients; 1 patient died as a result of the CVA, 2 survived with significant residual dementia and needed help from a family member to perform dialysis sessions and for every day self-care.
- Diabetes complicated the evolution of six DP elderly patients, but there was no need for insulin treatment.
- Heart failure has been registered in four patients, all with ischemic coronary disease and chronic atrial fibrillation. In the presence of a adequate Kt/V in all patients, heart failure was managed with diet and augmented ultrafiltration using high- glucose dialysate or extrarenal. After 6 month one patients had to be transferred on HD because of nonresponsive congestive heart failure.
- Other co morbidities – chronic viral hepatitis (1 patient), pulmonary chronic infections (COPD 2 patients), peptic ulcer (1 patient).

In HD elderly group, CCI increased from the moment of initiating HD; after 6 month, CCI stabilized for a period of 1-1,5 years; after this period, CCI continued to raise with a more rapid rhythm than in DP patients. The main co morbidities in elderly HD were:

- Congestive heart failure complicated the evolution of 27 patients; four died in the 3 years of study.
- CVA was recorded in 14 patients, 3 died.
- Acute myocardial infarction – 7 patients.
- Peptic ulcer disease or hemorrhagic gastritis – 12 patients.
- Chronic pulmonary disease, frequently because of chronic pulmonary status and pulmonary infection – 8 patients.
- Hepatic failure (viral or cardiac) – six

patients.

- Neoplasia – bladder 2 patients, pulmonary one patient, prostate three patients.
- Chronic lymphatic leukemia – 1 patient.

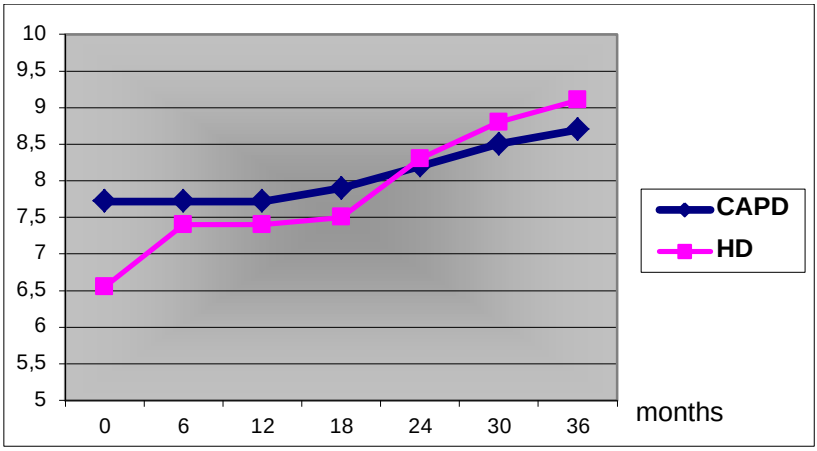

Fig. 5. Comparative evolution of CCI in HD versus PD elderly group

Comparative statistic analysis of the CCI evolution between the two groups revealed significant differences (*t* test): HD elderly patients experienced a greater increase of CCI in the first 6 month and in the second year of therapy, when compared with CAPD elderly patients (table III, table IV). These observations could have several explanations:

- Initiating HD, known to be a more aggressive therapy, may be accompanied, in elderly with hemodynamic instability, by several acute complications: digestive hemorrhage (regular heparin use in elderly with malnutrition and/or heart failure), intradialytic hypotension, angina or cerebral ischemia; CAPD is better tolerated from this point of view.
- Increase of co morbidities incidence in the third year of study in HD patients may be explained by progressive decrease of diuresis with several consequences: congestive heart failure, aggravation of hypertension, arrhythmia. Hepatic failure because of congestive heart failure of chronic viral hepatitis, hemorrhagic cerebral attacks because of uncontrolled hypertension and heparin use may occur in HD elderly quite often.

TABLE III

In the first 6 month after initiating dialysis, there was a significant more increase of CCI in HD elderly group (*T* test)

	CCI at the time of initiating dialysis			CCI at 6 month therapy			**Increase rate**		
	Median	SD	N	Median	SD	N	**Median**	**SD**	**N**
CAPD	7.72	1.90	18	7.72	1.90	18	**0**	**0**	**18**
HD	6.55	2.19	47	7.40	1.84	47	**0.85**	**0.59**	**47**

TABLE IV

Between 18-36 month of follow-up, CCI increased more in HD elderly group than in CAPD treated group (*t* test)

	CCI at 18 months			CCI at 36 months			**Increase rate**		
	Median	SD	N	Median	SD	N	**Median**	**SD**	**N**
CAPD	7.89	1.78	18	8.72	1.41	18	**0.83**	**0.79**	**18**
HD	7.51	1.93	47	9.11	1.05	47	**1.60**	**1.08**	**47**

CONCLUSIONS

This study demonstrates that PD is, in the majority of situations, a better option for dialysis in elderly. Of course, severe atherosclerosis – because of ageing, uremia and associated co morbidities – might be a problem for peritoneal capacity of ultrafiltration and/or toxins epuration. Even in this condition, peritoneal dialysis, providing a better hemodynamic stability, may be the optimal treatment for elderly uremics for a limited period (6 months, 1 year) in which it is obtained an improvement of general and nutritional status. Therefore, consulting a nephrologist from early stages of CKD in elderly would allow a better selection of dialysis mode, prevention of co morbidities and finally ameliorate the vital prognosis.

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EDITORIAL

CRYOPRESERVATION OF HUMAN EMBRYOS

The first pregnancy resulting from transfer of a thawed cryopreserved human embryo was reported in 1983 in Australia (1), and the first live birth following embryo cryopreservation was reported in 1984 in The Netherlands (2). After the first successful *in vitro* fertilization (IVF) treatment in 1978, the need for an effective cryopreservation program arose from rapid development and improvements of assisted reproductive technology (ART) protocols. Initially, all available embryos were transferred in IVF treatments owing to its low success rate, but improvements of clinical and laboratory aspects of IVF led not only to increased pregnancy rates but also to increased risk of multiple pregnancies. To prevent multiple pregnancies, fewer embryos were transferred and the supernumerary embryos cryopreserved for potential future use (3, 4).

Over the last decade the proportion of ETs derived from freeze-thaw cycles have increased substantially. According to the report by the US Centers for Disease Control and Preventions summarizing the number of assisted reproductive treatments in 2014, one in two of all embryos transferred in the United States had been cryopreserved. The European Society of Human Reproduction and Embryology has reported comparable trends (5). This rise in the number of frozen embryo transfers (FETs) performed can be ascribed to four major developments.

First, the introduction of ovarian hyperstimulation at the start of the 1980s resulted in an increased number of embryos available after each treatment cycle. During the same period rapid developments in laboratory techniques increased both the proportion of oocytes that generated viable embryos as well as the live birth rate (LBR) per embryo transferred. This reduced the need for multiple embryo transfer and increased the storage of supernumerary embryos for later use.

The second key development has been the widespread introduction of elective single embryo transfer (eSET). Extended embryo culture was introduced on the premise that only embryos of excellent quality with the highest potential for implantation will reach blastocyst stage selection (6). By prolonging the in vitro culture up to 5 days and allowing blastocyst stage embryos to develop, time and natural selection would aid clinical embryo selection. The LBR per blastocyst transfer has been demonstrated to be significantly higher compared with cleavage stage ET.

The third major contributor to the number of FET cycles performed has been the widespread replacement of slow freeze cryopreservation by vitrification (7). The slow freeze cryopreservation technique resulted in survival rates varying between 35% and 70%, compared with more than 90% after vitrification.

The latest element to extend the use of FET has been the introduction of "freeze-all" strategies that segment ovarian stimulation in a different cycle from that in which the embryo is transferred (8). This approach is argued to prevent many of the detrimental aspects of ovarian stimulation

on the health of the woman and endometrial function. Evidence from human and animal studies suggests that superovulation leads to histologic changes in the endometrium at the time of implantation. In animal models, superovulation has been shown to affect the depth of the surface epithelium, the number and length of microvilli, and the mitotic activity in the surface epithelium and stromal cells. Both human and animal studies have found that superovulation lowers the expression of specific integrins associated with the window of implantation (9). Freeze-all protocols may improve outcomes in women at risk of ovarian hyperstimulation syndrome and patients who develop an elevated P level at the time of hCG triggering (10). The possible detrimental effect of ovarian hyperstimulation on endometrial receptivity has been suggested to be intermediated by elevated progesterone. Indeed, progesterone elevation (PE) on the day of hCG administration in fresh embryo transfer cycles, a proxy of endometrial receptivity, has been associated with a decreased probability of pregnancy. More precisely, women with a PE of 0.8-1.1 ng/mL had a lower chance of pregnancy compared with women with progesterone levels <0.8 ng/mL (odds ratio [OR] 0.79, 95% confidence interval [CI] 0.67-0.95). In contrast, no effect of PE on pregnancy chances was observed when embryos that resulted from women exhibiting PE on the day of hCG were cryopreserved and transferred in a subsequent cycle (OR 1.03, 95% CI 0.79-1.34). The same results hold for embryo transfers in donor-recipient cycles (OR 1.18, 95% CI 0.76-1.84) (11). The results of several ongoing randomized controlled trials will clarify the place of freeze-all protocols in contemporary practice. However, if these studies show improved outcomes from freeze-all strategies compared with fresh transfer, a further rise in the number of FET cycles is to be expected.

Any women in reproductive age with a sufficiently high risk of developing future ovarian failure (gonadotoxic chemotherapy, radiotherapy, and/or ovarian surgery) may benefit from embryo cryopreservation though the tools of assessment of such a risk are still not very precise. Furthermore, the risk assessment can be influenced by many other factors such as the delay expected after chemotherapy and the number of children desired in the future. Embryo cryopreservation may be indicated in women with curable cancer where conception must be postponed until the resolution of the primary disease and in women with nononcological conditions where reproductive function is threatened. Breast cancer is the most frequent cancer diagnosed in women of reproductive age. Embryo cryopreservation is an attractive strategy for fertility preservation in breast cancer patients who have a partner or who are willing to use donor semen. The process of embryo cryopreservation requires ovarian stimulation, oocyte retrieval, and IVF, which typically requires a delay of 2 to 6 weeks. Because women with breast cancer generally have a window of approximately 6 to 8 weeks between surgery and the initiation of adjuvant chemotherapy, it is feasible to undergo controlled ovarian hyperstimulation.

Because hematological cancers, particularly Hodgkin lymphoma and acute lymphoblastic leukemia tend to occur in a younger population, a large proportion of patients will be candidates for fertility preservation. Each hematological malignancy has a unique constellation of fertility

considerations that relates to the disease itself, the gonadotoxic potential of common treatment protocols, and the age of the patient population.

Pelvic/abdominal radiotherapy is a well-established cause of premature ovarian failure and infertility. Radiotherapy to the ovaries causes DNA damage of somatic and germ cells that is not amenable to repair. Furthermore, the estimated lethal dose to destroy 50% of non-growing follicles present in the ovary is < 2 Grays. Gonadal damage occurs not only by direct exposure of the ovaries following total body, abdominal or pelvic irradiation, but also due to scattering radiation. Age, dose, extent, and type of radiotherapy are important prognostic indicators for development of ovarian failure. Single dose radiotherapy also seems to be more toxic than fractionated doses. These patients can benefit from fertility preservation procedures before treatment including embryo cryopreservation, or alternatively oophoropexy may be considered, especially if an abdominal surgery is already necessary for the treatment of the primary disease.

Surgery on the ovary due to endometriosis or any other benign ovarian condition may diminish ovarian reserve and lead to premature ovarian failure. Ovarian reserve can be further compromised, either due to extensive or progressive disease, or because of bilateral occurrence and repeated surgery. Therefore, fertility preservation procedures, such as embryo cryopreservation, should be considered before surgery in reproductive-age women at risk of ovarian failure.

Women with BRCA 1 and BRCA 2 mutations have a markedly higher cumulative lifetime risk of developing breast and ovarian cancer. While women with BRCA 1 mutation have an estimated 40% to 90% lifetime risk of breast cancer and a 10% to 40% lifetime risk of ovarian cancer, women with BRCA 2 mutations have an estimated 40% to 50% lifetime risk of breast cancer and a 10% to 20% lifetime risk of ovarian cancer. Therefore, prophylactic oophorectomy is suggested as soon as childbearing is completed, or by the age 35 to 40 years depending on the family history, to decrease the risk of ovarian and breast cancer. Embryo cryopreservation can be offered for women in reproductive age with available partner or for women willing to use donor semen who wish to delay childbearing beyond the age of 35 to 40 years. Moreover, women with BRCA mutation may have lower ovarian reserve, requiring multiple cycles to increase the embryo yield and improve subsequent pregnancy chances.

Fertility preservation with embryo cryopreservation is a safe and effective option in women at risk of premature ovarian failure due to medical treatment and interventions. As predicting the likelihood of infertility following gonadotoxic treatments is extremely difficult and this likelihood can be affected by unforeseen factors, fertility counseling should be offered to all females with reproductive potential. In most cases, ovarian stimulation protocols using GnRH antagonists and GnRH agonists for trigger should be preferred in fertility preservation cycles because of time limitations and to reduce the risk of ovarian hyperstimulation syndrome. Patients with estrogen positive receptor cancers can benefit from specific protocols of ovarian stimulation with aromatase inhibitors.

Frozen embryo transfer has been associated with an increased risk of neonates being born large for gestational age (12). This in turn may be associated with a high-

er incidence of perinatal complications, such as shoulder dystocia and the accompanying neonatal and maternal comorbidity. The cause of the reported increase in incidence of large for gestational age offspring after FET remains unclear. Indeed, follow-up studies have indicated a tendency for in vitro-generated embryos to produce slightly smaller babies compared with those spontaneously conceived. It has been proposed that the transfer of embryos into a uterus that has not been subject to the hormonal impact of ovarian stimulation may serve to "unmask" the previously reported tendency of in vitro-generated embryos to produce "large offspring syndrome". Moreover, there is some suggestion that the endometrial preparation method used in FET cycles can influence birth weight, with one study reporting a tendency to a higher birth weight after modified natural cycle FET (mNC-FET) compared with artificial cycle FET (AC-FET) (13). Placenta accreta is another possible obstetric complication that has been reported to arise more frequently after FET (14).

The question regarding the benefit of cryopreservation of all embryos with subsequent replacement in more physiologic cycles is not without controversy. As is the case when new hypotheses are generated, it is hard to filter through the early studies to determine whether there is a publication bias, bias and confounding due to patient selection, or unconscious (or conscious) reporting of only favorable results.

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NEWS

CONGENITAL THROMBOPHILIA AND PREGNANCY

A study of a group of researchers from Poland aims to evaluate the relationship between most common polymorphism genes involved in congenital thrombophilia and the incidence of IVH among newborns. This is a single center, prospective,study and includes 100 cases of preterm infants born from 24 to 32 weeks of gestation, exposed to antenatal steroid therapy and without congenital abnormalties, in Hospital of Gynecology and Obstetrics at the University of Medical Sciences in Poznan, Poland, between June 1,2014 and August 15,2016. IVH was diagnosed by routine cranial ultrasound and confirmed by two independent neonatologists.In conclusion, analysis showed a prevalence 4,5 times higher of IVH stages ll to lV in infants born with genotype CC of MTHFR 1298A>C gene polymorphism. In conclusion, this study confirmed that the MTHFR 1298A>C polymorphism is associated with the risk of IVH and did not confirm any significant prevalence of IVH development in other studied mutations polymorphisms. (Dawid Szpecht, Janusz Gadzinowski, Agnieszka Seremak-Mrozikiewicz, Grażyna Kurzawińska, Krzysztof Drews, Marta Szymankiewicz. The role of FV 1691G>A, FII 20210G>A mutations and MTHFR 677C>T; 1298A>C and 103G>T FXIII gene polymorphisms in pathogenesis of intraventricular haemorrhage in infants born before 32 weeks of gestation, *Childs Nerv Syst*, 2017; 33:1201–1208 DOI 10.1007/s00381-017-3460-8)

Maria Alexandra Tibeică

MODIFICĂRI TERMOGRAFICE ÎN POLINEUROPATIA DIABETICĂ

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CUTANEOUS THERMOGRAPHIC CHANGES IN DIABETIC POLINEUROPATHY (Abstract): **Aim**: to demonstrate the effectiveness of the thermography method in assessing and diagnosing peripheral polyneuropathy and risk of developing ulcerative lesions in diabetic patients planting. **Material and methods**: Study was prospective and included 90 patients of both sexes, aged between 18 and 70 years in the Neurology Clinic of the Rehabilitation Hospital Iaşi from January 2010-January 2011. The study group consists of 60 patients diagnosed with type 2 diabetes complicated with sensory- motor polyneuropathy. Control group consisted of 30 patients, selected from apparently healthy general population to assess the normal thermographic profile. To register thermic values it was used a thermal infrared camera FLIR A320 type of setting individual emission on the skin areas examined with automatic temperature correction reflected the distance or relative humidity. **Results**: Gender distribution of patients in the study group was predominantly male (63%), the ratio M / F = 1.7 / 1. Motor conduction velocity in the study group was significantly reduced both the SPE (41 ± 2.8 m/s) and SPI (40.5 ± 3.1m/s). Skin temperature was significantly higher in the control group compared with controls, both legs and the plantar surface (33.70 ± 0.80; 28 ± 0.50 t^0C). **Conclusions**: Thermographic changes in patients with sensory-motor neuropathy is an early sign in the detection and prevention of risk of injury planting.Thermography can be a useful method for diagnosis and dynamic evaluation of patients with peripheral sensory-motor polyneuropathy. **Key words**: TERMOGRAPHY CHANGES, DIABETIC NEUROPATHY, MOTOR CONDUCTION VELOCITY.

Termografia are multe aplicaţii în viaţa cotidiană, inclusiv în medicină. Primele încercări au fost făcute în anii 1960, folosind dispozitive mari şi zgomotoase (1). Acestea au fost utilizate în unele studii neinvazive ce implicau evaluarea temperaturii pielii (2). Unul din factorii cheie în termoreglarea organismului uman este pielea, care reprezintă interfaţa dinamică între corp şi mediul său. Imagistica în infraroşu furnizează informaţii cu privire la distribuţia temperaturii pielii. Având în vedere că mediul influenţează temperatura corpului, este important ca pentru orice examinare, metoda trebuie să fie cât mai neutră cu putinţă, şi standardizată (3). Un mare avantaj al termografiei este faptul că nu există contact al dispozitivului cu pielea, în procesul de formare a imaginii nu este utilizată nici o energie dăunătoare, termografia fiind o metodă repetitivă şi neinvazivă. Sistemul nervos periferic şi

central modulează termoreglarea. Implicarea sistemului nervos periferic şi a componentei autonome modifică diferenţiat şi uneori topografic termograma.

Polineuropatie diabetică este o neuropatie senzitivo-motorie, simetrică, care afectează iniţial extremităţile distale ale membrelor inferioare. Studiile arată că aproximativ 18% dintre pacienţi au semne de leziuni ale nervilor în momentul diagnosticului de diabet zaharat, sugerând faptul că scăderea toleranţei la glucoză, este asociată cu neuropatie. Semnele precoce ale neuropatiei diabetice reflectă, probabil, pierderea treptată a integrităţii atât a fibrelor mari mielinizate şi mici mielinice şi amielinice (4, 5).

În contextul acestor caracteristici ale polineuropatiei diabetice periferice, obiectivele acestui studiu au fost de a demonstra eficacitatea metodei termografice în evaluarea şi diagnosticarea polineuropatiei periferice şi a riscului dezvoltării leziunilor ulcerative plantare la pacienţii diabetici.

MATERIAL ŞI METODE

Am efectuat un studiu prospectiv observaţional pe un lot de 90 de pacienţi de ambele sexe, cu vârste cuprinse între 18 şi 70 de ani asistat în Clinica de Neurologie din cadrul Spitalului Clinic de Recuperare Iaşi, în perioada ianuarie 2010-ianuarie 2011. Pacienţii au fost împărţiti în 2 loturi, unul de studiu şi un altul martor.

Lotul de studiu format din 60 de pacienţi diagnosticaţi cu DZ tip 2 (conform criteriilor ADA din 1997 revizuite în 2003 şi 2010) complicat cu polineuropatie senzitivo-motorie (def. conform criteriilor ADA din 1997 revizuite în 2003 şi 2010). Au fost excluşi din acest studiu pacienţii cu polineuropatie de altă etiologie, arteriopatie obliterantă la nivelul membrelor inferioare, ulceraţii plantare, boală cronică de rinichi, insuficienţă cardiacă congestivă,vasculite, boală tiroidiană, afecţiuni cutanate, boală varicoasă şi sindrom edematous al nivelul membrelor inferioare de alte etiologii. Evaluarea lotului a cuprins (i) VCM, (ii) oscilometrie, (iii) termografie. Pentru înregistrarea valorilor termice a fost utilizată o cameră de termoviziune tip FLIR A320 cu setare individuală a emisiei pe ariile cutanate de examinat cu corecţie automată pentru temperatura reflectată, distanţa sau umiditatea relativă. Codul culorilor este albastru, verde, galben, roşu, alb. Prima culoare (albastră) reprezintă zona cea mai rece iar albul cea mai caldă. Culorile au un corespondent numeric echivalent al temperaturii cutanate. Imaginile termografice au fost preluate de la nivelul gambei şi plantei bilateral, făcându-se media temperaturilor obţinute. Pentru realizarea corectă a scanării termografice s-a recurs la următoarele măsuri generale: pacienţii au stat relaxaţi timp de 10 minute pentru acomodarea la temperatura camerei; nu au fost expuse la soare zonele de interes; nu s-a efectuat duş (baie) cu o oră înainte de examinare; nu s-au luat medicamente pentru durere sau vasodilatatorii cu 4 ore înainte de examinare; temperatura mediului ambiental a fost de 25^0 C.

*Lotul martor***,** a fost format din 30 de pacienţi, selecţionaţi din populaţia generală aparent sănătoşi în vederea evaluării profilului normal termografic. Pentru aceasta, am efectuat (i) viteza de conducere motorie, (ii) oscilometria şi (iii) termografia membrelor inferioare.

REZULTATE

Din punct de vedere a caracteristicilor epidemiologice se evidenţiază următoarele aspecte (tab. I):

- vârsta medie semnificativ mai mare la lotul de control comparativ cu lotul martor;

- nu s-au evidențiat diferențe semnificative între sexe;
- mediul de proveniență a fost preponderent urban la ambele loturi de studiu;
- valorile medii ale IMC au fost semnificativ mai crescute la lotul de control.

TABELUL I

Caracteristicile clinice ale loturilor studiate

	LOT STUDIU (*N* = 60)	**LOT MARTOR (*N* = 30)**	**Semnificație statistică**
Vârsta	51.4 ± 7.1 ani (IC95%: 38-69)	45.2 ± 8.3 ani (IC95%: 24-62)	**p < 0.001**
Sex Bărbați/Femei	39/21 (65/35 %)	18/12 (60/40 %)	p = 0.816
Mediul de proveniență U/R	33/27 (55/45%)	25/5 (83.3/16.7 %)	**p = 0.016**
IMC (kg/m^2)	26.4 ± 4.40 kg/m^2 (IC95%: 21-32)	22.8 ± 3.60 kg/m^2 (IC95%: 18,5-25)	**p < 0.001**
Tipul de tratament • **ADO** • **INSULINĂ** • **ADO + INSULINĂ**	 20 (33.3 %) 26 (43.3 %) 14 (23.3 %)	-	-

* *ADO* = antidiabetice orale.

Viteza de conducere motorie la lotul de studiu a fost semnificativ mai redusă atât la nivelul SPE cât şi SPI (tab. II). În cadrul loturilor, între sciaticul popliteu intern comparativ cu cel extern nu s-au evidențiat diferențe semnificative ale vitezei de conducere motorie (fig. 1).

Temperatura cutanată a fost semnificativ mai crescută la lotul de control comparativ cu lotul martor, atât la gambă, cât şi la plantă (fig. 2).

În cadrul loturilor s-au evidențiat diferențe semnificative ale temperaturii medii ale gambei comparativ cu planta (tab. III).

TABELUL II

Media vitezei de conducere motorie la cele 2 loturi

VCM (m/s)	**LOT STUDIU**	**LOT MARTOR**	**Semnificație statistică**
SPE	41 ± 2.8 (IC95%: 39-44)	52 ± 4.7 (IC95%: 47-69)	**p < 0.001**
SPI	40.5 ± 3.1 (IC95%: 39-45)	50 ± 5.3 (IC95%: 48-64)	**p < 0.001**
Semnificație statistică	p > 0.05	p > 0.05	-

* *VCM* = viteza de conducere motorie; *SPE* = sciatic popliteu extern; *SPI* = sciatic popliteu intern.

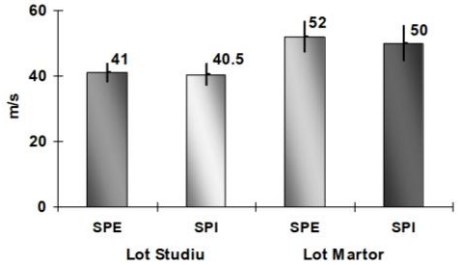

Fig. 1. Valori medii ale vitezei de conducere motorie pe loturi de studiu

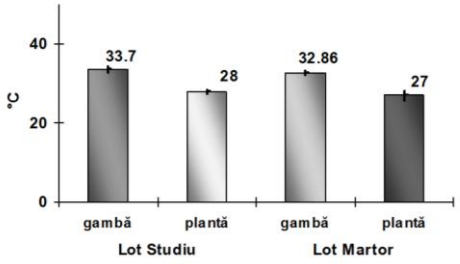

Fig. 2. Valori medii ale temperaturii cutanate pe loturi de studiu

Temperatura cutanată a fost semnificativ mai crescută la lotul de control comparativ cu lotul martor, atât la gambă, cât şi la plantă (fig. 2).

În cadrul loturilor s-au evidenţiat diferenţe semnificative ale temperaturii medii ale gambei comparativ cu planta (tab. III).

TABELUL III

Temperatura cutanată la cele 2 loturi studiate

T^0C	LOT STUDIU	LOT MARTOR	Semnificaţie statistică
Gambă	33.70 ± 0.80	32,86 ± 0,54	**p < 0.001**
Plantă	28 ± 0.50	27 ± 1.20	**p < 0.001**
Semnificaţie statistică	**p < 0.001**	**p < 0.001**	-

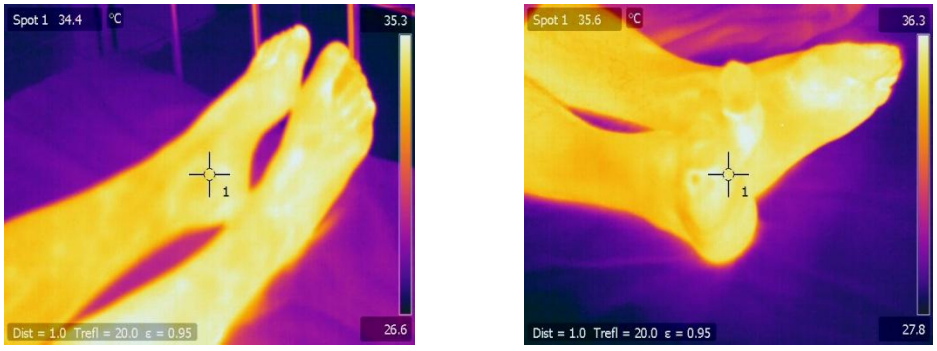

Fig. 3. Valori termice cutanate crescute în polineuropatia diabetică

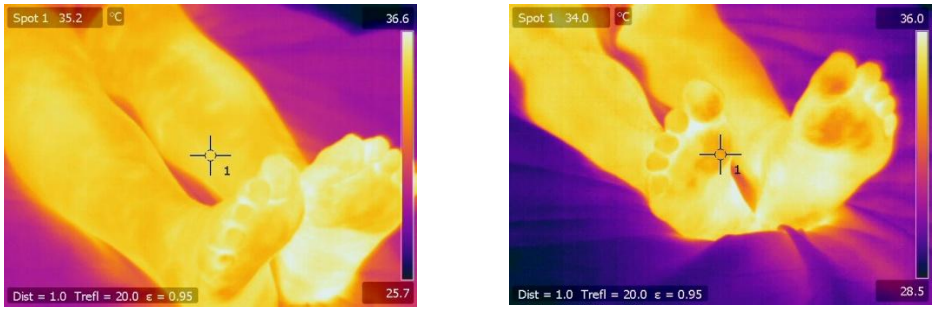

Fig. 4. Pacienţi cu modificări termografice şi viteză de conducere motorie normală

DISCUȚII

Evaluare termografică a fost realizată la ambele membre inferioare la nivelul gambei şi plantei, s-a obținut o temperatură medie pe pacient iar ulterior o temperatură medie pe un grup de pacienți.

Lotul martor format din 30 de pacienți, cu vârsta medie de 45,2 ± 8,3 ani, a fost examinat conform algoritmului prestabilit. Indicele oscilometric s-a înscris în intervalul de normalitate pentru 100% din cazuri iar VCM a înregistrat valori sub valoarea minimă normal. Evaluarea termografică a evidențiat temperaturi cuprinse între valorile, pentru gambă 30-31,9 şi pentru plantă 25,5-27.

Întrucăt literatura de specialitate nu conferă interval de referință pentru evaluările termografice am considerat aceste valori ca şi valori de normalitate, deoarece pacienții studiați nu au prezentat o altă patologie care să determine tulburări de termoreglare cutanată.

Viteza de conducere motorie la lotul de studiu a fost semnificativ mai redusă atât la nivelul SPE cât şi a SPI. În cadrul loturilor, între sciaticul popliteu intern comparativ cu cel extern nu s-au evidențiat diferențe semnificative ale vitezei de conducere motorie.

Temperatura cutanată a fost semnificativ mai crescută la lotul de studiu comparativ cu lotul martor, atât la gambă, cât şi la plantă.

În cadrul loturilor s-au evidențiat diferențe semnificative ale temperaturii medii ale gambei comparativ cu planta.

Măsurarea temperaturii plantare cu ajutorul radiațiilor infraroşii poate pune în evidență diferențele termice mici dincolo de limita vizibilă, în particular pentru pacienții fără semne inflamatorii sesizabile.

Disfuncția simpatică la nivelul extremităților inferioare duce la scăderea sudorației şi apariția pielii uscate, predispusă la apariția fisurilor şi ulcerațiilor.

Diferențele de temperatură la nivelul tegumentelor membrelor inferioare sugerează că fibrele nervoase mici joacă un rol preliminar în dezvoltarea alterării microcirculației periferice.

CONCLUZII

Temperatura cutanată a fost semnificativ mai crescută la lotul de studiu comparativ cu lotul martor, atât la gambă, cât şi la plantă.

Modificările termografice la pacienții cu neuropatie senzitivo-motorie reprezintă un semn precoce în depistarea şi prevenirea riscului apariției leziunilor plantare.

Termografia poate fi o metodă utilă pentru diagnosticarea şi evaluarea în dinamică a pacienților cu polineuropatie periferică senzitivo-motorie.

Sunt necesare mai multe studii pentru evaluarea specificității şi sensibilității metodei.

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DESPRE BETA-LACTAMAZE…PÂNĂ CÂND?

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BETA-LACTAMASES...UNTIL WHEN? (Abstract): Due to environmental factors and wide use of antimicrobials, only those species that developed their own defense mechanisms shall survive. Enzymatic inactivation is the most common cause of antibiotic resistance. Beta-lactamase production has been described early after the discovery of penicillin and it continues to diversify being now able to inactivate even the most advanced antibiotic. The association with other resistance determinants makes the infection with this kind of bacteria a major therapeutic problem. **Key words**: ANTIBIOTICS, CARBAPENEMS, SUSCEPTIBILITY

Microorganismele au supravieţuit milioane de ani datorită capacităţilor lor de a se adapta la schimbările mediului înconjurător. Astăzi, în era antibioticoterapiei larg răspândite, uneori nejustificată, se exercită o puternică presiune de selecţie asupra populaţiilor microbiene şi doar acele specii ce şi-au dezvoltat mecanisme proprii de apărare pot în continuare supravieţui.

În ultimii 70 de ani, bacteriile patogene şi nepatogene ce alcătuiesc microflora umană au fost expuse unor cantităţii impresionante de substanţe antimicrobiene; se estimează că în SUA se folosesc în jur de 1.600.000 kg de antibiotice anual pentru tratamentul şi profilaxia infecţiilor umane şi de peste 10 ori mai mult în zootehnie şi medicina veterinară.

În ultimul timp auzim vorbindu-se despre aşa-numiţii „super-microbi": stafilococul auriu meticilino-rezistent, microorganismele ESKAPE (acronim de la *Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* şi *Enterobacter spp*.). Mulţi dintre aceştia nu şi-au câştigat acest statut prin creşterea capacităţilor lor de patogenitate sau virulenţă, ci prin gradul înalt de rezistenţă la multiple clase de antibiotice şi prin dificultăţile terapeutice ce au rezultat de aici.

Cel puţin 8 mecanisme principale de rezistenţă a bacteriilor la acţiunea antibioticelor au fost descrise până în prezent: producerea de enzime inactivatoare, scăderea permeabilităţii peretelui bacterian pentru antibiotic, pomparea activă a substanţei antimicrobiene în afara celulei (eflux), modificarea, protecţia sau supra-producerea substratului, *by-pass*-ul lanţului metabolic inhibat sau deturnarea antibioticului de la adevărata ţintă.

Una din cele mai răspândite cauze pentru rezistenţa microorganismelor la antibiotice o reprezintă inactivarea enzimatică a acestora. Distrugerea inelului beta-lactamic de către enzime secretate de bacterii (beta-lactamaze) constituie principalul mecanism de rezis-

tenţă faţă de antibioticele din acest grup.

Beta-lactamazele (BL) au co-evoluat împreună cu bacteriile de mult timp ca factor de protecţie împotriva substanţelor cu acţiune antibiotică din natură, iar în ultimii au cunoscut o răspândire şi diversificare accelerată datorită folosirii pe scară largă a antibioticelor moderne.

Prima BL a fost descoperită în anii 1940, la puţin timp de la descoperirea şi introducerea în folosinţă a penicilinei, la o tulpină de *E. coli* rezistentă. Următorii ani au marcat extinderea rapidă a acestor enzime printre tulpinile de *S. aureus*. Printre bacilii Gram negativi apariţia rezistenţei la ampicilină a fost descrisă începând cu anii 1960 odată cu emergenţa unei BL cu transmitere plasmidică – TEM-1, denumită după numele unui pacient grec la care a fost iniţial descoperită. Acest tip de BL a devenit larg răspândit printre BGN în următorii ani. TEM-10, TEM-12 şi TEM-26 sunt cele mai frecvente pe continentul american.

Ulterior s-a remarcat apariţia unui alt tip de BL denumit SHV, ce sunt înrudite molecular cu cele din familia TEM, fiind transmise prin intermediul plasmidelor.

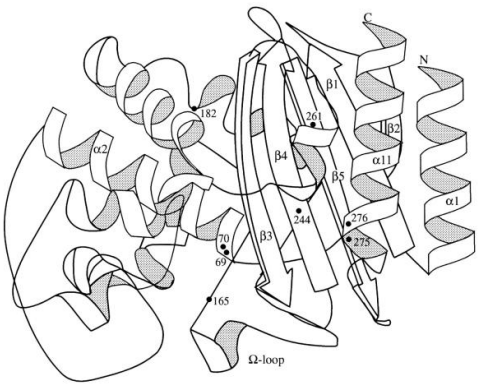

Fig. 1. Reprezentarea schematică sub formă de panglică a BL de clasă A – TEM

Sunt cunoscute mai mult de 50 de subtipuri de SHV, cele mai răspândite în America şi Europa fiind SHV-5 şi SHV-12. Industria farmaceutică a dezvoltat apoi cefalosporinele de generaţia a 3-a ce erau stabile la acţiune acestor beta-lactamaze, doar pentru a asista la apariţia unor alte BL cu spectru extins (ESBL).

La scurt timp după introducerea în folosinţă a carbapenemelor au apărut tulpini bacteriene secretoare de enzime capabile să le inactiveze, iar în prezent răspândirea carbapenemazelor reprezintă o îngrijorare serioasă în lumea medicală datorită tulpinilor de bacili Gram negativi multidrog-rezistenţi pentru care nu avem soluţii terapeutice adecvate.

BL sunt codate la nivel cromosomial sau sunt achiziţionate prin intermediul plasmidelor sau integronilor care pot fi purtătorii mai multor gene de rezistenţă la antibiotice.

În prezent au fost descrise peste 890 sute de BL produse de numeroase specii microbiene şi care pot inactiva selectiv sau în grup penicilinele naturale şi de semisinteză, cefalosporine şi carbapenemi, chiar în asociere cu inhibitori de BL.

Au fost propuse mai multe sisteme de clasificare a BL. Cel descris de Ambler le grupează în 4 clase (A-D) în funcţie de structura enzimatică, pe când cel descris de Bush-Jacoby-Medeiros le structurează în funcţie de tipul de antibiotic inactivat.

Clasele de BL A, C şi D inactivează inelul beta-lactamic al antibioticului folosindu-se de o serină în regiunea lor activă, pe când cele din clasa B sunt metalo-BL ce folosesc zincul pentru a inactiva antibioticul.â

În anul 1983 (Germania) a fost descrisă prima ESBL la o tulpina de *E.coli* dintr-o secţie de terapie intensivă, rezistentă la cefotaximă si ceftazidimă, urmată apoi în 1984 (Franţa) de o tulpina de *Klebsiella pneumoniae*.

TABELUL I
Clasificarea Ambler a beta-lactamezelor

Clasa	Site-ul activ	Tipul de enzimă	Substrat	Exemple
A	Serină	**Penicilinaze**		
		Spectru larg	Benzylpenicilin, aminupeniciline, carboxypenicilins, ureidopeniciline, cefalosporine	PC1 al *Staphylococcus aureus* TEM-1, SHV-1 la *Escherichia coli, Klebsiela pneumoniae*, alte BGN
		Spectru extins (ESBL)	Cefalosporine de generaţia a 3-a şi aztreonam	Enterobacteriaceae: TEM-derived, SHV-derived, CTX-M–derived; PER-1, VEB-1, VEB-2, GES-1, GES-2, IBC-2 la *Pseudomonas aeruginusa*
		Carbapenemaze	Cefalosporine şi carbapeneme	KPC-1, KPC-2, KPC-3 la *K. pneumoniae*; NMC/IMI, SME
B	Metalo-β-lactamaze (Zn^{2+})	**Carbapenemaze**	Cefalosporine şi carbapeneme	IMP, VIM, GIM, SPM, SIM la *P. aeruginusa, Acinetobacter* spp.
C	Serină	**Cephalosporinaze**	Cefalosporine şi carbapeneme	AmpC-type la Enterobacteriaceae, *Acinetobacter baumannii*
D	Serină	**Oxacilinaze**		
		Spectru larg	Aminopeniciline, ureidopeniciline, cloxacilin, methicilin, oxacilin, unele cefalosporine de gen I, II	OXA-family la *P. aeruginusa*
		Spectru extins	Cefalosporine şi monubactami	OXA-derived la *P. aeruginusa*
		Carbapenemaze	Cefalosporine şi carbapeneme	OXA-derived la *Acinetobacter* spp

TABELUL II
Clasificarea Bush-Jacoby-Medeiros a beta-lactamazelor

Grup	Tip	Inhibare de către clavulanat	Clasa moleculară	exemple
1	Cephalosporinaze	Nu	C	*Enterobacter cloacae* P99 (C), MIR-1 (P)
2a	Penicillinaze	Da	A	*Bacillus cereus, Staphylococcus aureus* (B)
2b	Cu spectru larg	Da	A	SHV-1 (B), TEM-1 (P)
2be	Cu spectru extins	Da	A	*Klebsiella oxytoca* K1 (C), TEM-3 (P), SHV-2 (P)
2br	Inhibitor-rezistente	Scăzut	A	TEM-30 (IRT-2) (P)
2c	Carbenicillinaze	Da	A	AER-1 (C), PSE-1 (P), CARB-3 (P)
2d	Cloxacillinaze	Da	D or A	*Streptomyces cacaoi* (C), OXA-1 (P)
2e	Cephalosporinaze	Da	A	*Proteus vulgaris* (C), FEC-1 (P)
2f	Carbapenemaze	Da	A	IMI-1 (C), NMC-A (C), Sme-1 (C)
3	Carbapenemaze	Nu	B	*Stenutrophomonas maltophilia* L1 (C), IMP-1 (P)
4	Penicillinaze	Nu	A	*Burkholderia cepacia* (C), SAR-2 (P)

B- cromosomial şi plasmidic; C - cromosomial ; P – plasmidic

În 1989 în Germania, Argentina, Franţa, Italia – se descrie la Enterobacteriaceae o nouă familie de ESBL - CTX-M ce conferă rezistenţă la cefotaximă si mai puţin la ceftazidimă. Acestea au apărut mai puţin prin mutaţii la nivelul unei gene existente, cât prin achiziţia plasmidică a unui material genetic de la specii de *Kluyvera* (specii de BGN comensali). Aceste ESBL-uri au structura genetică asemănătoare cu TEM sau SHV în proporţie de doar 40%. În prezent sunt cunoscute mai mult de 80 de subtipuri, unele mai active pe ceftazidim decât pe cefotaxim. CTX-M-14, CTX-M-3, CTX-M-2 sunt cele mai răspândite subtipuri, iar CTX-M-15 este cel mai comun subtip asociat *E. coli* din Anglia.

În 1993 (Turcia) se descrie un nou tip de ESBL - PER1 la o tulpină de *Ps. aeruginosa* sensibilă la beta-lactamine doar în asociere cu clavulanat, sulbactam sau tazobactam; în prezent este răspândită şi în Franţa, Italia, fiind frecventă la tulpinile de *Acinetobacter* multirezistente din Coreea.

ESBL de tip VEB1 sau VEB2 sunt descrise (1996) la tulpinile de *E. coli* în Asia de sud-est. Subtipurile GES1 şi GES2, răspândite în Franţa, Grecia şi Africa de sud sunt capabile să hidrolizeze imipenemul.

Subtipul OXA al BL (grup D) hidrolizează oxacilina şi derivaţii săi foarte eficient şi nu este inhibat de către acidul clavulanic. Familia de ESBL-uri OXA-derivaţi sunt produse în special de către tulpini de *Ps. aeruginosa* la care conferă un grad ridicat de rezistenţă faţă de oxymino-β lactami (cefotaxime, ceftazidime, ceftriaxone).

Alte subtipuri de ESBL-uri rare au fost descrise: SFO1 1998 (Japonia) - *Enterobacter cloacae* sensibil la clavulanat si imipenem, BES1 - 1996 (Brazilia): *Serratia marcescens* rezistentă la aztreonam si sensibilă la tazobactam si clavulanat, BEL1 – 2004: *Ps. aeruginosa* sensibilă la clavulanat si imipenem,TLA1 - 1993 (Mexic) la *E.coli*, TLA2 - 2002 (Germania).

BL de tip AmpC sunt codate cromosomial la multe bacterii Gram negative. Mutaţii punctiforme sub presiunea terapiei antibiotice determină o activarea transcripţiei şi rezistenţă la cefalosporinele cu spectru larg. Chiar dacă *E.coli, Salmonella şi Klebsiella* au nativ o exprimare scăzută a enzimelor AmpC, achiziţia plasmidică de materialului genetic le poate conferi rezistenţă la cefamycine şi oxyimino-betalactami, fără sensibilitate la inhibitorii de beta-lactamază.

Carbapenemazele (grup A, B şi D) sunt un grup divers de enzime care în prezent ridică cele mai multe probleme în practica medicală datorită faptului că pot inhiba nu doar carbapenemii ci şi cefalosporinele şi alte peniciline cu spectru larg.

Din grupa A, cel mai frecvent tip îl reprezintă enzimele de tip KPC, iniţial descrise în nord-estul SUA la tulpini de *Kl. pneumoniae*, cu răspândire geografică largă şi prezenţă şi la numeroase tulpini de *E. coli, Citrobacter, Enterobacter, Salmonella, Serratia* sau *Ps. aeruginosa.*

Carbapenemazele de grup B folosesc ionii de Zn^{+} pentru a desface inelul beta-lactamic, sunt susceptibile la chelatori de ioni (gen EDTA) şi nu sunt influenţate de acidul clavulanic, tazobactam sau sulbactam. Aceste enzime conferă rezistenţă la toate antibioticele beta-lactamice (cu excepţia monobactamilor). Sunt regăsite în mod natural la nivel cromosomial la tulpini nepatogene de *Aeromonas, Chryseobacterium* şi *Stenotrophomonas* din mediul înconjurător şi pot fi transmise prin elemente genetice mobile (integroni) la numeroase specii de BGN patogene pentru om. Cele mai importante tipuri sunt incluse în 5 familii: IMP, VIM, SPM, GIM, SIM.

Carbapenemazele de grup D aparţin sub-familiei OXA a BL, cele mai importante (OXA-23, OXA-24, OXA-40, OXA-58) fiind găsite la tulpini de *Acinetobacter baumannii* multirezistent.

În 2008 se izolează la New Dehli (India), de la un pacient suedez de origine indiană cu o infecţie urinară, o tulpină de *K. pneumoniae* rezistentă la toate antibioticele testate, cu excepţia colimicinei. Testele microbiologice şi moleculare efectuate au arătat că aceasta era secretoare de o nouă metalo-beta lactamază (denumită NDM-1) capabilă să hidrolizeze rapid penicilinele, cefalosporinele şi carbapenemele.

Gena ce codifică această nouă ESBL se află localizată la nivelul unei plasmide ce mai conţine o altă BL de tip CMY-4 şi determinanţi de rezistenţă pentru eritromicină, ciprofloxacină, rifampicină, cloramfenicol, cât şi o pompă de eflux şi promoteri ai creşterii microbiene.

Această plasmidă se transmite cu uşurinţă printre membri ai familiei enterobacteriacee, fiind găsită de cercetători indieni din Mumbai la 22 de tulpini carbapenem-rezistente de *Klebsiella* (10), *Escherichia coli (9), Enterobacter (2), Morganella morganii (1).* Studii mai recente au arătat că tulpini secretoare de NDM-1 sunt prezente în numeroase ţări de pe mai multe continente (Bangladesh, India, Pakistan, Anglia, SUA, Israel, Turcia, China, India, Australia, Franţa, Japonia, Kenya, Singapore, Taiwan, ţările nordice). Chiar dacă cazurile iniţiale au fost asociate cu călătorii în India, datorită uşurinţei cu care acest element genetic este transmis inter-specii se aşteaptă apariţia unor cazuri cu transmitere secundară locală.

DATE EPIDEMIOLOGICE

Cu toate că antibioticele beta-lactamice sunt folosite din abundenţă în întreaga lume, distribuţia geografică a BL nu este uniformă. Există spitale (în SUA) în care nu există tulpini bacteriene secretoare de BL, iar în altele până la 40% din cele de *K. pneumoniae* sunt ceftazidim rezistente ca urmare a secreţiei de ESBL.

ESBL-urile sunt cel mai probabil a fi prezente la tulpini de *K. pneumoniae, K. oxytoca* sau *E. coli*, dar au fost raportate şi la citrobacter, enterobacter, proteus, salmonella, serratia, la alte bacterii enterice şi nonenterice ca *Acinetobacter baumannii* sau *P. aeruginosa*. Rata cea mai mare de producere a BL o au tulpinile izolate din secţiile de terapie intensivă.

În Europa numărul tulpinilor de BGN rezistente la antibioticele beta-lactamice este în creştere. Din cele de *E. coli* izolate in hemocultura a fost descrisă rezistenţa la cefalosporine în 28% din ele în Bulgaria, 16% în Cipru şi România şi 12% în Portugalia. Studiul Mystic (Rusia şi Polonia) a arătat o creştere a rezistenţei la cefalosporine între 1997 şi 2004 a *E.coli* de la 2,1% la 10,8 % şi a *Klebsiella pneumoniae* de la 9% la 13,6% .

Studii din Spania au arătat o creştere (între anii 2000 şi 2006) a tulpinilor bacteriene comunitare secretoare de BL de *K. pneumoniae* de la 2.7 la 8% şi de *E. coli* de la 0.5 la 6%. În Italia (în 2003) 7.4% din tulpinile izolate în spital şi 3.5% din cele comunitare erau secretoare de BL.

Factorii de risc pentru colonizarea sau infecţia cu bacterii secretoare de beta-lactamaze sunt diferite de cei pentru achiziţia de infecţii nosocomiale.

Dintre cei mai frecvenţi întâlniţi în mediul spitalicesc (mulţi aflaţi în inter-relaţie) se pot aminti:

- Durată prelungită de spitalizare
- Internare prelungită în secţia de TI
- Gravitate crescută a bolii
- Folosirea de catetere (arteriale sau venoase) centrale
- Utilizarea de catetere urinare
- Ventilaţia asistată
- Hemodializa
- Proceduri chirurgicale abdominale efectuate în urgenţă
- Colonizarea anterioară a tubului digestiv

La pacienţii cu infecţii comunitare factorii de risc pentru prezenţa de tulpini secretoare de ESBL sunt diferiţi şi cuprind:

- terapie cu cefalosporine (II şi III)
- spitalizare in ultimele 3 luni
- terapie cu fluorochinolone
- terapie cu peniciline
- antibioterapie un ultimele 3 luni
- vârsta > 60 ani
- diabet zaharat

DIAGNOSTIC

Detectarea tulpinilor de BGN producătoare de BL se bazează pe laboratorul de microbiologie şi uneori pe constatarea eşecului clinic în terapia acestui tip de infecţii cu cefalosporine cu spectru larg.

Punerea în evidenţă a microorganismelor secretoare de BL poate fi dificilă pentru multe laboratoare datorită faptului că metodele de rutină pentru monitorizarea scăderii susceptibilităţii la oxyimino-cehalosporine şi aztreonam nu sunt suficient de sensibile pentru a detecta tulpinile secretoare de BL, în special de tip TEM-1, TEM-12 or SHV-2.

Există mai multe teste de screening pentru prezenţa beta-lactamazelor. Evidenţierea rezistenţei la ceftazidimă, indiferent de metoda folosită are o bună sensibilitate pentru secreţia de BL. Unii autori susţin folosirea rezistenţei la cefpodoxima ca test pentru producerea de BL.

Performanţele oricărui test ce foloseşte cefalosporine cu spectru larg este influenţată de zona geografică şi particularităţile locale de distribuţie a BL-lor. Într-un studiu al ESBL-urilor de tip SHV-3, toate tulpinile testate in vitro au fost rezistente la ceftriaxonă şi doar 50% la ceftazidim.

Recomandările actuale pentru detecţia producerii de ESBL-uri la tulpinile de *E. coli* sau *K. pneumoniae* sunt de a efectua un test screening iniţial cu 2 din următoarele antibiotice beta-lactamice: cefpodoxime, ceftazidime, aztreonam, cefotaxime sau ceftriaxone. Izolatele cu o CMI > 1 microg/ml trebuie confirmate fenotipic folosind ceftazidime plus ceftazidime/acid clavulanic şi cefotaxime plus cefotaxime/acid clavulanic. Orice tulpină la care se constată o scădere de minim 3 ori a CMI pentru oricare antibiotic folosit în asociere cu acidul clavulanic faţă de antibiotic izolat este considerată ca secretoare de ESBL.

Caracterizarea genotipică a tulpinilor detectate ca secretoare de ESBL este rezervată laboratoarelor specializate şi cercetării ştiinţifice.

MĂSURI TERAPEUTICE

Sensibilitatea in-vitro a microorganismelor producătoare de ESBL-uri variază şi chiar dacă sunt rezistente la unele antibiotice beta-lactamice, pot fi sensibile la altele.

Cele secretoare de BL de tip TEM şi SHV sunt aparent sensibile la cefepime sau piperacillin–tazobactam, cu toate că prezintă un efect de inoculum (scăderea sensibilităţii odată cu creşterea inoculumului de la 105 la 107).

Speciile secretoare doar de ESBL-uri sunt sensibile la carbapeneme şi cefamicine (cefoxitin şi cefotetan) fără a avea efect de inoculum. Cele secretoare de AmpC sunt rezistente la oxyimino-βlactami şi la cefamicine, dar îşi păstrează sensibilitatea la carbapeneme.

Tulpinile secretoare de carbapenemaze de tip IMP, VIM şi OXA rămân sensibile la aztreonam.

Din păcate, multe dintre speciile secretoare de BL au şi alţi determinanţi de rezistenţă pentru antibiotice non-betalactamice (în special pentru amino-glicozide şi fluorochinolone), ceea ce face terapia acestor tipuri de infecţie extrem de dificilă.

Există puţine studii in vivo care să determine tratamentul optim pentru infecţiile produse de tulpini secretoare de ESBL-uri. Pentru infecţiile determinate de *E. coli* sau *Klebsiella spp.* tratamentul cu imipenem sau meropenem s-a asociat cu cea mai bună rată de vindecare clinică şi microbiologică.

Cefepime sau piperacillin–tazobactam au fost mai puţin eficiente.

Tratamentul infecţiilor cauzate de tulpini (în special de *K. pneumoniae*) producătoare de carbapenemaze este o problemă dificilă şi de actualitate. Unele studii afirmă că în ciuda prezenţei acestor enzime, o asociere dintre un carbapenem la doze crescute şi un alt antibiotic activ ar putea avea rezultate pozitive. Alţi autori au folosit cu succes tigeciclina sau aminoglicozidele, pe când monoterapia cu colimicină pare să nu fie atât de eficientă.

În cazul sepsis-ului, VAP sau a rezistenţei la ertapenem se pot folosi imipepemul sau meropepemul.

Ertapenemul este indicat pentru infecţiile urinare complicate, infecţiile intraabdominale sau ale piciorului diabetic.

Fluorochinolone pot fio alternativă în cazul alergiei la carbapeneme; nitrofuratoinul sau fosfomicina pot fi o soluţie în terapia infecţiilor urinare joase şi necomplicate, iar tigeciclina, colistinul sau polimixina B la pacienţii alergici la beta-lactamine.

MĂSURI PREVENTIVE

La nivel individual:

- atenţie la tratament cu cefalosporine (III) aztreonam si cefuroxima
- atenţie la cateterisme
- igiena pacientului

La nivel institutional:

- restricţie pentru cefalosporine
- atenţie la internarea purtatorilor ESBL
- determinări pentru contaminarea cu ESBL

Limitarea transmiterii tulpinilor de BGN secretoare de BL ar putea fi realizată prin implementarea unor măsuri specifice de luptă împotriva infecţiilor nosocomiale. Spălarea frecventă şi corectă pe mâini a personalului de îngrijire, purtarea de mănuşi de unică folosinţă şi a halatului în asociere cu măsuri educative scad riscul răspândirii infecţiilor cu aceşti super-microbi.

Măsuri de control al folosirii cefalosporinelor cu spectru larg şi cunoaşterea spectrului de rezistenţă locală a patogenilor sunt indicate în toate secţiile de terapie intensivă. Interzicerea folosirii de primă intenţie a imipenemului, piperacillin–tazobactamului sau asocierea cefepime–amikacin a condus la scăderea frecvenţei infecţiilor cu tulpini producătoare de ESBL-uri.

Există studii care atestă limitarea acestor tipuri de infecţii prin folosirea de măsuri stricte de izolare pentru pacienţii diagnosticaţi în absenţa controlului antibioticoterapiei.

Folosirea în exces a carbapenemelor (imipenem) pentru tratarea infecţiilor cu BGN rezistenţi la cefalosporine poate duce la apariţia de tulpini secretoare de carbapenemaze (în special *Acinetobacter spp. , K. pneumoniae* secretoare de AmpC (ACT-1)).

În continuare, bacteriile depăşesc cu uşurinţă eforturile lumii medicale actuale de a le stăpâni răspândirea, iar industria farmaceutică este deseori neputincioasă în faţă multitudinii de mecanisme de rezistenţă pe care acestea le dezvoltă rapid şi apoi le transmit rudelor lor mai apropiate sau îndepărtate. Bacteriile ne sunt superioare ca număr, ca rapiditate a înmulţirii şi prin metodele ingenioase şi eficiente prin care pot schimba informaţia genetică necesară supravieţuirii într-un mediu ostil, populat de antibiotice folosite de multe ori excesiv şi fără discernământ de către medicina umană, veterinară sau în agricultură.

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NOUTĂȚI

PAUZA MINȚII: PERSPECTIVE TEMPORALE PENTRU ADULȚII CU ADHD

Încadrat în categoria tulburărilor comportamentale, ADHD apare la copil sub forma unor manifestări hiperkinetice cu deficit de atenţie, dar se apreciază că în proporţie de 60% persistă şi la vârsta adultă. Dacă la copil ADHD afectează un număr mult mai mare de băieţi decât de fete, la adulţi procentul devine egal, compromiţând cariera şi relaţiile cu cei din jur. Adultul afectat de ADHD este dezorganizat, copleşit de responsabilităţi, impulsiv, cu tulburări de memorie, nu se poate adapta şi este depăşit de termenele – limită; riscul său de a face depresie este de 5-6 ori mai mare decât al unei persoane normale. Studii recente relevă faptul că depresia şi anxietatea care se instalează atrag după sine diverse dependenţe: 40-44% din adulţii cu ADHD sunt fumători, dependenţi de alcool sau droguri. Perturbarea oricărei activităţi de planificare şi management a timpului rămâne principala dominantă la adult, iar terapia ridică numeroase semne de întrebare. (Carelli M.G., Wiberg B.Time Out of Mind : Temporal Perspective in Adults with ADHD. *J Atten Disord* 2011; 13(1):234-37).

Carmen Zamfir

TROMBOZA VENOASĂ PROFUNDĂ A MEMBRELOR SUPERIOARE: NOUTĂȚI ÎN DIAGNOSTIC ŞI TRATAMENT

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UPPER-EXTREMITY DEEP VEIN THROMBOSIS: CURRENT CONCEPTS IN DIAGNOSIS AND TREATMENT (Abstract): Upper-extremity deep vein thrombosis is a rare manifestation of venous thromboembolic disease. In the past few decades, the clinical importance of upper-extremity deep vein thrombosis has increased because of the wider use of central venous catheters and the development of ultrasonography as a simple and accurate objective diagnostic method. Primary upper-extremity deep vein thrombosis is a rare disorder (2 per 100,000 persons per year), which comprises (1) Paget-Schroetter Syndrome, also known as effort thrombosis, and (2) idiopathic upper-extremity deep vein thrombosis. Secundary upper-extremity deep vein thrombosis develops in patients with upper extremity central venous catheters, pacemakers or cancer and accounts for most cases of upper-extremity deep vein thrombosis. The imaging modes used for diagnosis are: duplex ultrasound, magnetic resonance. Contrast venogram is the standard diagnostic test for characterization of the anatomy. A staged, multimodal approach to Paget-Schroetter Syndrome can effectively restore venous patency, reduce the risk of rethrombosis, and return the patient to normal function. Primary care physicians should be aware of this condition and its atypical presentations, because delayed recognition in a high-functioning persons can be potentially disabling. **Key words**: DEEP VEIN THROMBOSIS, UPPER-EXTREMITY, PAGET-SCHROETTER SYNDROME, SUBCLAVIAN VEIN.

Tromboza venoasă profundă (TVP), denumită şi flebotromboză acută sau uneori tromboflebită profundă, este o afecțiune datorată producerii unui trombus, de obicei puțin aderent, într-o venă profundă. De regulă, este afectată o venă proximală sau distală a extremităților, bazinului, venele cave sau o venă profundă a gâtului, care sunt obstruate complet sau parțial de un trombus; TVP poate fi cantonată şi în sinusurile venoase sau la nivelul diferitelor organe, mai ales abdominale, inclusiv la nivelul venei porte (1).

Tromboza venoasă profundă a extremității superioare (UEDVT; TPMS) se referă la tromboza venelor axilară şi/sau subclaviculară. Din punct de vedere patogenic sunt clasificate ca primare şi secundare. TPMS secundară este mai frecventă (60% din cazuri) comparativ cu cea primară şi întâlnită la pacienți cu catetere venoase centrale, pacemakere sau cancere (2). TPMS primară este o afecțiune rară (2 la 100000 de persoane/an) şi cuprinde sindromul Paget-Schroetter (PSS) cunoscut ca şi tromboză de efort şi forma idiopatică

care poate fi asociată cu un cancer ocult (3). Într-un studiu, 1/4 dintre pacienții care s-au prezentat cu UEDVT idiopatică au fost diagnosticați cu neoplasme (cel mai frecvent cancer pulmonar sau limfom) în primul an de monitorizare. Majoritatea acestor cancere au fost descoperite în timpul primei săptămâni de spitalizare pentru tromboza venoasă (4).

Tromboza spontană a venelor extremității superioare a fost descrisă pentru prima dată de patologul francez Leon Jean Baptiste Cruveilhier (1791-1874) în 1816, în teza sa de doctorat, iar Sir James Paget, 1 st. Baronet, în 1875, o considera posibila cauză a durerii acute şi a tumefierii brațului (5). Primul care a relatat manifestările clinice ale ocluziei trombotice a venelor axilară şi subclaviculară a fost, în 1884, Leopold Schrötter Ritter von Kristelli (6). Termenul de maladie Paget-Schrötter a fost introdus mai târziu, după 1900, de E. S. Hughes (7). În literatură mai este denumită ca şi: Paget-Schrötter syndrome, Schrötter's syndrome, von Schrötter syndrome sau "tromboza indusă de efort".

ETIOPATOGENIE

Triada lui Virchow (1856), adică staza venoasă, leziunea peretelui venos şi hipercoagulabilitatea sângelui constituie cauza trombozei venoase. Cel mai adesea coexistă doi sau toți cei trei factori. În consecință, o varietate de situații clinice sunt asociate cu risc crescut de tromboză venoasă (tab. I) (8).

TABELUL I

Condiții asociate cu un risc crescut pentru dezvoltarea trombozei venoase

Chirurgie Procedee ortopedice, toracice, abdominale şi genitourinare
Neoplasme Pancreas, plămân, ovar, testicule, tract urinar, sân, stomac
Traumatisme Fracturi ale coloanei vertebrale, pelvisului, femurului, tibiei; leziuni ale măduvei spinării
Imobilizarea Infarct miocardic acut, insuficiență cardiacă congestivă, accident vascular cerebral, convalescență postoperatorie
Sarcina
Utilizarea estrogenilor (substituție sau contracepție)
Stări de hipercoagulabilitate Deficiențe de antitrombină III, proteină C sau proteină S; rezistență la proteina C activată; anticorpi antifosfolipidici; boli mieloproliferative; disfibrinogenemia; coagularea intravasculară diseminată
Venulita Trombangeita obliterantă, boala Behçet, homocisteinuria
Tromboză venoasă profundă precedentă

O echipă de medici ai şcolii medicale Harvard, Boston, a realizat un studiu multicentric prospectiv în rândul pacienților cu TVP din Bringham and Woman's Hospital. Au comparat caracteristicile demografice, simptomele, factorii de risc, profilaxia şi

managementul terapeutic şi au evidenţiat factorii predictivi independenţi pentru UEDVT neasociaţi cu CVC: vârsta < 67 ani, BMI < 25 kg/m^2 şi spitalizarea; cateterul venos central a fost cel mai puternic factor independent predictiv pentru UEDVT (odds ratio – OR=7,3; interval de confidenţă – CI = 95%, 5,8-9,2). Concluzia studiului a fost că factorii de risc ai UEDVT diferă de factorii de risc convenţionali ai TVP a membrelor inferioare (9).

Un alt studiu a evaluat influenţa parametrilor genetici şi dobândiţi ai trombofiliei la pacienţii cu UEDVT internaţi in clinica Hematologie a Spitalului Universitar Rotterdam "Dykzigt". Prevalenţa tulburărilor de coagulare nu a fost diferită semnificativ in rândul pacienţilor cu UEDVT spontană comparativ cu cei cu factor predispozant (CVC montat). Pe baza acestor rezultate s-a concluzionat că anticorpii antifosfolipidici sunt frecvent identificaţi la pacientii cu UEDVT (10). Mecanismul trombozelor venoase prin anticorpi antifosfolipidici este necunoscut; se presupune că ei acţionează pe plachete şi endoteliu vascular, producând ocluzie trombotică noninflamatorie a vaselor mari şi mici (11).

Mutaţia genei protrombinei a fost descoperită în 1996 de Poort şi colaboratorii săi. Aceasta se datorează unei substituţii nucleotidice (guanina este inlocuită cu adenina) la nivelul perechii de baze 20210, situată în regiunea 3' netranslatată a genei factorului II. Mutaţia se asociază cu niveluri de protrombină mai mari cu 30% decât normalul, care vor induce o stare de hipercoagulabilitate uşoară, ce poate predispune la tromboză venoasă. După mutaţia factorului V Leiden, mutaţia genei protrombinei G2021A reprezintă a doua cauză de trombofilie ereditară, fiind demonstrată în ~ 20% din cazuri şi este depistată la 6-8% dintre pacienţii aflaţi la primul episod de TV. S-a constatat, de asemenea, că riscul de TVP este de 16,3 ori mai mare la pacientele care folosesc contraceptivele orale şi sunt purtătoare ale mutaţiei, în comparaţie cu lotul martor (12).

Studiile au dovedit că contraceptivele combinate cresc cantitatea de factori ai coagulării VII, VIII (factorul von Willebrand), IX şi X şi cantitatea de fibrinogen. Astfel se explică frecvenţa TVP şi a emboliilor de circa 3 ori mai mare. Tendinţa la tromboză este legată de componenta estrogenică a medicaţiei (16), probabil prin dilataţia venoasă pe care o provoacă, diminuarea eliberării de activatori endoteliali ai plasminogenului şi scăderea concentraţiei de antitrombină III (AT III) (12).

Tumorile osoase benigne primare, incluzând osteocondromul, localizate la nivelul claviculei sunt rare. Departamentul de Ortopedie şi Reabilitare al Universităţii Iowa a raportat un caz atipic în care un osteocondrom s-a dezvoltat în partea medială a claviculei şi a cauzat sindrom de apertură toracică superioară cu tromboză primară a venei subclaviculare (sindrom Paget Schroetter), parestezii în teritoriul plexului brahial şi durere mecanică sternoclaviculară la un tânăr sănătos. Din informaţiile de la acea dată se pare că au fost primii care au raportat un caz de TOS (sindrom de apertură toracică superioară) produs de un osteocondrom al părţii mediale a claviculei. Celelalte două cazuri de tumori mediale de claviculă citate în literatură au fost asimptomatice (13).

DIAGNOSTIC

De obicei există o legătură temporală şi cauzală între activităţile implicând hiperabducţia membrului superior şi geneza

trombozei (14). Pacienții tineri, sănătoşi dezvoltă spontan UEDVT, de obicei în brațul dominant, după activități fizice intense, dar şi ca urmare a unei poziții nefiziologice prelungite a brațului. Efortul intens provoacă microtraume la nivelul intimei vasculare şi conduce la activarea cascadei coagulării. O importantă tromboză se poate produce dacă obstrucția mecanică a peretelui vascular este prezentă (11, 12). Hipertrofia musculară este o cauză neobişnuită de compresiune extrinsecă venoasă. Compresiunea venei subclaviculare apare de obicei la tinerii cu hipertrofie musculară coracobrahială şi a capului scurt al bicepsului care fac abducția completă a brațelor (13). Hiperabducția şi retroversia brațului, în special cu mişcări bruşte, pot luxa artera şi vena axilară şi subclaviculară determinând prinderea acestora între clavicula şi prima coastă. Aceasta determină lezarea intimei venoase şi inițiază tromboza venoasă (14).

Debutul în forma primitivă este imediat sau la interval scurt după un episod de abducție ipsilaterală şi rotație externă repetitive şi susținute, cu rigiditatea brațului şi edemul mâinii. Durerile sunt uşoare sau moderate. Ulterior edemul progresează şi apare circulația subcutanată vicariantă. Venele cutanate ale membrului afectat şi cele din regiunile pectorală şi scapulară se dilată. Vena axilară trombozată poate fi greu de palpat, cordonul venos devenind bine palpabil numai dacă tromboza se extinde şi la vena brahială. Emboliile sunt foarte rare, sindromul posttrombotic este mai frecvent (tendință la recidive), iar evoluția spre *phlegmasia caerulea* lipseşte (15).

Tabelul II descrie testele fizice de provocare utilizate în cursul examenului fizic al pacientului (3).

TABELUL II

Teste fizice de provocare

Test diagnostic	**Manevra**	**Test pozitiv**	**Mecanism**
Adson (scalen)	Pacientul este instruit să inspire profund şi să-şi țină respirația în timp ce gâtul este întins complet şi capul întors către zona afectată.	Pierderea sau scăderea pulsului radial saau reproducerea simptomelor neurologice sugerează un test pozitiv.	Testul Adon (scalen) provoacă îngustarea spațiului dintre mușchii scaleni anterior şi mijlociu, rezultând compresia arterei subclaviculare şi a plexului brahial.
Halsted (costoclavicular)	Pacientul este instruit să-şi aranjeze umerii într-o poziție militară (trași din spate şi în jos).	Pierderea sau scăderea pulsului radial saau reproducerea simptomelor neurologice sugerează un test pozitiv.	Testul Halsted (costoclavicular) este folosit pentru a diminua spațiul costoclavicular dintre prima coastă şi clavicula, determinând astfel compresia neurovasculară.
Wright (hiperabducție)	Pentru a realiza testul, brațul pacientului este în hiperabducție 180º.	Pierderea sau scăderea pulsului radial saau reproducerea simptomelor neurologice sugerează un test pozitiv.	Testul Wright (hiperabducție) determină compresia structurilor neurovasculare în regiunea subcoracoidă de către tendonul mușchiului pectoral, capul humeral sau procesul coracoidian şi mușchii/tendoanele inserate pe acesta.

Alte semne clinice pot fi observate în TVP a membrelor superioare, cum ar fi: subfebrilitate care poate fi atribuită trombozei sau febră mare sugestivă pentru o tromboflebită septică; tahicardia poate fi prezentă dacă există embolie pulmonară sau obstrucția venei cave superioare; sindrom Paget-Schroetter atipic, cu hipertensiunea pulmonară ca rezultat al emboliei pulmonare. Uneori, pacientul poate prezenta o tumefactie localizată a brațului și fetei, sau poate avea asociate simptome vizuale sau vestibulare, în funcție de locul obstrucției (16).

Un istoric de traumatism, sondă á demeure și status trombofilic trebuie excluse pentru a distinge sindromul Paget-Schroetter de forma secundară a trombozei venoase axilară/subclaviculară. Diagnosticul este confirmat de venogramă sau de ultrasonografia dublă. Rareori, tromboza secundară poate debuta în vena cavă superioară și se extinde retrograd. Emboliile sunt rare în absența transformării în phlegmasia caerulea, și ea foarte rară (17).

Eritemul localizat, sensibilitatea dureroasă la palpare și parestezиile sunt simptome nespecifice și pot apărea în celulite, limfedem, compresia neoplazică a vaselor de sânge, leziuni musculare sau tromboză venoasă superficială (tab. III). Mai puțin de jumătate dintre acești pacienți au dovezi imagistice pentru UEDVT. De aceea, este important a confirma sau exclude diagnosticul utilizând teste obiective (18).

TABELUL III

Semne și simptome ale trombozei venei axilare și subclaviculare versus sindromul aperturii toracice.

Afecțiunea	Simptome	Semne
Tromboza axilară subclaviculară	Disconfort vag al umărului și gâtului; edemal brațului și mâinii	Plenitudine supraclaviculară, cord palpabil, edem /cianoză a extremității, distensia venei jugulare, vene dilatate, imposibil accesul cateterului.
Sindromul aperturii toracice	Durere ce iradiază în braț; scăderea forței de prehensiune.	Sensibilitate dureroasă la nivelul plexului brahial, atrofia brațului/mâinii, test Adson sau Wright pozitiv.

Ultrasonografia dublă este primul test imagistic ales în scop diagnostic deoarece este neinvaziv și demonstrează o înaltă sensibilitate și specificitate pentru UEDVT periferică (jugulară, subclavicular distal, axilară). Rezultate fals negative se produc datorită umbrelor acustice ale claviculei care limitează completa vizualizare a venei subclaviculare (19).

Imagistica prin rezonanță magnetică a vaselor și a țesuturior moi este o metodă neinvazivă utilizată pentru a detecta trombii dintr-o regiunea anatomică dificil de abordat. În prezența trombozei, angiografia prin rezonanță magnetică are o bună corelație cu venograma de contrast când a doua este contraindicată (tab. IV) (20).

Venograma de contrast este testul diagnostic standard, ce poate fi efectuat preoperator în intervenții precum infuzia direcționată pe cateter a tromboliticelor, instrumente mecanice precum Angiojet, în angioplastie și urmărirea prin venograme pentru a aprecia răspunsul la intervenție (20).

Flebografia membrului superior este un examen radiologic ce presupune opacifie-

rea axului venos al acestuia prin injectare directă intravenoasă a substanței de contrast. În achiziția fluorografică, avantajul achiziției sub control scopic şi digitalizarea permit vizualizarea întregului ax venos cu o singură injectare (21).

TABELUL IV

Teste imagistice utilizate; riscuri şi beneficii.

Test diagnostic	Avantaje	Dezavantaje
Ultrasonografia dublă	Necostisitoare, reproductibilă, noninvazivă	Inconvenientul de a detecta trombul sub claviculă în vena subclaviculară.
Computer tomografia (CT)	Compresiune vasculară din exterior; poate descrie trombul central.	Necesită substanță de contrast; nevalidată în totalitate.
Rezonanța magnetică (MRI)	Detectează trombul central cu acurateţe; bună evaluare a colateralelor.	Nepotrivită pentru pacienții cu implanturi metalice sau pacemakere; claustrofobie.

Flebografia nu este practicată la femeia gravidă, pacienți alergici la iod, în caz de insuficiență renală, hepatică, etc. (21).

Această tehnică invazivă de explorare a circulației venoase se poate solda cu urmatoarele complicații: tromboflebită post flebografie (sau agravarea celei preexistente, cca 1%); infecție la locul puncției; extravazarea contrastului, ce poate duce la necroze tisulare locale (22).

TRATAMENT

Obiectivele principale ale tratamentului TVP sunt prevenirea embolismului pulmonar şi a sindromului posttrombotic. Pentru a realiza aceste obiective, tratamentul ideal ar trebui: 1) să reducă masa trombusului; 2) să mențină structura şi funcția sistemului venos a membrului afectat, inclusiv structura şi funcția valvulelor venoase; 3) să prevină embolizarea trombilor în plămâni. Aceste obiective sunt parțial atinse de tratamentul anticoagulant şi într-un mod aproape maximal de cel trombotic (23).

Anticoagularea este esențială în managementul TVP a membrelor superioare. Scopul este de a preveni eventuala propagare a trombului şi de a menține permeabilitatea venelor colaterale (23). Anticoagulantele nu lizează trombusul, ci previn extinderea acestuia, ameliorează simptomele acute şi indirect previn embolismul pulmonar, deci mortalitatea prin această complicație. Trombii venoşi sunt lizați parțial prin activitatea trombolitică naturală sau se epitelizează, dar structurile valvulare venoase rămân afectate, permițând apariția secundară a sindromului posttrombotic la aproximativ 50% din bolnavii cu TVP proximală. Astfel, terapia anticoagulantă este esențială în reducerea extensiei şi complicațiilor imediate ale TVP, dar este relativ puțin eficace în prevenirea sechelelor tardive, în special a sindromului posttrombotic (24).

Mai multe studii au validat utilizarea heparinei nefracționate cu greutate moleculară mică şi chiar a inhibitorului trombinei în terapia TVP ca o punte către terapia cu warfarină (22). Durata standard este de 6 luni de anticoagulante orale (21, 22, 24).

Agenții trombolitici lizează trombii venoşi prin activarea enzimei plasmatice inactive, plasminogen, în plasmină activă.

Aceasta degradează fibrina din trombus sau din cheagul hemostatic, în peptide solubile. De asemenea plasmina circulantă degradează fibrinogenul solubil, şi în parte, alte proteine plasmatice (21).

Pacienţii tineri şi activi cu sindrom Paget-Schroetter au un risc crescut pentru dezvoltarea consecinţelor pe termen lung (impotenţă funcţională) şi sunt de obicei trataţi mult mai agresiv decât utilizarea doar a anticoagulării convenţionale (21, 24, 25). Tromboliza restabileşte permeabilitatea venoasă rapid, minimizând prejudiciul endoteliului vascular şi reducând riscul complicaţiilor pe termen lung; aceasta este în special valabil pentru sindromul post-trombotic, caracterizat printr-o durere şi tumefacţie cronică a braţului şi mâinii. Contrar, tromboliza este rar utilizată pentru tratamentul TVP a extremităţilor inferioare deoarece aceşti pacienţi nu sunt, în general, suficient de preocupaţi de potenţialul risc al tumefacţiei cronice a piciorului. Cei mai buni candidaţi pentru tromboliză sunt tinerii, de altfel pacienţi sănătoşi cu TVP a membrelor superioare primară, pacienţii cu sindromul venei cave superioare simptomatic şi cei cărora li se impune păstrarea unui cateter venos central (25).

Contraindicaţiile includ sângerarea activă, intervenţiile neurochirurgicale în ultimele 2 luni, un istoric de infarct hemoragic, hipersensibilitate la agentul trombolitic şi intervenţii chirurgicale în ultimele 10 zile. Riscurile efectelor sistemice ale trombolizei sunt minimalizate folosind tromboliza dirijată prin cateter (26). Consensul general este de a folosi activatorul tisular de plasminogen obţinut prin metoda recombinării (rt-PA) sau urokinaza (23).

Intervenţia mecanică cu Angiojet poate evacua o mare cantitate a trombusului şi, în acest fel, limitează doza şi durata terapiei trombolitice (27). Pentru a preveni depunerea de fibrină în jurul cateterului în timpul tratamentului cu trombolitice, heparina este administrată în paralel. (20).

Dacă compresia venoasă extrinsecă provoacă PSS, majoritatea chirurgilor vasculari pledează pentru corecţia precoce a anatomiei (20, 26), care poate include rezecţia primei coaste şi/sau a claviculei, în funcţie de părţile implicate.

Arhiva spaniolă RIETE Registry în care sunt înscrişi pacienţii cu TVP sau EP, evidenţiază că pacienţii cu TVP a braţului prezintă un risc crescut de evenimente mixte (TVP recurentă, embolie pulmonară simptomatică sau sângerări masive) (OR = 3; 95% CI, 1,4-6,4) odată cu înaintarea în vârstă, cu apariţia neoplasmului sau dacă au prezentat la internare EP simptomatic (28).

Un studiu efectuat în perioada ianuarie 1997 - ianuarie 2007, la Departamentul de Chirurgie Vasculară al University School of Medicine, St. Louis, Washington, a evidenţiat că durata totală a conduitei terapeutice a fost semnificativ corelată cu intervalul de timp cuprins între diagnosticul venografic şi intervenţia chirurgicală ($r = 0,820$; $p < 0,001$), fiind mai lung la pacienţii cu simptome persistente ($p < 0,05$) sau retromboză înainte de indrumarea către medicul specialist chirurg ($p < 0,01$). Astfel, evoluţie favorabilă s-a datorat abordării multidisciplinare (29).

Un studiu efectuat la Departamentul de Chirurgie Cardiotoracică al Baylor University Medical Center, Dallas, Texas a demonstrat că stenturile intravenoase (IS) sunt contraindicate pacienţilor cu tromboză a venei axilare şi/sau subclaviculare (sindromul Paget-Schrotter). (30).

Un studiu efectuat în cadrul Departamentului de Cardiologie Interventională, University of Minnesota Medical School,

Minneapolis, USA, în perioada iulie 1985 - iunie 2006, evidentiază importanța tratamentului de urgență în sindromul Paget-Schrotter, constând în tromboliza imediată şi operație după evenimentul acut (31).

Unul dintre studiile efectuate în România despre tromboflebita profundă a membrului superior, publicat în 2006, evidențiază importanța terapiei anticoagulante precoce concomitent cu terapia adjuvantă în vindecarea fără sechele (32).

O echipă de medici germani de la Universität des Saarlandes, Hamburg a elaborat un studiu țintit pe circumstanțele în care tromboza primară a extremității superioare poate fi proclamată şi compensată de către societățile de asigurări fie ca accident de muncă, fie ca boală profesională. Concluzia a fost că sindromul Paget-Schroetter este o boală profesională si există relație între istoricul profesional şi manifestarea trombozei, eforturile fizice crescute sau mişcările din timpul activităților de la locul de muncă (33). În România, tromboflebita de efort a membrelor superioare este inclusă în lista bolilor profesionale cu declarare, cercetare şi evidență obligatorie, conform H.G. 1425/2006.

EVOLUȚIE

Pacienții simptomatici netratați vor dezvolta, datorită obstrucției venoase, o incapacitate pe termen lung având drept rezultat o pierdere importantă a productivității ocupaționale şi a calității vieții. Din acest motiv, cateterizarea precoce în scopul trombolizei, urmată de anticoagulare şi intervenție chirurgicală sunt recomandate în majoritatea literaturii recente. Tromboliza este cea mai comună formă de tratament, urmată de chirurgie, dacă se impune. Majoritatea literaturii susține o abordare multimodală, dar nu există un consens definit al managementului. Este nevoie de studii clinice randomizate care să stabilească cele mai sigure şi eficiente anticoagulante şi să definească durata optimă a tratamentului după tromboliză (34).

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CANCERUL GASTRIC LOCAL AVANSAT SAU METASTAZAT – NOI SOLUŢII TERAPEUTICE

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LOCALLY ADVANCED OR METASTATIC GASTRIC CANCER - NEW THERAPEUTIC SOLUTIONS (Abstract): Gastric cancer represents a major health problem worldwide. It is often diagnosed at an advanced stage, because screening, which would help an early detection is performed only in Japan. Gastric cancer rates may be decreased by reducing smoking and alcohol consumption and by increasing the number of fresh fruits and vegetables eaten. Eradication of Helicobacter pylori may also decrease gastric cancer incidence. Seventy-five percent of the patients are diagnosed in unresectable stage. They represent a challenge for clinicians as they have to choose between a strictly supportive approach or to expose patients to the side-effects of a potentially ineffective treatment. The treatment of advanced gastric cancer is essentially palliative. Therapy's main aim in this common group of patients with unresectable or metastatic disease is to achieve good symptomatic control, to improve quality of life, to avoid tumor progression and to prolong survival. **Key words**: ADVANCED GASTRIC CANCER, CHEMOTHERAPY, RETROSPECTIVE STUDY

Cancerul gastric constituie a doua cauză de mortalitate prin cancere în întreaga lume, cu toate că incidenţa globală este în scădere. Se estimează că în SUA, în anul 2009 au fost 21.130 cazuri noi şi 10.620 decese datorate neoplaziilor gastrice (1). Vârsta medie la diagnostic este de 71 ani în SUA şi o decadă mai devreme în Japonia (2).

Cauzele includ factori genetici şi factori din mediu. Din punct de vedere genetic sunt descrise: mutaţia genică p53, pierderea heterozigoţiei LOH a locusului APC, deleţia sau supresia genei FHIT etc. Factorii din mediu includ: dieta bogată în sare, nitraţii; produsele afumate, fumatul, infecţia cu *H. pylori* şi virusul Epstein Barr (EBV), obezitatea, consumul cronic de băuturi alcoolice, intervenţiile chirurgicale gastrice anterioare (3).

Cancerul gastric precoce este asimptomatic în 80% din cazuri (4). Când simptomele apar ele tind să mimeze ulcerul peptic. Cancerul gastric avansat este însoţit de scădere ponderală, dureri abdominale, greaţă, vărsături, saţietate precoce, disfagie, melenă, obstrucţie pilorică.

Diagnosticul este stabilit prin utilizarea de primă intenţie a endoscopiei digestive superioare (acurateţe diagnostică de 95%). În Japonia şi alte state cu prevalenţă mare se folosesc cromoendoscopia, NBI (narrow band imaging), endoscopia cu magnificaţie pentru detecţia cancerului gastric precoce.

Metodele de stadializare includ: ultrasonografia endoscopică, tomografia computerizată, rezonanţa magnetică, PET scan.

Prognosticul cancerului gastric rezecabil este influenţat de invazia peretelui gastric şi de implicarea ganglionilor limfatici regionali.

TRATAMENT

1. TRATAMENTUL CHIRURGICAL ŞI TERAPIA ADJUVANTĂ PERIOPERATORIE

Această opţiune reprezintă singura modalitate cu viză curativă a cancerului gastric. În Japonia este folosită cu succes rezecţia endoscopică mucozală pentru leziuni T1 de dimensiuni reduse. De asemenea, este folosită cu succes rezecţia gastrică laparoscopică pentru cancere precoce şi leziuni submucozale.

Cele 2 metode chirurgicale generale utilizate pentru cancerul gastric localizat sunt gastrectomia totală şi subtotală cu disecţia limfonodulilor regionali.

Rolul chimioterapiei adjuvante în CG este încă în curs de elucidare. Într-un studiu publicat în 2001, Lowy a demonstrat superioritatea radio-chimioterapiei (RT 45Gy + 5-fluorouracil 300 mg/m^2) urmata la 4-6 săptămâni de rezecţie chirurgicală şi radio-chimioterapie intraoperatorie (10 Gy). 11% din pacienţi au avut răspuns complet, iar răspunsul parţial a fost obţinut la 63% (5).

Într-un recent studiu de fază III, Stahl şi colaboratorii au constatat o creştere a răspunsului complet (15,5% vs. 2%) şi a răspunsului ganglionar (64,4% *vs*. 37,6%) în cazul pacienţilor cu adenocarcinom de joncţiune esogastrică local avansat, la care rezecţia chirurgicală a fost precedată de radiochimioterapie (cisplatin, 5- fluorouracil + 15-17 Gy) respectiv numai chimioterapie. Rata de supravieţuire la 3 ani a crescut de la 27,7% la 47,4% în cadrul braţului cu radiochimioterapie (6).

Chimioterapia adjuvantă intraperitoneală necesită evaluare şi investigaţii suplimentare în cadrul unor trialuri randomizate.

Trialul MAGIC (faza III) a evidenţiat faptul că în cazul pacienţilor cu adenocarcinoame esofagiene distale sau gastrice, chimioterapia perioperatorie (3 cicluri preoperatorii şi 3 postoperatorii constând în epirubicin, cisplatin şi 5- fluorouracil - schema ECF respectiv ECX când s-a înlocuit 5- fluorouracil cu xeloda) poate duce la descreşterea dimensiunilor tumorale şi la o îmbunătăţire semnificativă a progresiei şi a supravieţuirii globale de la 20 luni la 24 luni în favoarea braţului cu chimioterapie (7). În Marea Britanie şi în alte state europene, acesta este standardul de tratament la pacienţii cu cancer gastric rezecabil.

2. TRATAMENTUL ÎN CANCERUL GASTRIC LOCAL AVANSAT ŞI METASTAZAT

În practica zilnică suntem confruntaţi cu cereri contradictorii ale pacienţilor în ceea ce priveşte eficacitatea tratamentului (care este bineînţeles şi agresiv), dar şi menţinerea unei calităţi a vieţii corespunzătoare. CG este unul din cancerele care evoluează cu cele mai multe complicaţii generale: fracturi, tromboembolii, dureri, scădere în greutate, anorexie şi în plus multiple complicaţii digestive: ocluzii intestinale, perforaţii, sângerări şi disfuncţii viscerale motorii.

În acest moment în care "medicina bazată pe dovezi" reprezintă standardul de aur pentru o bună practică medicală şi există din plin literatură (review) publicată în jurnale respectabile, doar puţine din ele se referă la strategia din boala incurabilă, adică stadiul IV. De asemenea în practica zilnică, de cele mai multe ori, ne conducem în alegerea unui tratament după experienţa personală şi după posibilităţile centrului, şi mai puţin după datele din literatură.

În acest stadiu, scopul terapiei este reprezentat de un mai bun control al simptomatologiei, ameliorarea calităţii vieţii, întârzierea progresiei tumorale şi prelungirea supravieţuirii (8).

Modalităţi de tratament:

- stentare enterală în caz de obstrucţie; 7-22% din pacienţi însă necesită reintervenţii (9),
- rezecţia gastrică paliativă fără limfadenectomie D2 aduce o uşoară creştere a supravieţuirii comparativ cu lipsa gas-

TABELUL I
Monochimioterapia în cancerul gastric

Schema	RR	Observaţii
5- fluorouracil	21%	
Mitomicină C	30%	Mielotoxicitate tardivă (8 săpt.)
Doxorubicină	17%	
Cisplatin	19%	Lacave et al.
Taxani	17-29%	Alternativă la linia I
UFT (uracil + ftorafur)	28%	Adm. orală. RA: greaţă, vărsături, diaree
Capecitabină	19,4%	Adm. orală
Irinotecan	19%	
S1	49%	Utilizat doar in Japonia in prezent

TABELUL II
Regimuri de polichimioterapie folosite în cancerul gastric local avansat/metastazat

Schema	RR	Suprav. medie	Observaţii
FAM (5-FU, doxorubicin, mitomicină C)	40%		Standard până în 1980
FAMTX (5-FU, doxorubicină, leucovorin, metotrexat)	41%	42 săpt.	Standard în 1990
ECF (epidoxorubicin, cisplatin, 5-FU)	*71%*	*8,9 luni*	*Standard actual – toxicitate redusă*
EAP (etoposid, doxorubicin, cisplatin)	64%	9 luni	Posibile decese datorate toxicităţii
ELF (etoposid, 5-FU, leucovorin)	53%	11 luni	
Oxaliplatin, 5-FU, leucovorin	54%	11,4 luni	Efecte adverse: neutropenie, anemie
EPFL (epirubicin, cisplatin, 5-FU, leucovorin)	62%	8 luni	Mielotoxicitate importantă
XP(xeloda,cisplatin)	55%		Toxicitate scazuta grd3/4
Docetaxel, cisplatin	53%		Neutropenie 68%

trectomiei (9),

- by-pass gastric paliativ – gastrojejunostomă laparoscopică (9),
- radioterapia paliativă – determină amendarea sângerărilor locale şi a durerii date de invazia tumorală, fără a ameliora supravieţuirea,
- imunoterapia antineoplazică – nespecifică (BCG viu) şi specifică (antigeni tumorali sterilizaţi, IL, tulpini bacilare atenuate, anticorpi monoclonali etc.) – sunt metode aflate în studiu,
- chimioterapia paliativă aduce un plus de supravieţuire de 3-9 luni faţă de BSC. Trialurile randomizate care au comparat monoterapia (tabelul I) cu regimurile combinate au arătat rate de răspuns crescute pentru polichimioterapie, cu o rată de supravieţuire aproximativ egală.

În prezent, monoterapia nu mai este recomandată în managementul cancerului gastric local avansat/metastazat. Multe din trialurile care evaluează monochimioterapia sunt mici şi necontrolate, făcând dificilă elaborarea unor concluzii clare în ceea ce priveşte eficienţa. Metaanaliza a 11 trialuri randomizate care au cuprins un număr de 1472 pacienţi, din care 8 s-au desfăşurat înainte sau în anii 1990, a relevat faptul că terapia cu un singur agent citostatic nu este recomandată

TABELUL III
Polichimioterapia de linia a II-a

Tratament	Nr. pac.	RR	Timp până la progresia bolii	Suprav. globală (luni)
Docetaxel 75 mg/m² la 3 săpt. (16)	49	16%	2,5	8,3
FOLFIRI (17)	38	29%	3,7	6,4
Irinotecan 125 mg/m² z1,8,15 – la 4 săpt. (18)	37	20%	2,6	5,2
Irinotecan 160 mg/m² + Docetaxel 65 mg /m² (19)	49	20%	2,7	8,9

în CG local avansat/metastazat.

Trialul REAL 2 a dovedit noninferioritatea combinaţiei oxaliplatin+capecitabină faţă de 5-FU (fluorouracil) + cisplatin, supravieţuirea fiind aproximativ egală în cele 4 braţe ale studiului (ECX /EOX/ECF/ ECX) (10).

În acelaşi timp, la Royal Marsden Hospital din Londra a fost propus regimul ECF cu o perfuzie continuă de 5FU în doză mică, cu o rată de răspuns de 71% (din care 12% răspunsuri complete). Comparativ cu FAMTX, într-un studiu de fază III, ECF-ul a determinat o rată de răspuns de 45% vs 21% (p=0.0002) şi un timp mediu până la progresie de 7,4 luni *vs* 3,4 luni, cu o supravieţuire globală de 8,9 luni *vs* 5,7 luni (p=0,00090). Astfel ECF a devenit standardul în practica zilnică (Tabel II).

Într-un studiu coreean de fază III (xeloda 1000 mg/m²/zi, z1-14 + cisplatin 60-80 mg/ m², z1, repetat la 3 săptămâni) efectuat pe 42 pacienţi, rata de răspuns a fost de 54,8%, timpul mediu de progresie fiind de 6,3 luni şi fără efecte adverse importante (11).

Trialul randomizat multicentric ML 17032, la care au participat 13 ţări, a comparat eficacitatea şi profilul de siguranţă a combinaţiei Xeloda/Cisplatin (XP) cu regimul clasic 5FU/Cisplatin (FP). În braţul XP s-a administrat Xeloda 1000 mg/m² p.o. zilele 1-14 ţi cisplatin 80 mg/m² i.v. ziua 1, iar în bratul FP s-a administrat 5FU 800 mg/m²/zi perfuzie continua zilele 1-5 şi cisplatin 80 mg/m² i.v. ziua 1, la 21 zile interval. Rata de răspuns a fost superioară în braţul XP *vs* FP (41% *vs* 29%, p=0.03), iar supravieţuirea generală mediană (10.5 *vs* 9.3 luni) şi cea fără semne de boală (5.6 *vs* 5.0 luni) . Cele mai importante efecte adverse de grad 3-4 (XP *vs* FP) au fost neutropenia (16% *vs* 19%), vărsăturile (7% *vs* 9%), stomatita (2% *vs* 7%), diareea (5% *vs* 5%) şi anemia (5% *vs* 3%). Rezultatele susţin posibilitatea înlocuirii 5FU perfuzabil cu Xeloda în cancerul gastric avansat.

În ultimii ani au fost folosiţi pentru tratarea cancerului gastric local avansat sau metastazat: taxani (paclitaxel şi docetaxel), inhibitori de topoizomerază I (irinotecan), derivaţi noi de platină (oxaliplatin). Per ansamblu, folosirea acestor droguri noi în studiile de fază II a dus la o rată de răspuns de 65%, fără a se înregistra o creştere globală a supravieţuirii (circa 10 luni) (12,13,14).

Supraexpresia receptorului factorului de creştere epidermală EGFR, a receptorului factorului de creştere endotelială VEGFR şi a HER2 a fost asociată cu un prognostic mai prost. În trialuri clinice, trastuzumabul (anticorp anti-HER2), bevacizumabul (anticorp anti-EGFR), cetuximabul (anticorp anti--VEGFR) au fost testaţi în combinaţii cu diverse citostatice.

Studiul ToGA (15) care a comparat trastuzumabul (la pacienţii HER2 pozitivi) în combinaţie cu cisplatin + 5-FU vs. cisplatin + 5-FU a evidenţiat superioritatea regimului care a asociat trastuzumabul, supravieţuirea mediană fiind de 13,5 *vs*. 11,1 luni. Acest

TABELUL IV
Schemele de polichimioterapie folosite

Linia I de tratament:	
• *MAYO*	*25 pac.*
• *5-FU + CDDP*	*22 pac.*
Linia II de tratament	
• *Capecitabină + CDDP*	*5 pac.*
• *Taxol + CDDP*	*7 pac.*

fapt a dus la înregistrarea schemei ca standard de tratament la pacienţii cu cancer gastric local avansat/metastazat HER2 pozitivi.

Polichimioterapia de linia II a (tabelul III) – trialurile de fază II au arătat o rată de răspuns joasă la pacienţii selectaţi care pot beneficia de chimioterapie de linia a II-a. Nu există trialuri de fază III pentru chimioterapia de linia a II-a.

Există în momentul de faţă mai multe trialuri în desfăşurare :

(a) ST03 – MAGIC B : adenocarcinom gastric rezecabil sau Type III OGJ (3 cicluri ECX - epidoxorubicin, cisplatin, xeloda/chirurgie/3 cicluri ECX *vs.* 3 cicluri ECX + bevacizumab/chirurgie/3 cicluri ECX + bevacizumab) (20),

(b) CRITICS : adenocarcinom gastric rezecabil sau Type III OGJ (3 cicluri ECX/ chirurgie/3 cicluri ECX *vs.* 3 cicluri ECX/ chirurgie/ECX + RT 45Gy) (21),

(c) REAL3 : adenocarcinom avansat netratat sau carcinom nediferenţiat de esofag, joncţiune eso-gastrică sau stomac (EOX – epidoxorubicin, oxaliplatin, xeloda vs. EOX + panitumumab) (22).

Regimuri de chimioterapie folosite în cancerul gastric :

- **FAM** : 5FU 600 mg/m^2 iv z1,8,29,36+ Dox 30mg/m^2 iv z1,29+Mit C10mg/m^2 iv z1 repetat la 8săpt (12),
- **FAMTX** : 5FU 1500 mg/m^2 iv z1 la 1h după MTX+Dox 30 mg/m^2 iv z15+ MTX 1500 mg/m^2 iv z1 + leucovorin 15 mg/ m^2 po administrat la fiecare 6h,12 administrări, începând la 24 h după MTX, cu hidratare şi alcalinizarea urinii anterior de administrarea MTX, repetat la 28 zile (12),
- **ECF** : Epidoxorubicin 50 mg/m^2 iv z1 +Cisplatin 60 mg/m^2 iv z1, repetat la 3 săpt pentru max 8 cicluri +5FU 200 mg/ m^2 în pev continuă - 6 luni (23,)
- **ECX** : epidoxorubicin 50mg/m2ivz1+Cisplatin 60mg/m2 iv z1+Xeloda 2000mg/ m^2, po,z1-14, repetat ciclul în z22 (13)
- **DCX** : docetaxel : 60mg/m^2 iv z1+cisplatin 60 mg/m^2 iv z1+xeloda 2000 mg/ m^2, po z1-14 repetat la 3 săpt (11),
- **EOX** : epidoxorubicin 50 mg/m^2 iv z1 + Oxaliplatin 130mg/m^2 iv z1+Xeloda 2000 mg/m^2, po, z1-14, repetat ciclul în z 22,
- **EOF** : epidoxorubicin 50 mg/m^2 iv z1+ Oxaliplatin 130mg/m^2 + 5FU 200 mg/m^2 în pev continuă 6 luni, repetat ciclul în z22,
- **DC** : docetaxel 75 mg/m^2 iv,z1+cisplatin 75 mg/m^2 iv, z1, repetat la 3 săptămâni (14),
- **DCF** : docetaxel 75 mg/m^2 iv, z1+cisplatin 75 mg/m^2 iv, z1+5FU 200 mg/m^2 pev prelungită timp de 2 săptămâni, repetată la 3 săptămâni (14),
- **DCF(V325)** : docetaxel 75 mg/m^2 iv, z1+ cisplatin 75 mg/m^2 iv, z1+5FU 750 mg/m^2, z1-5 repetat la 3 săptămâni,
- **CF** : cisplatin 100 mg/m^2, z1+5FU 1g/m^2 în pev continuă z1-5, repetat la 4 săptămâni,
- **ILF** : CPT-11 80 mg/m^2 urmat de LV 500 mg/m^2 în 2 ore urmat de 5FU 2g/m^2

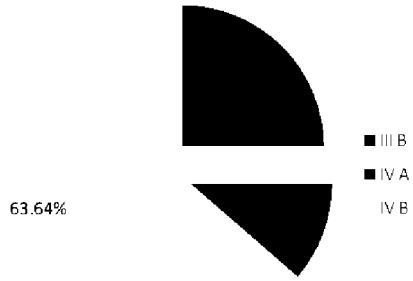

Fig. 1. Repartiţia pacienţilor în funcţie de stadiul bolii

pev de 22 ore, săptămânal timp de 6 săptămâni,

- **ECD**: epirubicin 50mg/m^2 iv, z1+docetaxel 60 mgiv/m^2, z1+cisplatin 60 mg/m^2 iv, z2, repetat la 3 săptămâni,
- **XELOX**: xeloda 2500 mg/m^2, z1-14 + oxaliplatin 85 mg/m^2, z1, repetat la 21 zile,
- **CPT-11**: 70mg/m^2 iv, z1, 15+ cisplatin 80 mg/m^2, z1, repetat la 4 săptămâni,
- **PCF**: paclitaxel 175 mg/m^2 iv, z1 + cisplatin 20 mg/m^2, z1-5 + 5FU 750mg/m^2 pev, z1-5, repetat la 3 săptămâni.

În cadrul Departamentului de Oncologie Digestivă a Institutului Clinic Fundeni am efectuat un studiu retrospectiv în perioada 2003-2004, asupra unui număr de 47 pacienţi cu cancer gastric avansat sau metastazat, confirmat histopatologic. Ne-am propus să urmărim toxicitatea schemelor chimioterapice utilizate, supravieţuirea globală şi supravieţuirea fără semne de boală.

Din totalul de 47 pacienţi, un procent de 25% prezentau cancer gastric local avansat, 63,64% stadiul IVA metastatic, respectiv 11,36% stadiul IVB metastatic (Fig.1).

Din punct de vedere histopatologic, toţi cei 47 de pacienţi au prezentat histologie de adenocarcinom.

Vârsta medie a lotului nostru a fost de 57 ani (28-76 ani), mediul de proveniență al pacienţilor fiind predominant urban (41 *vs*. 6 rural). 77% din pacienţi au suferit intervenţie chirurgicală cu viză paliativă, în timp ce la 23% nu s-a intervenit chirurgical.

33 pacienţi (70,2%) prezentau determinări secundare în momentul diagnosticului; 21 pacienţi (44,6%) aveau o singură metastază (12pacienţi – hepatică, 2pacienţi – pulmonară, 5 pacienţi – peritoneală, câte 1 pacient – ovariană, respectiv osoasă; 8 pacienţi aveau 2 localizări de metastaze, iar la 4 pacienţi au fost întâlnite peste 3 localizări de metastaze.

Schemele de polichimioterapie utilizate la aceşti pacienţi sunt prezentate în tabelul IV.

Din punct de vedere al efectelor secundare hematologice, pancitopenia a fost întâlnită predominant în cazul pacienţilor trataţi cu 5FU+CDDP. Toxicitatea digestiva (greaţă, vărsături, diaree, stomatită) a fost întâlnită în ambele braţe, cu o rată mai ridicată în subgrupul celor care au primit 5FU+ CDDP, alopecia urmând aceeaşi tendinţă.

De asemenea, toxicitatea renală a predominat în braţul celor trataţi cu CDDP şi 5-FU.

Supravieţuirea fără semne de boala a fost în medie de 6,31 luni (4,89-7,72 luni), iar cea globală calculată prin metoda Kaplan-Maier a fost în medie de 11 luni (7-14 luni).

Concluzii: (a) chimioterapia sistemică prelungeşte supravieţuirea globală; (b) menţinerea unui status de performanţă corespunzător a făcut posibilă administrarea liniei a II-a de tratament, ceea ce va creşte şi mai mult supravieţuirea globală; (c) probabil administrarea noilor agenţi chimioterapici va creşte nu numai ratele de răspuns, dar şi supravieţuirea globală, permiţând obţinerea unor remisiuni de lungă durată.

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EDITORIAL

COMPLICATIONS IN LAPAROSCOPIC SURGERY KEEP HAVING HIGH INCIDENCE

Unfortunately, Complications in Laparoscopic Surgery happen, and the fact is that they keep having high incidence, despite all interest and "calls for attention". As some consequences can be important, considering the sequels they may cause, the interest for understanding how they happen and the reasons why they show keeps having a high interest.

Nowadays, a very used term related with these problems is "Precision Surgery". As a matter of fact, it is, a summary of the kind of practice we all do, mostly, if not in all cases: performing surgery with utmost care and attention, step-by-step.

Another issue regarding these problems, is that it is also very difficult to convince surgeons to perform "better"; this is the kind of surgery every surgeon is convinced he (or she), "is already performing at "TOP QUALITY".

As a matter of fact, this happens in every case of surgery where the surgeon is more active, using it most commonly, and where he (or she) thinks they "excel".

Using, just as an example, the Iatrogenic lesions of the biliary tract, we can realize that, already in 1944, the great surgeon Grey-Turner was writing in the *Lancet*: "CBD lesions are, almost always, a result of an accident during surgery and, therefore, it can only be attributed to the surgical profession. These lesions cannot be a normal operative risk…"; (1) and A. R. Moossa, in a personal communication said: "we can generalize the 3 dangers of biliary lesions as:

1. Dangerous disease, like late acute cholecystitis, portal hypertension,
2. Dangerous anatomy, considering there are anomalies in 10-15% of cases,
3. Dangerous surgery, when the surgeon performs with technical deficiencies.

We can understand the appearance of complications, following the so-called Reason's Model of complications, or events". This is also known as the Swiss Cheese model (fig. 1). Considering a series of slices of swiss cheese, when all holes of it are in a line, meaning that we face a "lining" of possible errors or "mishaps", then, all conditions are arranged for the complication to happen.

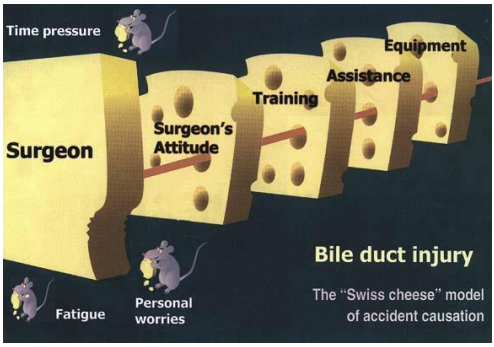

Fig. 1. The "Swiss Cheese" model of accident in laparoscopy

If we compare errors leading to complications in surgery, with the ones happening in high technology industries, we can see some similarities. They are based, mainly, in four orders of facts: Technical failures, Not following established rules, Ignorance of certain aspects of the process and Visual failures or misguidance for what one is

seeing.

There are certain General Factors, explaining these facts, and the General Etiology of Complications in Laparoscopic Surgery: starting with some technical situations, we have, inadequate placement of trocars, bad field exposition, bad light, bad anesthesia, surgeon (or team)'s inexperience, surgeon (or team)'s tiredness, lack of knowledge of eventual anomalies, patient issues, disease itself, inappropriateness (somehow) of the surgeon and/or surgical team, inappropriateness of the instruments..

Some points should be followed to minimize complications.

To start, let's not simplify Indications; these should follow the Guidelines for Indications for any kind of Laparoscopic surgery, not "forcing" the indications for each proposed patient.

Just because Laparoscopic Surgery is "simpler" do not see it as a reason for widening indications.

There are other problems related with the "Learning Curve" although this might be an issue to discuss separately, if one considers the concept of "Learning Curve" by itself. To start with, the "Learning Curve" for Laparoscopic Cholecystectomy, for instance, goes well beyond 50 cases and the operating time keeps lowering till 200 cases; on another hand, the improvement in the cognitive skills to deal with difficult cases continues for more than that. This according with Voitk *et al.*, as published in the *Am J Surg*, in 2001 (2).

But the risk goes beyond "first cases"; according to Morgenstern *et al.*, in a paper published in *Am J Surg*, in 1995, in a single institution, the first 1284 cases had a rate of complications of - 0.58% the following 1143 cases had a similar rate, - 0.50% (3).

Calvete *et al.*, published a paper in 2000, in *Surgical Endoscopy*, where they made an enquiry to 1500 surgeons and realized that about 30% of the rate of Bile Duct Injuries reported by those surgeons occurred after the first 200 cases (4).

What we can summarize is that surgeon's experience does not minimize the risk.

Way *et al*., published a very good paper in *Annals of Surgery*, in 2003, showing an interesting point for causes of Common Bile Duct lesions in Laparoscopic Cholecystectomies (5).

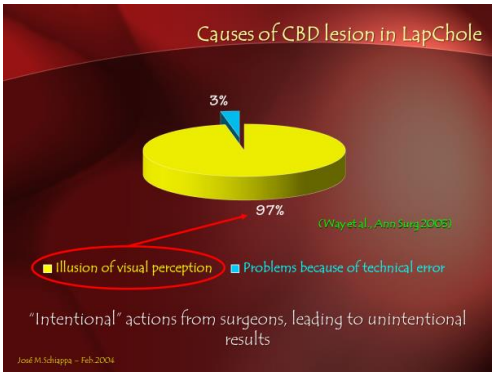

Fig. 2. Causes of Common Bile Duct lesions in Laparoscopic Cholecystectomies

This paper shows that only 3% of the causes of CBD lesions are technical; the other 97% are a result of what is called illusion visualization of reality (fig. 2). The brain, through a process called "Heuristic" shows its "laziness". As such the brain shows what it believes is the wish of the person to see. This leads to "Intentional" actions from surgeons, leading to unintentional results. We can show some examples of these processes: "Heuristic" Processes.

In this image, most people believe that they are seeing two triangles, a white one and a black one. In truth, there are no triangles at all: there are only three angles and

three "Pack Man" in such a disposition that they show the composed image one's brain processes as a composite one. This image is called - Kanizsa's Triangle (fig. 3).

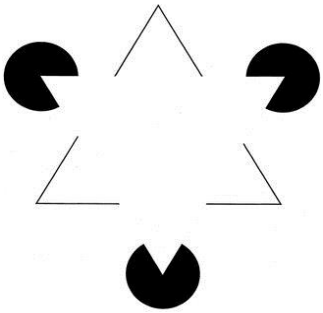

Fig. 3. Kanizsa's Triangle

Another example is the image above: depending on the first time one looks at it (and the fact that there is a mention of "first time" is because after this first viewing, it will be quite difficult to see it otherwise).

Another way of understanding "Heuristic" Processes is the watching of this turning image of a lady. Some persons will be able to see her turning Clockwise, some others turning Anti-clockwise: the authors of this image have found out that the ones seeing her turning Clockwise have a dominance in their brains, from the Right Brain, and the ones watching her turning Anti-Clockwise have a dominance from their Left Brain, as shown in the image (fig. 4). Now, turning into interpretation of this turning image: The Right Brain provides Anarchic Thinking, Intuitive ways of seeing life, Holistic, Synthetic and Subjectivity; while the dominance of the Left Brain, provides Logic Thinking, Sequential, Rational thinking, Analytic views, and Objectivity. Many problems arise, in fitting their ways of thinking, to the ones who can see the image turning both ways, as it may happen to a 5% number of persons, seeing it happen sequentially.

Fig. 4. "Heuristic" Process

A totally different aspect is Instrument Problems; of these we can consider two main ones: Use of Electrosurgery; when it is used too often for too long periods. The hook in general is very difficult to control, despite considerations by surgeons that in their hands all instruments are safe; when used to cut some structures, hooks can cut suddenly, being left free and almost uncontrollable and being able to transmit its heat to the organ it touches. Another issue is the fact (often forgotten) that all heating devices, including most of the thermal sealing and cutting instruments go to very high temperatures (up to 300ºC) and they take about 30 seconds to come back to zero. Very frequently surgeons are seeing using the instrument immediately after a "cutting session", to set aside or to dissect other organs.

Another situation arises from some disturbance in the electrical protection of the instrument, which usually is not visible, or looked for. One point more regards the use of the wrong instrument for a certain task; it is to be noted that any surgeon shall not be carried away, showing one's "abilities"

with every type of technology.

We can say that Lesions in Laparoscopic Surgery are serious lesions, but can be avoided (or, at least, minimized) by: a cautious approach when dealing with any kind of Laparoscopic surgery (1, 6).

Keeping a very liberal policy of conversion or asking for specialized help when facing any unexpected intra-operative problems and considering that by its specificity they should always be dealt with by experienced teams in reference centers, especially if the idea is to solve the problem still with a Laparoscopic approach.

Another precaution measure is to always use Prevention attitudes, and these may (or should) include: surgical access always adapted to patient's morphology, which can be different - do not use a "standard" approach; get always a good exposure of the surgical area and - again, always - get a good identification of structures, before any definitive measure (1,7).

Appropriate and correct dissection always, identifying all structures, clamp any vascular pedicles if in face of any big hemorrhage. Because of the reasons previously mentioned, take great care with the use of electrosurgery; do always use the highest human and surgical good sense (despite being what all surgeons say they do); have adequate knowledge of the local anatomy and of eventual anomalies. One very important point, often forgotten for several reasons is "When in doubt, stop and re-evaluate" and keep always keep a humble attitude.

Any surgeon must be aware of situations and of his (or hers) capacities; as such, every time one thinks it is advisable, ask for help...

Summarizing, one shall always be very aware of some Basic Rules (not to forget) (1, 8):

- No Surgeon is "protected" against this problem.
- Correct and complete training in Laparoscopy is mandatory.
- The leading Surgeon is responsible for the adequacy and quality of all the equipment and adequacy and quality of the team.
- THERE WILL BE MANY OPPORTUNITIES FOR DISASTER "...those who fail to remember the past, are doomed to repeat it..." (Santayana)

Tips for Safe Cholecystectomy (1, 8):

- Urinary bladder evacuated just before anesthesia and surgery;
- General anesthesia;
- Open insertion of first cannula;
- All instruments must be introduced, operated and removed under vision;
- Fundus retracted upwards towards right shoulder;
- Gall bladder neck retracted down and out;
- Cystic lymph node is an important landmark - keep to its right;
- No cautery in Calot's triangle - it should be used in gall bladder bed only;
- Clipping of the cystic duct flush with the common bile duct is not required;
- It is safer to leave a few mm of cystic duct than to encroach even l mm on the common bile duct;
- Double clips on the retained side of the cystic duct and cystic artery;
- Ligature, Haemoclip, Endoloop and stapler are useful devices to tackle a wide cystic, duct;
- Beware of wide cystic duct, long cystic duct, vertical cystic duct - it may be common bile duct;
- Very soft (normal) liver, fatty liver and cirrhotic liver can easily be injured

during retraction;

- Fundus first is a useful technique in difficult cases;
- Partial cholecystectomy is an option in case of difficulty;
- Panic should be avoided in case of bleeding;
- Pressure with a gauze or the mobilized gall bladder will control the bleeding or make it controllable;
- Desperate blinds attempt to clip or coagulate a bleeding point in a pool of blood must be avoided;
- Stumps (cystic duct/cystic artery) should be examined and re-examined for security of clips and any bile leak/bleed;
- Gall bladder bed should be irrigated and examined for any bleed/bile leak (from a cholecystic-hepatic duct);
- Presence of bile should make the surgeon stop and look for the source of bile - gall bladder or bile duct;
- Extraction of gall bladder under vision to make sure that there is no bile/stone spill;
- Spilled bile should be sucked out;
- Spilled stones should be looked for and removed;
- Cholecystectomy should not be considered a small/routine operation and should not be taken lightly/casually;
- Surgeons should avoid the temptation to get their names into books of records.

The day after cholecystectomy, the patient should be without pain, sitting up in the bed, having her breakfast and wanting to go home - vitals should be stable/ normal and abdomen soft; if not, observe the patient in the hospital for another day.

As general **conclusions**, it can be said that these are serious lesions. They can be avoided (or at least, minimized), by a cautious approach, a surgeon's liberal policy of conversion and asking for specialized help when facing unexpected intraoperative problems. No surgeon is "protected" against this problem, and correct training in laparoscopy is mandatory. These lesions, by their specificity, should always be dealt with by experienced teams in reference centers. Only this way, complications can be minimized, and quality and good outcomes guaranteed.

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NEWS

ULTRASTRUCTURAL ASPECTS OF VACUOLAR DEGENERATION OF CARDIOMYOCYTES IN HUMAN ENDOMYOCARDIAL BIOPSIES

Vacuolar degeneration of cardiomyocytes is a histological finding commonly encountered during routine light microscopic examination of human endomyocardial biopsy specimens. The vacuoles appear as intracellular clear areas lacking myofibers. By itself, this finding has little diagnostic value, but may have important clinical implications when the vacuolar contents are of etiological significance (e.g., accumulation of abnormal metabolites), and the clinical importance is increased when the disease is treatable. Thanks to its great resolving power, electron microscopy can often reveal the contents of the vacuoles and lead to a correct diagnosis. It can be used to differentially diagnose lysosomal storage diseases such as Fabry, Danon, and Pompe disease, doxorubicin cardiomyopathy, mitochondrial cardiomyopathy, autophagic degeneration, and accumulation of subcellular organelles (mitochondria, lipofuscin, glycogen granules, endoplasmic reticulum, etc.) as a nonspecific finding in failing cardiomyocytes. In conclusion, observing vacuolar degeneration of cardiomyocytes under a light microscope provides little information because it does not enable one to identify the contents of the vacuoles. On the other hand, electron microscopic examination can often reveal the contents of vacuoles and lead to a correct cardiac disease diagnosis. But even under an electron microscope, the contents of vacuoles cannot always be identified, and so undiagnosed cases remain. These may be patients with novel disease entities waiting to be discovered. Considering the great diagnostic value of electron microscopic examination, the authors strongly recommend fixation of a small piece of tissue samples for electron microscopy at every endomyocardial biopsy procedure (Takemura G, Kanamori H, Okada H, Tsujimoto A, *et al*. Ultrastructural aspects of vacuolar degeneration of cardiomyocytes in human endomyocardial biopsies. *Cardiovascular Pathology* 2017; 30: 64-71).

Doina Butcovan

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EVALUAREA PRIN MONITORIZARE CONTINUĂ A HIPERTENSIUNII ARTERIALE LA VÂRSTNICI

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AMBULATORY BLOOD PRESSURE MONITORING (ABPM) IN ELDERLY (Abstract). **Aim**: Comparison of blood pressure values measured by two methods. **Material and method**: 94 hypertensive patients (66 women and 28 men in relation to 2.36/1) were assessed classically and also by ABPM. For statistic evaluation we have used t – Student test, $\chi 2$ test, Pearson correlation coefficient and variation coefficient (cv%). **Results**: It shows significant differences between mean values of systolic and diastolic blood pressure obtained by the 2 methods. **Conclusions**: ABPM measured values are more accurate compared to clinic, bringing also information on pattern hypertensive therapy. **Key words:** ARTERIAL HYPERTENSION, BLOOD PRESSURE MONITORING, BLOOD PRESSURE MONITORING PARAMETERS

Hipertensiunea arterială (HTA) este considerată cea mai frecventă afecţiune în populaţie şi cu o rată scăzută de control terapeutic (~25%). In practica medicală curentă, tratamentul HTA este orientat frecvent în funcţie de valorile tensiunii arteriale (TA) obţinute manual, iar instituirea tratamentului se face în funcţie de experienţa clinicianului. Elementul esenţial pentru clasificarea HTA este nivelul tensiunii arteriale sistolice (TAs) şi tensiunii arteriale diastolice (TAd) întrucât morbiditatea şi mortalitatea cardiovasculară se află în relaţie continuă cu aceste variabile. Studiile epidemiologice au arătat că riscul de deces cardiovascular se dublează cu fiecare creştere cu 20/10 mmHg a TA, începând de la valori de 115/75 mmHg. Prin monitorizarea continuă a tensiunii arteriale (MCTA) se obţin valori exacte ale TA cu atât mai importante la vârstnici unde se întâlnesc multiple patern-uri hipertensive cu implicaţii terapeutice asupra controlului tensional .

OBIECTIV

Evaluarea hipertensiunii arteriale la vârstnici, prin utilizarea monitorizării continue a tensiunii arteriale.

MATERIAL ŞI METODĂ

Lotul a fost constituit din 94 pacienţi diagnosticaţi cu HTA, 66 femei şi 28 bărbaţi în raport F/M =2,36/1, cu vârste între 65 şi 83 ani. S-au evaluat prin MCTA: tensiunea arterială sistolică, tensiunea arterială diastolică, presiunea arterială medie, presiunea pulsului, încărcarea hiperbară şi indicele de hipertonie. Valorile maxime, medii şi minime se calculează pe 24 ore.

Sistemul de monitorizare utilizat a fost ABPM – 04 care foloseşte programul Medi-Base pentru prelucrarea statistică a datelor.

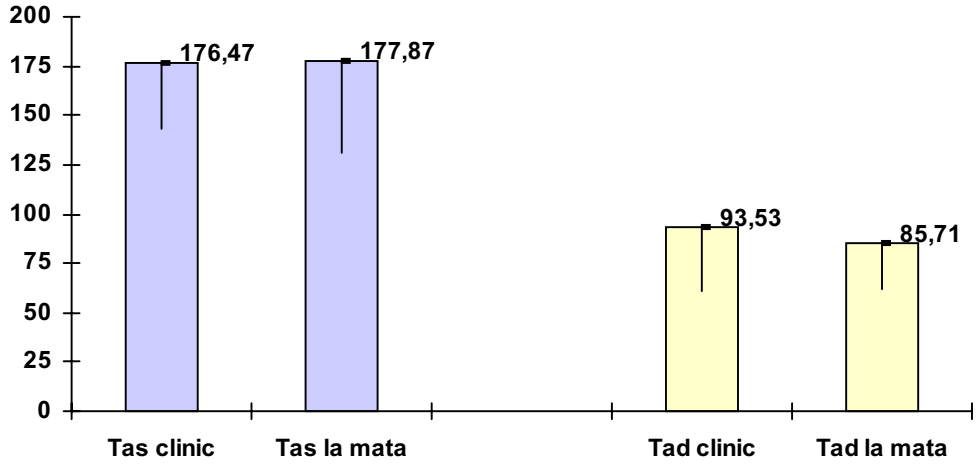

Fig. 1. Valorile medii ale TAs şi TAd.

Aparatul a fost setat pentru măsurarea TA din 15 în 15 minute în intervalul orar 6-22 şi din 30 în 30 minute nocturn (22-6). Rezultatele au fost afişate ca valori maxime, medii şi minime pe 24 h. Softul a calculat indicele diurn, indicele de hipertonie, încărcarea hiperbară pentru tensiunea sistolică, diastolică si medie.

Metode statistice aplicate: testul t-student, coeficientul de corelaţie „Pearson"(r) şi coeficientul de variaţie (CV%), prelucrate cu ajutorul funcţiilor statistice din EPIINFO şi EXCEL.

REZULTATE

Valoarea tensiunii arteriale sistolice medii măsurată clinic a fost de 176,47±26,68 mmHg, iar cea diastolică de 93,53±13,20 mmHg cu un procent de 20,2% din cazuri peste limita normală de 90 mmHg.

La monitorizarea continuă tensiunea arterială sistolică a prezentat maxime care s-au încadrat în intervalul 122 - 231 mmHg cu o medie de 177,87± 25,83 mmHg. TAd medie înregistrată a fost de 85,71± 10,61 mmHg, iar ponderea cazurilor care au depăşit limita normală a fost de 7,4% .

Tensiunea arterială medie (MAP) înregistrată a fost de 95,20±14,37 mmHg. Maximele MAP având o medie de 120,34± 22,50 mmHg iar MAP minima a avut o medie de 70,90±10,61 mmHg. MAP reprezintă măsura adevărată a debitului cardiac, definind statusul hemodinamic cu implicaţii asupra terapiei antihipertensive pentru controlul tensional specific fiecărui pacient, prin adăugarea de diuretic la pacienţii cu hipervolemie, de inhibitor al enzimei de conversie (IEC) sau un blocant de calciu la cei cu vasoconstricţie şi beta-blocant la cei cu hipertropism cardiac.

Presiunea pulsului (PP) medie înregistrată a fost de 63,12±15,46mHg, cu valori diurne cuprinse în intervalul 53 şi 153 cu o medie de 91,74±21,27 mmHg şi valori minime nocturne cuprinse în intervalul 19 la 77, cu o medie de 38,14±11,82 mmHg rezultând o afectare a sensibilităţii baroreflexe şi a balanţei autonome simpatico-parasimpatice, amplificate de amploarea fenomenelor aterosclerotice, ştiindu-se că riscul afectării cardiovasculare se asociază unei unde P peste 53mmHg (tab. I).

BP load, sau sarcina (cronică) de presiune, a înregistrat frecvente mari la TAs, în proporţie de 42,5%, în comparaţie cu TAd unde 91,5% dintre pacienţi au BP load < 100 % / 24 h (tab. II).

Sarcina de presiune de 100% denotă o creştere constantă a TA ce conduce la hiper-

TABELUL I
Diferenţe statistice ale valorilor medii a presiunii pulsului în funcţie de grupa de vârstă

Grupa de vârstă	PP medie	PP max	PP min
65-74 ani(n =65)	62,46±15,58	90,23±21,15	37,79±12,15
= 75 ani (n = 29)	64,83±15,40	95,76±21,60	39,10±11,14
t-Student	t = 0,69; p>0,05	t = 1,15; p>0,05	t = 0,51; p>0,05

TABELUL II
Distribuţia de frecvenţe a sarcinii cronice de presiune la pacienţii din lotul de studiu

BP load	TAS		TAD	
	n	%	n	%
< 100 mmHg*h/24h	54	57,5	86	91,5
100-500 mmHg*h/24h	32	34,0	8	8,5
> 500 mmHg*h/24h	8	8,5	-	-

trofie ventriculară. Din datele prezentate rezultă că un procent de 42,5% din pacienţi prezintă hipertrofie ventriculară stângă, procent confirmat şi prin ecografie.

Indexul diurn, exprimat ca raport al TAs pe interval de zi şi de noapte, a prezentat scăderi sub 10% la 43 pacienţi, între 10 şi 20% la 40 pacienţi, peste 20% la 9 pacienţi iar 2 pacienţi nu au prezentat variabilitate circadiană.

Tratament. După numărul agenţilor antihipertensivi, 45 pacienţi (47,88%) erau sub monoterapie, 27 pacienţi (28,72%) primeau biterapie, iar 22 pacienţi (23,40%) triplă asociere. După clasele de medicamente: inhibitori ai enzimei de conversie a angiotensinei (IECA) - 28 pacienţi; diuretic (D) 10 pacienţi; beta-blocante (BB) -5 pacienţi; blocante ale canalelor de calciu (BCC) -2 pacienţi; IECA+D – 1 pacient; BB+D – 3 pacienţi; BCC+D -4 pacienţi; IECA+BB+D -14 pacienţi; IECA+ BCC+D -8 pacienţi.

După tratamentul ghidat prin monitorizare continuă a pacienţilor din lotul de studiu timp de 24 de ore, tensiunea arterială sistolică medie a fost de 137,40 mmHg, iar tensiunea arterială diastolică a fost de 82 mmHg valori apropiate de cele recomandate de diferite studii (tab. III).

TABELUL III
Valorile normale ale tensiunii arteriale în funcţie de studiu

Studii	TA pe 24 ore
Ghid ABPM - BHS	<130/80
GHID OMS/ISH	125/80
Manual Meditech	130/80
Staessen	133/82

DISCUŢII

Ţinta terapeutică în HTA a vârstnicului este aceiaşi cu cea pentru pacientul tânăr adică mai mică de 140/90 mmHg dacă este bine tolerată. Pentru aceasta mulţi pacienţi necesită unul, două sau mai multe droguri antihipertensive dar reducerea sub 160 mmHg pentru HTAs izolată poate fi dificil de realizat. Tratamentul medicamentos poate fi iniţiat cu diuretice tiazidice, antagonişti de calciu, blocanţi ai receptorilor angioten-

sinei şi beta blocanţi în conformitate cu recomandările ghidurilor. Unele trialuri ce au specific tratamentul HTAs izolate au arătat beneficiul tiazidelor şi antagoniştilor de calciu, iar alte trialuri au arătat beneficiul blocantelor de receptori angiotensinici. Datorită posibilităţii crescute pentru efectele nedorite la dozele iniţiale, dozele trebuie crescute mai lent în special la persoanele foarte vârstnice. Totuşi la subiecţii de 80 de ani şi peste beneficiile terapiei antihipertensive sunt încă neconcludente. De asemenea nu există dovezi pentru întreruperea unui tratament de succes sau bine tolerat când pacientul atinge vârsta de 80 de ani să fie întrerupt.

CONCLUZII

Rezultatele studiului pun în evidenţă superioritatea controlului tensional la pacienţii cu terapie ghidată după monitorizare continuă, cu aceleaşi clase de medicamente antihipertensive dar orientată după componenta dominantă a hipertensiunii arteriale (volumul intravascular crescut, hiperinotropism sau vasoconstricţie) şi a paternului hipertensiv, micşorând numărul de cazuri de hipotensiune, de rezistenţă la tratament, de reacţii adverse ale diferitelor grupe de medicamente antihipertensive, a riscului cardiovascular total, precum si o complianţa mai bună a pacientului la tratament.

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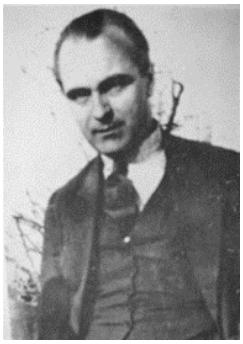
EDITORIAL

THE IAŞI SOCIETY OF PHYSICIANS AND NATURALISTS AND THE MEDICAL-SURGICAL JOURNAL DURING THE GREAT WAR

Romanian physicians – both military and civilians – were fully aware of their mission on the 27th of August with Romania's entry into the Great War joining the Entente which finally led to the unification of all provinces inhabited by Romanians. Many of those physicians were doctors and medical undergraduates from Iasi and Bucharest who unconditionally joined the army. The majority of the physicians who took part in the Great War were members of the "Sf. Spiridon" Hospital Council of Administration (Epitropy of "Sf. Spiridon") and members of the Society of Physicians and Naturalists in Iași. Some of these physicians had previously published their literary output in the main journals of the prewar period.

Among them, **Grigore T. Popa** a fresh graduate from the Faculty of Medicine in Iaşi and future professor of Anatomy and Dean of the Faculty of Medicine, future president of the Society of Physicians and Naturalists, **Mihai Ștefănescu-Galați**, founder of the Iași School of Urology, future Dean of the Faculty of Medicine, **Eugen Mironescu** future professor of Dermatology. Other physicians fought as simple soldiers or as specialists in endemic diseases (1, 2, 3).



Prof. dr.
Grigore T. Popa

Prof. dr.
Mihai Ștefănescu-Galați

Prof. dr.
Eugen Mironescu

It is but natural to feel that is our duty to identify and honor those who fought and even gave their lives to the great task of uniting all the provinces inhabited by Romanians. Among those great victors we identified some names that deserve being presented here:

Professor **Ernest Juvara** (1870-1933), the founder of the First Surgery Clinic at "Sf. Spiridon" Hospital. The gifted surgeon was mobilized in 1916 with the rank of Major and appointed to at the 4th Hospital for Evacuation that followed the 4th Army Corps at Hangu, with two complementary sections, at Bicaz and Broşteni. He moved to another fronts Piatra Neamţ, Neamţ Monastery and Botoşani. He continued to operate on the wounded soldiers' day and night.

Prof. dr. Ernest Juvara

He was promoted to the rank of colonel and was transferred to the Evacuation Hospital no. IX that followed the army corps at Mărășești. He was followed by two other celebrated surgeons: E. Christidi and Gh. Olaru. Soon he became known all over Moldavia. He was an outstanding traumatology and orthopedist who easily employed spinal anesthesia in operation rooms of the military hospitals. His team moved from hospital to hospital and performed operations that nobody ventured to think of at the time. His contributions to war surgery were included in a book: his approach to diaphysis fractures proved to be useful to say nothing of the external fixator which has been used ever since (3).

Professor **Paul Anghel** (1869-1937), also a surgeon, was mobilized in 1913 as colonel in the campaign in Bulgaria and who continued the fight until 1918.

Other physicians who had strong ties with the medical school of Iași were: dr. **George Bogdan** (1859-1930), who for the first time taught medical deontology in Iasi, and who served during the 1913 war and between 1916 and 1919. He was awarded the Order of Commander of the French Legion of Honor; dr. **Mihai Manicatide** (1867-1953), founder of Iasi School of Pediatrics in "Sf. Spiridon" Hospital, participated in the First World War on the Moldavian front; dr **Constantin C. Dimitriu** (1894-1960), founder of the Iași School of Semiology and Gastroenterology took part in the First World War as a trainee and as an undergraduate in the Iaşi hospitals; Dr. **Alexandru Ionescu-Matiu** (1883-1975), an outstanding pharmacist, head of led the Central Chemistry Lab of the Romanian Army; **C. Ionescu-Mihăieşti**, specialized at the Pasteur Institute of Paris, fought during the war in the campaign against cholera, in 1913, in Bulgaria and then against exanthema, working in the Experimental Laboratory of Medicine evacuated to Iasi (1916) and Harkov (1917), mainly in making serums and vaccines needed by the allied armies in the fight against all sorts of epidemics. Some of the doctors who worked in this field were: **Prof. dr. Ion Cantacuzino**, prof. dr. Alexandru Slatineanu, dr. Mihai Ciucă, dr. Petru Condrea, dr. Stefan Nicolau (3).

Prof. dr. Ion Cantacuzino

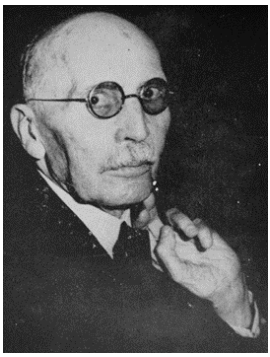
Alexandru Slătineanu (1873-1939) was decorated in 1914 with the Military Virtue for the organization of the Laboratory of Warfare Bacteriology. With his own means, private and public subsidies, he started a laboratory that could successfully face the needs of the country during the Great War. Moreover in 1917 he set up a

new method of preparation of anti-cholera vaccine that could be administered in a single dose at the Institute of Bacteriology in Harkov. In 1916, when Romania joined the Entante he was Head of the Second Army Sanitary Service and fought against cholera on the Dobrodgea front and among the war prisoners in Galaţi. In 1917, he joined, under Professor Cantacuzino`s command, the fight against exanthemas typhus. For his war contributions he was awarded the Order of Queen Maria (1st Class, 1917) Commander of the Spoon Crown and Knight of the French Legion of Legion (1918) (4).

Prof. dr. Alexandru Slătineanu

In 1918, Professor Slătineanu hosted the Experimental Medicine Laboratory of Professor Ioan Cantacuzino that had to be evacuated from Bucharest. In his modest laboratory in Iasi, 18 Muzelor St. the serums and vaccines were prepared not only for the Romanian, Russian and French armies operating on this front but also for the local civilian population and refugees who fled the foreign occupation, saving in this way import huge sums of money.

After the outbreak of the Great War a lot of other physicians served in the Romanian Army. **Mihai Ciucă** (1883-1969) fought against numerous epidemics, as Head of the Laboratory of the 1st Corps of Army and then as Head of the Military Hospital no.2 in Iasi. He had to fight against smallpox, tetanus, gangrene, recurrent fever, influenza and exanthemas typhus.



Prof. dr. Mihai Ciucă

Petru Condrea (1888-1967), professor of bacteriology, took part in the First World War as a prosecutor of all hospitals and head of the screening departments of the contagious hospitals in Piatra Neamt (1916-1918). In 1918-1919 he worked on the production of serums and vaccines in Professor Slatineanu's first laboratory of bacteriology, where he hosted the experimental medicine laboratory of Professor Ioan Cantacuzino evacuated from Bucharest.

Gheorghe Năstase (1887-1975), an intern, the future professor of dermatology, was, in the summer of 1913 sergeant who was responsible cholera cases in the campaign in Bulgaria. He began his medical activity on the front, treating the wounded. He was mobilized until March 1st, 1918 (3).

Stefan Nicolau (1896-1967), future professor of microbiology, as a young graduate, participated in the First World War on the Moldavian front (4).

Vasile Răşcanu (1885-1978) was mobilized when he graduated from the Faculty of Medicine and sent to the front in the campaign in the Balkans. He participated in the vast anti-cholera campaign initiated and

led by professors Ion Cantacuzino, Alexandru Slatineanu and Mihai Ciucă. With Romania's entry into the Great War in 1916, he was again mobilized and sent to the front, being directly involved in the fight against the exanthematical typhus epidemic that broke out in the winter of 1916 and then in 1917.

Other doctors, members of the Society of Physicians and Naturalists of Iasi died on the front: **Alexandru Brumă, Iacob Ţăranu. Mircea Gane, Victor Imervol, August Muler** (5)**.**

About 350 doctors and over 2,000 health workers in Romania gave their lives to the great task of unifying Romania in the First World War.

During the war, the scientific activity of the Physicians and Naturalists Society was interrupted, and the journal ceased to be published for about 5 years. The headquarters of the Society were used by the Faculty of Medicine for courses, its premises were rebuilt as a hospital and then bombarded.

It was not until 1920 that the scientific medical movement began to return to normal life by bringing together the members of the Society of Physicians and Naturalists and by planning scientific meetings and presentations of clinical and laboratory cases. The volume that bought together the scientific activity in 1920-1921 meant a step forward in the scientific and cultural life Iasi. This issue gave scientists the chance to gather together the energies of Iasi scientists that for several years were hindered to publish. Their energies were put instead to the service of the country.

The foreword was an appeal to medical colleagues asked to contribute with all their love of science to attend the meetings of the Society and to come with contributions able to maintain the regular publication of the journal by means of lectures and presentations. At the same time, it is stressed the fact that the newsletter published in Romanian was a means of meeting the needs of the Bessarabian students who were not proficient in French the language, in which the Bulletin was published.

The foreword also asked colleagues to pay tribute to all physicians who sacrificed their lives in the fight for the Great Union of Romania (6).

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EDITORIAL

MOLECULAR AND CELL BIOLOGY TRANSLATIONAL RESEARCHES IN THE MANAGEMENT OF ORAL PREMALIGNANT LESIONS

Traditionally, according to international standards, research in general and implicitly medical research is divided into two categories: basic and applied. Basic medical research is focused on better or fuller understanding of both normal cellular and molecular mechanisms as well as those of different diseases, the findings not being always developed into useful clinical applications. Applied research is directed towards a specific aim and/or solving a problem, such as new therapies and/or diagnostic methods, etc. (1, 2).

In this respect, the purpose of translational research is to connect basic research to patient care (from laboratory to bedside), to integrate molecular and cell biology research with clinical research, facilitating the development of laboratory findings into clinical applications. Thus, clinical observations on the occurrence and progression of some diseases and/or efficacy of various therapies should be the starting point of basic research aimed to provide clinicians with answers, biomarkers as specific and useful as possible for prognosis and/or early diagnosis, select targeted therapies and assess the efficacy of therapy. This constant feedback may lead to improved management of some diseases with major population impact (2, 3).

A good example of translational research in the field of human pathology is represented by the studies on malignant transformation in different locations. In the oral mucosa, they pointed out that the development of oral squamous cell cancer is a two-step process indicating the initial presence of a precursor, a premalignant lesion (3, 4).

According to statistical studies, oral cancer is the most common neoplasm and is a major public health problem, in developing countries being the leading cause of death. It accounts for 2-4% of the malignancies in the Western countries and 40% of all cancers in India. A key factor in the increasing prevalence of these tumors is related to their diagnosis and treatment only in advanced stages (3-5).

Clinical diagnosis is often difficult because subjective and objective manifestations are nonspecific and do not correctly reflect the degree of damage to epithelial and connective tissues. Moreover, unfortunately patients present to the dermatologist and/or dentist when the disease is in a late-stage, transformed (6,7).

In over 50% of cases, this type of carcinoma develops on premalignant lesions, of which the most common are leukoplakia, actinic cheilitis, oral lichen planus, and oral submucous fibrosis (8).

Malignant transformation rate of these lesions is approximately 17% with a mean period of 7 years after diagnosis. The highest transformation rate is seen in heterogeneous erythroplakia and erythroleukoplakia with dysplastic changes (4). However, some studies show that 16% to 62% of oral leukoplakic lesions show characteristic changes of squamous cell carcinoma at the time of diagnosis (3, 9, 10).

Despite advances in surgical, radiological and chemotherapeutic treatment, the 5-year survival rate for oral cancer has not improved significantly in recent decades

still being around 50-55% (11-13).

Given the constant exposure to various internal and external environmental factors, oral mucosa is frequently under inflammatory stress, explaining its vulnerability to infectious/inflammatory processes such as those involved in carcinogenesis (14).

The results of numerous translational researches of molecular and cell biology support the idea that epithelial-mesenchymal transition (EMT), an important event in epithelial cell malignant transformation, may be induced by a series of proinflammatory cytokines (15).

The identification by translational studies of the molecular changes during the early stages of premalignant oral lesion development before they become clinically manifest is an important step in understanding how cellular response is regulated during carcinogenesis at this level and for identifying biomarkers useful for the early diagnosis of these lesions (16).

In this respect, a series of studies aimed at identifying the involvement of such molecules as tumor necrosis factor (TNFα), interleukin-6 (IL-6), metalloproteinase-2 (MMP-2) and cyclooxygenase-2 (Cox-2) in creating a micro medium suitable for malignant transformation at oral mucosa level (17,18).

It has been found that evaluating the role of IL-6 and TNFα cytokines in the early stages of oral carcinogenesis, before carcinoma becomes invasive, requires identifying and establishing correlations between their expression and/or activation/inhibition of some signaling pathways (18).

Thus, TNFα influences both angiogenesis by IL-6 and vascular endothelial growth factor (VEGF) stimulation and tissue remodeling by metalloproteinase stimulation (19-20). Increased TNFα expression in oral premalignant lesions influences IL-6 and MMP-2 levels, thus revealing the intricate roles of these molecules in what may be called the molecular mosaic specific to oral premalignant lesions (20).

IL-6 is considered a marker of malignancy, with sensitivity of 60-70% and specificity of 58-90%. However, studies on the diagnostic and prognostic value of this molecule are scarce and fairly controversial (17-20). In oral squamous cell carcinomas, it is believed to act as immunosuppressive molecule that facilitates the "eludation" of the immune system control mechanisms, the increased serum IL-6 levels being negatively correlated with prognosis (18).

Also, some studies indicate that on one hand this cytokine is stimulated early in oral premalignant lesions, prior to their transformation into carcinoma, and, on the other hand, its expression is much increased in premalignant compared to malignant lesions (19 -21).

As to Cox-2, researches revealed increased expression in both oral premalignant lesions and malignant tumors, depending on the intrinsic morphological characteristics and with prognostic relevance (20-23).

However, the association of COX-2 overexpression with poor prognosis remains controversial, although it is known that increased levels of prostaglandin E2 (PGE2), metabolite product of Cox-2 catalytic action, are associated with an increased risk of invasiveness and angiogenesis in the early stages of carcinogenesis (19,20).

Several cell and molecular biology studies indicate that cell interactions specific to these premalignant lesions are influenced by the expression of matrix metalloproteinases (MMPs). These are expressed by both tumor cells and stromal macrophages and endothelial cells. Some authors also suggest that patients in which increased MMP expression in stromal cells is detected have a poor prognosis because of its association with the

risk of lymphatic metastasis (1, 16, 20).

The results of the published studies, some of which were conducted in the Department of Cell and Molecular Biology of the Iasi "Grigore T. Popa" University of Medicine and Pharmacy revealed that in all leukoplakic lesions the increase in TNFα and IL-6 levels are associated with elevated Cox-2 and MMP-2 levels. Lesions with a mild degree of dysplasia exhibit decreased expression of TNFα, IL-6 and Cox-2 and increased expression of MMP-2 compared to lesions with a higher degree of dysplasia. Thus, Cox-2 expression is directly proportionally stimulated by proinflammatory cytokines TNFα and IL-6 and is associated with the severity of dysplasia. In contrast to these results, it was found that MMP-2 expression is inversely correlated with both TNFα, IL-6 and Cox-2 levels and the degree of dysplasia. IL-6 positively affects Cox-2 expression and negatively TNFα and MMP2 expression. The same correlations have been identified in other premalignant lesions, such as erosive oral lichen planus and erosive actinic cheilitis.

In clinical practice, despite the numerous data documenting its efficacy chemoprevention is still in its infancy. New therapeutic strategies targeting several molecules whose functional intrication has been proven by the cell and molecular biology researches briefly reviewed above are needed so that this type of lesions to be diagnosed early and treated effectively (23).

Imiquimod, a topical preparation commonly used in the treatment of oral premalignant lesions, may stimulate the production of proinflammatory cytokines like TNFα and IL-6, thus explaining such side effects as oral aphthous ulcer. Hence the need of combining imiquimod with other treatments such as corticoids with inhibitory effect on TNFα expression (22).

As shown above, Cox-2, overexpressed in various types of premalignant lesions may be a molecular target in their prevention and therapy. Clinical studies have shown that treatment with selective Cox-2 inhibitors reduces the formation of intestinal, esophageal, tongue, breast, skin, lung and bladder tumors (20-22).

Selective Cox-2 inhibition is also a method proposed for chemoprevention of malignant transformation of premalignant lesions. Cox-2 inhibitors have important advantages because they are active on the oral mucosa and have few contraindications (15, 19).

The current therapeutic management of oral premalignant lesions and oral squamous cell carcinoma is based on clinical data, often nonspecific, and some anatomopathological parameters insufficient to differentiate between severe and mild or moderate lesions. Therefore, the use of therapeutic, chemical and physical agents is often accompanied by numerous adverse effects, their incompletely elucidated explanation can be improved by the correlated study of the involved molecules. Under these conditions, it is once again revealed the importance of identifying through molecular and cell biology translational research of the specific molecular markers necessary for identifying and developing targeted and personalized therapeutic strategies.

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SINDROMUL HEPATO-PULMONAR (SHP)

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HEPATO-PULMONARY SYNDROME (Abstract): The hepato-pulmonary syndrome (HPS) is a rare complication of liver cirrhosis, with poor outcome; the term includes liver disease, increased alveolo-arterial gradient and intrapulmonary vascular dilations, described by Fluckiger, Kennedy and Knudson. HPS impairs survival in cirrhotic patients and the posttransplant outcome is altered in correlation with severity of HPS. Combined determination of SaO_2 in clino- and orthostatic position by a pulsoximeter is a simple test for HPS identification in patients with chronic liver disease or non-cirrhotic portal hypertension. **Key words**: HEPATO--PULMONARY SYNDROME, LIVER CIRRHOSIS, PORTAL HYPERTENSION

SHP este o complicaţie rară, cu prognostic rezervat în ciroză. În 1884, Fluckiger a descris pentru prima dată o femeie cu ciroză hepatică, cianoză şi hipocratism digital. Termenul de SHP (triada = boală hepatică, gradient alveolo-arterial crescut şi evidenţierea dilataţiilor vasculare intrapulmonare) a fost reluat în 1977 de către Kennedy şi Knudson (1). Aceste anomalii vasculare predomină în câmpurile pulmonare inferioare. Deoarece gravitaţia induce creşterea fluxului sanguin spre câmpurile pulmonare inferioare, hipoxemia se accentuează când se trece de la poziţia clinostatică la ortostatism.

Date privind incidenţa SHP sunt limitate şi estimările prevalenţei variază pe măsură ce sunt folosite definiţii diferite pentru SHP (1,3%). Rydell şi Hoffbauer (1956) au descris un băiat de 17 ani cu ciroză, vase pulmonare dilatate şi fistule arteriovenoase la autopsie (3). Hales descrie şi el câţiva pacienţi de vârstă mică având aceleaşi caracteristici, deci SHP poate apare şi la copil. Bolile hepatice acute şi cronice au fost asociate cu SHP (4,5,6). Cel mai frecvent, SHP apare în bolile cu progresie spre ciroză în special criptogenică, alcoolică, ciroza biliară primitivă şi HVB sau HVC. Totuşi, apariţia SHP la cei cu hipertensiune portală (HTP) noncirotică sugerează că ciroza nu este condiţia esenţială pentru dezvoltarea SHP. În unele studii există sau nu o corelare între SHP şi testele hepatice (bilirubina, timp Quick, albumina). De asemenea, alte studii găsesc asocierea SHP cu spider nevi şi alte manifestări cutanate. Hipoxemia uşoară apare la aproximativ 1/3 din toţi pacienţii cu boală cronică hepatică şi este multifactorială, din cauză că alte anomalii cardiopulmonare (efuzie pleurală, ascită) sunt frecvente la aceşti pacienţi şi pot coexista cu SHP. Principalele caracteristici ale SHP sunt platipnea (dispnee indusă de ortostatism şi ameliorată de clinostatism) şi ortodeoxia (deoxigenare arterială indusă de ortostatism şi ameliorată de clinostatism). Deşi aceste fenomene sunt patognomonice pentru SHP, ele sugerează net acest diagnostic în contextul disfuncţiei hepatice.

Simptomele respiratorii sunt tot mai frecvente la pacienţii cu boli cronice hepatice astfel încât 50-70% dintre aceştia acuză „scurtarea respiraţiei" (1). Diagnosticul diferenţial al dispneei este extensiv la aceşti pacienţi, fiind necesară enumerarea unor cauze numeroase. În ultimii 15 ani anomaliile vasculare pulmonare au devenit recunoscute ca entităţi clinice importante care influenţează supravieţuirea şi candidatura la transplant hepatic.

Cea mai comună anomalie, SHP, apare când vasodilataţia pulmonară alterează oxigenarea arterială. Acest sindrom este actualmente recunoscut la mai mult de 15-20% din pacienţii aflaţi în curs de evaluare pentru OLT (transplant hepatic ortotopic).

Prezenţa SHP creşte mortalitatea cirozei şi poate influenţa frecvenţa şi severitatea complicaţiilor HTP (3).

DEFINIŢIE

SHP se defineşte clasic printr-un gradient crescut alveolo-arterial al oxigenului ($AaPO_2$) în aerul din cameră (>15 mmHg sau >20 mmHg la pacienţii cu vârsta mai mare de 64 ani) cu sau fără hipoxemie care rezultă din vasodilataţia intrapulmonară în prezenţa disfuncţiei hepatice sau a HTP. Din punct de vedere practic identificarea pacienţilor cu $PaO_2 < 70$ mm Hg este utilă pentru recunoaşterea celor cu SHP clinic semnificativ. Studii recente subliniază că este necesară excluderea tuturor celorlalte cauze de disfuncţie cardiopulmonară pentru a stabili diagnosticul SHP. Totuşi, este tot mai evident că SHP poate coexista cu alte anomalii cardiopulmonare şi contribuie semnificativ la anomaliile schimburilor gazoase.

În plus, $AaPO_2$ creşte în mod normal cu vârsta şi variază semnificativ, chiar la adulţii sănătoşi. Deci utilizarea valorilor peste intervalul de confidenţă de 95% pentru $AaPO_2$ corectat în funcţie de vârstă este importantă pentru a evita supra-diagnosticarea SHP(1).

FIZIOPATOLOGIE

1. Mecanisme la om

O problemă fundamentală în patogenia SHP este faptul dacă mecanismele alterărilor vasculare pulmonare sunt similare cu cele implicate în alterările sistemică şi splanhnică din statusul circulator hiperdinamic din ciroză.

SHP apare mai frecvent în ciroza de diferite etiologii . Totuşi există controverse dacă prezenţa sau severitatea vasodilataţiei intrapulmonare şi a SHP se corelează cu severitatea bolii hepatice subjacente iar studiile au găsit SHP în ambele cazuri-ciroză compensată sau în stadiul final. Recent SHP a mai fost recunoscut la pacienţii cu HTP în absenţa cirozei (tromboza venei porte, hiperplazia regenerativă nodulară, fibroza hepatică congenitală şi sindromul Budd-Chiari) şi a fost raportat în hepatitele acute şi cronice în absenţa HTP. Acestea arată că SHP se dezvoltă şi în absenţa bolii hepatice avansate iar evenimentele fiziopatologice care survin în microvasculatura pulmonară a pacienţilor cu SHP sunt relative faţă de circulaţiile sistemică şi splanhnică.

Markerul patogenic al SHP este dilataţia microvasculară în cadrul circulaţiei arteriale pulmonare. Aceste modificări pot rezulta din scăderea tonusului arteriolar pre-capilar (izolat) sau se pot datora unor mecanisme adiţionale (angiogeneză, remodelare, vasculogeneză) sugerate mai recent (3). În SHP la om vasodilataţia rezultă din producţia excesivă vasculară de vasodilatatori, în special oxidul nitric (NO). Acest lucru se bazează pe observaţia că nivelele de NO expirat, care măsoară producţia pulmonară de NO, sunt crescute la pacienţii cirotici cu SHP şi se normalizează după OLT, odată ce SHP se remite. În plus, la un caz raportat în literatură s-a arătat că inhibarea acută a producţiei de NO sau administrarea N^G-nitro--L-arginin-metil esterului (L-NAME) sau a albastrului de metilen inhibitor de GMP ciclic ameliorează tranzitoriu SHP (1,3,4).

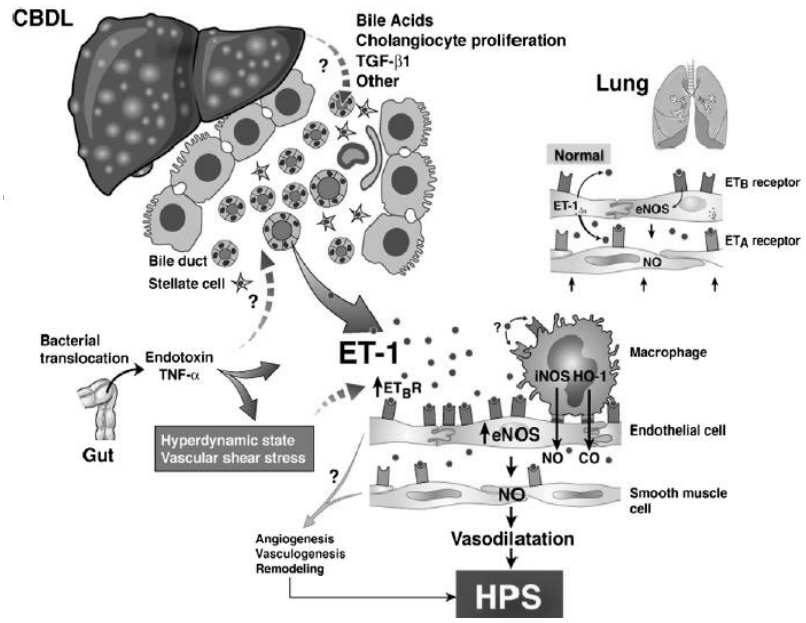

Fig. 1. Mecanisme şi tratamente posibile în SHP experimental, după Palma DT, Fallon M

Totuşi, un studiu recent a arătat că administrarea L-NAME inhalator nu a ameliorat net vasodilataţia pulmonară, suspicionând faptul că alţi factori decât efectele NO derivat din NOS asupra tonusului vascular contribuie la SHP.

Mecanismele exacte ale hiperproducţiei endogene de NO şi relaţia cu prezenţa HTP, cu circulatia hiperdinamică şi gradul leziunii hepatice, rămân necunoscute. În plus, nu s-a stabilit încă dacă alţi mediatori, cum ar fi monoxidul de carbon derivat din hemoxigenază,(3), ar putea contribui la vasodilataţia intrapulmonară şi ar explica lipsa ameliorării SHP odată cu inhalarea NO la unii pacienţi.

2. Mecanismele SHP experimental

CBDL (ligatura cronică a canalului biliar comun) la şoareci este singurul model experimental ce reproduce caracteristicile fiziologice ale SHP la om (fig.1). Este unic printre modelele de rozătoare cu ciroză şi/sau HTP prin faptul că alte modele folosite frecvent cum ar fi ciroza prin thioacetamidă şi ligatura parţială a VB (vezica biliară) nu duc la dezvoltarea SHP. Studii recente la animalele cu CBDL s-au concentrat pe rolul vasoconstrictor al eicosanoizilor şi asupra creşterii celulelor macrofage-like intravasculare (1, 3). Studii subsecvente au identificat creşterea sintetazei endoteliale vasculare pulmonare a NO (eNOS) ca sursă majoră de producţie pulmonară de NO (6) şi au demonstrat că administrarea de L-NAME a ameliorat hipoxemia după CBDL (7). Studiile ulterioare au arătat că o creştere a producţiei hepatice de endotelină 1 (ET-1) cu eliberarea ei în circulaţie este un mecanism trigger al creşterii eNOS pulmonare şi al debutului vasodilataţiei după CBDL. Acest efect poate fi mediat de o creştere a expunerii receptorului endotelinei B (ETB) endoteliale vasculare pulmonare, indusă de stress, care duce la creşterea producţiei de NO endotelial prin ET-1. Conform acestui fapt, administrarea unui antagonist selectiv al RETB la animalele CBDL va scade eNOS endotelială pulmonară şi nivelele RETB cu ameliorarea semnificativă a SHP.

Date recente susţin faptul că epiteliul biliar este o sursă importantă de ET-1 hepatică după CBDL şi pot explica susceptibilitatea unică a anomaliilor CBDL la SHP. Odată cu progresia SHP experimentale există o acumulare de macrofage vasculare. Aceste celule produc tranzitor sintetaza inductibilă de NO (INOS) (6,7) şi progresiv hemoxigenaza 1 (HO-1). Aceste evenimente contribuie la vasodilataţia suplimentară prin producerea de NO din INOS şi CO derivat din HO-1. Astfel, inhibarea HO ameliorează SHP experimental. În plus, tratamentul prelungit al animalelor CBDL (început din momentul ligaturării) cu Norfloxacină pentru a inhiba translocaţia bacteriană şi producţia de TNF-alfa va scade acumularea macrofagelor şi va preveni creşterea tranzitorie a INOS, susţinând faptul că TNF-alfa contribuie la acumularea macrofagelor. Mai mult, pentoxifilina, inhibitor fosfodiesterazic nonspecific care creşte nivelele AMPc intracelular şi inhibă TNF-alfa produs în macrofage (8), poate preveni debutul sau poate scade severitatea SHP. Ambii agenţi administraţi la debutul leziunii hepatice influenţează dezvoltarea statusului hiperdinamic şi pot modifica expresia R-ETB şi evenimentele de semnalizare legate de ET în microvascularizaţia pulmonară. Alte date noi despre modelul CBDL arată că o secvenţă de evenimente legate în parte de creşterea stresului vascular şi de producţia hepatică de ET-1 poate declanşa debutul SHP experimental. Observaţia că nivelele hepatice şi plasmatice ale ET-1 cresc în prima săptămână după CBDL) sugerează că producţia şi eliberarea ET-1 hepatice apare la un nivel modest al proliferarii ductelor biliare. Descoperirea că macrofagele se acumulează în microvasele pulmonare şi că pot fi influenţate de inhibarea TNF-alfa susţine faptul că aceste celule pot de asemenea contribui la vasodilataţie. Figura ilustrată mai sus include posibile ţinte terapeutice pentru SHP conform datelor experimentale.

MANIFESTĂRI CLINICE

Caracteristicile clinice ale SHP constau tipic în acuze respiratorii şi manifestări asociate bolii hepatice cronice. Debutul insidios al dispneei, de tip expirator, este cea mai comună problemă, deşi nespecifică. Platypnea (platipnea) (scurtarea respiraţiei exacerbată de ortostatism şi ameliorată de clinostatism) şi orthodeoxia (hipoxemie exacerbată în ortostatism) sunt clasic descrise şi rezultă din creşterea gravitaţională a fluxului sanguin prin vasele dilatate de la bazele pulmonare (1). În timp ce orthodeoxia a fost observată în unele situaţii patologice, cum ar fi post-pneumectomie, tromboembolii pulmonare recurente şi defecte septale atriale (foramen ovale permeabil), este înalt specifică pentru SHP în condiţiile bolii hepatice (8). "Sensibilitatea" orthodeoxiei pentru SHP este relativ scăzută dar creşte sever în cazurile de SHP. Tusea este un simptom rar. Angioamele stelate sunt frecvent raportate în SHP dar apar mereu la ciroticii fără SHP. Un studiu a observat că pacienţii cu aceste leziuni cutanate aveau vasodilataţie pulmonară mai mare şi gradienţi de oxigen alveolo-arterial mai mari decât la cei fără angioame stelate ($AaPO_2$ de 20 mm Hg versus 8mm Hg). În sfârşit, clubbing fingers (degete hipocratice) şi cianoza distală, când apar în condiţiile de hepatopatie sau HTP, ar trebui să ridice suspiciunea de SHP (1).

DIAGNOSTIC

Caracteristicile diagnostice ale SHP includ boala hepatică sau HTP, gradient al oxigenului alveolo-arterial ($AaPO_2$) crescut, dovada vasodilataţiei intrapulmonare. În prezenţa unei boli cardiace sau pulmonare asociate, stabilirea diagnosticului SHP poate fi dificilă. Există un algoritm pentru diagnosticul SHP (fig. 2). O evaluare logică a dispneei la pacientul cu boală hepatică sau HTP începe cu un istoric atent şi o examinare fizică. Astfel, clinicianul poate face distincţia între

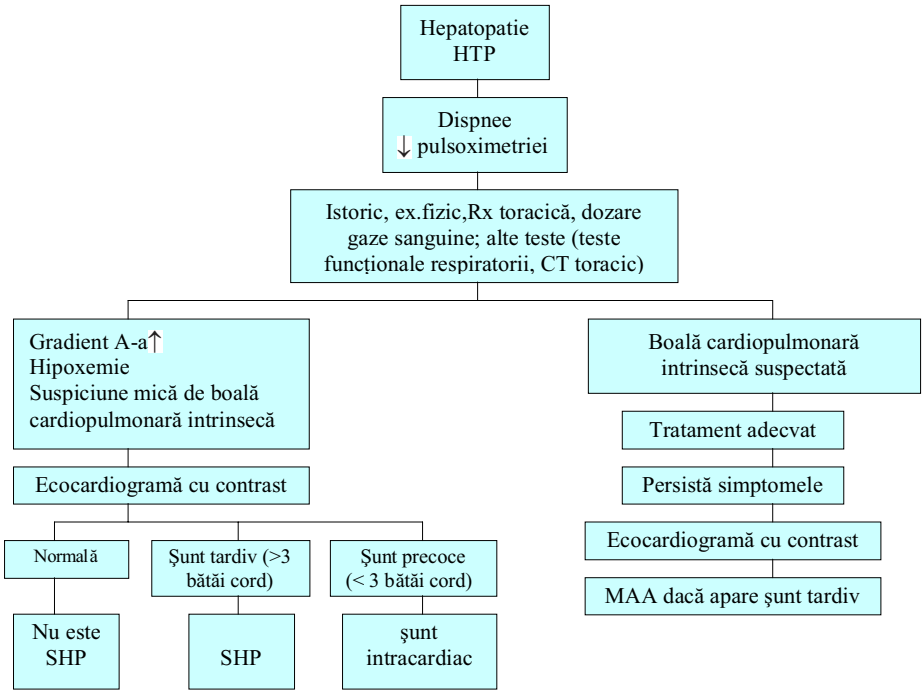

Fig. 2. Diagnosticul SHP, după Palma DT, Fallon M

BPOC, insuficienţa cardiacă cronică sau ischemie miocardică. Totuşi, dacă se pot exclude cauzele obişnuite de dispnee şi, în mod particular, dacă platipnea sau hipocratismul digital sunt prezente, evaluarea suplimentară pentru SHP este inerentă.

1. Evaluarea oxigenării arteriale

La pacienţii cu boală hepatică şi dispnee sau hipocratism, sau la cei propuşi pentru transplant, pulsoximetria este un test simplu, neinvaziv, de screening, pentru hipoxemie iar SaO_2 scăzută ar trebui să implice analiza gazelor sanguine arteriale (AGSA). Totuşi, interpretarea unei SpO_2 ca „normală" ar trebui făcută cu prudenţă, deoarece pulsoximetria poate supraestima SaO_2 la aproape 50% din pacienţii cu ciroză(1). Astfel, pentru a detecta hipoxemia, ar trebui considerată analiza gazelor arteriale (AGA), când valorile SpO_2 sunt mai mici sau egale cu 97%. În plus, dacă hipoxemia sau SHP sunt suspicionate pe baza istoricului şi a examenului fizic, AGA trebuie efectuată în timpul respirării aerului din cameră, în afara pulsoximetriei. În SHP, analiza gazelor relevă un gradient crescut alveolo-arterial ($AaPO_2$) cu sau fără hipoxemie. Limita superioară a $AaPO_2$ normală pentru aerul din cameră la o vârstă dată (interval de confidenţă $>95\%$) poate fi calculată prin ecuaţia: $AaPO_2 = ş0{,}26$ x vârsta-0,43ţ+10 (2). Dacă sunt decelate anomalii de schimb gazos sunt necesare radiografia şi testele funcţionale pulmonare pentru a evalua prezenţa altor anomalii pulmonare. Deoarece tulburările cardiopulmonare fără legatură cu hepatopatia sau cele legate de ascită sunt mai frecvente decât SHP, tratarea acestora înainte de evaluarea suplimentară pentru SHP este mai rezonabilă, în absenţa unei hipoxemii semnificative ($PaO_2 < 70$ mm Hg). ERS Task Force a propus un sistem de clasificare care utilizează presiunea arterială a oxigenului (PaO_2) pentru a stadializa severitatea SHP. Conform acestui sistem, o $PaO_2 < 50$ mm

Hg indică SHP foarte sever, $PaO_2 = 50$ şi < 60 mm Hg sugerează SHP sever iar $PaO_2 = 60$ şi < 80 mm Hg corespunde unui SHP moderat. Stadializarea severităţii SHP este importantă în predicţia supravieţuirii (3) şi determinarea momentului şi riscurilor pentru OLT (3,6,8).

2. Ecocardiografia cu contrast

Dacă se suspicionează SHP, ecocardiografia cu contrast transtoracică cu microbule este testul screening preferat pentru vasodilataţia intrapulmonară (1). Aceasta se realizează prin injectarea intravenoasă de ser fiziologic agitat în timpul ecocardiografiei transtoracice normale, producând microbule vizualizate sonografic. Acest bolus opacifiază ventriculul drept (VD) în câteva secunde iar în absenţa şuntului dreapta-stânga, bulele sunt absorbite de plămâni. Dacă este prezent un şunt intracardiac, agentul de contrast intră în VS în timpul a 3 bătăi cardiace (sunt precoce). Dacă şuntul intrapulmonar caracteristic SHP este prezent, VS se opacifiază la cel puţin 3 bătăi de cord după VD (şunt tardiv). În timp ce mai mult de 40% din cirotici au ecografia de contrast pozitivă (3), numai un subgrup din aceşti pacienţi au o suficientă vasodilataţie pentru a determina un schimb gazos anormal şi îndeplineşte criteriile pentru SHP. Dacă un pacient cu boală hepatică sau HTP şi hipoxemie are o ecocardiogramă de contrast pozitivă, în absenţa unei boli semnificative cardiopulmonare, diagnosticul de SHP este stabilit.

A apărut un sistem de scor semnificativ pentru evaluarea şuntului intrapulmonar în timpul ecocardiografiei de contrast, deşi rămâne neclar dacă gradul şuntării se corelează cu gradul anomaliilor de schimb gazos.

3. MAA scan (macroaggregated albumin scan)

La pacienţii cu hipoxemie care prezintă vasodilataţie intrapulmonară şi boală cardiopulmonară intrinsecă, MAA scan (scintigrafie cu macroagregate de albumină marcate cu technetium) poate fi utilă în definirea contribuţiei SHP la anomaliile de schimb gazos. În această investigaţie, agregate de albumină radiomarcate, cu diametrul de aproximativ 20 μm sunt injectate în sistemul venos. În mod obişnuit, particulele de această mărime sunt captate în microvasculatura pulmonară iar scintigrafia relevă o captare aproape normală în plămâni. În prezenţa unei şuntări intrapulmonare semnificative, o fracţie din microagregatele de albumină trece prin plămâni spre circulaţia sistemică. Scintigrafia mai indică o captare în alte organe, permiţând calcularea fracţiei de şunt. Într-un studiu, MAA scan a fost pozitivă doar la pacienţii cu SHP şi $PaO_2 < 60$ mm Hg şi nu la cei cu BPOC şi cu grad similar de hipoxemie(3). Totuşi, MAA scan este mai puţin sensibilă decât ecocardiograma de contrast şi poate fi cea mai utilă în a vedea dacă SHP contribuie la hipoxemie la pacienţii cu boală pulmonară obstructivă concomitentă.

4. Explorări funcţionale pulmonare

În timp ce testele funcţionale pulmonare anormale sunt frecvent observate în SHP, acestea nu au o specificitate mare. În absenţa unei boli concomitente obstructive sau restrictive pulmonare, valorile capacităţii pulmonare totale şi ale ratei de flux expirator de la pacienţii cu SHP sunt în general normale. Afectarea difuziei este frecventă în SHP. Într-un studiu, capacitatea de difuziune a monoxidului de carbon (DLCO) era cu 80% mai mică decât valoarea predictivă la 15 din 18 pacienţi cu SHP (9). Totuşi, prezenţa DLCO scăzută cu spirometrie normală nu este specifică pentru SHP şi este observată de rutină la pacienţii cu boală pulmonară interstiţială precoce, boală vaso-ocluzivă şi anemie severă.

ALTE TEHNICI DE DIAGNOSTIC

Angiografia pulmonară este scumpă şi

Fig. 3. I.S., 9 ani, ciroza hepatica de etiologie neprecizata complicată cu sindrom hepato-pulmonar (colecţia Clinicii a II-a Pediatrie Iaşi – Prof. Dr. Evelina Moraru)

invazivă şi are o sensibilitate mică în decelarea vasodilataţiei intrapulmonare, deci nu este folosită de rutină în diagnosticul SHP.

CT de înaltă rezoluţie a toracelui şi evaluarea timpului de tranzit sanguin pulmonar sunt modalităţi diagnostice mai noi de evaluare a SHP. Într-un studiu recent gradul dilatării microvasculaturii pulmonare observată pe CT toracic se corela cu severitatea anomaliilor de schimb gazos într-o cohortă mică de pacienţi cu SHP, sugerând că este posibilă cuantificarea vasodilataţiei intrapulmonare. Într-un alt studiu, timpul de tranzit pulmonar al eritrocitelor, măsurat prin analiza ecocardiografică a complexelor serice umane albumină-aer-microbule din inimă, de asemenea s-a corelat cu anomaliile de schimb gazos, într-un grup mic de pacienţi cu SHP. Rămâne de văzut dacă aceste tehnici vor fi utile pentru diagnosticul SHP.

ISTORIE NATURALĂ ŞI PROGNOSTIC

Istoria naturală a SHP nu este complet elucidată. Cei mai mulţi pacienţi par să dezvolte vasodilataţie intrapulmonară progresivă şi schimburi gazoase deteriorate în timp (9), iar ameliorarea spontană este rară. Un studiu prospectiv recent a evaluat istoria naturală a SHP pe o cohortă de 111 pacienţi cu ciroză, din care 27 (24%) aveau SHP. Supravieţuirea medie a pacienţilor cu SHP a fost semnificativ mai mică (10,6 luni) faţă de cei fără SHP (40,8 luni). Mortalitatea a rămas mai mare la cei cu SHP după aprecierea severităţii bolii de bază a ficatului şi după excluderea pacienţilor ce fuseseră transplantaţi hepatic în timpul urmăririi. Cauzele morţii la pacienţii cu SHP au fost datorate în special complicaţiilor prin disfuncţie hepatocelulară şi cele ale HTP şi s-au corelat cu severitatea hipoxemiei în SHP.

Aceste date sugerează posibilitatea ca prezenţa SHP să fie un factor important care influenţează progresia bolii hepatice şi riscul complicaţiilor legate de HTP. În sfârşit, chiar şi o hipoxemie modestă legată de SHP se poate agrava în somn, pe baza observaţiilor cum că saturaţia nocturnă a oxigenului scade la un grup mic de pacienţi cirotici fără SHP (10).

Mortalitatea după OLT pare de asemenea să fie mai mare la pacienţii cu SHP comparativ cu cei fără SHP. Utilitatea severităţii SHP ca predictor al evoluţiei după OLT a fost evaluată prospectiv pe o cohortă

de 24 pacienţi cu ciroză şi SHP (10). Autorii au arătat că mortalitatea după OLT a fost mult crescută în SHP sever, în parte datorită apariţiei unor complicaţii rare postoperatorii recunoscute la cei cu SHP. O presiune PaO_2 preoperatorie =50 mm Hg izolată sau combinată cu o fracţie de şunt a albuminei macroagregate = cu 20% sunt predictorii cei mai „solizi" ai mortalităţii postoperatorii.

SHP poate afecta supravieţuirea la pacienţii cu ciroză iar prognosticul posttransplant se alterează proporţional cu severitatea SHP. Determinarea combinată a SaO_2 în clino şi ortostatism utilizând un pulsoximetru este un test simplu de identificare a SHP la pacienţii cu boală cronică hepatică sau HTP noncirotică. În studiul prospectiv numai 4/316 (1,3%) aveau SHP. Şunturile pot fi cuantificate prin scintigramă pulmonară şi se corelează mai bine cu valorile SaO_2 decât ale PO_2. Această corelaţie poate permite o estimare a severităţii şunt-volumului fără efectuarea scintigrafiei. În practică, determinarea saturaţiei O_2 arterial cu pulsoximetrul poate fi utilizată ca test screening simplu şi ajută la identificarea mai multor pacienţi cu SHP în scopul studiului patogeniei şi al dezvoltării strategiilor terapeutice.

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EDITORIAL

GENOME EDITING: ALI BABA'S TREASURE OR PANDORA'S BOX ?

At the beginning of this decade a series of discoveries in the field of bacterial genetics has enabled the development of molecular biology technologies capable of changing the genetic structure of higher organisms. Overall, these technologies are known as genome editing and are based on the use of different nucleases that use the natural mechanisms to correct DNA mutations. Thus, such technology allows the correction of DNA sequences by inserting or removing fragments of interest.

The attempts to change the genetic material of a living organism are not new, but the methods used since the '90s of last century were marked by many difficulties and failures.

The first system used was that of nucleases of the type ZFNs (zinc finger nucleases) which have two parts: a domain binding to the DNA ("zinc finger" type) and a nuclease derived from the restriction enzyme *FokI* (1). This system is able to recognize a DNA sequence of 9 to 18 base pairs, but its use has difficulties, like a limited number of target sequences, a high rate of off-target effects and an increased cytotoxicity. The only advantage is the small size that allows the incorporation of such system in viral vectors such adenoviruses (2).

A second system applied was the transcription activator-like effector nucleases (TALENs) identified in *Xanthomonas* species. It also consists of two components: a nuclease and a DNA binding system (3). The nuclease is an enzyme derived from restriction enzyme *FokI*. The binding to DNA is provided by a repeating unit of 33-35 amino acid sequence. Of these the amino acids residues from positions 12 and 13 are variable and represent elements which ensure the specific recognition of DNA sequences containing a thymine at the 5' end. Compared with ZFNs, TALENs is characterized by a much lower rate of off-target effects and a low level of cytotoxicity (4).

The last genome editing system discovered at the beginning of this decade, is the more efficient. It consists of combination of Clustered Regularly interspaced Short Palindromic Repeats (CRISPR) and CRISPR Associated (Cas) proteins. This system, originally identified in *Streptococcus pyogenes*, is a natural defense mechanism against infections with plasmids and viruses in arches and bacteria. Naturally, Cas9 nuclease guidance is provided by two sequences of RNA: CRISPR RNA (crRNA) and transactivating CRISPR RNA (tracrRNA) that are complementary to different viral sequences of approximately 20 nitrogenous base pairs. In genome editing the two pieces of RNA are combined into one single guide RNA sequence (sgRNA) much easier to synthesize and use (5). Cas9 target DNA sequences must have a protospacer-adjacent upstream motif (PAM) that contains the sequence NGG (N could be adenine, cytosine, thymine or guanine), at which level Cas9 allows an enzymatic cut that produces a double strand breaks (DSB). The main advantages of using this editing system are: the low price, adaptability to different target sequence, high-throughput and possibility of multiplex editing (6).

Genome editing mediated by Cas9 nuclease is carried out by DBS, followed either by a randomly insertion/deletion via error-prone non-homologous end-joining pathway, or a controlled insertion of a foreign

DNA sequence via homology-directed repair (HDR) pathway, which generate a precise gene knock-in (7). Modifying the Cas9 nuclease (by controlled mutagenesis) enabled an enzyme which preserves the DNA binding ability but does not induce DNA breaks - dCas9 (*Cas9 death*). In this way, different groups are attached to dCas9 and they can modify the transcription of certain genes, provide the labelling of certain DNA sequences (for example, fluorescent labelling of telomeres) or change the epigenetic control of some gene's domains (8).

Genome editing via HDR or DSB is characterized by unwanted events (generation of insertions and deletions (indels) at target and off-target sites) so attempt have been made to develop more accurate technologies. An example is base editing, a system formed by a chimeric protein that consists of a DNA targeting module and a catalytic domain capable of deaminating of cytidine or adenine base. In this way is changed a DNA base along with the manipulation of the DNA repair machinery to avoid faithful end repair of the modified base (9).

Genome editing has had an explosive development, with applications in all fields of human pathology, from oncology to assisted reproduction.

In oncology, it has been attempted to change all specific processes of oncogenesis (6). Thus, it has tried to inactivate oncogenes (10), to activate repressed tumor suppressor genes (11), to develop Chimeric antigen receptor (CAR) T cells targeted against certain tumors (12), to develop methods of identification of telomeres lengths (13), to repress the activity of cytokine receptors (14), to develop methods of screening the genes implied in metastasis (15), to remove binding sites of microRNAs in order to block neoangiogenesis (16), to develop methods for monitoring tumor heterogeneity (17), to inhibit apoptosis by viral gene (18), and to develop methods of screening of new metabolic regulator (19).

Another field where the genome editing can be applied is the modification of human stem cells, thereby producing a virtually infinite number of corrected pluripotent cells. For this were used embryonic cells, marrow stem cells and cancer cells. In principle, it can be used in two major directions: the introduction of a pathogenic mutation into a normal stem cells line or the correction of a pathogenic mutation into a stem cell line obtained from a patient with a particular monogenic disease. The gene mutations correction was tried for *CFTR* gene in cystic fibrosis (21) *HBB* gene in sickle cell anemia and beta-thalassemia (22, 23) *JAK3* gene in SCID (24) *RPGR* and *MERTK* genes in retinitis pigmentosa (25, 26) *DMD* gene in Duchenne muscular dystrophy (27), *PKD1* or *PKD2* genes the polycystic kidney disease of adults (28) .

The application of genome editing in degenerative pathologies of the central nervous system provides therapeutic opportunities for the treatment of diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease or autism spectrum, but practical application is still limited by our ability to introduce cells with corrected genes in nervous central system (29).

Genome editing was used to destroy HIV-1 in latently infected cells, but unfortunately the virus quickly developed resistance against genome editing at a single antiviral target site (30).

Currently, through genome editing it is possible to change the embryos bearing various pathogenic mutations. Thus, this method was applied to correct the mutation in *HBB* gene which determines the beta-thalassemia (31) or *MYBPC3* gene involved in the pathology of hypertrophic cardiomyopathy (32). However, such an approach raises numerous bioethical issues mainly

related to genomic editing errors such as incomplete editing, inaccurate editing (off- and on-target effects) possible interference with other genetic events (pleiotropy, penetrance or intervention of environmental factors or epigenetic mechanisms). On the other hand, bioethical dilemmas are correlated with a possible change of human genome in the direction of eugenics, such as improving the biological quality of certain individuals with an emphasis on social inequalities (33, 34).

Also, genome editing was used for improving various biotechnology, functional biology or gene therapy, allowing the modification of a single pair of nitrogenous bases, genomic and epigenomic modification, genome-wide screening, production of new protein variants, animal or vegetable researches with the aim to improve food quality, the development of animal models for human genetic pathology, improvement of xenotransplantation, production of performant antimicrobial or antiviral treatment or development of innovative therapies in medicine (9, 34).

All the above provide the premises of an extensive development of genome editing with multiple applications in biotechnology and medicine, but on the other hand generate concerns about the inappropriate use of these methods, because of the possibility to change the genome of certain embryos and modify the development potential of some people in the detriment of individuals with no genomic changes.

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EDITORIAL

NONALCOHOLIC STEATOHEPATITIS: THE MAINSTAY OF CLINICAL HEPATOLOGY

Nonalcoholic fatty liver disease (NAFLD) is a major cause of chronic liver disease and has now become a global epidemic affecting 1 in 4 adults worldwide (1). NAFLD encompasses two distinct conditions with distinct histologic features and prognosis: 1) nonalcoholic fatty liver (NAFL), which includes steatosis only, and 2) nonalcoholic steatohepatitis (NASH) characterized by steatosis and inflammation with hepatocyte ballooning, with or without fibrosis, which can progress to liver fibrosis, cirrhosis, hepatocellular carcinoma (HCC) and liver transplantation (2).

The global prevalence of NAFLD is about 25% (1) and the prevalence of NASH among NAFLD patients ranges from 10% to 59% among patients that underwent liver biopsy (1), meaning that millions and millions of people worldwide are at risk of cirrhosis and its complications. However, the true prevalence of NASH is not yet known, due to the need for liver biopsy (LB) to set the diagnosis, but current data show that 1 in 3 patients with NAFLD have NASH (1), while the global prevalence of NASH in general population ranges between 3%-5% (3). Even more alarming, an increase in NASH prevalence will soon parallel the rising rates in obesity, type 2 diabetes mellitus (T2DM) and metabolic syndrome - all three being the most important risk factors for NASH (4,5). Thus, in a recent study using a Markov model for predicting NAFLD/NASH progression in the USA showed an exponential increase in burden of disease, estimating that 33.5% of adult populations will have NAFLD, among which 27% will present with NASH, and 29% of patients with NASH will have advanced fibrosis by 2030 (6). Projected NAFLD cases will increase to 21%, from 83.1 million (2015) to 100.9 million (2030), while prevalent NASH cases will increase by 63%, from 16.52 million (2015) to 27.00 million cases (2030) (6). A Markov model was also used to estimate NAFLD/NASH disease burden modelled from 2016 to 2030 in eight countries (USA, United Kingdom, Japan, China, Germany, France, Italy and Spain) including a quarter of the world's population and showed that the prevalence of both conditions is expected to constantly rise in parallel with the epidemics of obesity, diabetes, and the metabolic syndrome (7). The highest rate of NAFLD population (26.3% in 2016) was estimated for the USA and is expected to increase by 18.3% (28.4% prevalence) to 100.9 million cases by 2030, whereas the population with NASH was estimated to increase at 56%, rising from 17.32 million cases (2016) to 27.00 million cases (2030). In China, the overall NAFLD population was estimated to increase from 17.6% in 2016 (246.33 million cases) to 29.1% in 2030, with 314.58 million cases. Among the European countries, the estimated NAFLD population in 2016 ranged from 18.45 million cases in Germany to 10.53 million cases in Spain, and by 2030 will have increased to 20.95 million cases in Germany, and 12.65 million cases in Spain. By the year 2030, the highest prevalence of NAFLD population was estimated in Italy (29.5%) and the lowest in France (23.6%). As to the NASH population, the highest number of cases in 2016 was re-

ported in China (32.61 million cases) and projected to increase to 48% (48.26 million cases) by 2030, while in the USA, the number of NASH cases was estimated to reach a peak of 56% (from 17.32 million cases in 2016 to 27.00 million cases in 2030). Spain is projected to have the highest proportion (29.5%) of NASH cases (792.110) by 2030 (7).

There are several other studies, reviews, and meta-analyses reporting the prevalence of NAFLD/NASH in different countries worldwide. A very recent one showed that in the European Union, the prevalence of NAFLD was estimated to 20-30%, with 3% having NASH, Romania and Hungary being mentioned with the prevalence of 20% and 22%, respectively (8). In Asia, prevalence of NAFLD ranges from 15% to 40%, and NASH ranges from 2% to 3%, while in Australia, NAFLD/NASH has become the most common liver disease, affecting a third of population. In North America, the prevalence of NAFLD reaches 24%, less than in South America (32%), while the Middle East has 31.8% - exceeding that in the USA. In Africa, the prevalence of NAFLD/NASH was estimated to be 13.5%, but it should be underlined that there is a lack of studies on the epidemiology of this disease in this continent. An almost similar prevalence of NAFLD/NASH was reported by Younossi *et al.* in a meta-analytic study (1) and in a recent review (9).

Of note, NASH is found not only in adults, but also in children and adolescents and to a high degree, paralleling the growing epidemic of childhood obesity, with an estimated of 7 million children/adolescents affected in USA only (10). NASH at a young age can progress to cirrhosis and end-stage liver disease (ESLD); one study reported that adolescents with NASH have 16 times higher chances to develop ESLD by the age of 20 compared to American youth with similar age and gender (11).

As mentioned previously, NASH prevalence is higher in patients with T2DM, obesity, and metabolic syndrome than in general population, largely due to a matching growth in the prevalence of this group of diseases. A recent study reported that the number of obese adults increased from 108 million (1980) to 671 million in 2016 (5). A similar increase in diabetes prevalence in adults - from 108 million (1980) to 422 million in 2014 - has been reported by another study (12).

In Romania, the first published study, in 2008, assessing the prevalence of NAFLD using ultrasound examination in hospitalized patients without liver diseases, reported a rate of 20% (13), and was followed more recently by other three studies (14-16) and a review (17).

To summarize, the prevalence of NASH has seen a tremendous growth over the last decades, turning it into an epidemic alongside obesity, diabetes, and the metabolic syndrome. However, it should be underlined that because of liver biopsy (LB) requirements for diagnosis, there are no accurate data on the prevalence of NASH at a global level given variations in assessment and definitions(18).

LB is the "gold standard" for the diagnosis of NASH and is regarded as the only method to distinguish NASH from NAFL (19). Still, its invasive nature, high cost, inter-and-intra-observer variability of histopathological interpretation, sampling errors, and the risk of rare but potentially life-threatening complications (20), make it unsuitable for disease diagnosis and monitoring in clinical practice, unrealistic and even impossible to be performed in millions of patients. The above-mentioned drawbacks of LB have led to the development of several noninvasive methods for liver fibrosis evaluation ranging from serum markers assays to advanced imaging techniques, representing a major advancement in clinical

hepatology, while some of them are already included in clinical practice guidelines for NAFLD including NASH (21). Intensive debates have been carried out in the past two decades between pros and cons (22), including the case of hepatitis C, a field where LB had been an important component in patient care and clinicians were reluctant to the introduction of noninvasive methods as an alternative to biopsy. Nowadays, LB is not any longer performed in the evaluation of liver fibrosis for decision making in chronic HCV antiviral therapy, being replaced by noninvasive methods. No doubt, this will be the case in NASH evaluation.

The economic burden of NAFLD/ NASH is huge, as evaluated by different methodologies. Thus, in a steady-state prevalence model to quantify the expected increase in the economic burden of NAFLD/NASH in the USA and four European countries (United Kingdom, Germany, France, and Italy), projections showed that in USA over 64 million people have NAFLD/NASH at a medical cost of about $103 billion annually, while in the Europe – in the 4 mentioned countries - there will be 30 million people with this disease, and with an annual cost of about 35 billion euros (23).

Younnosi *et al.* constructed lifetime Markov models for all stages of NASH, and a separate model to identify the increased burden of advanced NASH (fibrosis stage >3) and found that, in the USA, there are 6.65 million adults with NASH, with an estimated $222.6 billion cost during their lifetime, while the population with advanced NASH will cost $95.4 billion (24).

The natural history of NASH is not yet entirely known. What is known is that NASH is a main cause of chronic liver disease affecting millions of people worldwide. NASH is associated with an increased mortality, as it is the most important cause of death due to cardiovascular disease followed by malignancy, and liver-related disease (25). There is a higher incidence of HCC in patients with NASH than in general population (26) and NASH is the leading cause of HCC-related liver transplantation (2).

NASH was first described as a distinct entity by Ludwig *et al.* in 1980 (27) and remained for several years an obscure liver disease neglected by physicians, until two decades ago when it started to be the focus of attention, becoming nowadays a hot research topic and of great concern for the hepatologists, due to its high prevalence worldwide and its role in the development of liver cirrhosis and HCC.

Until five years ago, viral hepatitis (B and C) had been the focus of clinical research in hepatology. The advent of therapies that suppress hepatitis B replications and the highly effective direct acting antivirals curing HCV-infections in almost all patients, giving hope to see these infections eliminated by 2030 (28), switched the focus towards NAFLD and to one of its types, NASH - in particular, which has virtually replaced viral hepatitis and changed the perspective in hepatology.

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HEPATITELE VIRALE – ÎNCOTRO ?

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VIRAL HEPATITIS-WHERE TO ? (Abstract) : Viral hepatitis is a serious health problem all over the world. The aim of the study is to present the actual achievements in the therapeutically field and the general knowledge concerning the subject. Are presented the etiological agents of the acute and chronic hepatitis with the focus on hepatitis B and C, which has become a priority of WHO, with an incidence of the diseases of 360 billion/year for hepatitis B and an sub estimated level for C hepatitis. The most used drugs are presented and the therapeutical combination meant to decrease biological and virusological markers (ALAT, viral load) and to ameliorate the histological aspects of the liver. In conclusion, acute and chronic viral hepatitis represents a challenge for epidemiologists who try to stop the spread of the disease, but also for the infectious diseases specialists and gastroenterologists. **Key words** : VIRAL HEPATITIS, ALAT, VIRAL LOAD, DRUGS.

EDITORIAL

INTESTINAL MICROBIOTA AND INFLAMMATORY BOWEL DISEASES

Introduction. Inflammatory bowel disease (IBD) includes two distinct disorders, hemorrhagic colitis also known as **ulcerative colitis** (UC) and **Crohn's disease** (CD). These are chronic inflammatory diseases of the digestive tract characterized by alternating periods of exacerbation and remission. In UC the lesions caused by inflammation are continuous, without areas of normal mucosa, affecting the rectum and extending proximally to the colon, but do not involve the ileocecal valve. Histologically, the inflammation is restricted to the mucous and submucous membrane, described as inflammatory infiltrate in lamina propria and crypt abscesses. Unlike UC, CD can affect any segment of the digestive tract, from the oral cavity to the anus, the lesions being discontinuous, asymmetric, and transmural. Histologically, granulomas are pathognomonic findings. The most common location of lesions is in the terminal ileum ("terminal ileitis").

Epidemiology. The incidence of IBD (6-20/100,000 population for UC and 5-6/100,000 population for CD) has increase in the recent years, with geographical variability in the US, England, Scandinavian countries, where this increase is alarming, compared to Central and South Eastern Europe (including Romania), Latin America, Asia, Africa where the incidence is much lower. It is estimated that over 2.2 million individuals have IBD in Europe, and it was found that industrialization and adoption of the Western lifestyle led to a higher increase in the frequency of CD compared to UC (1). In Romania, the incidence of IBD is 6.5/100,000 population, with slight south-west epidemiological and phenotypic differences (2).

Etiopathogenesis. The pathogenesis of IBD is not fully elucidated, this implying the interaction between a number of such elements as individual genetic predisposition, environmental factors, immunological factors and intestinal microbiota.

Genetic factors. Studies on monozygotic twins have shown a concordance rate of 40% for CD and 10% for UC. Approximately 10-20% of IBD patients have family members with the same disease, the risk of disease among first degree relatives being of 7% (1, 2). Genomic studies have identified over 200 susceptibility loci for IBD, the transmission being polygenic, and the most commonly known polymorphism being that in NOD2/CARD15 gene (3).

Environmental factors. The Western diet low in fiber, high in processed foods rich in carbohydrates and fats, stress, the use of oral contraceptives and non-steroidal anti-inflammatory drugs, all increase the incidence of IBD in developed countries. Smoking and appendicectomy are protective factors against UC but are considered risk factors for CD. Often the development of UC can be triggered by quitting smoking.

Immunological factors. The gut mucosal immune system has an increased level of tolerance to food or bacterial antigens. At the time of contact with a foreign antigen, Th-1 lymphocytes produce proin-

flammatory cytokines, such as gamma interferon and IL-2. In its turn, Th-1 will activate through cytokines the macrophages that release other pro-inflammatory cytokines: tumor necrosis factor-alpha (TNF-α), IL-1 and IL-6, thereby amplifying the inflammation. Chemokines control the recruitment of numerous circulating cells at the site of inflammation, with the release of prostaglandins, leukotriene, oxygen-free radicals, proteases and nitric oxide that will amplify tissue destruction. In the normal gut, the Th-2 lymphocyte-mediated inhibition mechanism becomes immediately active, and with the help of anti-inflammatory cytokines like interleukin-10 and TGF-beta inhibits Th-1 and macrophages, stimulating mucosal healing (4, 5).

The therapeutic response to corticosteroids or anti-TNF may provide evidence of the immune pathogenesis of IBD.

1. Intestinal commensal flora. Microbiota composition is highly diversified among individuals, but generally similar in the same species. Intestinal microbiota represents an ensemble of more than 10 trillion microorganisms that colonize the digestive system, including approximately 1,000 species of bacteria, viruses and fungi with more than 1 million genes, 150 times more than human genome. One-third of the intestinal microbiota is common to most people, but two-thirds are specific to each individual, demonstrating the different responses of patients to pathogens and treatment. Vaginal birth plays an essential role in gut colonization with commensal bacteria, *by crossing*/through the direct contact with the pelvic genital tract and then with maternal perineal area. Thus, babies born vaginally have different gut bacteria than those delivered by cesarean section.

Typically, the composition of intestinal microbiota in babies delivered by cesarean section is less diverse but is characterized by a high interindividual variability (6). As the infant consumes increasingly more complex dietary substrates, the gut microbiota undergoes changes. It tends to be relatively stable throughout early childhood (7). Two major microbial phyla - Firmicutes and Bacteroidetes - account for 99% of the bacteria in the gut. Despite its high interindividual variability, the gut microbiota shows a high functional uniformity regardless of age or sex (8).

2. Structure of the intestinal flora. Intestinal microbiota contains more than 1000 different species of microorganisms, weighing about 1 kg. The proximal part of the digestive tract, the stomach and small intestine are less populated by bacteria_due to the low oxygen concentration at these levels. Most of the microorganisms colonize the large intestine. The vast majority of bacteria belong to two classes: *Firmicutes* and *Bacteroidetes*.

From an anatomical point of view, bacterial species are distributed as follows: in the stomach there are normally between 0-100 bacteria of the genera *Lactobacillus*, *Candida*, *Streptococcus*, *Helicobacter*, *Peptostreptococcus*; the duodenum and ileum contain approximately 100 bacteria of the *Streptococcus genus/* Streptococcaceae and *Lactobacillus genus/* Lactobacillaceae families. Starting from the proximal ileum up to the colon, the bacterial population begins to increase in number and vary: at the level of proximal ileum there are 1000 bacteria of the genus *Streptococcus* and *Lactobacillus*, in the distal ileum 10^7-10^8 bacteria of the genera *Clostridium*, *Streptococcus*, *Bacteroides*, *Actinomycinae*, *Corynebacterium*, and in the colon 10^{11}-

10^{12} bacteria of the families *Bacteroides/* Bacteroidaceae, *Clostridium* groups IV and XIV, *Bifidobacterium/* Bifidobacteriaceae and *Enterobacteriaceae*.

Normally, a healthy human intestine hosts 10^{14} microbes-10 times the number of cells in the human body (9). Due to the major and bidirectional influence, the intestinal microbiota has a significant impact on the host and also the host influences bacterial adaptation and evolution. The relationship between the host and intestinal flora is extremely complex and important, and thus the assertion "the gastrointestinal microflora, especially that gained in the initial period of life, influences so deeply the morphological and physiological characteristics of its host, that many of the characteristics of the adult, which appear to be the expression of genetic endowment, are determined in reality by its microbial environment" is supported today by the existence of more and more disorders problematic in terms of therapeutic possibilities and patient response. Especially in the intestine, microbiota influences the structural development of lymphoid tissues, ensures the integrity of the epithelial barrier, the function and development of innate and acquired immunity (9).

Factors influencing the intestinal microbiota

Bacterial interactions. Bacterial interactions can act either antagonistically or synergistically trough the low secretion by aerobic bacteria of oxidation-reduction compounds, which will favor the growth of anaerobic microorganisms, enzymatic mechanisms and the production of growth factors.

From an antagonistic point of view, the barrier effect that is of particular importance to prevent digestive tract colonization with exogenous bacteria is highlighted. Due to the fact that in the intestine the commensal flora occupies some specific spaces, the exogenous microorganisms cannot penetrate. This effect can be exerted both between different species and within the same bacterial species. One of the characteristics of this barrier is the rapid elimination, by peristalsis, destruction, of the bacteria that try to penetrate the intestine.

Of importance for clinical practice is the fact that the barrier effect can be reversed in the course of an infection with pathogenic bacteria, which has several advantages through the virulence factors and do not have regular clearance, and thus may cause various diseases through changes in commensal flora structure "because of host-related factors that allow transient colonization with non-pathogenic species" (10).

Adhesins (bacterial adhesion factors) play a major role in bacterial colonization. These are proteins that recognize a certain carbohydrate sequence in the structure of glycoproteins or glycolipids present in the cell membranes, and which allow bacteria to adhere to the surface of the mucosa they colonize (7).

Host dependent factors. The relationship between the intestinal microbiota and the human body is symbiotic, reflecting a dynamic balance in order to maintain a physiological bacteriological structure of the digestive tract and, more precisely, a state of *eubiosis*.

Factors that keep the intestinal bacterial population under control are saliva, gastric acid secretion, self-defense capacity of the small intestine through mucus secretion, enzymatic activity, intestinal immune system, normal intestinal peristalsis and ile-

ocecal valve.

Saliva, through lysozyme, has an antibacterial role against the proliferation of bacteria belonging to of *Lactobacilli, Staphylococci, Streptococci* species.

Gastric acid secretion prevents the development in the digestive tract of exogenous bacteria originating from food or having the source in the secretions of the upper respiratory and digestive pathways. At the same time, gastric secretion is sensitive to acid-sensitive bacteria, which is demonstrated by the different structure of the intestinal flora in a patient with normo- or achlorhydria.

The self-defense capacity of the small intestine is the most important mechanism against the proliferation of exogenous bacteria. This self-defense capability is represented by gastrointestinal mucus (physical barrier to bacteria and their toxins) and intestinal mucosa participating in the defense process through innate and acquired immunity. In the case of gained immunity, secretory IgA, and IgG and IgM antibodies are secreted, which contain bactericidal antibodies and thus highlight specific responses from the gastrointestinal immune system. On the other hand, innate immunity produces in the gastrointestinal tract a group of antimicrobial peptides called defensins. The defensins in the human body are alpha and beta type; Paneth cells in the small intestine contain alpha-defensins and have bactericidal activity against *Listeria monocytogenes, E. coli, Salmonella typhimurium, Candida albicans* (6, 8, 9).

Normal intestinal peristalsis is an important mechanism of bowel clearance that counteracts the development of a pathogenic flora at this level by transporting the food and bacteria distally and thus preventing stagnation and multiplication.

Beneficial effects of intestinal flora at local and systemic level

Morphological effects. The host-flora interaction has a major impact on the morphology of intestinal mucosa, through antigenic stimulation, which can confer the digestive mucosa the appearance of chronic inflammatory response (3).

Immunological effects. Intestinal microbiota is involved in immune system homeostasis, so the body has the ability to tolerate the microorganisms it comes into contact with. Intestinal flora is the stimulus for the maturation of the immune system, by involving humoral and cell-mediated immunity, contributing to the expression of class II of the major histocompatibility complex molecules, development of a normal antibacterial humoral immunity, as well as of physiological tolerance to soluble intraluminal antigens: microbial or viral (1, 7).

The intestinal mucosal surface is one of the main entry points of pathogens into the human body, but in the healthy state the cells of the intestinal wall mucosa, interact with those of the commensal microbial flora, providing an effective barrier against pathogenic microorganisms. Intestinal epithelial barrier, consisting of a continuous layer of epithelial cells, closely attached to each other and in contact with the immune cells that underlie lamina propria (organized lymphoid tissues, Peyer plates), mucus-producing cells and overlapped mucus layer together with the commensal microbiota form a complex that protect the host against pathogen invasion, colonization and superinfection. For a long time, it was considered that the role of the thick mucin layer was exclusively of limiting the exposure of epithelial cells to microorganisms. However, an active functional role of

mucin in immunity has been demonstrated, aiding the recruitment of luminal antigens by the subjacent dendritic cells (DC) through a layer of small intestinal mucus gates and the depreciation of inflammatory responses of DC to bacterial antigens by co-stimulation with hyperglycosylated MUC2 mucin.

The commensal intestinal microbiota is an important obstacle against pathogen invasion through several mechanisms, including the struggle for metabolic growth substrates, production of antimicrobial compounds, occupation of intestinal niches and potentiation of host immune responses (i.e. the production of IgA and IgM) (8). A well-controlled and regulated bacterial contact is essential for maintaining an optimal immune function at the intestinal level. Similarly, intestinal microbiota, in particular through the production of butyrate resulting from fermentation processes, contributes to intestinal wall integrity, providing approximately 50% of intestinal mucosa energy needs, thus ensuring both the optimum proliferation of mucous cells and the epigenetic adjustment of cellular differentiation and apoptosis.

Understanding how intestinal microbiota modulates the intestinal immune system is the result of studies on murine models that have shown that the lack of antigenic stimulation leads to underdevelopment of gut-associated lymphoid tissue (GALT), as well as of systemic immunity, including impaired development of lymphoid organs, impaired recruitment and differentiation of lymphocytes. These alterations ultimately lead to the occurrence of immune-mediated inflammatory diseases (9, 10). It has been shown that, in humans, antibiotic treatment during childhood results in a higher risk of developing conditions such as: allergies, asthma and IBD (6, 9).

Human gut microbiota is a constant stimulus for the host immune system. To cope with this diversity, the host immune system recognizes only certain highly preserved phylogenetic antigens. This recognition is mediated by epithelial cells (EC) at the mucosal surface and dendritic cells (DC). The underlying dendritic cells play an important role in differentiating the naïve T cells in the mesenteric lymph nodes (MLN) into regulatory T cells that subsequently express proteins in the gastrointestinal mucosa where they mediate immune homeostasis (5, 6). It also directs T Helper cell responses to Th1 and Th2. Th1 immune responses are dependent on IL-12 production and are characterized by the production of interferon (IFN)-γ and IL-12. Th2 responses involve IL-4, IL-5, IL-6 and IL-13 with important role in humoral immunity. DCs can induce differentiation of regulatory T lymphocytes, with an important role in immune response modulation (6).

The bacterial components recognized by the host are usually nucleic acids, cell wall fragments via microbial antigen receptors, recognition receptors (PRRs) which recognize molecular motifs shared by different types of microbes, such as pathogen-associated molecular patterns (PAMPs), or surface molecule-associated molecular patterns, such as teichoic acid, polysaccharides, flagellin, DNA and double-stranded RNA. These microbial components serve as elements for differentiation of microorganisms by the immune system. Moreover, intestinal mucosal microorganisms appear to possess many different channels of communication with the host immune system and activate different PRRs, resulting in synergistic action and functional redun-

dancy between PRRs (5, 6, 8). PPRs include toll-like extracellular receptors (TLRs), Nod-like receptor family and the CD14 receptor for bacterial lipopolysaccharide. TLRs are signaling PRRs, which activate the transcription factor NF-κβ and recognize cell wall components of Gram-positive and Gram-negative bacteria, such as lipopolysaccharide (TLR-4), teictoic acid, and peptidoglycan (TLR-2) and bacterial flagellin, a common feature of intestinal pathogens (TLR-5) and unmethylated CpG DNA (TLR-9). Unlike TLRs, Nod proteins occur cytoplasmically rather than on the cell surface, allowing recognition of intracellularly translocated microbial components. Three important Nod-like receptors, namely Nod1, Nod2 and IPAF factor are encoded by caspase recruitment domain (CARD)4, CARD15 and CARD12, respectively. Nod1 recognizes gamma-D-glutamyl-meso-diaminopimelic acid, a peptidoglycan degradation product; Nod2 binds to muramyl dipeptide (MDP) - another peptidoglycan component - and IPAF binds to flagellin. Nod1 and Nod2 domains interact with receptor-interacting protein 2 kinase (RIP2/RICK) that mediates inhibitor kappa B (IκB) activity and NF-κB-dependent gene expression of proinflammatory cytokines. Certain microbial ligands can be identified by several PRR receptors. Typical examples are bacterial flagellin and peptidoglycan, which can be recognized by TLR5 and TLR2, respectively on the cell surface and by intracellular IPAF and Nod. Toll-like extracellular receptors have been shown to release cytokines from myeloid cells, resulting in the amplification of the resulting immune response. Stimulation of PRRs immediately triggers the activation of signal cascades for antimicrobial immune responses and downstream transcription of inflammatory modulators. Therefore, by recruiting/stimulating immune effector cells through signaling molecules and antigen presentation by DCs to B cells and MLN T cells, and by mucosal IgA production respectively, it can be argued that both innate immunity as well as the acquired one are activated. Detection of PRR-PAMP in the gastrointestinal tract also has the function of maintaining immunological tolerance and intestinal homeostasis (10, 11).

Therefore, dysbiosis can cause abnormal immune responses, thus being correlated in recent years with a variety of autoimmune and allergic diseases. Garett and al. demonstrated by a study on murine models that the depletion of the T-bet transcription factor in the RAG resulted in the development of spontaneous colitis. This suggests that microbiota is sufficient to induce intestinal inflammation (9). According to findings from animal studies, but also in humans, inflammatory bowel diseases (IBD) have been associated with dysbiosis. Children living in developing countries and which come in contact with a larger intestinal bacterial diversity have a lower incidence of inflammatory conditions in adulthood, including IBD. Statistically significant relationships have been also found between early exposure to antibiotics and the occurrence of IBD in these individuals in adulthood (2, 5, 12).

It has been shown that the continuous sampling of antigens and their presentation to naïve T-cells leads to the development of adaptive immunity to microbial invasion of the intestinal mucosa. Recent studies have shown that butyrate and propionate can inhibit histone-deacetylase (HDAC) not only in colon cells, but also in DC, through a mechanism involving the sodium-coupled

monocarboxylate transporter SLC5A8. According to current findings, butyrate and propionate block DC maturation and therefore inhibit the intestinal immune response and inflammation, through a DC mechanism dependent on SLC5A8 and HDAC. It has been shown that intestinal commensal bacteria are necessary to induce the expression of the short-chain fatty acid (SCFA) transporter SLC5A8 for DCs. (13, 14). DCs are involved in the potentiation of immune responses and increased antigen uptake by inducing the TLR expression (9).

Dysbiosis and inflammatory bowel disease

The native, resident microbiota is acquired in the first year of life and is a unique combination for each individual, such as digital footprint. Transient microbiota colonize the intestine and consists of pathogenic and non-pathogenic organisms.

Colon microbiota plays an essential role in the body by its participation in the achievement of important functions such as: prevention of pathogenic microorganism invasion, improving the function of intestinal cells, strengthening the immune system, vitamin synthesis, stimulate intestinal peristalsis to ensure transit and reduce unpleasant discomfort and bloating, avoiding inflammatory bowel processes (15).

Bacteria which are in colon microbiota composition are classified into several categories depending of their function: - Immunomodulatory microbiota: regulates the functioning of the intestinal immune system; - Protective microbiota: prevents colonization by pathogenic bacteria; - Muconutritive microbiota: maintains the integrity of the intestinal mucosa; - Proteolytic Microbiota: is beneficial in a small amount but harmful in a large amount. They are joined by lees which have a minor beneficial role but are harmful when forming the micelles.

However, there are other species, which are not numerically significant, but are important for homeostasis: *Proteobacteria*, *Actinobacteria*, *Fusobacterium* and *Verrumicrobia*.

In IBD patients, quantitative changes were revealed in bacteria belonging to the classes of *Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria* (16, 17). For example, a reduction in the Clostridiales species of the *Firmicutes* class is observed in the fecal microbiota of patients with IBD (5, 8, 10, 18). An increase in *Ruminococcus gnavus* in the fecal microbiota was also observed among these patients. Other bacteria considered "beneficial" for the host have been shown to be reduced quantitatively in the fecal microbiota of these patients. An increased amount of bacteria of *Streptococcus* genus has been observed, as opposed to *Ruminococcaceae bacteria* (genus *Faecalibacterium*), which appear to be particularly deficient, especially in patients with CD. Salem *et al*. demonstrated in a recent study, using molecular methods for identifying bacteria, that *Faecalibacterium prausnitzii* was one of the most deficient species of *Faecalibacterium* genera in MAM of IBD patients (compared to healthy subjects) (19). As common elements, both in patients with CD and in those with ulcer hemorrhagic rectocolitis (UHRC), a decrease in *Clostridium, Coprococcus, Eubacterium* genera (*Firmicutes* class) was highlighted. Through studies in which both bacterial cultures and molecular methods were used, an increase in the population belonging to *Bifidobacterium* genus, highlighted in the fecal microbiota, as well as in intestinal mucosa biopsies of patients with IBD, and especially in patients

with CD was shown. Also, in many CD patients an increase in abundance of the bacterial population from the *Enterobacteriaceae* family, *Escherichia* and *Shigella* genus was identified (6, 8, 10).

Metabolic effects and the role of short-chain fatty acids in inflammation

The metabolic activities of intestinal microbiota are multiple and complex, acting as a "metabolic body" through the synthesis of vitamin B12, folic acid, vitamin K, proteins (hydrolysis, amino acid degradation), lipids (lipid hydrolysis, synthesis of long-chain hydroxylated fatty acids.

Action is also observed on endogenous products (deconjugation, dehydroxylation, dehydrogenation of bile acids), deconjugation of bilirubin and conversion of bilirubin into urobilinogen, cholesterol degradation, deconjugation of sex hormones (ensuring enterohepatic circulation), urease activity (ammonia production) and it produces pH changes and a decrease in redox potential (10, 20). In other words, the intestine is a multifunctional organ involved both in immunity and metabolism.

The intestinal epithelium has two differential gene networks, one regulating immunity and the other governing the lipid metabolism, the two being inversely expressed.

SCFAs are the end products of bacterial metabolism, mainly derived from the anaerobic fermentation and hydrolysis of polysaccharides and undigested proteins, which exert important physiological effects (8, 10). The main SCFA products of the colon are acetate, propionate and butyrate in a ratio of 2: 1: 1. They can be absorbed by the host, securing up to 10% of the basic energy needs. While acetate is used in cellular respiration and cholesterol synthesis in the liver and brain, propionate and butyrate are mostly used by colonocytes and liver as a source of energy. SCFAs may influence the recruitment of leukocytes, in particular neutrophil chemotaxis, by binding to the coupled receiver to protein G (GPR 43) and activation of protein kinase (MAPK), of protein kinase C (PKC) and transcription factor (ATF-2) (8, 10). SCFA, particularly butyrate, has been shown to reduce the production of monocyte chemoattractant protein-1 (MCP-1) an important factor that binds to monocytes and macrophages. Macrophages play a role in the secretion of proinflammatory mediators in chronic inflammation states, such as insulin resistance and obesity, atherosclerosis and also rheumatoid arthritis and neurodegenerative diseases, as well as in IBDs. Therefore, the production of MCP-1, IL-6 and INF-γ by intestinal epithelial cells influences the inflammatory and immune response of intestinal SCFAs, particularly butyrate and propionate, also reduce proinflammatory cytokines such as TNF-α and nitric oxide (NO) in neutrophils. SCFAs also influence phagocytosis and the production of reactive oxygen species by neutrophils and macrophages (10).

Studies on microbiota open up new perspectives on autoimmune diseases, proving that the microbiota is closely associated with host immune system, this interaction being based on epigenetic mechanisms. This way, new therapeutic strategies and more sensitive and specific biomarkers and potential therapeutic targets can be developed.

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EDITORIAL

MALNUTRITION, SARCOPENIA AND FRAILTY IN LIVER CIRRHOSIS

Cirrhosis is the end stage of chronic liver diseases, associated with high mortality, and responsible for about 170,000 deaths yearly in Europe alone as reported by the World Health Organization (1), as well as the second cause of death associated with digestive diseases, after colorectal cancer (2). It is well known that cirrhotic patients are particularly prone to the development of malnutrition which has been shown to be an independent predictor of survival in these patients (3), mortality increasing in direct proportion to the severity of malnutrition, reaching almost 80% in those with sarcopenia and frailty (4, 5). Consequently, all patients with cirrhosis should be screened for malnutrition in order to diagnose this complication and its degree of severity.

Malnutrition is common across patients with liver cirrhosis, with an estimated prevalence of 40% in compensated cirrhotic patients and more than 95% in those with decompensated cirrhosis (4). In general population, malnutrition is defined as loss of muscle mass, while in patients with liver cirrhosis, malnutrition is defined as loss of skeletal muscle mass and body weight, decreased subcutaneous and visceral fat mass.

By definition, malnutrition occurs when diet is poor (decreased protein, fat and carbohydrate intake), unable to provide the necessary number of calories to maintain nutritional status or secondary to malabsorption due to liver disease and exocrine pancreatic insufficiency. In addition to poor dietary intake and malabsorption, there are several other causes of malnutrition in cirrhosis such as low protein synthesis, increased intestinal loss, cirrhotic hypermetabolism (hypermetabolic state is present in at least a quarter of cirrhotic patients), small intestine bacterial overgrowth, and gastrointestinal dysmotility (6). Decreased nutrient intake in patients with decompensated liver cirrhosis can also be accounted for by anorexia ─ a symptom that is present in over 90% of patients ─ early satiety related to compression from ascites (7). It should be underlined that the most frequent form of malnutrition in cirrhotic patients worldwide is poor nutritional status.

According to some studies, alcoholic cirrhosis is associated with more severe nutritional state than virus-related cirrhosis, and the malnutrition is more common in cirrhotic men than in women (8, 9). Several studies have reported that malnutrition in cirrhotic patients is correlated with the severity of liver disease and particularly with the development of complications such as hepatic encephalopathy, hepatorenal syndrome and ascites (10).

Assessment of nutritional status in cirrhotic patients should include a complete medical history (weight and appetite fluctuations, dietary intake, nausea, vomiting, and diarrhea episodes) and physical examination (to detect ascites, peripheral edema, muscle wasting and subcutaneous fat loss). Subjective Global Assessment (SGA) combines several elements of nutritional assessment among which muscle wasting is considered the most important (11).

SGA is recommended by the European Society of Parenteral and Enteral Nutrition (ESPEN) as a practical bedside method to evaluate the nutritional status in cirrhotic patients. The SGA method allows us to easily classify the severity of malnutrition in 3 grades: 1 (mild), 2 (moderate), 3 (severe) (11). In addition, there are several other clinical methods for assessment such as mid-arm circumference, hand-grip strength, and skinfold. The International Society for Hepatic Encephalopathy and Nitrogen Metabolism (ISHEN) recommended The Royal Free Hospital – Nutritional Prioritizing Tool (RFH-NPT) as the method to evaluate nutritional risk of cirrhotic patients (12). The RFH-NPT score was reported to correlate with severity of disease, clinical complications and survival (13).

Sarcopenia, defined as generalized loss of skeletal muscle mass, strength, and function, is widely prevalent in patients with liver cirrhosis, affecting more than 70% of these patients (14). In addition, sarcopenia is a progressive disorder, leading to physical disability and increased mortality related to respiratory and cardiac muscles involvement (15, 16).

In adults, a 0.5% loss of skeletal muscle mass occurs annually, this percentage rising after the age of 65, so that by the age of 80 an individual has only 40% of his/her peak of muscles mass (17).

In addition to sarcopenia of skeletal muscle, cirrhotic patients may present cardiac sarcopenia, characterized by left ventricular dysfunction in the absence of any overt cardiovascular disease (16), and respiratory sarcopenia increasing the risk of lung infections (15). The prevalence of sarcopenia in cirrhosis is much higher than in other chronic disease due to metabolic complications induced by hepatocellular failure.

There are several diagnosis methods in sarcopenia. Muscle wasting, defined as the progressive and generalized loss of muscle mass is the main clinical diagnosis criterion, whereas skeletal muscle cross-sectional imaging with computed tomography (CT) or magnetic resonance imaging (MRI) is the gold standard objective method for diagnosis (18). Functional evaluation of sarcopenia includes hand grip strength and 6-minute walk test (19).

Frailty can be defined as multi-system impairment associated with increased vulnerability to stressors (20). There is a significant overlap between sarcopenia and frailty, defined by Fried et al, both conditions including low gate speed and low grip strength as well as weight loss, self-reported low physical activity and exhaustion (21). The most reliable method for measuring frailty in clinical practice is the Fried Frailty Phenotype which combines five elements: exhaustion, slow gait speed, unintentional weight loss, low physical activity, and weak hand grip strength. A score of 0 is non-frail, 1 to 2 is defined as pre-frail and 3 to 5 is defined frail.

The management of malnutrition, sarcopenia and frailty improves survival and quality of life in cirrhotic patients. The European Association for the Study of the Liver (EASL) Clinical Practice Guidelines on nutrition in chronic liver disease recommends in cirrhotic patients at least 35 kcal/kg daily, with a 1.2-1.5 g/kg protein intake and supplementation with branched-chain amino acids (BSAAs) (22). It should be noted that this recommendation is not in line with the traditionally protein- restricted diet in cirrhotic patients aiming to avoid the risk of developing hepatic encephalopathy.

However, according to ESPEN only cirrhotic patients with stage III or IV encephalopathy require restricted protein intake (11). Oral intake should be recommended in all patients, and when this is not possible, artificial nutrition may commence either through a nasogastric, nasoduodenal or nasojejunal tube (enterally) or intravenously (parenterally).

The management of malnutrition in clinical practice includes the use of the BSAAs leucine, isoleucine and valine administered orally, enterally or parenterally. Although some studies have reported improvement in the degree of encephalopathy, their usefulness remains controversial, as according to ESPEN, enteral feeding enriched with BCAAs should be reserved to patients who develop encephalopathy with enteral feeding despite appropriate treatment.

Finally, the management of malnutrition, sarcopenia and frailty in patients with liver cirrhosis should include physical activity and exercises tailored to the patient's ability, beginning with moderate intensity, to improve muscle mass and function (22).

However, long-term studies are needed to demonstrate that by combining nutritional needs (at least 35 kcal/kg daily, 1.2-1.5 g/kg daily protein intake) and physical activity, we can achieve growth in muscle mass and function, as well as in the survival rate of such patients.

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NEWS

HYPERHOMOCYSTEINEMIA IS AN INDEPENDENT RISK FACTOR FOR INTRACRANIAL ATHEROSCLEROTIC STENOSIS

Stroke has become one of the main causes of disability and death among the population. Intracranial atherosclerotic stenosis (ICAS) is the most common cause of stroke. It is essential to identify, prevent and treatment he modifiable risk factors of ICAS. Hyperhomocysteinemia (HHcy) is considered a risk factor for cardiovascular disease and atherosclerosis. In addition, the exiting data in literature on the existing literature on the relationship between serum homocysteine level and stroke is still controversial. A new study published in *AGING* examines the association of homocysteine concentration with ICAS. According to statistical and processing data, HHcy could be an important marker of vascular injury and a potential target for the prevention of vascular injury, especially for intracranial artery. Therefore, the research group´s conclusion was that increased homocysteine may be an independent risk factor for intracranial atherosclerotic stenosis (Ying Liu,Jing-Hui Song, Xiao-He Hou et al.Elevated homocysteine as an independent risk for intracranial atherosclerotic stenosis. *Aging* 2019, Vol. 11, No. 11).

EDITORIAL

COVID-19 AND CARDIOVASCULAR STORM

COVID-19 pandemic is one of the biggest challenges confronting communities and nations, having a tremendous impact on public health services all over the world. In the past years, people have faced several worldwide epidemic infections, such as Severe Acute Respiratory Syndrome (SARS), avian influenza, influenza, but SARS-Cov-2 is rapidly becoming the most threatening global enemy, endangering not only the health systems' stability, but transforming into a social and economic crisis.

SARS-CoV-2 is part of a group of viruses, including severe acute respiratory syndrome CoV (SARS-CoV) and Middle East respiratory syndrome CoV (MERS-CoV) (1), well known in the past two decades and infecting almost 9,000 people worldwide (2).

Coronaviruses are single stranded RNA, presenting (when symptomatic) with almost the same symptomatology, consisting of fatigue, fever, and dry cough at the beginning (3). The severity of symptoms, alone or in combination with modified blood biochemistry represent prognostic markers in COVID-19 patients (4) and they may vary from absence of symptomatology or mild pneumonia to severe respiratory impairment: dyspnea, tachypnoea, low blood oxygen saturation or lung infiltrates in 24-48 hours.

The severity of lung injury may lead rapidly to acute respiratory distress syndrome (ARDS), an entity that is usually associated with high mortality rates since the early reports from the American-European consensus conference in 1994 (5), despite new ventilation protocols with new generation of ventilators.

The ARDS evolution may be complicated by respiratory failure, septic shock, or multiple organ dysfunction with an increased risk of death. Moreover, studies suggested that in ARDS, cardiovascular complications such as fulminant myocarditis and vascular thrombotic events should be considered (6).

COVID-19 and cardiovascular complications

The thrombogenic status in COVID- 19 creates the premises of developing pulmonary thromboembolism, acute myocardial infarction, or strokes. Since February 2020, several studies reported a high incidence of myocardial involvement in critically ill patients with COVID-19 with or without previous cardiac impairments (7).

Previous conditions may represent risk factors for the infection with the new coronavirus (8), data confirmed also in a systematic review that included 41 studies and 27670 patients which demonstrated the higher risk of death in patients with preexisting cardiovascular diseases, immune and metabolic disorders, respiratory diseases, neoplasia, cerebrovascular, renal, and liver diseases (9).

The main target of SARS-CoV-2 is represented by ACE2 receptors, that are highly expressed in the lungs and heart, a mechanism that rapidly became controversial in terms of using or not Angiotensin Conversion Enzyme Inhibitors (ACEIs) or Angiotensin Receptor Blockers (ARBs) in COVID-19 hypertensive patients. This discussion was clarified by the most recent guidelines recommendations, which stated that the use of ACE inhibitors is safe and

should be continued as indicated (10).

In a systematic review and meta-analysis that analyzed 27 studies with 10197 patients taking renin-angiotensin drugs in COVID-19 there was nonsignificant associations between ACEIs/ARBs and death or ICU admission, risk or severity of SARS-CoV-2 infection and hospitalization, but a significant association between RAAS drugs usage and hospitalization was found, in the subgroup analyses of the United States of America included studies (11).

Furthermore, there are several studies that show higher rates and severity of the infection in men versus women due to a higher expression of proteins that bind the virus and genome disparities (12), meanwhile a study performed in the United States of America revealed racial and ethnic disparities, African American/Black and Hispanic populations having a higher rate of SARS-CoV-2 infection and mortality compared with non-Hispanic White population, but with similar rates of case fatality (13).

COVID-19 and
acute coronary syndromes

When acute coronary syndromes are taken into consideration, the endothelium plays a major role. It provides normal vascular homeostasis by regulating vascular tone, cells adhesion, thrombosis, and inflammation, meanwhile lymphocytes represent a major regulatory point in maintaining homeostasis and against exaggerated inflammation after infection (14).

Therefore, due to the intense activation of endothelium caused by a high number of cytokines associated with low levels of T-lymphocyte lines, the COVID-19 patients present an increased prothrombotic status associated with an intense inflammatory response (15).

The magnitude of the inflammation is well corelated with the cardiac involvement, which can be revealed by high levels of troponin and NT- pro BNP at admission. In consequence, these patients present a high risk of coagulopathy, heart failure or a mortality up to 50% (16).

Other biomarkers that can be used for therapy efficiency are endothelial extracellular vesicles (17) and endothelial progenitor cells, that can be also correlated with disease severity (18) and with vascular injury (19).

It is well known that atherosclerosis and inflammation represent important risk factors in developing acute coronary syndromes (20). But several studies also reported a relation between acute coronary syndromes and COVID-19 caused by coronary artery thrombosis, even without a pre-existing atherosclerotic lesion or with nonobstructive lesions (21), most likely due to microvascular involvement (22).

A study that included 305 patients with COVID-19 from 7 hospitals in New York City and Milan illustrates the significance of the cardiac involvement. Thereby, from the 305 patients included, 190 had evidence of myocardial injury and from them 26.3% had right ventricular dysfunction, followed by 23.7% with left ventricle wall abnormalities, 18.4% with left ventricle global dysfunction, 13.2% with diastolic dysfunction grade II or III and 7.2% pericardial effusions (23).

In terms of paraclinical methods, these patients require classical echocardiography, but cardiac MRI mapping techniques are particularly useful providing evidence of diffuse myocardial infiltration even in asymptomatic cases (24).

Regarding the acute coronary syndromes therapy strategies, the ESC Guidance for the Diagnosis and Management of Cardiovascular Disease during the COVID-19 pandemic stipulates that the invasive cardiac procedures in emergency situations, such as ST-

elevation acute myocardial infarction, non-ST elevation acute myocardial infarction in very high risk and high-risk patients and also in cardiogenic shock must not be postponed. The reperfusion therapy remains unchanged in terms of strategy in STEMI patients that must be managed as if they are COVID-19 positive in the absence of a previous RT- PCR test for SARS-Cov-2.

The reperfusion therapy remains the primary PCI, with a maximum delay from diagnosis to reperfusion of 2 hours, procedure that may be delayed no more than 60 minutes due to care and protective measures implementation. Fibrinolysis will be maintained as second option if the reperfusion target time cannot be achieved and if it is not contraindicated (25).

COVID-19 and arrhythmias

Several studies demonstrated the presence of cardiomegaly and right ventricle enlargement in COVID- 19 patients, with the presence of arrhythmias caused probably by myocardial infiltration and severe inflammation linked to ion channel dysfunction and both electrophysiological and anatomical remodeling (26).

Besides these, adding the pharmacological agents that COVID- 19 patients require, antiviral drugs such as Hydroxychloroquine and Azithromycin that are well known to prolong QT interval, requires a special attention. Repeated ECGs, electrolytes balance and kidney function are necessary and the combination with Amiodarone should be avoided. Moreover, all modifiable QTc prolonging factors must be corrected, including kalium and calcium levels, magnesium supplementation and bradycardia avoidance, with the necessity of ceasing concomitant QT- prolonging drugs if needed (23).

On the other side, elective procedures such as ablation or cardiac device implantation should be postponed, urgent procedures being performed only in exceptional cases.

COVID-19 and myocarditis

Myocarditis is well known to provide heart failure and sudden cardiac death (27), being also potentially caused by COVID-19.

There are four presumed mechanisms that may explain the cardiovascular involvement in COVID-19: a direct viral effect against cardiomyocytes, myocardial interstitial fibrosis, secondary hypoxia induced by pulmonary impairment and the inflammatory immune response that generates the cytokine storm (28).

Fulminant myocarditis represents a high mortality cardiac condition, in some registries with a short-term survival rate of only 58% (29), but with a higher long-term survival after discharge rate reported in some retrospective studies compared to non-fulminant myocarditis forms (30).

Chen *et al.* observed that in their center, which treats critically ill patients with SARS CoV-2 complicated by severe cardiac injury, the main cause of death was fulminant myocarditis. From the 120 patients included in the study, 27,5% had high levels of NT-pro BNP and 10% c Tn I (6).

In SARS-Cov-2 infection the virus binds on ACE2 receptors located in the myocardium and the endothelium, sometimes developing an overwhelming immune reaction that can lead to cytokine storm, with powerful damage of the endothelium that leads to multiorgan failure and death (31).

The risk factors and incidence of myocarditis in COVID-19 are still unclear, patients receiving in these cases anti-inflammatory therapy alongside with high dose parenteral glucocorticoid or even immunoglobulins, but standard care therapy may reach its limits, therefore mechanical circulatory support therapy may be neces-

sary for these patients. Adding venous-arterial ECMO (V-A ECMO) to standard therapy may cause a positive impact on patient's survival.

The first case of COVID-19 complicated by fulminant myocarditis reported in China was a male patient with high levels of troponin I, and NT pro BNP, with diffuse myocardial dyskinesia and a low left ventricular ejection fraction, successfully recovered after 3 days on ECMO (32).

Also, myocardial injury may be present even in convalescent patients with no previous cardiac symptoms or conditions (33).

In case of cardiogenic shock, if there is a suspected or positive COVID-19 patient, the European Society of Cardiology recommends prioritizing the health- care workers' protection, being also more restrictive with mechanical circulatory support; however, if needed, the use of ECMO should be considered. In case of negative COVID- 19 patients they will be treated as usual.

COVID-19 and pulmonary embolism

Even if pulmonary embolism is an uncharacteristic evolution of COVID-19, more patients present at the time of admission with both pathologies, coincidental or not (34).

The signs that must alert the medical team of an acute onset of a pulmonary embolism can be a sudden respiratory worsening, the occurrence of a new tachycardia , hypotension, hypovolemia or sepsis and ECG changes suggestive for pulmonary embolism or deep vein thrombosis signs revealed on clinical examination.

In a systematic review of 42 studies enrolling 8271 patients, deep vein thrombosis was found in 20% of the COVID-19 patients, meanwhile pulmonary embolism occurred in 13% of the cases. The study concluded with a pooled mortality rate of 23% in patients with thromboembolism versus 13% in patients without thromboembolism, showing an increased risk of death in COVID-19 associated with this complication (35).

The incidence of pulmonary embolism seems to be higher that it was thought and is revealed also in a study that analyzed 3011 patients, being the most common condition, with 198 cases (6.6%), followed by 55 patients with heart failure, 15 acute coronary syndromes, 14 ventricular arrhythmia, 4 endocarditis and 3 myocarditis. Even though the study showed a low incidence of cardiac complications during hospitalization (36), the high rates of pulmonary embolism must alert the medical team in a fast diagnosis and treatment in these patients. Another evidence showed the magnitude of venous thromboembolic embolism in COVID patients, translated in terms of survival, the incidence of severe condition being more likely to occur, especially in those with lower levels of lymphocytes (37).

But since other studies reported similar in- hospital mortality in critically ill COVID-19 patients with or without the presence of pulmonary embolism (38), further prospective randomized studies are needed.

The most common measures that must be taken in embolism prevention is to consider anticoagulation at standard prophylactic doses in all COVID- 19 patients. Also, due to several possible interactions between non-vitamin K antagonist oral anticoagulants (NOACs) and antiviral drugs such as Lopinavir or Ritonavir, the use of NOACs must be avoided, preferring the use of heparins instead. The risk stratification is accordingly to the current ESC guidelines.

Ongoing randomized clinical trials still represent the most powerful tool to provide high quality clinical care and offer best opportunities to manage the pandemic crisis and they started offering us the hope that we needed.

CONCLUSIONS

COVID-19 still represents a difficult enemy to deal with, but in the past weeks we started to see the light over horizon by developing vaccines and by continuously analyzing best specific and supportive treatments for the SARS-CoV-2 infection.

An important lesson learnt from the COVID-19 pandemic is that there must be a conjugated effort from the Heart Team, that now includes the infection disease doctor and the epidemiologist, in eradicating this virus and assuring the patient survival.

Only a persuasive collaboration between the entire medical team, patients, and health public policies alongside with every single social and economic aspect that is to be improved, may shape the victory against any health menace that may endanger humanity.

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EDITORIAL

THE IMPACT OF COVID-19 PATHOLOGY ON THE SENIOR POPULATION

The outbreak of coronavirus in December 2019 has completely reshaped urban life due to the need to limit the spread of the disease and provide essential services to the city's most vulnerable residents, such as senior patients. Current epidemiology suggests that approximately 80% of patients with COVID-19 have mild symptoms, 45% have severe symptoms that require hospitalization, while 5% become critically ill, requiring mechanical ventilation. Differences in reactivity are due to the degree of viral load, the host's immune response and, more importantly, the patient's age in the presence of generic co morbidities (such as hypertension, diabetes, and thrombotic events), and geriatric syndromes (such as frailty, malnutrition, risk of falling, cognitive impairment) (1).

Older adults rank in the most at-risk segment of the population because the basal functional resilience is fading (2), and because it is physiologically associated with frailty, cognitive decline (3), and progressive alterations of motor performance (4). All these alterations intimately coexist in the frailty syndrome (5), which is known to be associated with disability, traumatic falls, and hospital admission (6). On top of that the wearisome settings of hospital wards provide poor incitements to the older patients and often aggravate nutrition-related or activity-related sarcopenia (6, 7). Multidisciplinary interventions, such as Age-Friendly Hospitals or the HEPAS approach (Healthy Eating, Physical Activity, and Sleep), are models for dealing with elders' multiple issues simultaneously. Despite this knowledge, contemporary society, and health services put the older adults in the background (8).

As expected, it became immediately evident that the majority of deaths from COVID-19 occurs in the older patients, especially in those suffering from two or more comorbidities, cardiovascular diseases, diabetes mellitus, chronic obstructive pulmonary disease, malignancy, and chronic kidney disease being the most frequent (9). Additional factors worsening the COVID-19 emergency within geriatric population may include intrinsic multidimensional features such as disabilities, cognitive and mood disorders, polypharmacy, social isolation, nutritional deficits, and extrinsic factors, such as widespread ageism, the generalized lack of geriatric culture and alleged errors in the management of long-term care facilities (10).

It is well documented that advanced age is frequently associated with impaired nutritional status and a recent meta-analysis indicates the prevalence of high malnutrition risk in Europe to be 28%, 17.5% and 8.5%, for hospital, residential care, and community settings, respectively (11). There are many causes that concur to malnutrition in older age, such as malabsorption, increased nutrient losses, augmented metabolic demands, and reduced dietary intake. Malnutrition is a major geriatric syndrome and an important cause for muscle wasting and weight loss, which is strongly related to frailty, sarcopenia, and negative outcomes (12, 13, 14).

Nutritional status influences the im-

mune system of senior population through impairment in cell mediated immunity, cytokine production and phagocyte functions. In addition, aging *per se* is characterized by a process of progressive dysfunction of several compartments of the immune system, named immunosenescence, which includes smoldering inflammation and immunodeficiency (10). The complex relationship between immunity and nutrition in older population is clinically relevant and negatively impacts antibody production, response to vaccination and susceptibility to infections (15, 16, 17). Notably, the altered response to vaccination should be seriously considered and evaluated by randomized control trials now, that an efficient vaccine against SARS-CoV-2 is available.

It is important to emphasize that both arms of the immune system are altered in elderly: decreased B-lymphocytes production (exposing to severe infections) and T-lymphocytes activity, decreased cell-mediated and humoral-mediated immune response (16, 17). Due to this age-dependent alterations of the immune system, a relevant percentage of older patients progresses to insidious systemic inflammation, mainly affecting the lung, heart, renal function, and coagulation system. This second stage of hyper inflammation is characterized by massive production of pleiotropic cytokines (e.g., IL-6) by lung resident (e.g., macrophages) and circulating immune cells (18).

In a retrospective, single-center study, performed in the Renmin Hospital of Wuhan University in Wuhan, China (19), that included 339 cases of COVID-19 over 60 years old, the data reported that lymphocytopenia was found in 211 cases (63.2%), and the counts of $CD4^+$ and $CD8^+$ cells were all decreased compared with normal values. Very old elderly (aged 80 years or above) was shown to increase the likelihood of death compared to young old patients (60-70 years old). Symptoms of dyspnea and the presence of co morbidities including cardiovascular disease, cerebrovascular disease and COPD were all predictive of fatal outcomes. SARS-CoV-2 caused a much more severe pneumonia in the aged people than younger patients. Of the 339 elderly COVID-19 patients included, over 70% were severe or critical, and the case fatality rate was 19% (19).

Cough and fever were the most commonly seen symptoms in patients with COVID-19 and were mortality risk factors (20). While cough was equally observed in all age-groups, fever was more likely to be absent in older patients. This phenomenon is not uncommon and is, perhaps overlooked. A recent study reviewing the clinical features of COVID-19 patients admitted to Hainan General Hospital found significant difference in the prevalence of fever between young, middle-aged, and elderly patients (1). It is likely because of the lower baseline body temperature in elderly due to aging (21, 22, 23) which may translate to a lower maximum temperature of fever (24). This suggests that the usual temperature threshold for fever should not be used as a case definition of COVID-19 in geriatric patients. In fact, a study on a total of 111 nursing home residents found that lowering the threshold for a clinically significant fever increased the sensitivity of detecting infections (22). However, it is not recommended to lower the temperature threshold in the case definition of COVID-19 in geriatric patients because an individual's baseline body temperature is also determined by a range of demographic

factors, comorbid conditions, and physiological measurements (21). To assist surveillance against the current pandemic, older patients should measure on a regular basis the baseline body temperature that can be used to define the threshold temperature for fever. If this is not feasible, a baseline body temperature of 36.5 degrees Celsius might be used instead (23).

There is compelling evidence that several nutrients, including vitamins (A, B group, C, D, E) and minerals (zinc, iron, selenium, magnesium, and copper), exert a key role in supporting the immune systems. Micronutrients contribute to the development and integrity of physical barriers, participate to neutrophil/macrophage maturation and functions, and play a role in modulating inflammation. Additionally, adaptive immunity is influenced by nutritional deficits, especially lymphocyte maturation and differentiation, memory cell generation, and cytokine and antibody production. Nutritional support has been shown to improve immune responses in older malnourished patients (10, 18, 25).

Zinc deficit is common in malnourished elderly individuals and it is related to increased risk of infections. Accordingly, adequate zinc support counteracts the alteration in the immune response through the enhancement of both total number and function of T cells (26, 27). Moreover, zinc supplementation has been reported to increase natural killer cell activity (28).

Vitamin A is a key factor for the development of the immune system, for epithelial and mucosal barrier generation, and contributes to support the efficacy of T cell-based viral vaccines (29). Vitamin E stimulates T cell function and confers protection against upper respiratory tract infection (30). Recent data supports that probiotics have a role in modulating the immune system, with probiotic supplementation recognized as an effective nutritional intervention (31).

Vitamin D modulates several aspects of the innate and adaptive immune system and its deficiency is highly prevalent in the elderly worldwide. Vitamin D supplementation appears to reduce the risk of infections through pathways that can reduce both viral replication and the production of pro-inflammatory cytokines. These are the reasons why it has been proposed to administer high doses of vitamin D in COVID-19 patients (10, 32, 33). A recent study that was performed in a nursing-home dedicated to old residents with physical disabilities and major neurocognitive and psychiatric disorders evaluated the effects of an oral bolus of 80,000 IU vitamin D3 administered either in the week following the suspicion or diagnosis of COVID-19, or during the previous month. The main finding was that bolus vitamin D3 supplementation during or just before COVID-19 was associated with less severe COVID-19 and better survival rate in frail elderly (34). A recent trial has assessed the effects of vitamin D supplementation on influenza vaccine response in elderly vitamin D-deficient subjects (35). Surprisingly, no effect has been detected on antibody production, thus concurring to underline the complex metabolic-immunological substrate of the geriatric population.

Vitamin C influences immunity through regulation of innate B and T cell proliferation and differentiation, their migration to sites of infection, and promoting antimicrobial activity and antibody production (10). It is well known that vitamin C tends to be depleted during infections, particularly in respiratory infections, and that is why

trials started to investigate the efficacy of intravenous vitamin C in COVID-19 patients. The data showed that administration of 15 g/day of IV vitamin C for 4 days may decrease mortality in older patients with complications such as sepsis-related ARDS (36). This new line of investigation is supported by a previous observation indicating a low severity of pneumonia in senior patients supplemented with vitamin C, especially in those with smaller initial concentrations (37).

Malnutrition, frailty, and sarcopenia are key factors in the increased risk of falls and fractures in older population. The frail seniors face a risk of 1.2 to 2.8 times for falls and fractures and a risk of 1.2 to 1.8 times for hospitalization. During the pandemic, surgery on subjects who suffered trauma from a fall was a matter of debate. In the post-COVID-19 era, the saturation of health services can only be the tip of the iceberg in relation to the dynamics of fragility, the COVID-19 pandemic causing a cycle of decline in functional capacity, with a higher risk of traumatic falls and hospitalizations for fractures on frailty in the near future (38, 39).

As with other diseases, COVID-19 shows atypical or peculiar manifestations in elderly patients. In the event of a new COVID-19 outbreak, the occurrence of sudden behavioral changes and subsequent *delirium* deserve special attention, as it may be considered a prodromal phase of the disease, especially in the presence of lymphopenia; widespread inflammation or hypoxia may appear later (40). It is possible that SARS-CoV-2 might have early and particularly harmful effects on the brain functions of older patients with dementia, manifesting as delirium which might be frequent in the prodromal phase of the infection when other typical symptoms have not yet emerged. As also suggested by other authors, (41, 42) we believe that a symptom-based strategy centered on the identification of typical symptoms is not an effective approach for preventing the spread of the virus in this specific population and should therefore be revised to include delirium and other atypical symptoms.

It has also been shown that older patients with Parkinson's disease and a history of lifelong psychiatric symptoms may be exposed to increased vulnerability to the stressful effect of the COVID-19 outbreak. Psychiatric characteristics are *per se* multimorbid, characterized by great intra- and interindividual variability in clinical presentation and can be greatly influenced by stressful events (43). The COVID-19 pandemic is an important stressor associated with exacerbation of psychiatric symptoms. Recent data have shown higher levels of psychological distress among the general population and an increased risk of recurrence and worsening in patients with neuropsychiatric disorders during the outbreak. As a result, recent preliminary reports have shown higher levels of stress, depression, and anxiety in patients with Parkinson's disease. The endangerment of the quality of life during social restrictions has also been confirmed (44, 45).

Depression turned out to be the most common psychiatric symptom reported by patients with clinical aggravation. Current findings suggest that interventions aimed at reducing mood irritability and instability, such as the use of mood stabilizers, could have an indirect effect on the health and well-being of older patients with psychiatric diseases during the pandemic (42, 44, 46, 47).

CONCLUSIONS

Aging comes with a multitude of psychological, social, and environmental vulnerabilities. Frailty in older adults carries the risk of various infections and decreases all forms of immune response, which makes the progression of the disease and the risk of death three times higher than in other age groups. In addition, seniors have multiple co morbidities and increased number of hospitalizations, which increases the chance of contracting the infection during a pandemic. They have cognitive and sensory deficits that make it difficult for them to understand and follow precautions. Many of them are institutionalized, being exposed to the risk of overcrowding, poor hygiene, and lack of adequate supervision. Pandemics have a significant psychosocial impact over them: anxiety, panic, adjustment disorders, depression, chronic stress, and insomnia are the main branches. Misinformation and uncertainty give rise to the massive alert.

In these contexts, the elderly are particularly vulnerable. Social distancing, although a major strategy for combating COVID-19 is also a major cause of isolation, especially in settings such as hospitals or nursing homes, as an independent risk factor for depression, anxiety disorders, and suicide.

While identifying methods to treat and prevent COVID-19 infection is an urgent priority, it is also important to consider that the current pandemic is the third major outbreak of a new coronaviral infection in humans in the last 20 years. However, the complex protection of the vulnerable senior population is still sorely missing, and strategies to address this issue should be a first and new intention.

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EDITORIAL

CONTEMPORARY WITH THE PARADIGMS OF A PANDEMIC

The COVID-19 pandemic marked our existence, and for over a year we faced various experiences, some of them dramatic.

Over time, humanity has gone through such moments that have resulted in suffering, loss of lives, economic crises, and social change. Epidemiological theories predict that at an interval of 100 years pandemic events may evolve as a consequence of the phenomena of adaptation of microorganisms and the crossing of barriers between species, some of which cause devastating diseases in the human population. By prospecting the history of these events, in the last hundred years there have been several crisis events with a tendency towards the evolution of new pandemics or epidemic phenomena with pandemic risk. The Spanish flu pandemic in 1918 was followed by the Asian flu pandemic (1957), the seventh cholera pandemic (1968-1975), the Hong Kong flu pandemic (1968), the HIV/AIDS pandemic (since 1981), a new type of influenza A (H1N1) pandemic (2009), but also some epidemic events that had all the chances to evolve towards pandemic manifestations such as: the epidemic of acute bovine spongiform encephalitis with a tendency to spread to humans (1996), localized avian influenza A (H5N1) epidemics (1997), Severe Acute Respiratory Syndrome - SARS (2002/2003), Middle East Respiratory Syndrome - MERS (2021), West African Ebola epidemic (2013/2014), Zika virus infection (2015). All these culminated in 2019 with the entry into circulation of a new coronavirus, SARS-CoV-2, different in varying proportions from SARS (genetic similarity of 80%) or MERS (genetic similarity of 50%) (1, 2). These global alerts have surprised the often-unprepared population and an unfortunate experience was quickly forgotten for various reasons but especially by the fact that the 21st century has installed itself in an effervescence of changes through an alert pace of technology, population movement and information explosion.

Since the beginning of this COVID-19 pandemic, we have faced several paradigms. Starting from the definition of "paradigm" (a pattern or model, a system of rules and regulations, a set of specifications or criteria for setting boundaries), from December 2019 until now, we have experienced many paradigm shifts exemplified by changing to a set of rules to other rules, what was acceptable in the past will no longer be tolerated in the future, what was successful in the past will not be successful in the future.

The new EPIDEMIOLOGICAL PARADIGMS brought into discussion several aspects, some of them still remaining in the field of controversy. SARS-CoV-2 infection has been considered a less severe respiratory disease than influenza… but the mode of spread and the number of cases or deaths contradict this first presumption from the beginning of the phenomena (3). Transmission is direct and occurs through "droplet nuclei" with dimensions of < 5μm… but transmission through respiratory secretions is made directly during coughing, sneezing, speaking or singing but also indirectly through air currents that cause septic secretions, surfaces or contaminated objects because the persistence of the virus can last for hours and days (4, 5, 6). It was

considered that asymptomatic patients do not transmit the… virus, but the initial WHO statement was disputed by subsequent scientific evidence and epidemiological arguments. The increased rate of asymptomatic and presymptomatic (up to 40% of the infected population) contagious over a period of at least 14 days should be considered in establishing infection detection and control strategies (7, 8).

The virus reduces its circulation during the season by the fact that temperature and humidity can influence the viability of the virus through their effect on the properties of surface viral proteins and lipid membrane… but research using mathematical modeling methods, which introduced climate factors variables, have failed to establish with certainty that there is a direct link between the spread of the virus and high temperatures. The proof of the lack of braking effects of the high temperatures of the summer season for the circulation of the virus is direct and concrete by registering an increasing evolution of the number of cases in the months with high temperatures or in the territories with warm climate (9).

It was initially considered that only the elderly or adults with comorbidities are affected… but although these categories of people have a higher risk of developing severe forms of illness and death (the fatality rate is 10-27% in those over 85 years and 3-11% respectively at 65-84 years, compared to 1-3% for 55-64 years), severe forms were later reported in people without comorbidities, from young age groups, and regulations on the clinical management of cases emphasized the need to take into account the possible unfavorable development in young people and even children (10, 11). Thus, a paradigm shift has been established that any person, regardless of age or preexisting morbidity, should be considered at a potentially increased risk of developing a moderate to severe form of the disease.

Another paradigm shift started from the premise that natural collective immunization can be seen as a possible strategy to accelerate the limitation of the spread of the virus… but the early relaxation policy applied in Sweden and the United Kingdom through which schools and most jobs continued their activities, on the principle that the rapid natural immunization and as many people as possible will ensure a halt to the phenomenon, did not work (12). Approximately 6 months after the onset of the pandemic, low levels of seropositivity in the general population have been reported in most EU / EEA Member States. A study conducted in a region of Austria, which was severely affected, showed a seroprevalence of antibodies of 42% among its residents (1,500 people evaluated in week number 17) and in Spain, in a cohort of 60 983 people in the general population, the presence of IgM and IgG antibodies was identified in only 5% of those investigated (week 18-19) (13). By using models to assess the prognosis of the evolution of the pandemic, it was considered unlikely that the level of population immunity will reach, by winter 2020-2021, sufficient levels to achieve extensive protection (14), which time has shown. October - December 2020 the third pandemic wave brought back to a state of crisis and panic through the large number of cases but especially serious forms and deaths.

The new TECHNOLOGICAL PARADIGM has changed a lot the educational framework, the health systems and the business or economic environment. The education sector already used digital platforms at the beginning of the pandemic, which could make it more dynamic in the implementation of distance or hybrid edu-

cation. Telemedicine platforms are not new, but the COVID-19 crisis has shown the need to digitize this sector. E-Health and M-Health platforms (telemedicine through monitoring devices, mobile phones) with very different objectives proved to be necessary. These platforms can range from patient care activities, diagnostic or care medical management, etc. making possible several facilities such as: saving time, avoiding unnecessary transfers, optimizing resources and reducing costs. The limits of social contact, especially during lockdowns, have forced the implementation of operational and economic solutions through remote technologies in various sectors of life (15).

The new PARADIGM OF THE HEALTH SYSTEM has changed the concept of curative policies in infectious pathology based on the *"Pasteurian paradigm"* which states that every disease is due to a pathogen; for each disease there is a sure treatment that targets the cause responsible for its occurrence. The COVID-19 pandemic has triggered unprecedented measures worldwide, which have often been taken in an "emergency" manner. Alternatively, COVID-19 should be assessed as part of a global health problem that requires a "systemic approach" that allows interaction with other known and preventable health conditions that currently receive a disproportionately low level of attention. The shift from a specific reactionary paradigm to a "systemic paradigm", holistic, coordinated and preventive, has become a necessity in the current context. The globalized world is currently facing a "syndemic" - which means a synergy of epidemics that occur in different times and places, interact with each other to produce complex sequelae and have in common underlying social factors (16).

The examples are numerous, and the attention directed only to the virus has blurred the interest for capturing some essential changes in the personal life of each one as well as that of the community, in the demographic, political and social evolution of the whole humanity. The pandemic will be gradually brought under control but the question that needs to be concerned is whether these paradigm shifts in the COVID-19 pandemic have made us more competitive or better prepared for a possible new challenge.

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NEWS

SCREENING OF MULTIPLE BIOMARKERS ASSOCIATED WITH ISCHEMIC STROKE IN ATRIAL FIBRILLATION

Atrial fibrillation (AF) is a common arrhythmia and constitutes a major health problem worldwide mainly because of its associated increased risk of stroke. In individuals with AF, the risk of ischemic stroke is substantially reduced by oral anticoagulation; however, residual risk remains. Several clinical characteristics are associated with an increased risk of ischemic stroke in patients with AF regardless of oral anticoagulation, most importantly older age, prior stroke, and cardiovascular comorbidity. In recent years, protein biomarkers, such as cardiac troponin, reflecting myocardial damage, and NT-proBNP (N-terminal pro-B-type natriuretic peptide), reflecting cardiac stress and dysfunction, have been shown to be more important for prediction of ischemic stroke than all clinical information, except prior stroke, in patients with AF. Biomarkers reflecting pathways of inflammation, renal function, coagulation, and platelet activity have also been investigated, although without consistent evidence of association with risk. To better understand the remaining risk of ischemic stroke in anticoagulated patients with AF, there is a need to further explore additional mechanisms. To explore the pathophysiological features of ischemic stroke in patients with atrial fibrillation (AF), the authors evaluated the association between 268 plasma proteins and subsequent ischemic stroke. The authors explored this new biomarker screening approach to identify plasma biomarkers associated with subsequent risk of ischemic stroke or systemic embolism (SE) and to improve the mechanistic understanding of ischemic stroke risk in patients with AF on oral anticoagulation (Hijazi Z, Wallentin L, Lindback J, *et al.* Screening of Multiple Biomarkers Associated With Ischemic Stroke in Atrial Fibrillation. *J Am Heart Assoc* 2020; 9: e018984).

EDITORIAL

THE UNFINISHED WAR - MICROORGANISMS AGAINST HUMANS SARS-CoV-2 - THE ENEMY FROM NOWHERE

Throughout history, humanity was confronted with many epidemics which were generated by different types of microorganisms: parasites, bacteria or viruses. However, in the last two centuries the fight against these invisible enemies has advanced through the implementation of antiseptic measures, vaccines or different medicines (especially antibiotics). In the last hundred years only, a few epidemics were classified as pandemics: the Spanish flu - 1918-1920 (minimum 50 million deaths), the Asian flu - 1957-1959 (2 million deaths), the Hong Kong flu - 1968-1969 (2 million deaths), and HIV - in the last four decades (35 million deaths) (1).

At the end of 2019 a few cases of a particular form of pneumonia with an unknown origin were discovered in Wuhan city in China. The presumption of a viral origin in these cases was confirmed by the identification of a new form of a coronavirus, named SARS-CoV-2. This virus presents a high virulence and is characterized by a rapid spread from those infected to the healthy population. For these reasons, at the beginning of 2020 World Health Organization (WHO) declared SARS-CoV-2 infection a public health emergency and three months later this epidemic was classed as a pandemic. In the last two years, the cases of COVID-19 spread around the world, approximately 219 million people became infected, and 4.54 million individuals died (2).

The big question is why the epidemic spreads so quickly and why the infection is so virulent? The answers are difficult to find, but certainly the particularities of the virus are intricate with some features of the host.

Coronavirus represents a class of viruses found in animals and humans, but its implication in human pathology has been limited, the first report of a disease caused by a coronavirus was in 1965 and only seven Coronavirus strains were associated with human pathology. Three of these viruses determined epidemics: SARS, MERS and COVID-19. In all three cases the viral reservoir is represented by different types of bats and the transmission from bat to human implied intermediate hosts: palm civet (SARS) dromedary camels (MERS) and minks and pangolins (COVID-19) (3-6).

Coronavirus belongs to the *Coronaviridae* family of the *Nidovirales* order, *Orthocoronavirinae* subfamily, *Sarbecovirus* subgenus and genus beta *Coronavirus*. The microscopic aspect of this virus is a spiked sphere, a shape that allows attachment to host cells. The genome of Coronavirus is an RNA type, and its' multiplication requests the use of translation machinery of the host. By this way, the host cells allow the multiplication of Coronavirus and production of many viral entities able to infect other cells, but also to spread to other people. The genome of COVID-19 is big for a viral genome and contains around 30 kilobases. Some genes of this genome codify structural proteins (essential for formation of virus - spike [S], envelope [E], membrane [M] and nucleocapsid [N]) while others - open reading frames of the

replicase complex (orf1ab) - codify different proteins and enzymes necessary for the multiplication of the viral genome or intermediate interactions virus-host, by modulation of production of pro-inflammatory cytokines. The dimension of the viral genome offers a large base for the accumulation of new mutations, and also its recombination is very important (6-8).

The pathogenic mechanism of SARS-CoV-2 is complex and implies a linked connexion between virus and host. Fixation of the virus by spike protein at host cell is allowed by the membrane receptor for ACE2 (angiotensin converting enzyme 2) but this process depends on the presence of serine protease TMPRSS which acts as primer for viral protein S. Since some human tissues like the lungs, kidneys, liver, intestines, heart and T lymphocytes have a high number of ACE2 receptors on cell membrane, these tissues represent the most important way for SARS-CoV-2 to enter (7, 8).

After the release of the viral genome in the host cell cytoplasm, translation and replication begins. Firstly, the viral non-structural proteins are synthetized by translation. These proteins are involved in a discontinued transcription of viral RNA, by which new RNA strands are produced, and also is produced the synthesis of structural proteins: S, E, M and N. The nucleocapsid incorporates viral RNA and is joined together with S, E and M into the membrane of the endoplasmic reticulum. After the complete viral structure is formed, Coronavirus passes into the Golgi vesicle. At the end of the process, the mature virus is released into the extracellular environment by exocytosis (1).

Another challenge in the fight against SARS-CoV-2 is the polymorphic symptomatology that ranges from cases with minimally ill patients to complicated cases that require intensive care and respiratory ventilation (9, 10). The frequency of asymptomatic infections is yet to be determined and the differentiation between those and pre-symptomatic cases should be considered (9, 11). In contrast with other infections with Coronavirus when fatality rate was high, infection with SARS-CoV-2 is characterized by an overall fatality rate of 2.3%. Despite this low rate of fatality some groups of patients present a very high rate of fatality: 14.8% in older patients (aged over 80) or 49-61% for individuals that required ventilator support in intensive care unit (9). The most important risk factors are old age, arterial hypertension, diabetes mellitus, obesity, cardiovascular, pulmonary, and cerebrovascular pre-existing diseases (12). The onset of COVID-19 is insidious, with non-specific signs and symptoms of upper airway viral infection (fever, fatigue, cough, and dyspnea, anorexia, myalgia, and productive sputum), which evolves to a particular form of pneumonia, sometimes complicated with acute respiratory distress syndrome that requests mechanical ventilation or extracorporeal membrane oxygenation. In other cases, a gastrointestinal symptomatology was described, with nausea, vomiting, and diarrhea (9, 13, 14). This particular pattern of symptomatology is probably correlated with the presence of a high number of ACE2 receptor in the epithelium of respiratory and gastrointestinal tracts.

Many patients with COVID-19 present a neurological symptomatology that ranges from anosmia and dysgeusia in less severe cases, to meningitis, encephalitis, myelitis, and Guillain-Barre syndrome as components of severe COVID-19. Even, respiratory failure could present a neurogenic part (by viral invasion of the respiratory cen-

ters) added to local modifications of the lungs (15). Another particularity is a tendency for coagulopathy, thrombosis, microthrombosis, and even a disseminated intravascular coagulation during severe COVID-19 (16). This coagulopathy could be accompanied by septic shock and metabolic acidosis. Coagulation issues are associated with an excessive inflammation and a cytokine storm triggered by the presence of the virus in the body and an inhibition of the adaptive immune response by reducing the IFN-γ secretion. All these allow a high rate of fatality, especially in elderly patients and those with underlying disorders (9, 17, 18).

Pediatric symptomatology of COVID-19 is usually extremely low, but some children have presented a severe clinical picture with auto-inflammation and vasculitis, similar to Kawasaki disease. The low severity of disease in childhood could be linked to innate immunity (intermediate by IFN-γ secretion) that slows down the viral infection before the adaptive immune response (9, 18, 19).

The differences in the hosts' response to the aggression of SARS-CoV-2 infection derive from genetic polymorphisms of the human beings. Such variations concern different genes, but the most important are genes involved in the immune defense system (MHC/HLA or CCR5) and those that allow the virus to enter the human cells (gene for *ACE2* or genes for *TMPRSS2* and lysosomal cathepsins). For example, the expression of ACE 2 proteins in human cells is higher in Asian males than females (especially in lungs) and this is correlated with a high degree of severity of COVID-19 (20). On the other hand, the expressions of *ACE 2* gene and also of *TMPRSS2* gene in nasal and lung epithelial cells are low in children and this explained partially the reduced severity of COVID-19 disease in this population group (8). The polymorphism p.Arg514-Gly of the *ACE2* gene is associated with severe cardiovascular and pulmonary complications in the African or African American population with COVID-19 disease (21). Individuals with blood type A are more vulnerable to SARS-CoV-2 compared to those with type blood O (22). The HLA polymorphisms are also important in the COVID-19 disease: HLA B*46:01 confers vulnerability to disease while HLA B*15:03 generates protection (23). Another genetic polymorphism is located 3q21.31, when there is a cluster of six genes (*SLC6A20*, *LZTFL1*, *CCR9*, *FYCO1*, *CXCR6*, and *XCR1*), derived from the Neanderthal genome. The polymorphism rs11385942-GA of this region is associated with severe disease and respiratory failure and its frequency is high in modern populations that conserve part of the Neanderthal genome (especially the Asian population) (24). Other genetic polymorphisms were described in the *ApoE*, *IFITM3*, *TLR7* and *TMEM189- UBE2V1* genes (25).

On the other hand, females, especially during their reproductive years, are more resistant to infections than men, a feature mediated mainly by estrogen. However, the differences between men and women could be explained by some environmental factors, like higher levels of smoking and drinking among men compared to women or the more responsible attitude of women toward the COVID-19 pandemic than men (20).

The confirmation of the SARS-CoV-2 presence in patients with COVID-19 imposes the use of different molecular methods like rapid diagnostic tests, real time - polymerase chain reaction (RT-PCR) multiplexes respiratory virus infection assays,

clustered, regularly interspaced short palindromic repeats (CRISPR), and metagenomics next gene sequencing (mNGS). By using such techniques, a final result is obtained in maximum one day. The RT-PCR test has become the standard method for diagnosis of this infection, and it targeted different sequences of the COVID-19 genome (orf1ab, S, E, M and N genes). However, RT-PCR has many limitations, the most important being the false-negative rates linked to high genetic diversity of viral genome. In cases with negative results of RT-PCR tests, it is useful to apply shotgun mNGS including short-read and long-read sequencing. Also, CRISPR or electron microscope technology could offer an early diagnosis, but its routine use is not yet applicable (26, 27).

The onset of epidemics with COVID-19 finds humanity without any therapy or vaccine against SARS-CoV-2. In these conditions, a variety of approaches was triggered in order to design and develop a novel drug and/or vaccine. Searches of a useful treatment were directed to the application of existing broad-spectrum antiviral drugs, screening of libraries that contain existing chemical compounds and the redevelopment of new specific drugs based on the genome of coronaviruses. The most promising therapeutic approaches were viral receptor blockers, monoclonal antibodies or gene silencing. Antibodies against the receptor-binding domain of the spike protein or antibodies that bind to the ACE2 receptor represent potential therapies. Monoclonal antibodies against the spike protein allow the block of virus attachment, fusion, and entry to the host cells. An immunotherapy that uses plasma from convalescent COVID-19 patients has also been used (8, 28, 29).

In addition, different strategies were applied to develop a vaccine: epitope-based peptide vaccines, viral vector-based vaccines and use of bioinformatics and immunoinformatics to design more efficient targets. More than 300 vaccines for COVID-19 are being developed, and many of these have already passed the clinical trial phase. The most revolutionary approach was the development of "genetic" vaccines, based on genetic engineering that use some part of SARS-CoV-2 genome (especially S gene) or allow a synthetic defective mRNA from SARS-CoV-2 to trigger immune response. Such mRNA vaccines have a low risk of integrating into the host's genome and thus present a high advantage reported to other type of vaccines. Worldwide, the development of vaccines for COVID-19 was very quick, and this particularity can be correlated with the existence of previous knowledge about the pathogenic mechanism of Coronavirus (obtained from studies conducted during previous epidemics with SARS and MERS) and with record-breaking speed which the SARS-CoV-2 genome was sequenced with (25, 28-30).

The last two years have brought many successes in the battle against SARS-CoV-2, but many questions remain unanswered. Some features like the differences of severity in different population groups (children vs. elderly; men vs. women), the wide range of severity from asymptomatic people to the seriously ill in individuals without associated conditions, the ethnic differences of severity, the presence of individuals with a long period of RT-PCR positive values, people who develop a tiny immunity after disease, the differences in response to therapy or vaccine and also the differences in recovery after COVID-19, have only partial explanations. Some answers were provided by studies of genetic polymorphisms, but

deciphering the full enigma requires further research on the viral and human genomes and interactions between these two genomes. In addition, past mistakes should not be repeated, such as stopping research on Coronavirus in SARS and MERS epidemics, which prevented the development of high-performance vaccines. We will probably have to live with COVID-19, and the best protection option will be the regular application of active vaccine variants against the new virus strains.

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EDITORIAL

THE MEDICAL-SURGICAL JOURNAL OF THE SOCIETY OF PHYSICIANS AND NATURALISTS OF IASI ON THE ANNIVERSARY OF 125 YEARS OF PUBLICATION AND 135 YEARS SINCE ITS FOUNDATION

The Society of Physicians and Naturalists of Iași and its first periodicals

The Society of Physicians and Naturalists of Iași. Established on January 11, 1830, as "The Iasi Circle of Medical Reading" (*Jassyer medizinische Leseverein*), the Society of Physicians and Naturalists of Iasi (SPN) was due to the initiative of Dr. Iacob Cihac and Dr. Mihail Zotta, supported by both by the Doctoral Council (Doctoral Commission) structured in 1829 in the capital of Moldavia, as well as by representatives of the political parties and progressive intellectuals. It was soon transformed into an academic society. Indeed, in the spirit of the Organic Regulations (Organic Laws), on March 18, 1833, the Government of Moldavia approves the creation of the "Society of Physicians and Naturalists of the Principality of Moldavia" (SPN). It was the first scientific association in the Romanian Lands and in South-East Europe. Besides the specific goals related to the development of medicine and pharmacy, health care and health education of the population, SPN promoted national emancipation through culture, political union of the Romanian Countries and cooperation between the Romanians from everywhere, but it also encouraged the use of the Romanian language, which was adopted by all the publications of this association. From the very beginning, the Society had a well-defined European personality and its work largely consolidated European scientific spirit in Moldavia and Romania.

1844-1845. The Advisor of Health and Economy. In this context, under Dr. Costache Vârnav's editorship, the *"The Advisor of Health and Economy. Periodical Leaflet for the Romanian People" (Povățuitorul sănătății și a economiei. Foaie periodică pentru poporul românesc)* was printed in Iași on March 1, 1844. It was the first medical popularization journal in Romanian, a real landmark in this field as Dr. Victor Gomoiu specified. Published between 1844-1845 under the SPN auspices, "The Advisor" dealt with the state of rural hygiene and economy in this corner of the world. The motto of the "Periodical Leaflet for the Romanian People" was: "To keep one's health and to spare one's wealth is the effort of a wise man." Due to his tenacity to impose this medical-sanitary, economic and veterinary periodical, Dr. Vârnav contributed to the formation, improvement and dissemination of Romanian medical terminology, to the popularization of general data on human and veterinary domestic medicine and the spread of the home economy in the Principality. In 1848, according to some authors, the "Medical-historical-natural and agronomic gazette" was to be printed in the Moldavian capital, but it probably remained a mere and ephemeral attempt, or just a simple intention.

1851-1853. The Leaflet of the Society of Physicians and Naturalists of the Principality of Moldavia. After many "projects and prospectuses", the perseverance to create a high standard scientific journal of the SPN, whose activity to be reflected in detail, materialized by the appearance, between July 1, 1851, and June 1, 1853, of

the weekly "Leaflet of the Society of Physicians and Naturalists of the Principality of Moldovia" (*Foaia Societăţii de Medici şi Naturalişti din Principatul Moldovei*). It was the first periodical in Romanian edited by a national scientific association, a landmark in the history of the Romanian medical press, as Dr. Victor Gomoiu mentioned in his iatro-historical writings (1). With great effort, the edition was coordinated by the same dedicated Dr. Costache Vârnav, in charge of this responsibility by Dr. Gheorghe Cuciureanu (Cuciuran), SPN President in office. Among the constant collaborators of the periodical there was a skilled physician, pharmacist and botanist, Dr. Josif Szabó, able to distinguish, classify and describe the medicinal plants of the Moldavian flora. A note of the Editorial Board in the first issue let readers know that the publication was going to include "articles on natural sciences, and especially folk and domestic medicine, dietetics, hygiene, veterinary medicine, and home economy." In addition, *"The Leaflet"* was equally concerned with agriculture in terms of soil maintenance and promotion of new vegetables, especially maize and potatoes, without overlooking domestic business and trade (1, 2). Details about the SPN activity and members were published as well.

A "serious, competent and useful" periodical of the Society

1887. The Bulletin of the Society of Physicians and Naturalists of Iasi. In 1885 *"The Medical Newspaper of Iaşi"* (*Gazeta Medicală din Iaşi*) was edited in the Moldavian capital of culture and science by Professor Dr. Vasile Bejan, as its director. Always overcoming the vicissitudes of times and the opposition or indifference of the people, on March 21st, 1868, Dr. Gheorghe Iuliano, an experimented dermatologist, and the famous geologist Professor Dr. Grigore Cobălcescu outlined once more the need for a periodical of the Society. On April 24, 1874, the proposal was reformulated by the SPN president, Professor Dr. Anastasie Fătu. Equally commendable was the resumption of work in 1876, at the insistence of the same Prof. Dr. Cobălcescu, supported by Dr. Aristide Peride, professor of anatomy and embryology, and several other members of the Society. A periodical entitled "The SPN Archives", a high-level scientific journal, was intended to be edited. But it was not until November 28, 1886, that "after long debates and incessant obstacles", under the chairmanship of Professor dr. Ioan Ciurea outstanding SPN members made the firm decision to publish a "serious, competent and useful" periodical of the SPN. This resolution was adopted by Dr. Ioan Ciurea, professor of forensic medicine, SPN President, following the proposal reiterated by Prof. dr. Peride, convincingly supported by the Academician Cobălcescu, notable professor of geology, together with Dr. Gustav Otremba, chief doctor of the Army in Iași, Dr. Eugen Rizu, professor of *Materia medica and the Art of formulation*, and with the help of many enlightened SPN members among the "most distinguished professors of the Iași Faculty of Medicine".(3) On January 1, 1887, the first issue of the "Bulletin of the Society of Physicians and Naturalists of Iasi" was printed, under Professor dr. George Bogdan's Presidency of the Society of Physicians and Naturalists of Iași. Dr. Peride, Prof. Dr. Cobălcescu, Dr. Otremba, Dr. Rizu, Dr. Bottez and Dr. Konya formed the first Editorial Board. It was a monthly *Bulletin*.

From 1888 to 1900, the publication appeared under Prof. dr. Bogdan's successful direction (2).

1887-1924: The first period of the Bulletin. Initially, the content of the *Bulletin* encompassed more domains, like e.g., natural sciences, geology and philosophy. Romanian and French became its official languages. Not only forging an enriched Romanian scientific vocabulary was targeted, but integration, communication and exchanges with the international medical and scientific communities were envisaged as well. It was only in January 1887 that the legacy of Costache Vârnav and his generation was dimensioned by the publication of this new journal, "able to determine a beneficial movement". After a short period of calm or "languor" from 1860 to 1885, the Society entered an "era of regeneration" with the publication of its scientific *Bulletin* (2, 3). *The SPN Bulletin* appeared with remarkable regularity and information from it were reported in foreign journals, as SPN President, Dr. Otremba, noted with satisfaction in 1890: "We started with scarce, very modest means, I have no doubt that we will make progress - and we will show our descendants the path they must follow" (4). The first four issues were sent to Dr. Iacob Cihac at Aschaffenburg, and, in a warm letter to the Society of Physicians and Naturalists he had founded in Iasi, he immediately expressed his joy and satisfaction that his dream could finally come true. Cihac specified that he intended to communicate this good news to foreign Societies of Natural History in order the relationships with them to be resumed (2).

From its very beginning, *The SPN Bulletin* was exchanged with many other national and international publications. The Society of Physicians and Naturalists thought it was "far from fulfilling its purpose if the published works could not be known and controlled by the whole scientific world" (4).

This viable SPN periodical could only appear almost a decade after the Faculty of Medicine in Iași was inaugurated in 1879, when a more professional medical stuff got involved in this work, and when both the number of scholars and the scientific and cultural level of the intellectuals in Iași greatly increased due to the existence of the first modern University of the country, established there in 1860. This aspect was already mentioned in the *Foreword* of the first issue of the *Bulletin*: "To the Readers" – this publication and "the proliferation of scientists and scientific societies" occurred also "thanks to the progressive development of the Faculties of Science and of Medicine - which, although recently established and in its infancy, still has a focus on scientific activity".

In 1897, the *Bulletin* was printed only in Romanian, but it continued to be viable, even when exclusively the Romanian language was used, a fact that obviously restricted its international access. Paradoxically, a Society with only 49 "ordinary members" in 1896, managed to support a valuable *Bulletin*, the proof being, among other things, the fact that no less than 92 scientific journals were received in exchange from abroad (2). Among the close collaborators of the *Bulletin* there were famous Romanian scientists of Iași, such as Grigore Cobălcescu, Leon Cosmovici, Samuel Konya, Nicolae Leon, Leon Papp, Emanuil Riegler, Gabriel Socor, Ludovic Russ junior, Vasile Negel, Theodosie Philippescu, Constantin Bottez, Theodor Bastaki, Victor Imervol, Constantin Thiron, or the philosopher Vasile Conta. From Bucharest, e.g., Zaharia Petrescu and Alexandru Șaabner-Tuduri had their research published in the *"Iași SPN Bulletin"*.

With sacrifices and efforts, the Society's periodical was published in its first form, from 1887 to 1912, when it was re-

named *"Bulletins et mémoires de la Société des Médecins et des Naturalistes de Jassy".* The 58th and 75th anniversaries of the SPN foundation in the fall of 1890 and in 1909/1911, respectively, were accompanied by jubilee editions of the *Bulletin*. The content of this scientific tribune of the Iași Medical-Naturalist Society included summaries of SPN manifestations, annual administrative and scientific reports, minutes of meetings, original communications with a wide range of topics, reports and reviews on papers or communications published in other journals and up-to-date scientific topics under debate in the country and abroad, meteorological tables for Iași.

1912-1923. Although in its first decades, SPN lived in the aura of the German culture due to the decisive influence of Dr. Iacob Cihac and other German-speaking graduates from foreign educational institutions, and, after 1866, due to the new wave of German-speaking Romanian intellectuals, the Society added on the shelves of its Library a dominant number of French language books and journals.

Works by French scholars were also offered to the *Society* for reviews to be printed in its *Bulletin*.

On the eve of the First World War, SPN received a large number of journals donated or sent in exchange for "our modest Bulletin", as Dr. Gustav Otremba put it. Francophone literature obviously predominate (2, 3). The periodicals exchanged with the *SPN Bulletin* came from Bucharest, Iași and abroad: France, Germany, Switzerland, Italy, Belgium, Russia, USA, Spain, Portugal, Greece, Poland, Vienna and Prague (Austro-Hungarian Empire), the Netherlands, Turkey, Uruguay, Brazil, Argentina.

Between 1912 and 1923 the name of the SPN periodical was changed to *"Bulletins et Mémoires de la Société des Médecins et des Naturalistes de Jassy",* being edited both in French and Romanian.

The publication of the *Bulletin* ceased in 1916, during World War I, when only four issues appeared, and it was resumed some years later, in 1923, with the cumulative volume XXXI.

Prominent professors and physicians of the Iași Faculty of Medicine were members of the Editorial Board and authors of papers: Alexandru Brăescu, Panait Zosin, Gheorghe Demetriade, Constantin Bacaloglu, Constantin I. Parhon, Paul Anghel, Alexandru Tzaicu, Mihai Ciucă, Alexandru Slătineanu, Elena Pușcariu, Mihai Manicatide, Gheorghe Tudoranu, Vladimir Bușureanu, Gheorghe Plăcințeanu, Nicolae Hortolomei, Emil Hurmuzachi, Alexandru Moruzi, Corneliu Șumuleanu, Leon Ballif, Ion Bălteanu, Ion Alexa, Dumitru Brănișteanu, among many others (5).

1924-1949: The second period of "The Medical-Surgical Journal of Iași". From January 1, 1924, Dr. Răşcanu, SPN President in office and professor of Physiology, modified again the publication title in order to point out its exclusive medical character. Hence its 35th volume became *"The Medical-Surgical Journal of Iași. Bulletin of the Society of Physicians and Naturalists".* The title was expressed either in French - *"Revue Médico-Chirurgicale. Bulletin de la Société des Médecins et des Naturalistes de Jassy*" - or in Romanian: *"Revista Medico-Chirurgicală din Iași. Buletin al Societății de Medici și Naturaliști".* It was published every two months, i.e., each volume comprised six issues per year, summing up about 600 pages (6). Its subtitle – *SPN Bulletin* – reminded the tradition and continuity of the original denomination: *"Bulletin of the Society of Physicians and Naturalists of Iasi".* The volume XXXV, the first of the new series,

had 44 pages, signed by outstanding specialists who remained in the history of the Iași Faculty of Medicine and of Romanian medicine, like, e.g, Gheorghe Tudoranu, Vladimir Bușureanu and Gheorghe Plăcințeanu.

Interesting clinical, medical-surgical and laboratory studies, original articles, meteorological tables, biostatistical and epidemiological assessments regarding especially Moldavia, inaugural lectures, commemorations and obituaries were published, together with summary presentations of both SPN activities, along with reports on different manifestations in the country and abroad. Papers from foreign contributors, book and article reviews published around the world were integrated, too. The *Journal* also consisted of book and article reviews, minutes of the SPN meetings, and popularization of various aspects concerning the medical life of Iași. This was a period of consolidation and high productivity of the *Journal* and evolution of modern medicine during those years was reflected.

In the interwar and post-war decades**,** from 1924 to 1949, *The Bulletin* ..., respectively *The Medical-Surgical Journal* ... greatly improved its quality, the topics being deeply rooted in the regional and national everyday medical practice and theory, and concurrently synchronized to international events and discoveries. International congresses, mainly held in France, were announced in *"The Medical-Surgical Journal".* This editorial policy maintained the European profile of the publication.

The specialized scientific Romanian vocabulary was substantially enriched and acquired an increasingly natural allure.

The SPN Bulletin continued to be exchanged with foreign journals. In 1929, for example, out of the 500 copies of the *Bulletin*, 63 were sent abroad, and 34 periodicals were received. In 1931, old numbers of the *Bulletin* were requested by the British Museum to complete its collection. In 1932, *"The Medical-Surgical Journal"* was considered by the Hygiene Department of the League of Nations in Geneva to be one of the "great medical publications" (4).

The SPN *Bulletin* was still printed during the Second World War, under the title of "*Revue Médico-Chirurgicale. Bulletin de la Société des Médecins et des Naturalistes de Jassy*" or *"Revista Medico-Chirurgicală din Iași. Buletinul Societății de Medici și Naturaliști".*

But "1944-1945 was a difficult time for Iași, which lost its usual intellectual turmoil, to become a theater of territorial and corporal turmoil. Our war-torn society suffered severe damage. The printed material for the last issues of 1943 was destroyed before being handed over. The headquarters of the Society were hit by 11 bombs, 4 of which directly affected the building, partially demolishing it and destroying a large part of the collections gathered and arranged there with great effort (...). And yet, after this material turmoil, its ruins and the time of a totally new spiritual and social turmoil, we dare to concentrate our strength again and reappear. (...) We hope for peace and good times"- the editors confessed in the *Foreword* to volume 57, no.1, 1946.

Apart from topics related to war surgery or the onset of epidemic diseases, the content did not betray the tragedy of the moment. In the pages of the *Journal* there were articles related to medical research and practical experience, synthetic reports, inaugural lectures of great professors, deontological approaches belonging to Iași and other Romanian medical and pharmaceutical centers (4).

For a while, after 1948, the interde-

pendence between the SPN, its *Journal* and the Faculty of Medicine was clearly stated on the publication cover, where it was specified: "*Published under the scientific direction of the teaching staff of the Faculty of Medicine*".

1949-2012: The third period of "The Medical-Surgical Journal of Iași" went through several stages including its suppression and rebirth. Between 1943 and 1946, years of the Second World War, the SPN postponed its meetings. The newly established communist government was obsessed with centralizing the press and controlling information. In 1949, for political reasons *The Medical-Surgical Journal*… ceased to appear. After 1949, under the constraint of the radical changes that took place in post-war Romania, including its scientific life, both the SPN and its publication were in danger of being prohibited or losing their identity. Eventually the SPN became the local section of the unique Romanian Society of Medical Sciences (SSM) based in Bucharest, whereas its *Bulletin* was suppressed. However, through the energetic intervention of some personalities of both the Faculty of Medicine of Iași and SPN members, among which Acad. Prof. Dr. Vasile Rășcanu, efficiently supported by Acad. Prof. Dr. Constantin I. Parhon and Acad. Prof. Dr. Nicolae Hortolomei, former famous professors of the Iași medical school and presidents of this academic Society, the SPN was able to keep not only its name, but its publication, too, even after it became the Iasi Branch of the Medical Sciences Society in Romania (SSM) (6). Founded in June 1949 in the country's Capital, the SSM was transformed into the Union of the Medical Sciences Societies in Romania (USSM) in 1962.

On April 27th, 1950, it was stated that the Iași Branch of the SSM represented the continuation of the Society of Physicians and Naturalists created in 1830. Consequently, it was renamed: *"The Society of Physicians and Naturalists. The Iași Branch of the Medical Sciences Society".* The problem of restoring the former title of the SSM Branch in Iași and the resumption of the publication of the *Medical-Surgical Journal* were re-discussed during the debates on the SSM Statute in September 1955, when the SSM was organized into sections. On that occasion, the SPN - The Iași Branch of the SSM also proposed medical periodicals to be expanded, allowing all SSM Branches existing in the university centers of the country to edit specialty journals, because the suppression of such local-regional *Societies* and Journals in 1949 had significantly restricted the scope, dissemination and valorization of research activities (6). The pleadings were won on June 21, 1956, when a festive plenary session was held. Finally, the *Medical-Surgical Journal* reappeared as an organ of the SPN, the Iași SSM Branch.

Consequently, interrupted in 1949, the publication of the *Medical-Surgical Journal* was restarted six years later, in 1956, when its 60th volume was edited. Acad. Professor Dr. Vasile Rășcanu, "the second founder of the Society" (i.e., after Cihac), was the editor in chief of the *Journal* and SPN President (1956-1973). The editorial signed by Acad. Professor Dr. V. Rășcanu and entitled "The historical and scientific significance of the Medical-Surgical Journal of Iași" was a plea for the continuity of the periodical. The editorial board included Acad. dr. V. Rășcanu (editor-in-chief), Prof. Dr. Șerban Brăteanu and Prof. Dr. Vladimir Bușureanu (associate editors), Assoc. Prof. dr. Theodor Economu, Assoc. Professor Dr. Gheorghe Popovici and Professor Dr. Ernest Ungureanu (editorial

secretaries). Ten years after the resumption of the publication, 20 million pages had been printed and there were 2,000 subscribers.

In these post-war series from 1956 to 1989, the title of the publication was *"The Medical-Surgical Journal of the SPN of Iași, Branch of the SSM in the Romanian People's Republic*", and after 1965, "Medical-Surgical Journal" of the SPN of Iași, Branch of the USSM in the Socialist Republic of Romania. Meant to outline its long history and permanence, the previous versions of the SPN periodicals were illustrated on the cover page of the new edition: *"The Advisor of Health", "The SPN Leaflet"* and *"The SPN Bulletin".* As usual, covers II-III-IV mentioned the exchanges with foreign publications. Once again, the tradition of the longest-lived Romanian medical periodical was emphasized. The name of *"The SPN Medical-Surgical Journal"* has been preserved to this day.

Although the post-war language was exclusively Romanian until 1990, the articles were accompanied by abstracts in foreign languages: French, Russian, English or German. As customary, the *Journal* recorded research results, clinical cases, reviews and reports of the SPN activity usually written by representatives of the medical and pharmaceutical stuff in Iași and Moldavia. Contributions by foreign scientists achieved independently or in collaboration with Romanian colleagues were sometimes included, too. The *Journal* was offered in exchange for various publications from abroad, mostly European ones. It was an excellent and economic way to keep the SPN library updated in a period of extreme isolation and inadequate funding.

However, paradoxical as it may seem, from 1965 to 1989 "*The Medical-Surgical Journal"* and the SPN itself went through their most prolific years not only in terms of the number of articles published and of undertaken activities, but equally in terms of their scientific quality and variety of topics. The *Journal* turned into a real methodological forum. In 1975, Professor Dr. Grigore Teodorovici was elected editor-in-chief, and he proved to be one of the best directors ever of the SPN publication, which could benefit from his competence and editorial skills until the end of his life, in 1992. He towered over this third golden period of the *Journal*. His best collaborator was Professor Dr. Traian Mihăescu.

The centenary. *„The Medical-Surgical Journal",* volume 91, no.1, January-March 1987, only remembered the100th anniversary of the publication of the first issue of the *Bulletin of the Society of Physicians and Naturalists of Iasi* that had occurred on January 1, 1887. But, since in some years the *Bulletin* did not appear due to real difficulties, volume 100, marking a century of effective appearance, corresponded to the year 1996.

December 1989-2012. So, it was in 1996 that the centennial volume of the SPN periodical could be really celebrated. A hundred years after its appearance, *"The Medical-Surgical Journal"* was the best general medical periodical in the country, being exchanged with 275 journals from 43 countries, and indexed in 22 databases, the most prestigious of whom was PubMed.

However, after the revolutionary events of December 1989, a new period of "languor" followed by another period of "regeneration" occurred. Little by little the SPN got inside the penumbra, because of the scientists' need to communicate their own results in high-profile publications indexed in international databases, but also in publications acknowledged and well rated by the Higher Education National Council of

Scientific Research in Romania. This fact-maintained scholars' interest in *"The Medical-Surgical Journal of the SPN"* to a certain extent but at the same time urged the *Journal* to keep pace with changes and impelling demands of the day. The SPN periodical reconfirmed its role and achieved a remarkable and constant recovery in the last decades. Succeeding Professor Dr. Teodorovici, since 1992 editor-in-chief was Professor Dr. Valeriu Rusu, who fulfilled his duty with competent efficiency and dedication until his death. Articles, monographs, treatises of major publishers continued to be reviewed, the SPN Library being thus enriched.

The openness produced in the Romanian society and the diligence of the editorial boards soon rendered the *Journal* one of the most appreciated national publications the CNCSIS finally rated in the B+ category. The printing of contributions in international languages has intensified, and from 2012 on, all articles are published exclusively in English. In 2008, the number of pages for all the four issues was 1160 and there were 1000 subscribers.

2012 to nowadays: ***the fourth period of the Journal***. Considered by international databases, such as Index Medicus, Excerpta Medica, Medline and PubMed, *"The Medical-Surgical Journal"* has been monitored since March 3rd, 2011, for evaluation according to Thomson-Reuters / ISI criteria. Since the last issues in 2012, the Iași SPN periodical appeared exclusively in English.

On October 28, 2016, at the end of its 120th editorial year, *"The Medical-Surgical Journal"* has been indexed in the Thomson Reuters Web of Science (*Emerging Sources Citation Index-Journal List*) - current Clarivate - Analytics Web of Knowledge, even though no impact factor has been calculated for it as yet. A special merit for this turn belonged to Professor Dr. Aurel Ivan, who endeavored and succeeded to have this publication acknowledged by Clarivate - Analytics Web of Knowledge.

Due to Professor Dr. Aurel Ivan and his team *"The Medical - Surgical Journal"* maintained its format and cover, acquired a better editorial quality, and began to observe stricter rhythmicity of its issues. It has a national and international editorial board and publishes articles from Iași and other Romanian university centers, as well as from abroad.

The *Iași SPN Journal* continues to have the same generalist character as always, covering various fields: basic bio-medical sciences, surgical specialties, medical specialties, dental medicine, pharmacy and medical bioengineering. Since 2021, general reports, reviews and clinical cases have been abandoned.

The historical and scientific significance of the Medical-Surgical Journal of Iași. The Society of Physicians and Naturalists of Iași and its *Journal* went through two world wars, a communist dictatorship, suffered during transition, but survived, proving that *„the great works of humanity are done by people who manage to overcome the situational incapacities of the times"*- said Professor Dr. Gheorghe Scripcaru, one of the SPN Presidents. As always in its long history, now, once again, united forces are needed so that the soul of this Society and implicitly of its periodical remain vivid, so that its projects could still inspire vitality and find constant echo both in the Romanian and medical communities worldwide. Globalization and European integrative requirements of the 21st century have imposed the adoption of the English language for the *SPN Journal* to be available and accepted in the international circuit of values.

Time, facts and people, the stylistic matrix of the Romanian nation always had to make the right choices between complementary identities, embracing progress and multiculturalism. Consulting today *"The Medical-Surgical Journal"* collection, one of the few in the world that spanned over 100 years, gives us a glimpse into the long way the Romanian society travelled in order to get synchronized to universal scientific progress and welfare.

Concurrently it illustrates a reliable source of documentation that highlights the specificities of the medical school of Iași, its problems, glorious moments and brilliant personalities, important for Romania, but for science as a whole, as a transnational entity, too.

The close cooperation of the 191-year-old SPN with the 142-year-old Faculty of Medicine, and later with the Institute and then the University of Medicine and Pharmacy of Iași, along with the 264-year-old "Sf. Spiridon" Establishments, constituted the key point of their together evolution, resistance and adaptation in the achievement of social implementation of knowledge, medicalization of the society and professionalization of medical and pharmaceutical practitioners and researchers.

In 2021, on the occasion of its 125th anniversary, *"The Medical-Surgical Journal"* was awarded a Diploma during the "Editorial Harvest Days" Book Fair in Iași.

Evoking and honoring the 125-year-old *"The Medical-Surgical Journal of the Society of Physicians and Naturalists of Iași"* also means understanding the importance of this pioneering national publication for the Romanian scientists and the Romanian society, as a hole, as well as the necessity to continue, following in our most noteworthy predecessors' footsteps. It is in this capacity to adapt successfully to challenging circumstances without losing identity that lays the source of the perpetual renewal of our values.

The *Society of Physicians and Naturalists of Iași* and its long-lived *"The Medical-Surgical Journal"* are such admirable paradigms in the Romanian history of science.

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EDITORIAL

POST-COVID-19 CARDIOPULMONARY REHABILITATION

For more than two years the entire activity of world healthcare systems carries on under the sign of COVID-19. Beyond the acute problem, SARS-CoV-2 infection frequently associates long-term persistent symptoms and complications. After COVID-19, the patients may develop persisting dysfunction of almost any organ, resulting in a very wide range of symptoms and signs. These result from both organic sequelae and various functional imbalances, mainly of respiratory and cardiovascular nature, but also involving other systems, from the central nervous system to the peripheral muscles. Several months after the infection, persistent dyspnea, muscle weakness, fatigue, sleep disturbances, depression and anxiety often persist (1). Long term COVID-19 sequelae and complications are more commonly observed in patients older than 65 years, frequently associating cardiovascular diseases, diabetes or other chronic comorbidities. However, the consequences of COVID-19 are highly severe, even in young patients, if they require disease management in the Intensive Care Unit (ICU), for complications such as respiratory distress syndrome (ARDS) or multiple organ failure (2).

In the context of the epidemiologic implications and of the therapeutic particularities, including oxygen therapy and ventilation, COVID-19 patients remain bedridden for long periods, thus leading to muscle strength and mass reduction, increased risk of deep vein thrombosis and to an altered capacity of sputum clearance (3). Extended immobilization in ICU patients also determines more important complications such as neuromuscular complications, severe peripheral muscular weakness and fatigue, dysphagia, even quadriparesis, resulting in a significant quality of life alteration (2, 4). In addition, psychological problems, cognitive and emotional complications are common in COVID-19 patients, treated in ICUs (5).

RESPIRATORY SEQUELAE AND REHABILITATION RECOMMENDATIONS

Clinical evidence resulting from the limited COVID-19 experience, but also from previous outbreaks of Coronavirus resulting in Severe Acute Respiratory Syndrome (SARS), showed long term exercise capacity reduction, in the context of persistent pulmonary function impairment: restrictive ventilatory pattern, altered diffusing capacity in the alveolar capillary membrane, respiratory muscles dysfunction (6, 7). In addition, cardiorespiratory and musculoskeletal performance impairment determines an important quality of life alteration, after COVID-19 (8).

Pulmonary rehabilitation is a multidisciplinary personalized intervention, providing comprehensive care, in order to improve both the physical and psychological condition of patients with respiratory diseases (9). Pulmonary rehabilitation is based, but not limited to exercise training, including also psychosocial support, and measures of education and behavioral mod-

ification. Pulmonary rehabilitation increases the functional capacity, reduces symptoms as dyspnea and improves quality of life, even in patients with irreversible organic abnormalities of the lung. Although there are not enough studies regarding the benefits of pulmonary rehabilitation in post-COVID-19 patients, this intervention should be considered, as these patients may present some degree of functional limitation, partially due to the respiratory dysfunction (8).

The Stanford Hall consensus statement for post-COVID-19 rehabilitation, recommends as low intensity exercise (≤ 3 Metabolic Equivalents, METs) to be considered initially, particularly for patients who required oxygen therapy. Exercise training should be performed under the careful monitoring of vital signs and parameters (pulse oximetry, heart rate and blood pressure). Exercise intensity should be gradually increased, based on symptoms and perceived exertion (8). Aerobic exercise training such as walking, jogging, swimming is recommended 3 to 5 times per week, for 20 to 30 minutes each session. Intermittent exercise can be used in patients with a lower exercise capacity. Breathing training is recommended in patients with dyspnea, wheezing, difficult sputum discharge. This training should include breathing rhythm adjustment and breathing muscle group mobilization. Sputum training should aim to diminish sputum production and to reduce energy consumption with coughing. Some patients may need to be assisted through positive expiratory pressure or oscillatory techniques. Strength training may also be associated to aerobic training, by progressive resistance training, 2 to 3 times per week, for a 6-week period (2).

A comprehensive clinical and functional assessment should be done, before initiating the respiratory rehabilitation program, in order to conduct exercise training in safe conditions. Physical examination will be followed by lung imaging and laboratory testing, including inflammatory markers. Respiratory functional assessment includes spirometry, respiratory muscle strength measurement, diffusing capacity of the lung for carbon monoxide (DLCO) testing. Aerobic exercise capacity should be assessed by exercise testing, preferably by cardiopulmonary exercise testing. This allows a rigorous individualized determination of the workload level for aerobic exercise training, resulting in minimizing the risk and maximizing the benefits of physical activity (9).

In post-COVID-19 patients, respiratory rehabilitation has important benefits in relieving symptoms of dyspnea and cough and reducing anxiety and depression, resulting in significant quality of life improvements and in long term complications prevention.

CARDIOVASCULAR SEQUELAE AND REHABILITATION RECOMMENDATIONS

Cardiac complications and sequelae should be considered in all post-COVID-19 patients, regardless of severity. Most common cardiac complications of COVID-19 are arrhythmias and myocardial injury, determined by multifactorial mechanisms, i.e., like systemic inflammation, direct viral myocardial injury, hypoxia, or even drug toxicity (10). Advanced age, prior cardiovascular comorbidities and diabetes mellitus are important risk factors for cardiac complications (11). All post-COVID-19 patients should undergo an assessment of cardiovascular symptoms and some basic

or more advanced investigations may be necessary: ECG, 24-hour HOLTER ECG monitoring, echocardiogram, cardiopulmonary exercise testing, serum biomarkers of myocardial injury, cardiac magnetic resonance imaging (MRI). In case of post-COVID-19 myocarditis, a period of rest for some weeks, depending on symptoms and severity, will reduce the risk of heart failure (8).

Cardiac rehabilitation represents a priority for patients with prior cardiac pathology such as heart failure, myocardial infarction, coronary revascularization (12). The cardiac rehabilitation program should be individualized depending on the main cardiovascular pathology and cardiac complications of COVID-19. As in respiratory rehabilitation, aerobic exercise training represents the core component of the cardiac rehabilitation program. Exercise testing represents the best modality of prescribing the individualized workload level of the exercise. In addition to exercise training, management of cardiovascular risk factors, health behavioral change and education are other essential components of the cardiovascular rehabilitation programs (13). Current international guidelines show the benefits of cardiac rehabilitation not only by relief of cardiac symptoms, increased exercise capacity and improved quality of life, but also by reducing cardiovascular mortality and hospitalizations (12, 13).

MULTIDISCIPLINARY ASPECTS IN POST-COVID-19 REHABILITATION

Cardiopulmonary post-COVID-19 rehabilitation programs should be initiated in a hospital setting, as supervised interventions, especially for patients treated in ICU and for patients requiring oxygen therapy. However, after a comprehensive clinical assessment, rehabilitation can be continued as outpatient or even home-based programs. An important issue is the timing of initiating the rehabilitation of COVID-19 patients. There are some evidences that early bedside rehabilitation facilitates long term recovery reduces the duration of hospitalization and the time of assisted ventilation, in patients with important respiratory disease (4). On the contrary, there are situations when early respiratory rehabilitation is not well tolerated because it may determine significant rapid desaturation. For this reason, it is important to adapt the timing for initiating rehabilitation, depending on the individual response of the patient (2). Classically, an inpatient pulmonary rehabilitation program lasts between 6 and 9 weeks. However, there are not clinical studies on the post-COVID-19 period regarding the optimal duration of rehabilitation (14).

Clinical experience of the acute phase COVID-19 showed a high incidence of other medical consequences (hepatic, hematological, renal, gastrointestinal, endocrine), beyond those involving the lung and heart, as a direct result of the infection as well as an indirect result of medical interventions. It is unclear whether and which of these persist as long-term complications of COVID-19. However, post-COVID-19 rehabilitation should not ignore the multiorgan involvement. In this perspective, post-COVID-19 rehabilitation becomes a multidisciplinary patient-centered intervention, tailored to individual COVID-19 complications, as well as to individual comorbidities, needs and preferences of the patient.

A large number of patients with COVID-19 require ICU treatment, often

lasting for several weeks. Some of these patients developed a combination of physical, cognitive and psychological issues, defining "the post-intensive care syndrome": dyspnea, depression, anxiety, impaired exercise capacity, pain and altered quality of life (15, 16). Patients that have long ICU hospitalizations, frequently experience intensive care acquired weakness, resulting from rapid muscle mass loss. This worsens in case of multi-organ failure. Physical weakness may also occur in patients not admitted to ICU, in the context of deconditioning, which appears particularly in frail patients. These patients are more likely to require support by rehabilitation after the COVID-19 hospitalization (17).

Prone positioning for many hours per day represents an important measure for respiratory failure associated with COVID-19, in both ventilated and non-ventilated patients. This position determines an increased risk of compression neuropathies and peripheral neural damage. Neuromuscular blockades in orotracheal intubated patients may increase peripheral neural damage. Neuropathies and muscle atrophy are better managed together, by musculoskeletal post-critical care rehabilitation and specific physiotherapy programs (17).

Mental health issues are common in patients admitted in ICU for acute respiratory distress syndrome: 40% associated anxiety, 30% experienced depression, 20% had post-traumatic stress disorder (18). Mild cognitive impairment is also very common in the patients with acute respiratory distress syndrome discharged from ICU and may persist up to one year. Specific cognitive assessment at discharge is important, because otherwise a mild cognitive impairment may remain unidentified. Cognitive impairment may impact on professional performance and on societal participation.

For the efficient management of post-intensive care syndrome, a holistic approach is mandatory and should involve a physiotherapist, psychologist and social worker in addition to a cardiologist, pneumologist, and other medical specialists (19).

Nutrition intervention is an essential part of the rehabilitation program for COVID-19 patients, especially if they suffered pulmonary and cardiac distress or in critically ill patients, considering the weight loss, sarcopenia and frailty associated with these conditions. Individually tailored nutrition support is necessary (20). Despite significant muscle loss, an important percentage of post-COVID-19 patients are overweight or obese, indicating sarcopenic obesity, a category with the potential for misidentification of malnutrition.

CONCLUSIONS

Post-COVID-19 rehabilitation represents an important intervention for patients with persistent symptoms and complications after the SARS-CoV-2 infection, especially in those with post-intensive care syndrome. Beyond respiratory and cardiovascular components, post-COVID-19 rehabilitation should be a multidisciplinary patient-centered intervention. Aerobic exercise represents one of the core components of cardiopulmonary rehabilitation, increasing demand upon the cardiopulmonary system and resulting in significant benefits on general fitness and on a wide range of post-COVID-19 problems, such as fatigue, lack of performance in professional and recreational physical activities and

emotional disturbance.

In the context of the paucity of evidence-based guidelines on post-COVID-19 rehabilitation, further clinical research regarding long-term complications of COVID-19 is needed.

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NEWS

PREGNANCY-ASSOCIATED PLASMA PROTEIN-A - A LINK IN BREAST CANCER PROGRESSION

Pregnancy associated plasma protein A (PAPP-A) is a zinc-containing metalloproteinase produced by the syncytiotrophoblast whose concentrations increase rapidly in early pregnancy with a doubling time of 3 days before slowing to a more gradual increase until term. PAPP-A regulates the concentration of free IGF-II by cleaving insulin-like growth factor binding proteins (IGFBP), predominantly IGFBP-4. PAPP-A circulates as a large heterotetrametric composed of two subunits of PAPP-A and two subunits of pro-major basic binding protein, which is produced by the cytotrophoblast cell layer of the placenta. PAPP-A measurements are performed exclusively as part of first trimester screening. Although PAPP-A is present in men and nonpregnant women, concentrations are typically < 1 mIU/L, this protein is highly expressed in pregnancy-associated breast cancer tissues. A recent study showed that PAPP-A was upregulated in pregnancy associated breast cancer tissues and breast cancer cells. Overexpression of PAPPA promoted cell proliferation, motility, invasion, and epithelial-mesenchymal transition. Also, the study shows that the PAPP-A is an oncogenic protein highly expressed in PABC tissues and promotes breast cancer progression. The levels of PAPPA in mARN were significantly higher in pregnancy-associated breast cancer (PABC) tissues than in normal breast tissues (n = 30). The protein level of PAPPA was detected by western blot in breast cancer cell lines (T47D, MCF-7, BT549, MDA-MB-231, and MDA-MB-468) and in normal breast epithelial cell MCF-10A. These overexpression's promoted proliferation, migration and invasion of breast cancer cells. Finding a way to suppress the action of this protein may serve as a novel therapeutic target for breast cancer (Zhang J, Zhang Y, Li L, Nian Y, Chen Y, Shen R, Ma X. Pregnancy-associated plasma protein-A (PAPPA) promotes breast cancer progression. *Bioengineered* 2022; 13(1): 291-307. doi: 10.1080/21655979.2021.2000724).

EDITORIAL

CHALLENGES OF THE DENTAL MEDICAL PRACTICE DURING COVID-19 PANDEMIC AND POST-PANDEMIC PERIOD

The COVID-19 pandemic represented a change of paradigm for dental health care providers as this category of professionals faced unexpected and unforeseen challenges regarding moral decision-making (1).

The first challenge was the interruption of the ongoing dental treatments; dental professionals were limited to telephone consultations, and antibiotic prescriptions sent by e-mail or *WhatsApp*, and ensured only major dental emergencies (uncontrolled bleeding; diffuse soft-tissue bacterial infections with intra-oral or extra-oral swelling or trauma involving facial bones that potentially compromises the patient's airway) and dental urgencies (swelling of gum, cheek or face which; acute oral pain; mouth ulcers prolonged for more than two weeks; bleeding that lasts more than 20 minutes to a patient with recent tooth extraction; fractured tooth which is causing pain and damage to cheek or tongue; traumatized tooth; bleeding due to facial trauma). These measures were requested by higher risk of COVID-19 infection within dental professionals due to face-to-face contact, and the presence of COVID-19 in aerosols (liquid and solid particles suspended in air) and splatter (mixture of air, water, solid substances) frequently formed during routine dental procedures when high-speed instruments are used (2-3). As 91.7% of subjects with COVID-19 have virus in saliva samples (4), dental procedures with high volume of aerosols make ineffective the standard protective measures for patients in the incubation period, unaware of their infection status, or those who conceal their infection (5). Regardless of the degree of community spread, dentists must screen all people who enter the dental setting for symptoms of COVID-19. Dental treatment had to be provided after considering both the risk of delaying dental health care and the risk for dentists of healthcare-associated disease transmission (6). Conservative treatments were the most affected dental procedures during COVID-19 pandemic, followed by periodontal diseases treatment, where almost half of the planned treatments could not be performed (7). The patients between 35 and 74 years were the most affected by the absence of dental caries treatment, endodontic therapy, and periodontal diseases treatments (7). Blockage of routine dental activity has been associated with increased vulnerability of patients to other conditions (caused by increased consumption of antibiotics), as well as the occurrence of functional, biological, or aesthetic complications caused by delayed dental procedures, prosthetic treatments, orthodontic treatment sessions (7). Poor oral health can accelerate the development of other systemic conditions as follows: cardiovascular system pathology, neurodegenerative diseases, diabetes, low birth weight, osteoporosis, and kidney disease (8).

The second challenge was related to the transition to the alert state. Clinicians have adopted a series of measures recommended

by the Romanian College of Dentists, measures aimed at preventing and limiting the spread of COVID-19, as well as protecting members of the professional body; these measures were inspired by similar measures adopted by professional dental associations in the USA, Canada, France (9, 10, 11). The implementation of these measures required the reorganization of the circuits and work protocols in the dental offices that generated the increase in expenses by the additional purchase of protection and disinfection materials (for example, surgical ultraviolet lamps and High Efficiency Particulate Arrestance Filters). In addition, there was a decrease in the number of patients treated per day due to the increase in time required for the preparation of the office and medical staff but also the postponement of elderly patients with associated general pathology. To restrain and mitigate the COVID-19 infection. It was proposed the introduction in dental public and private clinics of screening tools, either rapid molecular testing or saliva point-of-care technology, which should be effective in identifying and isolating the potential contacts and carriers (12).

The new economic conditions have contributed to the reduction of patients' addressability to complex oral rehabilitation treatments. At the same time, the higher percent of population with untreated needs and lower income has increased the burden on public health systems and, at the same time, has reduced the revenues of private dental practices.

In this context, the projects regarding the oral health status of the population in the post-pandemic period must address factors such as socio-economic status, social environment, cultural norms, and the health insurance system. In this post-pandemic period, we must consider the recommendations of World Health Organization for the integration of the oral health into general health promotion strategies and the assessment of the oral health needs through socio-dental approaches, as well as the promotion of oral health within disadvantaged population groups (13).

Complex oral rehabilitation remains, in the post-pandemic period, an area of interest for patients who request the complete restoration of the functions of the stomatognathic system. In this context, it is requested an interdisciplinary management of edentulous patients through the collaboration of specialists in the fields of periodontology, implantology, prosthetics, oral surgery, and orthodontics.

Clinicians are currently focusing on factors that influence the success rate of classical and implant-prosthetic prosthetic treatments: biomaterials, design and surface bioactivity of dental implants, biomechanical factors, quality and volume of bone tissue, surgical technique. Decisions regarding the selection of techniques and materials used in prosthetic and implant therapy imply uncertainties regarding the prognosis of therapy, in relation to the experience and preferences of the practitioner, the preferences and values of patients, and the costs involved (14).

The long-term success of the mucosal support reconstruction techniques is highly related to the individualized analysis of the specific systemic, loco-regional, local parameters and the focus on therapeutic algorithms adapted to each individual case.

Modern imaging techniques used for the diagnostic and assessment of muco-osseous support allows the understanding of the predictive factors and the adoption of adequate informed decisions regarding the implant-prosthetic therapeutic solutions (15).

The development of individualized protocols and algorithms for alveolar reconstruction techniques and addition materials, in relation to the particularities and complexity of clinical cases, is a necessity in the therapeutic management of patients with severe alveolar resorption.

The effectiveness of the oral complex rehabilitation procedures can be significantly increased by the introduction of the digital workflow, from the treatment planning and designing, from implant surgery procedures to the fabrication of individualized prostheses produced by computer-aided design/computer aided manufacturing (CAD/ CAM) technologies (16). Implant-prosthetic therapy assisted by digital systems favors the use of early loading (post-implant 3-6 months) or immediate loading (post-implant first 48 hours) protocols that reduce the number of sessions, reduce the post-operative healing time, and increase patient post-operatively comfort (17). The digital tools and technologies also give a strong boost to various minimal invasive surgical techniques used in the implant stage.

Despite these advantages, before COVID-19 pandemics the current use of the digital technology was still lacking in the clinical dental practice, situation that was linked to the slow adoption of digital technology (18).

COVID-19 pandemic period imposed a larger adoption of the digital devices and technologies. We can predict further enrolling of the dental practitioners in digital courses aiming to gain in-depth knowledge of digital applications and expert systems that optimize diagnosis and treatment plan, as well as support clinical decisions based on causal and probabilistic reasoning in theoretical decision-making schemes.

The widespread use of dental technology in the pandemic period provides patients with the opportunity to fulfill the esthetic demands, to preview the future prosthetic solutions and, by the use of the minimally invasive surgical procedures, to shorten the time required from diagnostic to final implant-supported prosthetic restoration for uninterrupted social and professional life of the edentulous patients.

COVID-19 pandemic period was followed by clinical, economic, legal and social consequences with future impacts on dental practice (19). In this context, professional scientific associations, government authorities, public and private dental care services must design new rules and policies for similar long-term pandemic periods.

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EDITORIAL

PULMONARY REHABILITATION BETWEENA PAST IN SHADOWS AND A BRIGHT FUTURE

BRIEF HISTORY OF PULMONARY REHABILITATION

During the last century numerous data and innovative ideas regarding pulmonary rehabilitation (PR) have accumulated worldwide, coupled with the development of an arsenal of new techniques for investigating and evaluating the respiratory function. It is an important advance in modern medicine and a concern for an expanding pathology. In parallel with humanity's efforts to modernize, aiming for a high degree of civilization, the pathology is also growing. With the industrialization and urbanization of lifestyle, the human body, and especially the respiratory system, is subject to risk factors that are ever growing in harm.

The roots of the PR treatment have their origins in distant history. In the writings of the ancient peoples (Assyrians, Egyptians, Jews, Greeks and Romans) there is evidence of the use of natural physical agents for therapeutic purposes such as air, water, heat and light.

Laennec first described the concept of PR in 1821 and Charles L. Denison, a former patient with pulmonary tuberculosis, was one of the pioneers of respiratory physiotherapy. In 1880, Dr. Denison recommended breathing exercises for the rehabilitation of patients with tuberculosis in his work "Mountain Resorts with Therapeutic Effects" and in 1985 he also published an article titled "Exercises for the Lungs with Disabilities".

In 1942, the National Rehabilitation Association (NRA), the oldest professional member organization in the United States, defined rehabilitation as a restoration of the individual in terms of the medical, mental, emotional, social, and vocational potential of which the patient is capable of. PR is extremely effective for lung diseases and the basic prototype it was founded upon was the patient with chronic obstructive pulmonary disease (COPD).

In 1981, the American Thoracic Society officially supported the PR of COPD patients. In 1984, after extensive study, Dr. John Hodgkin published the paper "Pulmonary Rehabilitation - Guidelines for Success" which included the official definition of PR and the structure of practical PR programs. With the publication of these papers, the interest and involvement of physicians and medical associations has grown, and their contribution has been observed to this day: "American Association for Respiratory Care", "American Association for Pulmonary and Cardiovascular Rehabilitation", "American Thoracic Society (ATS)", "European Respiratory Society (ERS)".

The concept of rehabilitation also entered Romania in 1969, when the statistics of the Ministry of Health estimated that 3% of the active workforce had lost their work capacity and recovery and thus reintegration measures were required. In the 1970s, the managements of the County Sanitary Directorates from Iasi and Cluj offered to

the Ministry of Health a solution to the problem by setting up rehabilitation hospitals in these two university centers that would fully meet the health requirements of modern medicine. By Decree 296 of 1973, the Ministry of Health approved the construction of rehabilitation hospitals in the municipalities of Iasi and Cluj.

In 1974, at the fifth session of the Romanian Academy of Medical Sciences, the first guidelines for PR were drawn, and in recent years we are witnessing an explosion of PR services and various techniques and devices used in the rehabilitation of lung patients. These respiratory therapies complement the pharmacological treatment and represent the cornerstone of the management of these patients.

The year 2006 was a significant year in the history of pulmonary rehabilitation due to internationally publishing the *Joint Declaration on PR by ATS / ERS* while in Romania the *Working Group on Respiratory Rehabilitation of the Romanian Pneumology Society* was created at the proposal of the undersigned, and with the support of the Romanian Pneumology Society Board, as a representative of the Rehabilitation Clinical Hospital and the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi. Research in this field is in full swing worldwide and in our country (1).

PULMONARY REHABILITATION-OBJECTIVES, EVALUATION

PR treatment occupies a central place in patients with chronic lung disease, being recognized by all specialists in the field. The current definition of PR, according to the *Official Report of the American Thoracic Society Workshop* published in May 2021, is based on definitions previously developed, on scientific evidence, by PR specialists, which were published in 1974 and updated in 2006, 2007, 2013 and 2015, respectively. According to this report, "pulmonary rehabilitation is a comprehensive intervention based on an announced patient assessment, followed by patient-friendly therapies, which includes, but is not limited to, training, medical education and behavior change designed to improve the physical and psychological condition of people with chronic respiratory disease and to promote the long-term adherence to health-enhancing behaviors" (2).

Pulmonary rehabilitation is an *extremely effective treatment for people with chronic lung disease*, including post-COVID-19 respiratory sequelae, which is still underused worldwide. The conditions that can benefit from pulmonary rehabilitation are:

a) *lung diseases with obstructive ventilatory dysfunction*: COPD, asthma, bronchiectasis, cystic fibrosis, bronchiolitis obliterans;

b) *lung diseases with restrictive ventilatory dysfunction*: interstitial lung diseases (pulmonary fibrosis, occupational or environmental lung diseases, sarcoidosis, scleroderma, lymphangiomyomatosis, post-COVID-19), thoracic wall deformities (kyphoscoliosis, spondylitis ankylosis) restrictive ventilatory dysfunction;

c) *other pathological conditions*: lung cancer, pre / post lung transplant conditions, pre / post thoracic and abdominal surgical conditions with ventilatory dysfunction, pre / post LVRS (Lung Volume Reduction Surgery) conditions, ventilator dependence, pediatric patients with respiratory diseases, respiratory diseases related to obesity, autoimmune diseases with pulmonary involvement, pulmonary hypertension (3, 4, 5).

Initial assessment of patients for inclusion in the PR program involves: detailed history and a correct clinical examination, assessment of contraindications for the rehabilitation program, assessment of smoking status, assessment of nutritional status, measurement of peripheral muscle strength, assessment of dyspnea and fatigue, performance evaluation of daily activities, quality of life assessment, medical education assessment, respiratory function assessment, exercise capacity assessment, electrocardiogram, echocardiogram, chest imaging assessment, additional needs assessment ± other investigations depending on the patient's comorbidities.

Comprehensive and thorough patient assessment is essential for personalizing the PR program and for effectively addressing the objectives of individual PR programs tailored to the patient's needs. In recent years, new models of PR programs have emerged, which aim to improve patient access, tailored to their needs, including telerehabilitation and low-cost models useful for PR at home. Quality assurance processes for PR programs are important to ensure that any PR service delivers optimal outcomes for patients and health services. The success of these PR models is assessed by the achievement of the essential components of the PR programs and the expected results of the patient, including improved exercise capacity, reduced symptoms, especially dyspnea, fatigue, cough, sputum, chest pain, anxiety and depression, reduced hospital admissions and improved health-related quality of life (6, 7, 8).

PULMONARY REHABILITATION AND LONG-COVID SYNDROME

The onset of SARS-CoV-2 coronavirus in December 2019 in Wuhan, China, and its rapid spread to over 216 countries has had a profound impact on humanity, leading to an unprecedented global crisis, both economically, socially and medically while also representing the biggest challenge we faced after the Second World War.

Experience, as well as studies conducted during this pandemic, have shown us that although COVID-19 is primarily a respiratory disease, the virus can affect other organs and systems of the body, which are manifested by characteristic symptoms. This is mainly due to the ubiquitous distribution of the SARS-CoV-2 virus receptor, a virus that causes systemic disease, with possible involvement of the heart, liver, pancreas and kidneys, causing changes in circulating lymphocytes and the immune system.

The impact of the severity of acute respiratory syndrome-coronavirus-2 may persist in some cases months after the initial illness, requiring individualized assessment and long-term follow-up for early detection of lung sequelae and correction of imbalances.

Long-COVID syndrome is defined as the syndrome that marks what is known so far: the persistence of COVID-19 disease symptoms 6 to 12 months after the acute phase, which are not explained by any other alternative diagnosis. It is characterized by the existence of a symptomatic corollary such as: fatigue, dyspnea, chest pain, cough, anosmia, dysgeusia, migraines, joint and muscle pain, cognitive dysfunction, etc., which proves that there are multiple organs involved (lung, cardiovascular, skin, neuropsychic, etc.), of which the lung suffers in two important areas, pulmonary fibrosis with or without traction bronchiectasis, pulmonary nodules and pleurisy, being the head of the poster,

followed in the second time by lung infections.

There are frequent cases that pass the acute period and are transferred from *COVID-19* to *non-COVID-19* services, due to the persistence of these accusations or their aggravation, the dyspnea being severe, with immobilization in bed and oxygen requirement between 10-15 L/min or non-invasive ventilation. It is true that in terms of symptoms, after post-COVID-19 recovery, 87.4% of patients have at least one persistent symptom, only 12% are asymptomatic after 2 months and in 51% of patients the quality of life is affected. These expressions are only the tip of an iceberg that can take a turn of gravity at any time (9).

In a clinical approach to post-COVID-19 status, a key concept is highlighted, namely the universal issue of rehabilitating post-SARS-CoV-2 sequelae, which is a complex concept that is not limited to the Real Time Test-The Polymerase Chain Reaction (RT-PCR) negative or the presence of anti-SARS-CoV-2 antibodies. *The same approach points out that there are still many unknowns related to COVID-19 disease, especially in the post-acute phase, in terms of the persistence of symptoms and the severity of long-term respiratory sequelae, as well as other complications (neurocognitive, cardiac, renal, etc.)* (10).

To develop a rehabilitation program for post-COVID-19 patients, the PR team has a well-recognized and widely accepted option: *reproducing the PR algorithm for patients with chronic pulmonary disease (COPD).* However, these patients need precise triage and a set of basic fundamental assessments to provide special rehabilitation programs (in-hospital, outpatient, tele-coaching, telerehabilitation, home rehabilitation), because the trajectories of phenotypes are very different and are not always related to hospitalization in the intensive care unit.

The benefits of PR programs for various chronic lung diseases have been demonstrated in numerous studies over time, and increased findings are currently being made on the favorable short- and long-term effects of including post-COVID-19 patients in these programs, *highlighting the crucial role of rehabilitation specialists* (11).

Long-term follow-up is a new challenge and is strongly recommended in these patients to detect time-consuming lung sequelae and appropriate management for their dysfunction. *Monitoring of post-COVID-19 patients* in specialist outpatient clinics and/or hospitals allows early assessment of symptoms (fatigue, dyspnea, cough, chest pain, depression and anxiety, etc.), of physical performance and lung function, which allows the application of the PR program, as well as the organization and tracking of this complex treatment. Early complex and comprehensive PR intervention in these patients certainly demonstrated a significant reduction in symptoms at 1, 3, 6 and 12 months, respectively, after the acute episode and an increase in physical and cognitive performance, and overall quality of life (12, 13).

PULMONARY TELEREABILITATION

The last two years have presented us with a brand-new challenge: *telemedicine*, which is defined by the World Health Organization as "the provision of healthcare services, where distance is a critical factor, by all healthcare professionals, through the use of information technologies and communication, for the exchange of valid in-

formation for the diagnosis, treatment and prevention of diseases and injuries" in which *pulmonary telerehabilitation* (tab. I) occupies an important place (14).

TABLE I.

Pulmonary telerehabilitation: advantages and disadvantages (15, 16)

Advantages	Disadvantages
• The patient is in a family environment; • Allows the patient to be autonomous, confident; • The rehabilitation process is facilitated, and a real acceleration can be observed; • Telemedicine / telerehabilitation removes geographical and distance barriers; • Everything can take place on secure medical communication platforms, where data confidentiality is strictly maintained; • Reduce healthcare costs; • Saving the time needed to travel home; • Wider access to the PR program; • The PR program can be monitored;	• Patients need to know technology; • Requires internet connection; • Inability to perform the PR program without the presence of a qualified and trained medical staff for what it means to assist in pulmonary rehabilitation; • There is currently no legal framework in Romania to regulate the activity of telemedicine; • Lack of transparency regarding data security; • Lack of information and trust regarding specific eHealth solutions among patients, citizens and healthcare professionals.

Telerehabilitation at home is a useful option for patients who are in the period after an acute illness and cannot be taken over by the rehabilitation centers but need to start the rehabilitation program as soon as possible. Although pulmonary rehabilitation is a well-established therapeutic method, with proven benefits, 8-40% of patients referred for hospital PR programs do not adhere to them, while 10-30% of those who start the program do not complete it.

Telerehabilitation is a therapeutic tool that can be used successfully in patients with chronic respiratory diseases. Studies comparing telerehabilitation with standard rehabilitation have shown favorable results in increasing exercise capacity and reducing symptoms, as well as improving the quality of life of patients with chronic lung disease (17).

Telemedicine has proved its worth in the midst of the pandemic, when health and distance rules made it inaccessible for patients to participate in rehabilitation programs, including patients with SARS-CoV-2 infection, both in the acute phase and the post-COVID-19 state, manifested predominantly by respiratory symptoms during rest and exertion, for which PR is required. In this context, telerehabilitation has been an extremely important aid. It has been used successfully in Wuhan hospitals, where patients with COVID-19, isolated in wards, have been given, through a telephone application, models of aerobic exercise, resistance training with elastic bands, and exercises for training the respiratory muscles, in the form of videos or written recommendations. The exercises were also recorded, the application having a symptom monitoring system, which was transmitted from patients to doctors.

In a multicenter study conducted in Australia, which assessed the access of patients with chronic respiratory diseases to technology, out of 254 participants, 92% had access to at least one electronic device,

70% a tablet or a computer and 85% a mobile phone; 60% of patients were willing to access telerehabilitation (18).

Technologies used to perform pulmonary telerehabilitation include home video conferencing, the use of smartphones, tablets, web platforms and pedometers connected to the internet, virtual reality and augmented reality. Progress in telemedicine focuses on augmented reality, which is based on the concept of real-time, barrier-free interaction. Augmented reality differs from virtual reality by creating a real-time physical environment using four computer-generated sensory inputs (sound, video, graphics, or GPS data), while virtual reality detaches contact from reality. *The future of medicine is geared towards augmented reality, although there is currently very little evidence of its use in rehabilitation* (19, 20).

The use of technology provides patients with chronic respiratory diseases with extensive access to pulmonary rehabilitation. The technological devices are multiple and can be used singularly or combined and are in a permanent development.

CONCLUSIONS

The history of pulmonary rehabilitation is interesting. What appeared to be a field or an uncertainty with obscure benefits, it is now supported by evidence classified by experts to be of the highest standard. There is also hoped that the upcoming years will provide even newer and more exciting ways for a wider and more important role of PR in a futuristic version of the field.

Specific assessment of each patient to achieve adequate results, long-term effects and maintaining the benefits of PR in the individual patient, the most appropriate access of PR to the early onset of disability, overcoming potential barriers, raising awareness of this science to people/patients and to professionals, the incorporation of new technologies and the principles of e-health are all real frontiers to be explored in order to improve PR.

This is an interesting and opportune time for pulmonary rehabilitation, which can bring new opportunities and allow the exploration of new areas in search of improvement. Overall, the future of pulmonary rehabilitation will include more patient options and greater program customization, while comprehensive patient assessment should continue to be a benchmark for all programs and models.

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EDITORIAL

TYPE 2 DIABETES MELLITUS: ANOTHER ONGOING SILENT PANDEMIC

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder, with an increasing prevalence in the past two decades being associated with impaired insulin secretion, resistance to peripheral actions of insulin, or both which cause persistent hyperglycemia (1). In patients with T2DM, persistent hyperglycemia can damage different organ systems in combination with other metabolic abnormalities, which can result in life-threatening and disabling health complications. The most common of these complications are microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular ones, which increase cardiovascular disease risk by two to four times (2). According to the World Health Organization (WHO), approximately 422 million adults between the ages of 20 to 79 years had T2DM in 2022 affecting 6% of the global population (3). In Romania, the prevalence rates are similar, approximately 5% of the population had this metabolic disorder. Moreover, 7 out of 10 patients with T2DM in our country, had been diagnosed with long-term complications of diabetes (e.g., peripheral arterial disease, chronic kidney disease, retinopathy or blindness) (4).

Obesity, metabolic syndrome (MetS), and non-alcoholic fatty liver disease have all become more common in recent years, along with T2DM prevalence. Type 2 diabetes (T2DM) and non-alcoholic fatty liver disease (NAFLD) are linked in both directions; NAFLD causes insulin resistance (IR) and T2DM through several pathways, but T2DM also promotes liver disease as a separate risk factor (5). Although NAFLD was first considered to be the hepatic manifestation of MetS, there is now convincing evidence that NAFLD is a major contributor to MetS, and that hepatic involvement is one of the components of multisystemic organ involvement (6). NAFLD is now considered a global epidemic that affects 1 in 4 persons worldwide, with a prevalence estimated to range between 25% and 30%. The fact that the prevalence of NASH is rising along with the rates of MetS and its components is even more concerning with the main risk factors for NASH being obesity, T2DM, and MetS (7). Moreover, NAFLD is more common in people with T2DM-roughly 75% more common than it is in people without diabetes. Patients with severe steatosis had a higher chance of acquiring T2DM, and those with NAFLD had a risk of developing it that was at least twice as high as that of subjects without NAFLD. Also, patients with NAFLD with T2DM had a 2.2-fold greater risk of all-cause mortality (8).

The guidelines recommendations are in conflict when it comes to screen T2DM patients for liver steatosis and fibrosis. Therefore, according to European Guidelines, patients with NAFLD can be screened for T2DM by measuring their fasting blood sugar or hemoglobin A1C (9). In addition, diabetic patients with increased ALT or hepatic steatosis should be screened for NAFLD, according to the American Diabetes Association (10). In-

stead, the American Association Society of Liver Disease disagrees with the routine screening of T2DM patients (11). Due to its simplicity and low cost, abdominal ultrasonography (US) is one of the screening methods that is most frequently used. According to a meta-analysis, this approach had a specificity around 80% compared with liver biopsy for detecting mild and severe steatosis. The US has the drawback of relying on the operator to determine the degree of steatosis in obese individuals or those with moderate steatosis (12). On the other hand, vibration-controlled transient elastography (VCTE) with controlled attenuation parameter (CAP) is a quantitative method that has a higher sensitivity and specificity for assessing hepatic steatosis. Additionally, assessments of the CAP numerical values correlate with the histological degree of steatosis (13).

Weight gain and obesity are the most important predictors factors for developing T2DM. These relationships most likely originate from inflammatory stimulation of adipose tissue, which causes insulin resistance (14). Recently, WHO estimated that 650 million people globally are ob ese and 2 billion adults worldwide are overweight (15). By 2025, it is anticipated that 2.7 billion adults would be overweight, nearly 1 billion individuals will be obese, and 177 million persons will be extremely obese if current trends continue. According to predictions, 1 in 5 women and 1 in 7 men would have obesity by the year 2030 (16). Obesity is defined by the WHO as an excessive buildup of body fat that is clearly related with health concerns; because of its ease of use, the body mass index (BMI) is most frequently used to measure obesity. By dividing the body weight in kilograms by the square of the height in meters, the BMI is determined. Grade 1 obesity is defined as having a BMI $\geq$ 30 kg/m^2, grade 2 obesity as having a BMI $\geq$ 35 to 40 kg/m^2, and grade 3 obesity as having a BMI $\geq$ 40 kg/m^2 (17). There is a widespread understanding that obesity, whether it be a condition that predisposes to disease or a disease in and of itself, needs to be treated and, more importantly, prevented (particularly in children), given its serious comorbidities, mortality, and financial implications (18). The prevalence of NAFLD and T2DM, two chronic disorders linked to obesity, has increased as this silent pandemic has spread around the world. NAFLD is the obesity-related noncommunicable illness with the greatest rate of growth, and it is a reliable indicator of liver and cardiovascular mortality (19). The global food system has seen significant changes over the last few decades, and billions of people are now daily exposed to processed foods and beverages high in energy that multinational firms have increasingly sophisticatedly marketed (20). As a result, it is challenging to imagine how individual-focused initiatives, like weight-loss programs or healthy eating campaigns, can be successful without laws that change the underlying environmental factors (19).

Several studies that were recently published found that Romanian patients with T2DM, had a prevalence in NAFLD of approximately 73%, and the main prognostic factor of steatosis was the severity of liver fibrosis (21, 22). Using VCTE, Trifan *et al*. found that the prevalence of advanced fibrosis was 11.7%, and the prevalence of cirrhosis was 13.6%. Moreover, 40.8% of the subjects included in the study had a BMI$\geq$ 30 kg/m^2 and was associated with severe steatosis and fibrosis (advanced

fibrosis and cirrhosis) (21). In another study, which included medical students by Nastasa *et al*. found that half of the obese subjects had NAFLD (23).

T2DM, obesity and NAFLD are regarded as risk factors for each other. This causes us to be cautious about the potential effects of early detection of one disease in the presence of another. Additionally, the progression and consequences of each disease are influenced by the presence of NAFLD, T2DM and obesity (24). The same lifestyle adjustments, oral hypoglycemiant drugs, and bariatric surgery as a last-resort therapy option are the key components of their prevention and management plans, which have been found to have an impact on the management of NAFLD, T2DM and obesity.

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EDITORIAL

OBESITY: THE COVERED RISK OF CANCER

Almost all developed and developing nations now experience a sharp increase in the incidence of overweight and obesity, which affects 60-70% of the adult population in industrialized nations and is more common in females and metropolitan regions (1). Overweight and obesity are now more common over the world than ever before-in adults, by 27%, and in children, by 47% (2). When a person's caloric intake exceeds their metabolic and physical activity-related energy expenditure, obesity results. Fat is deposited and accumulates as ectopic fat tissue, increasing the risk for several disease entities as a result of excessive or aberrant fat tissue formation that surpasses genetically and epigenetically established adipose tissue stores (3). The World Health Organization defines obesity as having a body mass index (BMI) ≥30 kg/m^2 (≥30 kg/m^2 as grade 1, ≥35 kg/m^2 as grade 2, and ≥40 kg/m^2 as grade 3), with normal values being between 18.5 to 24.9 kg/m^2 and overweight being intermediate values of 25 to 29.9 kg/m^2 respectively (4). According to these criteria, roughly 35% of adults in the USA over the age of 20 are already overweight, and the prevalence of obesity is expected to rise to 42% in persons over the age of 18 by 2030 (5).

With an estimated 14.1 million incident cases and 8.2 million fatalities per year, cancer ranks as the second largest cause of death globally (6). Obesity is a recognized risk factor for a number of cancers, in addition to the well-established cancer risks factors such as genetic predisposition, ionizing radiation, nicotine use, infections, unhealthy diet, alcohol intake, sedentary lifestyle, and other environmental exposures (7). Due to the rising prevalence of risk factors, particularly obesity and metabolic syndrome, cancer incidence will continue to rise (8).

According to global estimates, obesity and overweight were linked to 5.5% of cancer cases in the UK, while others asserted that the relative risk of cancer death linked to obesity was roughly 14.2% in men and 19.8% in women (9). The link between obesity and cancer is fairly strong, particularly when it comes to gastrointestinal (GI) tract tumors, where being overweight increases the risk of cancer by 1.5-2.4-fold. The types of cancer that are most strongly linked to obesity have been defined by several studies to include breast cancer in postmenopausal women, colorectal cancer (particularly in men), hepatocellular carcinoma, endometrial cancer, ovarian cancer, esophageal adenocarcinoma, gastric cancer, pancreatic cancer, gall bladder cancer, thyroid, kidney cancer, multiple myeloma and meningioma (10).

Numerous theories have been put out to explain how obesity may raise the risk of some malignancies. As a result of the release of mitogenic signals, the local microenvironment that supports tumor growth also drives cell survival mechanisms and the development of tolerance in cytotoxic host T cells (11). The overproduction of estrogen by fat tissue, also known as adipose tissue, has been linked to an increased

risk of developing breast, endometrial, ovarian, and other cancers. Increased blood levels of insulin and insulin-like growth factor-1 are common in obese people (IGF-1). Insulin resistance causes high insulin levels, or hyperinsulinemia, which precedes the onset of type 2 diabetes, another proven cancer risk factor. Increased insulin and IGF-1 levels have been linked to an increased risk of endometrial, renal, prostate, and colon cancers (12, 13).

Chronic inflammatory diseases like gallstones or non-alcoholic fatty liver disease are frequently seen in obese people. These circumstances can result in oxidative stress, which damages DNA and raises the risk of developing biliary tract and other malignancies (14). Adipokines, which are produced by fat cells, are hormones that can either promote or inhibit cell growth. For instance, as body fat accumulates, the blood level of an adipokine called leptin rises as well. Leptin levels above a certain point can encourage the development of abnormal cells. Adiponectin, another adipokine, may have antiproliferative properties that inhibit the development of tumors but is less prevalent in obese individuals than in individuals with a healthy weight. Mammalian targets of rapamycin (mTOR) and AMP-activated protein kinase are two more cell growth and metabolic regulators that fat cells may directly and indirectly affect. Obesity may also increase the risk of cancer by reducing tumor immunity and altering the mechanical characteristics of the tissue that surrounds growing tumors (13). Obesity can complicate screening and management in addition to having biological implications. Thus, women who are overweight or obese are more likely to get cervical cancer than women who are of a healthy weight, most likely as a result of less efficient cervical cancer screening in these people (15).

According to a nationwide cross-sectional study using BMI and cancer incidence data from the US Cancer Statistics database, from 2011 to 2015, about 74,690 new cases of cancer in women and 37,670 new cases of cancer in men aged 30 and older, respectively, were attributable to excess body weight (overweight, obesity, or severe obesity). As high as 51% for liver or gallbladder cancer and 49.2% for endometrial cancer in women and 48.8% for liver or gallbladder cancer and 30.6% for esophageal adenocarcinoma in males were among the percentages of cases where increased body weight was blamed (16). According to a recent study by Sung *et al.*, increased body weight contributed to almost 3.9% of all cancer cases globally in 2012 (544,300 cases), with women bearing a greater share of the burden (368,500 cases) than males (175,800 cases). Less than 1% of malignancies related to excess body weight occurred in low-income nations, while 7% to 8% occurred in several high-income Western nations as well as Middle Eastern and Northern African nations (17).

In several studies, it was discovered that obese individuals who reduced weight had lower rates of breast, endometrial, colon, and prostate cancers. For instance, a major prospective study of postmenopausal women found that an intentional weight loss of more than 5% was linked to a decreased risk of malignancies linked to obesity, including endometrial cancer. Unintentional weight reduction, however, was not linked in this study to a higher risk of cancer (18). Teras *et al.*, found that sustained weight loss was linked to a decreased risk of breast cancer among women more than 50 years old (19). Other scientific research has shown that bariatric surgery reduces

the risk of cancer overall, of hormone-related cancers including breast, endometrial, and prostate cancers, and of obesity-related malignancies such as postmenopausal breast cancer, endometrial cancer, and colon cancer in people with obesity, especially in women (20-22).

In conclusion, there is considerable evidence linking cancer, insulin resistance, adipokines, and chronic inflammation brought on by obesity. Determining the ontological function of adipocytokines and their interplay in obesity-related cancer etiology requires additional basic and translational study. It is anticipated that more prospective and long-term research would identify a wide range of obesity-related biomarkers and assess their clinical value in cancer prognosis and monitoring. With regard to public health, lifestyle therapies like weight loss, increased physical activity, dietary changes, and bariatric surgery may be able to reverse the malfunction and inflammation of the adipose tissue that is linked to obesity and may slow the development of cancer.

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EDITORIAL

ARTIFICIAL INTELLIGENCE IN CARDIOLOGY

"Artificial Intelligence is the new electricity"
Andrew Ng

Traditional medicine was, and still is, based on history, physical signs, routine tests, imaging and molecular data. In each stage of the process for the final diagnosis and the treatment plan medical thinking is a real help. Medical thinking over time has evolved in parallel with the evolution of medical science.

Training for effective medical thinking need years of study, and the elaboration of the diagnosis also consumes time, and in some fields, we need experts (other preparation time, another time to elaborate the diagnosis). Of course, we have to admit that medical thinking has a subjective side.

The great medical achievements of the 20th century was the following: electrocardiogram-1903-Einthoven; cardiac catheterization -1929/1941 – Forssman / Cournand; echocardiography -1954 - Elder and Hertz; coronary angiography -1958-Sones; invasive cardiology -1978 - Gruentzig. Computed tomography, magnetic resonance imaging, optical coherence tomography can be added...

The 21st century, in the first 2 decades, is dominated by two scientific achievements: genetic and artificial intelligence.

What is artificial intelligence?

There are many definitions, but this one seems to be the clearest: "... computer systems performing the tasks that normally require human intelligence" (1).

The avalanche of data, the "flood of data", which exceeds the power of human imagination, is stored by computers with a capacity that is hard to imagine (every day 2.5 exabytes (2.5×10^{18}bytes) are created and can thus be stored over ½ of all the words ever spoken in the whole world (including telephone conversations) (2) .

This data has been called "**big data**". (fig 1)

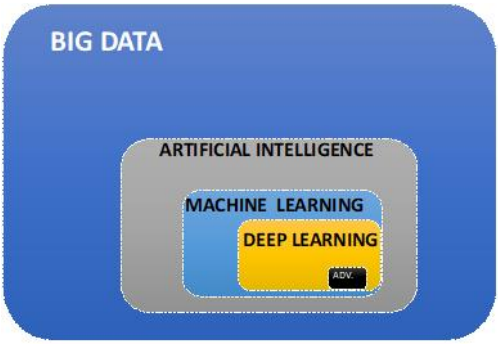

Fig. 1. Components of the artificial intelligence. ADV-advance deep learning

In this „avalanche of data" **Artificial intelligence** (AI), is programmed to imitate human behavior, so AI works with human-made programs and algorithm. Within AI has been developed so called automatic learning, **machine learning**, when computers learn without programs, through examples from the hundreds and thousands of received information (e.g., electrocardiograms, computed tomography or other data).

Here man has only the role of supply of data in the sense that he provides the examples to the system. The human being trains the system, and the system no longer needs programs to function.

Later a complex network of neurons, artificial neurons, was developed, neurons that transmit information from one to another, gathered from the immensity of data, information that they are processed by the artificial neuron network without being understood by human intelligence. This stage is known as **deep learning.**

In deep learning the information collected from different sources enters the system, the information is processed and exchanged with other neurons from many layers and finally conclusions (output) are drawn, and decisions are made (fig. 2).

Conclusions are drawn, quite good, even very good. Nobody knows, nobody understands until today how to get a certain conclusion. For this reason, this part is known as **black box** and creates a big headache for everyone. **As AI becomes more advanced, it is less understood by humans**. Recently a new sub-group called **advanced deep learning** has emerged ! (3).

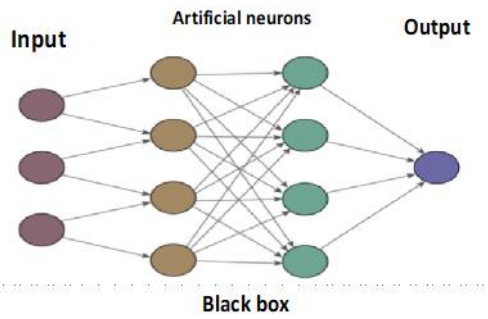

Fig 2. ***Deep learning***. The data entering the system are processed by the neural network and the result is released. It is not known how the result is reached (3)

Although this concept of AI was started in the 1950s by Alan Turing and amplified by Jack Good in the 1970 its development has been done in recent years (because the computers were not up to store and process a big data) with increased velocity (4).

Today AI has 3 major applications in medicine: a) disease prevention and diagnosis; b) faster development of medicines and vaccines (see the anti-COVID-19 vaccine) and c) gene editing.

We will discuss here the relationship between AI and cardiology.

AI has many applications in cardiology in this time and especially in the near future.

Thus, AI can read images or analyses ventricular function, to diagnose valvular heart diseases, arrhythmias, congenital heart diseases reading electrocardiography (fig. 3).

Electrocardiography. Its interpretation can be time-consuming and challenging at times. Automated ECG interpretation is widely used, from many years, with various limitation and over-reading.

Some years ago, in 2002, with my colleague Dr. Ioana Stoian, we have published a book on electrocardiography (5) where one chapter (64 pages), out of the 4 chapters of the book, was dedicated to the electrocardiographic traces read by the computer. We were drawing attention to the erroneous interpretations of computer algorithms and inviting doctors to learn the electrocardiogram well.

Today things are different, apart from the automatic good reading of the electrocardiogram, AI can recognize valvular heart diseases, congenital heart diseases, rhythm disorders, cardiomyopathies and even ventricular function !

Atrial fibrillation is one of the most

common arrhythmias.

Today various mobile devices can be used for detection of atrial fibrillation, these include smartphones, smart bands or smartwatches and earlobe sensors (6).

AI can interpret electrocardiograms during sinus rhythm and was able to find out whether a patient had experienced atrial fibrillation previously and whether it would occur latter (7). This, like other "achievements" of AI, is very curious and still difficult to understand for me.

For many years AI has been a real help in electrophysiology studies.

AI and ECG can recognise left ventricular ejection fraction. Z. Attia *et al.* tested a set of 52.870 patients with ventricular dysfunction (EF <35%), with 12-lead ECG and showed sensitivity 86.3%, specificity 85.7% and accuracy 85.7%. (8).

The ability of the algorithm to read an ECG and see if the FE is low is very important for daily practice, for prevention and for medical research.

Another example: one program was instructed to identify the presence of a low left ventricular ejection fraction from 100,000 ECG (plus other bonus data).

The result was 0.93 (area under the curve), very good! In a recent randomized trial published in Nature Medicine, Yao and colleagues illustrate the power of utilizing AI-enabled ECGs to identify individuals with reduced heart function (9).

Now, using AI-assisted interpretation of ECGs are able to tell us much more. The electrocardiogram with the help of AI can indicate a certain valvular heart disease, for example aortic stenosis (10) mitral stenosis and can evaluate pulmonary hypertension (11, 12) (fig. 3).

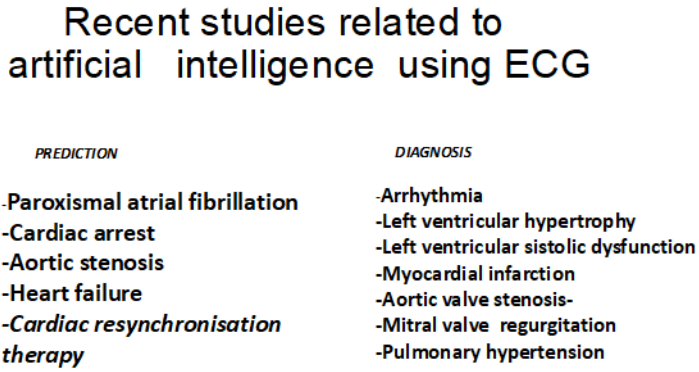

Fig. 3. Recent studies related to artificial intelligence using electrocardiography

Cohen-Shelly *et al.* (10) developed and validated an AI model for detecting aortic stenosis using electrocardiography, they have shown that AI using ECG can identify patients with moderate or severe aortic stenosis and might be able to predict developing aortic stenosis! They have screen ECG and Echo 258,607 patients. In the test group, (102,926 (40%) randomly selected subjects) the AI-ECG patients was positive with the area under the curve of 0.85. The sensitivity, specificity, and accuracy were 78%, 74%, and 74%, respectively (fig. 4).

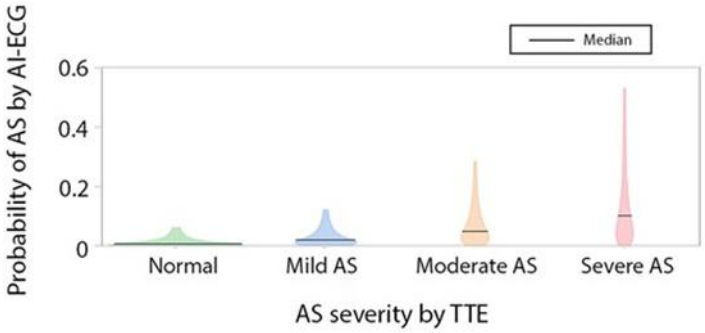

Fig. 4. The probability of aortic stenosis positive labelled by AI-ECG compared with Echo (25) AS-aortic stenosis; TTE- transesophageal echocardiography

Cardiovascular imaging

The first applications of AI in cardiology were possible in digitally acquired images including Echocardiography, Cardiac CT (Computed Tomography) and MRI (Magnetic Resonance Imaging) as well as nuclear imaging (13).

Echocardiography

Echocardiography as a technique has advanced very quickly thanks to computers and advance as an interpretation thanks to artificial intelligence. The results of the echocardiographic examination depend, till now, on the echocardiographer and for this reason this examination has an important subjective part.

Today AI has changed that. Cavity size, wall thickness, ejection fraction and multi-parametric Doppler are automatically calculated and displayed (14).

Now with the aid of AI some diseases are also recognized, such as hypertrophic cardiomyopathy, constrictive pericarditis. Very important for a clinician is the differential diagnosis between constrictive pericarditis and restrictive cardiomyopathy (very difficult diagnosis) and between hypertrophic cardiomyopathy and athletic myocardial hypertrophy.

Cardiac computed tomography (CCT)

CCT is used often for coronary angiography and specially to determine the location and extent of atheromatous plaques in the coronary vasculature as well as for calcium scoring.

Motwani *et al.*, have studied 10,030 patients with suspected coronary artery disease with CT angiography and follow-up the patients during a 5-years. 25 clinical and 44 CT angiography parameters were measured. The AI approach was a significantly better predictor of a 5-year mortality, in comparison to the clinical or CT angiography alone (16).

Recently, in a multicenter study, was developed and validated a deep system for measures of plaque volume and stenosis severity (17). Another research has been used AI for automatic estimation of CT coronary angiography fractional flow reserve (FFR) with good agreement between AI derived values and those which were invasively measured (18).

Cardiac Magnetic Resonance (CMR) is used for the diagnosis of cardiomyopathies, congenital heart disease, valvular heart disease, ischemic heart diseases, pericardial lesions, and cardiac tumors. AI can help to a more efficient scanning and accurate interpretation process: image construction,

image segmentation and image quality control (19).

In a recent trial of 1,348 patients with hypertrophic cardiomyopathy, a new AI driven CMR technology named virtual native enhancement (VNE), was used to generate images identical to the standard Late Gadolinium Enhancement (LGE), without the need of a gadolinium-based contrast agent. VNE resembles conventional LGE but does not require intravenous access or administration of contrast ! (20).

*Cardiac resynchronization therapy (CRT***)**

Conventionally, patients eligible for CRT implantation have greater benefit on reduction of mortality and should have an ECG morphology with Left Bundle Branch Block and QRS duration ≥ 150 ms. Around 30% of patients meeting these criteria and receiving an implant, do not experience clinical benefit from CRT. A model, with the aid of AI, for better discrimination of the risk (mortality) were developed for prediction of evolution post-CRT and with a 2-fold change in survival (21).

Other applications

An algorithm for heart murmur detection was developed: pathologic cases were identified with sensitivity of 93%, specificity of 81%, and accuracy of 88%. AI has a potentially useful screening tool for valvular heart disease in a general population.

A recent interesting example: a group of Chinese scientists developed a convolutional neural network (CNN) which can detect coronary artery stenosis > 50%, (stenosis documented by angiography)), *via* analyzing the patient's facial photo (!) to 5,796 patients with an 85% sensitivity and 54% specificity (higher than the standard prediction scores) (22).

Single-photon emission computed tomography (SPECT) stress testing. AI methodology has been applied in this modality, to improve image acquisition, image reconstruction and automated quantitation (3)

I would like to make a clarification here. I don't want it to be remembered that I have unlimited confidence in AI, that all the data is correct, unchangeable. I am convinced that AI brings support to our activity, to life in general and to medicine in particular, but we must be careful about limits, exaggerations, dysfunctionalities and the impact on people and, in our case, on doctors. AI should act to support and not replace the physician, but as Erick Topol says that "doctors who will use new technologies & AI will replace doctors who will not use it" (23)

Remains to be discussed who is responsible for the AI wrong results.

The application of AI in Cardiology is still in its infancy. It is expected clinical validation, implementation as well as regulation. Food and Drug Administration (FDA) has released a regulatory framework to establish safe and effective AI- based medical devices. (24). The European Union has also proposed a regulatory framework on the use of AI - technology (25).

In the future, AI will have an important role in the prevention of diseases and in the treatment of the diseases as early and efficiently as possible.

Cardiologists should educate themselves in the development of AI and utilize them in their practice.

There are many ongoing studies/trials regarding AI and cardiology in different fields such as: preventive cardiology, cardiovascular imaging, electrocardiography, heart failure, interventional cardiology and we are waiting for the results in the follow-

ing years (26)

The role of doctor in the era of AI

Doctors will have to remain the ones to make the final decision

For patients doctors will provide explanations, humanity, empathy.

The question is whether in the future will be better than today? Possible, if we consider that the medical error is a third cause of death after cardiovascular diseases and cancer (27)

In conclusion, medical thinking still proves its value, and we have an obligation to develop it and induce it to the younger generations.

It is clear that AI exhibits significant potential in the field of cardiology, it can improve diagnostic accuracy and support clinical decisions for many diseases.

Artificial intelligence in the future will be an integral part of medicine.

It is important to prepare the new generation of doctors and the new generation of teachers regarding the concept of AI and the usefulness of this concept.

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EDITORIAL

SUDDEN DEATH IN THE CARDIOLOGIST'S VIEW - BETWEEN LITERATURE AND EPIDEMIOLOGY

I have chosen a topic that is most often talked about in whispers. Why ? Because both, whispering and silence hide fear. All of us, we must admit, are afraid of death. Death is a problem for us doctors, only to the extent that we face it most often, being obliged to "ascertain" and "declare" it.

I have been a cardiologist for over 30 years in an emergency hospital where, from the first time witnessing a dying patient, I had the strange feeling that death is lurking around the corner and that, in fact, we are working with it next to us.

The problem of death and its analysis as a phenomenon, concerned both the philosophers of antiquity and those of our days, writers, poets, psychoanalysts. Allow me to revive one of the now famous quotes of Seneca which said that "from the moment we are born, time begins to take back our life". I've lived ever since dominated by the same feeling that sometimes, no matter what we do, what we think, we know how to do, and even if we are doing everything, death comes when it wants. "Nothing is more certain than death, nothing more uncertain than its moment" (said one the most famous Romanian writers - Liviu Rebreanu).

Many centuries later after Seneca, Sigmund Freud, who is considered the founder of psychoanalysis, stated that "The purpose of life is death", a phrase which, beyond the philosophical substrate, in turn caused a wave of confusion. Sometimes we experience the feeling of uselessness in a world where everything seems to be organized by others and we just keep moving forward. Sometimes the disease has its own course that doesn't seem to be influenced by "how well prepared" we are. "I did everything" seems meant to excuse us only in front of our own conscience, in front of those around us, in the eyes of divinity. In fact, no one really knows what "Everything" means and where it stops.

"I think I got deathly ill the day I was born" - the poet Marin Sorescu said very beautifully in his poem entitled "Illness". Comparing his own life with a relentless, incurable disease, the poet does not express his feelings, but suggests them. Moreover, Sorescu's poetry is impregnated not only with symbolic elements, but also with Parnassian elements. Life, death and the state between the two are deeply and precisely sectioned and presented. Borrowing the ideas and concepts of the great philosopher Artur Schopenhauer, from ideas published in "The World as Will and Representation", he does not consider that there is a clear opposition between life and death, but on the contrary - they intertwine: death contaminates life, without necessarily ending it.

From a medical point of view, sudden death is defined as a "natural, cardiac death, characterized by a sudden, rapid and unexpected loss of consciousness, occurring within the first hour after the onset of symptoms of an illness, in persons with known illnesses or unknown". Pre-existing

heart disease may be known but the time and manner of death are unexpected. Controversy exists and remains as to the circumstances in which an unexpected death can be called "sudden" and how the cardiac nature of the cause death can be proven. Sometimes sudden death can be preceded by prodromal phenomena, vaguely defined as malaise, weakness, sometimes palpitations, convulsions but sometimes they are absent, especially in the case of deaths that occur during sleep. Cardiac arrest is not equivalent to death. The latter means brain death or in medical terms - failure of resuscitation, absence of electrical / mechanical activity of the heart and absence of brain activity after cardiorespiratory resuscitation maneuvers (1).

The fundamental concepts on which the definition of sudden death is based are the non-traumatic nature of the event and the fact that the death must be unexpected and instantaneous (quick, max one hour). In a very simple classification for current medical practice, sudden cardiac death can be "coronary" or "non-coronary", starting from the premise that myocardial infarction continues to remain the most common cause of sudden death in adults.

The most important and unequaled cause of death in the adult population in the industrialized world is sudden cardiac death caused by coronary heart disease. In approximately 5-10% of cases, sudden cardiac death (SCD) occurs in the absence of ischemic heart disease or congestive heart failure.

They are excluded from the definition of sudden death - "Instant deaths" that occur quickly and unexpectedly in the evolution of severe diseases in which death is predictable (e.g., neoplasia, cirrhosis) or of states of hemodynamic or biological imbalance, as well as "Violent deaths" by accident or post-traumatic etc.

If we talk about the incidence, according to the statistics in the USA, a number of 300,000 - 350,000 SCDs are estimated annually, and according to the reports of the European Society of Cardiology, sudden cardiac death has an incidence between 0.36 and 1.28 per 1,000 inhabitants, with the mention that only victims examined or resuscitated by emergency medical services were included in these studies and that the data underestimate the true incidence in the general population (1). The incidence is also higher among men aged 60 to 69 with a history of cardiovascular disease, with rates of up to 8 per 1,000 per year reported. In Maastricht, a population-based study that followed all cases of cardiac arrest occurring in victims between 20 and 75 years of age recorded a total annual incidence of SCD of 1 in 1,000 per year. Overall, 21% of all deaths in men were sudden and unexpected compared to 14.5% in women. According to the same statistics, 80% of out-of-hospital cases occurred at home and approximately 15% on the street or in public spaces, and 40% of all cases of SCD occurred without witnesses. The incidence is higher in industrialized countries (which seems logical, especially related to the stress factor associated with an unhealthy lifestyle) and lower in underdeveloped or developing countries (2, 3).

Statistics estimate that 65% of SCD occur outside the hospital, of which 80% occur at home or during transport and 15% - in public places. There is also talk of a circadian mood, SCD being more frequent in the early hours of the morning (6-10 a.m.). The annual incidence in the general population is estimated at 1-2/1,000 people, but it increases 10 times in those with in-

creased cardiovascular risk. The maximum incidence registers 2 peaks: the first between birth and the age of 6 months (sudden infant death syndrome), and then drops steeply, remaining at a low level throughout childhood and adolescence and begins to increase in young adults, reaching a second peak between 45 and 75 years old. Aging is an important risk factor for sudden cardiac death. From 1 to 13 years, only one in five sudden natural deaths is due to cardiac causes. Between 14 and 21 years old, the proportion increases to 30%, and then to 88% for middle-aged and elderly people. Young and middle-aged men and women have different susceptibilities to SCD, but the gender difference disappears with advancing age. The total male/female ratio is approximately 4:1, but in the 45-64 age group, SCD in men increases and the ratio is almost 7:1. This percentage drops to 2:1 between 65 and 74 years old (2, 3).

The difference in SCD risk parallels the risk for other manifestations of coronary heart disease in men and women. There are also differences by race, with the incidence of SCD being higher in Caucasian men than non-Caucasian men for the 55-64 and 65-74 age groups (2-4).

If we were to systematize the production mechanisms of SCDs, which are otherwise very complex, we can talk about 2 types of anomalies: pre-existing cardiac structural anomalies that form in the vulnerable substrate and transient functional factors that can transform a long-term, clinically latent structural anomaly , in a manifesto.

We are also talking about factors that are associated with an increased risk of SCD: advanced age, male sex, black race, family history of ICD, elevated LDL-cholesterol, hypertension, smoking, diabetes, increased heart rate, increased heart rate variability, excessive alcohol consumption, ECG/echo signs of LVH. Risk categories are described: Severe left ventricle (LV) dysfunction (LVEF below 35%), ischemic heart disease, history of AMI, history of malignant ventricular rhythm disorders.

In recent years, there has been discussion about the intervention of the genetic factor constituting what we call - The genetic bases of SCD, which are incriminated in a series of syndromes such as long QT syndrome, Brugada syndrome, obstructive-hypertrophic cardiomyopathy, arrhythmogenic right ventricle dysplasia, catecholaminergic polymorphic ventricular tachycardia , certain types of dilated cardiomyopathy. "The family environment, including dietary, psychological and developmental factors" may play a particular role in determining this aggregated susceptibility in family members. "Rare" variant of DNA, one or more polymorphisms (variants common DNA) (5).

"*Death stops both love and fights. Everyone remains only with how much he loved or how much he fought. He still has time, maybe, just to regret that he didn't love and fight enough..." (Octavian Paler)*

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EDITORIAL

COLORECTAL CANCER: IT'S TIME TO A CALL FOR ACTION AMONG YOUNG ADULTS

Currently, colorectal cancer (CRC) is the fourth most prevalent malignancy in both genders in United States of America, with approximately 150,000 new cases identified each year. However, over the past four decades, the overall incidence rate of CRC has considerably decreased (1, 2). Colonoscopy, the primary screening method for CRC, enables the identification and removal of colon polyps before they become malignant. The incidence rates of CRC in persons over 50 years have mostly been attributed to interrupting the adenoma-carcinoma sequence (3). Malignant colorectal malignancies could be found and removed at an early stage, together with the removal of the polyps, to reduce colorectal cancer mortality, by reducing the occurrence of precancerous lesions that would otherwise turn into cancer. In fact, studies using flexible sigmoidoscopy (18-26% in intention-to-screen analyses and 31-35% in per-protocol analyses, in RCTs), colonoscopy (68% in a meta-analysis of observational studies), and fecal immunochemical tests (10-22% in observational studies) have also shown decreased colorectal cancer incidence (4). In contrast, the prevalence of CRC in young patients has been progressively rising every year. In the past ten years, patients with CRC who were diagnosed while they were under 50 years old have formed the young-onset or early-onset CRC patient cohort. The term "young-onset" or "early-onset" colorectal cancer (CRC) refers to CRC that appears in individuals under 50 years who, until recently, did not meet the typical age criteria for average risk screening in the United States (3). This is concerning for a number of reasons. Firstly, this patient group is not routinely examined for colorectal cancer (CRC), as the conventional suggestion is for CRC screening to begin at age 50. Secondly, as we have learned over the past ten years, the concern extends beyond a delay in diagnosis and considers if there are several genetic and environmental factors that distinguish this group of young CRC patients (5).

When CRC incidence was examined using epidemiological data from 1974 to 2013, it was discovered that adults aged 20 to 39 had an annual increase in incidence of 2.4%, while adults aged 40 to 49 had an annual rise in incidence of 1.3% in the mid-1990s. There was a 3.3% yearly drop in the incidence rates of CRC in people over 65 between 2012 and 2016. During the same time period, the incidence rate of CRC among patients under 50 increased by 2.2% year, with people between the ages of 20 and 34 experiencing the largest increases (6). The second most common type of cancer in men under 50, and the fourth most common in women, is CRC at the moment. Recent estimates indicate that by 2030, the incidence rates of rectal and colon cancer in this population will both increase by 124.2% and 90%, respectively. Rectal cancer incidence rates have increased annually by 2.3% in adults aged 40 to 49 over the 1990s and by 3.2% in people aged 20 to 39 when compared to proximal colon cancer incidence rates. In patients under the age of 50, approximately 1 in 10 cases of colon cancer and 1 in

4 cases of rectal cancer are predicted to be diagnosed by 2030 (7). Despite a decline in CRC mortality rates among older persons, an estimated 1.9 deaths per 100000 people were projected to have occurred in 2018. Several countries, including Canada, United Kingdom, Sweden, Switzerland, Italy, Australia, Egypt, and Asia, have all documented international tendencies that are similar to the aforementioned USA trends (2, 8).

There are significant racial and ethnic differences in the incidence of CRC in the United States (5). Wallace *et al.* reported that in contrast to 8.6% of non-Hispanic whites and Japanese, 21% of South Asians and 20% of Southeast Asians were diagnosed with colorectal cancer (CRC) before to the age of 50. The study examined patterns in the most racially and culturally diverse country's CRC frequency. When they were diagnosed, two thirds of those under 50 had a late-stage illness (regional or distant). Additionally, the research found that non-Hispanic Black Americans had lower overall survival rates and a higher than expected frequency of CRC diagnosis (9). When it came to late-stage sickness, non-Hispanic black Americans had a 40% higher prevalence than non-Hispanic white Americans. Between 1990 and 2014, South-East Asian communities saw a rise in the incidence of colorectal cancer, in contrast to the general population (10).

The biggest identified risk factor for the disease is a family history of CRC. There was a family history of CRC in 23–39% of patients with young-onset CRC, according to estimates. There is a two- to four-fold increased risk of having CRC in those with first-degree relatives who have the condition, and this risk increases with younger age at diagnosis (11). CRC risk is increased by having numerous affected first-degree and distant relatives with an adenoma's family history (12). Globally, the incidence of CRC in young adults has increased, which is assumed to be due to a major birth cohort impact, which effects occur when age-specific incidence rates vary by age group as a result of a change in exposure to variables (behavioral, cultural, lifestyle, and environmental variables that affect disease risk) (13). A typical "Western" diet, which is high in fast food, processed meat, and red meat, as well as a lack of exercise and consumption of fruits and vegetables, is linked to an elevated risk of CRC (1). The screening level of CRC risk was achieved 5 years faster in diabetic patients than in a 50-year-old man or woman without diabetes, according to a national longitudinal study including 12.6 million Swedish inhabitants between 1964 and 2015. This result enabled researchers to estimate how much faster patients with diabetes would develop the 10-year cumulative risk of CRC than a 50-year-old man or woman without diabetes (14).

According to a study by Glover *et al.*, patients with colorectal cancer (CRC) who were between the ages of 20 and 39 had higher rates of medical comorbidities, such as diabetes, obesity, or smoking, and the greater prevalence of malignant lesions in youth may or may not be caused by behavioral variables (15). The risk of CRC is also increased by a factor of two to three by inflammatory bowel disease (IBD), and the likelihood of APC mutations is lower in young-onset CRC patients with IBD and higher in those with mucinous or signet ring histology (16).

Caucasian women are more likely than black women to develop CRC at an early age. Although colorectal cancer (CRC) usually has no symptoms, there are warning signs that could include changes in stool

diameter, unexplained anemia, and rectal bleeding. The most common initial signs of CRC with a young onset include rectal bleeding/hematochezia, altered bowel habits, stomach discomfort, anemia, decreased appetite, and weight loss. Per the research, the diagnosis of colorectal cancer (CRC) is postponed in those under 50 years of age by seven weeks to two years. Clinicians who treat or refer patients for anorectal evaluation (e.g., hemorrhoids) or who think the condition is benign, similar to irritable bowel syndrome, may have a low degree of suspicion for colorectal cancer (17). Often, younger-onset colorectal cancers (CRCs) originate away from the rectum or splenic flexure and are less likely to have a prior adenomatous lesion (11). Younger people with colorectal cancer also seems to be diagnosed with synchronous or metachronous malignancies more frequently than older patients with colorectal cancer, and they also appear to be diagnosed at a later stage (presenting with distant or regional metastases) (16).

The American Cancer Society announced recommendations for CRC screening for persons at average risk starting at age 45 in May 2018 (18). Moreover, other Americans oncological societies most recently increased its recommendations for CRC screening standards to encompass individuals between ages 45-49. In additions they advise starting screening for persons who have a family history of CRC 10 years before the youngest affected relative is diagnosed with the disease or at age 40, whichever comes first (5). In European countries, the implementation of screening programs begins around age of 50 or above, since older persons are more likely to get colorectal cancer, a population screening program for those under 50 years old may not be practical (19). Moreover, a recent study published by Cardoso *et al*. found that nations with long-standing screening programs and coverage of more than 50% of the eligible population saw significant decreases in colorectal cancer incidence. In nations with no screening programs in place, incidence rates were either steady or gradually rising (4).

It has been shown that young people with CRC experience a delay in diagnosis and are identified at later stages of the disease. Clinicians must keep a close eye out for the warning signs of CRC, as well as any family history and genetic abnormalities, in order to effectively assess, identify, and treat CRC in young adults. Therefore, population-based studies are required to both examine the significance of conventional risk factors in young-onset CRC and to find lifestyle and environmental factors linked to the disease. Finding the fraction of young people who might benefit from colorectal screening while minimizing the cost burden on society is the challenge of the future.

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EDITORIAL

ANTIBIOTIC RESISTANCE: A NEW RISING SILENT PANDEMIC

Antimicrobial resistance (AMR) poses a significant global public health threat, ranking in the top 10 challenges faced by humanity. The primary factors contributing to the emergence of antimicrobial resistance in pathogenic bacteria are the inappropriate usage and excessive use of antimicrobials, which leads to the proliferation of drug-resistant infections. Inadequate infection control, clean water, and sanitation deficiencies exacerbate the establishment and dissemination of AMR bacteria (1). The presence of antibiotic resistance results in extended hospital stays, increased death rates, and a substantial financial burden. The presence of resistant bacteria has hindered our capacity to effectively address novel and emerging infections. The rate at which microorganisms develop resistance mechanisms is affected by the excessive usage and incorrect use of antibiotics, in addition to their natural acquisition of resistance (2).

While antibiotics are widespread substances, it is unlikely that environmental bacteria, commensals, and human and animal diseases will encounter the same varieties of antibiotics. Pathogenic bacteria are more likely to encounter therapeutic doses of antibiotics that are of medical significance. In their study, Darmancier *et al.* conducted a thorough bioinformatic analysis of 16,632 full bacterial reference genome sequences. The objective was to determine if there is a correlation between bacterial virulence and antibiotic resistance. They discovered data indicating that certain types of genes related to disease severity and resistance to antibiotics could be simultaneously favored and that mobile genetic components, such as integrative and conjugative elements, could have a significant role in facilitating their transfer. The emergence of antibiotic resistance in human infections, which necessitates specialized medications for treatment, has become a significant barrier to effectively treating diseases that pose a hazard to humans (3). This collection compiles papers on antibiotic resistance in several significant emerging infectious pathogens, including *Mycobacteroides* abscessus, *Klebsiella pneumoniae*, *Salmonella sp*., *Francisella tularensis*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. It also covers antibiotic resistance in other human pathogens such as *Neisseria gonorrheae*, *Staphylococcus aureus*, and *Escherichia coli*. Most of these diseases are classified as high priority by the National Institute of Allergy and Infectious Diseases, and they are considered internationally and medically significant (4).

Despite continuous endeavors to reduce the excessive utilization of antibiotic treatments, the prescription of antibiotics remains unavoidable, particularly in severe instances such as bacterial co-infections or secondary infections accompanying another illness, to guarantee the complete eradication of the pathogen load. Lower- and upper-respiratory tract viral infections greatly increase the likelihood of bacterial infections in patients. This is due to several factors: the viruses create more opportunities for bacteria to attach to surfaces, they weaken the immune system's ability to respond, and they cause damage to cells and tissues, which makes it easier for bacteria to spread and cause severe infection (5, 6).

Secondary bacterial infections, such as those caused by *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *S. aureus*, and *Haemophilus influenzae*, can further exacerbate viral disorders. It should be emphasized that bacterial co-infections are less common than secondary infections (7). Out of a sample size of approximately 1,400 individuals with COVID-19, Wang *et al.* found that 2.7% of individuals were identified as having a concurrent bacterial infection with the viral illness (8). Another study by Chen *et al.* described that 5.1% of the cases (5 out of 99) of COVID-19 had co-infections, namely involving *Acinetobacter baumannii* and *Klebsiella pneumoniae* (9). In a comprehensive study conducted by Blasco *et al.*, involving a total of 221 patients diagnosed with COVID-19, it was shown that 25.8% of these patients (57 out of 221) had co-infections. Furthermore, among these co-infected cases, 29.8% were specifically identified as having bacterial co-infections (10). Therefore, there has been a rise in the occurrence of hospital-acquired infections and sepsis during the COVID-19 pandemic, highlighting the significance of readily available testing and diagnostics for both co-infections and secondary infections. However, the availability of such testing has been restricted during the pandemic. An important obstacle lies in the inconsistent rate of diagnosed co-infections and secondary infections, which highlights the lack of effective diagnostics for these illnesses. This is particularly evident in the absence of rapid identification of bacteria and resistances, even when hospitals are under strain or testing is required in settings with limited resources (11).

Although antibiotic treatments have proven to be effective, the heightened utilization of antibiotics in COVID-19 hospitalizations during this pandemic is expected to exert significant selective pressure on bacterial pathogens, prompting them to develop resistance. Consequently, there will likely be a rise in the occurrence of drug-resistant bacterial infections (12). The bacterial infections reported in COVID-19 patients consist of Streptococcus pneumoniae, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. According to the Center for Disease Control and Prevention of the United States of America, these diseases are also among the most common and life-threatening cases of antibiotic resistance (13).

Engineering assays that promptly differentiate between viral and bacterial infections would reduce the wasteful use of antibiotics by preventing their preemptive prescription. This is in addition to the development of diagnostics for microbial pathogens. Traditional diagnostic techniques for bacterial infections include genotypic methods that identify resistance genes or immunological assays like ELISA (enzyme-linked immunosorbent assay) that rely on interactions between antigens and antibodies. A significant limitation of conventional methods is their time-consuming nature and the need for centralized laboratory facilities (14). In addition, genotypic approaches cannot identify antimicrobial susceptibility and growing resistance patterns in cases where a specific gene has not been identified. Biosensing technologies have shown considerable promise in enabling fast point-of-care testing using affordable devices (15).

The articles in this special edition concentrate on newly identified and recurring infections, as well as the antimicrobial resistance (AMR) linked to them. From our perspective, comprehending AMR and its present progression is of the highest priority. The discoveries in these publications could additionally aid in the advancement of diag-

nostics, therapeutics, and prevention measures to control and alleviate the risks posed by infectious diseases, while also ensuring the sustainability and safety of food and the environment. We anticipate that these publications will help and provoke thought among readers engaged in the topic of antimicrobial resistance (AMR).

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EDITORIAL

THE MEMORY OF PROFESSOR VALERIU RUSU, M.D., Ph.D., GUIDING LIGHT IN THE DEVELOPMENT OF NUCLEAR IMAGING DOMAIN: TEN YEARS OF A FULFILLED LEGACY IN THE FIELD OF NUCLEAR MEDICINE AT IASI

It has already passed, as if in an instant, ten years since the premature disappearance of the distinguished Professor Valeriu Rusu M.D., Ph.D., the mentor of nuclear medicine in Iasi. Many things remain encrypted in his mind, an unwritten encyclopedia, which gone, unfortunately, with the being. But there are also many essential things that have remained here, with us, through the school he formed around him.

The master's words are our legacy: *"I have other projects, also, but their completion does not depend only on me... I don't know if time will be patient".* And even if time ran out of patience, unfairly quickly, the teacher's words turned into a duty of honor for the collaborators he trained, professionally and morally, and the Professor's projects were and are being carried forward, developed and successfully completed.

The Nuclear Medicine Laboratory *"Professor Valeriu Rusu, M.D., Ph.D.*" currently bears his name and keeps his memory alive. It could not have been otherwise, because it was developed and formed by the great professor to be the place where, from the whole country, all SPECT vector molecules imported into Romania are always used the most and the newest techniques in the field of nuclear imaging are introduced, for the final benefit of the patient.

It was a difficult 10 years period for the laboratory which initially, through the immense effort of the Professor, was equipped with two SPECT gamma camera devices, innovative for the endowment years, 1990-2000, which allowed it to develop important projects and publications in the field, at a level that quickly brought the Iasi university center to first place in the country in the field of functional imaging research, devices that, obviously, would wear out physically and morally in over 20 years. There followed memos upon memos, attempts, often unsuccessful, repeated with the perseverance learned from the mentor, so that, in the end, they were successful in renewing the equipment to world standards.

The laboratory has developed and, today, is equipped, as a result of a project co-financed from the European Regional Development Fund through the Competitiveness Operational Program 2014-2020, with the only SPECT-CT gamma camera in our university center and one of the 2 in Moldova; along with a SPECT gamma camera, maintained with seriousness and care by the laboratory staff, as the teacher taught us, currently used for Pediatric Nephrology - the center being the only one in the country where the use of functional imaging for the benefit of pediatric nephrology pathology is developed at international standards. Within the laboratory, the first center in the country was established, which aims to use artificial intelligence for the benefit of precision medicine, starting from imaging data, MEDIMAG IA (Regional Center of Excel-

lence for Personalized Diagnosis and Artificial Intelligence in Medicine and Imaging), which creates the premises for international collaborations that will contribute both to increasing the visibility of our university and of region, for the final benefit of the patient.

These are standards that keep nuclear medicine from our university center where our master taught us that it should be: a spearhead in fundamental and applied research in the field of nuclear imaging in our country and in understanding images through biophysical uptake mechanisms of radiopharmaceuticals, according to the French model - the most logical model, France being the country where the professor completed his professional training, through the famous governmental scholarship, Bourse de Collège de Médecine des Hôpitaux de Paris, and where he, later, guided the steps of 3 collaborators, 2 of them currently continuing in the field, demonstrating care for the formation of a school of nuclear medicine that will continue the development of the field, in Iasi. It remains, even currently, the laboratory with the most teaching staff in the field, in the country and one of the only 2 centers in the country that coordinate doctoral studies.

As a result of all these arguments, starting from the foundations created by the Professor and through the openness that the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi and the "Sf. Spiridon" County Clinical Emergency Hospital had in encouraging this action, Iasi is currently the only university center where functional imaging is the nucleus around which the only cluster dedicated to medical imaging in the country has developed, the IMAGO-MOL Cluster, which militates, together with University and through the collaboration of over 70 members (universities, research institutes, private members), for increasing the visibility of nuclear imaging in synchronism with artificial intelligence, through European projects and publications in prestigious journals, and through international collaborations, starting with student exchanges from the most distant countries (such as Brazil, Mexico or Japan), through involvement in international scientific consortia.

We would not have succeeded in fulfilling this duty of honor, regarding the endowment at world standards, in one year, as long as the project lasted, if we had not learned from our mentor the seriousness of a job well done, tenacity and perseverance doubled by professionalism, teamwork based on communication and mutual respect, surrounded in a formative way by young specialists, students, residents, PhD students in the domain, the openness to collaboration with the applied fields of nuclear imaging and, perhaps most importantly, the professional enthusiast.... all representing the legacy of the soul that the Professor left us.

The same legacy can be found in another field dear to the Master – *"The Medical-Surgical Journal of the Society of Doctors and Naturalists from Iasi"*, the oldest medical society in South-Eastern Europe. It is the place where Professor Valeriu Rusu was the Editor in Chief, for 20 years (1994-2014), of the first medical journal in Romania (1833), marking, for sure, in a positively determining way, the development of the journal. Here, the periodic meetings of the Editorial Board represented, at the same time, real lessons in critical medical reading, particularly valuable for the young part of the Editorial staff; it is also the place that has often been a silent witness to some tasty discus-

sions full of intellectual contortionism between great masters from the medical world, discussion partners of the master, discussions watched with looks full of professional enthusiasm by the other members of the collective.

The brilliance achieved by the fields he touched explains, without a doubt, what was so special in the personality of Professor Valeriu Rusu, what attracted and conquered the hundreds of students who filled his amphitheaters and all those who knew him, from where the "aura" of the so respected and beloved teacher came from.

All of them were the expression of his personality: an erudite, an encyclopedic mind that managed to give sparkle to the fields he developed, positively marking their evolution even beyond passing into non-existence. Seriousness + extreme self-demand = Professionalism, to which the teacher gave color through professional enthusiasm developed in a philosophically erudite manner, this was his essence, which he managed to induce to those he mentored, also. Because, above all, the teacher remains a great lover of the profession of educator and trainer of the minds of the many students, Ph.D. students and young specialists whom he mentored.

A righteous teacher, who was only sometimes, influenced by his own heart, a teacher who managed to find the way to the misunderstood part of each of his students, chiseling, sometimes demandingly, but always with exceptional results, their skills for medical research.

Even if time ran out of patience and the Professor left us too soon, he will remain alive in our memory, of those he guided and mentored and of all those who knew him, and through the fulfillment of the projects he had. Our mentor's projects have become the moral duty that we always try to carry out and to develop in an innovative and formative way, for those who will follow and, always, for the final benefit of the patient.

At every step, the teacher's words and memory remained our guiding light.

He looks at us from the painting on the wall of the Nuclear Medicine Laboratory, and seems to be smiling slightly, we hope satisfied with what has been achieved so far, but, as if also a bit admonitory, to take care to tirelessly continue the development of nuclear imaging, of the laboratory whose value he cherished and shepherded her all his life and of all other fields that he loved so much.

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EDITORIAL

LIVER TRANSPLANTATION: WHERE ARE WE?

Modern surgery was born in the second half of the 19th century, with the introduction of general anesthesia. Gradually, surgeons began to become more and more daring, performing operations that were not possible until then. In this context, at the beginning of the 20th century, the old dream of medicine was revived, that of completely replacing organs whose function was destroyed, with healthy ones.

The decisive step was taken by the great scientist Alexis Carrell, laureate of the Nobel Prize for medicine in 1912, who described the technique of vascular anastomoses (as it is easy to imagine, the new organ is connected to the receptor using such anastomoses).

From this moment begins a spectacular race that contributed to a huge extent to the progress of medicine in general, allowing not only the development of surgery at an unprecedented level, but also of other specialties such as anesthesia and intensive care, immunology, infectious diseases and not in lastly, internal medicine specialties such as nephrology, cardiology, hepatology, pneumology, etc.

The driving force of this race, which today is in full swing, were the surgeons, the ones who stubbornly demonstrated that the operations to replace irremediably compromised organs can be performed.

The operation was not the biggest problem in the case of the kidney, which was the first organ successfully transplanted in December 1954 at the Peter Bent Brigham Hospital in Boston by Joseph Murray and his team (the surgeon being rewarded with the Nobel Prize in 1991). Instead, at least at the beginning, it seemed that in the case of the liver, the technical problems are so great that there were opinions that the operation to replace this organ with the survival of the patient will never be possible.

At the beginning there were two great surgeons who tried to contradict these opinions: Thomas Starzl in Denver and Francis Moore in Boston (the same place where the first kidney transplant had been performed). Both surgeons performed the first liver transplant operations in the first half of 1963, but none of the transplanted patients survived. It seemed that the pessimists would be right! Nevertheless, Francis Moore was selected by Time magazine as the most important personality of that year and made the already famous cover.

Meanwhile, anesthesia and intensive care had made remarkable progress, which had considerably increased the chances of success of the operations.

On the other hand, advances in immunology allowed the identification of the phenomenon of "rejection", set up against the transplanted organ by the recipient's immune system which recognizes the new organ as "non-self".

The first therapeutic protocols for the control of rejection, based on cortisone and azathioprine (Imuran), had limited success and it was obvious that something more efficient would be necessary.

After four years in which none of the transplanted patients survived postoperatively, in 1967 Thomas Starzl obtained the great victory: the first liver transplantation with patient survival!

At the end of the 1960s, an ad hoc

Committee (including besides doctors and ethicists as well as a priest) from Harvard University sets up the criteria for declaring "brain death", which regulated organ donation from people whose brain was irreversibly destroyed.

The 60s saw moments of enthusiasm for xenotransplantation, the transplantation from an animal (chimpanzee or baboon) to a human, but the immunological barrier proved very strong and the method was abandoned until more effective immunosuppression protocols were found.

The real success of the liver transplant occurred only after 1980, the year in which Ciclosporin was introduced. This was a powerful and effective immunosuppressive drug discovered by chance in a sample of Norwegian soil by Jean Borel in the laboratories of the Sandoz company and tested in the clinic by the great surgeon and pioneer of liver transplantation in England, Roy Calne, at Cambridge University.

Also, in the 80s, the Belzer solution (or UW-University of Wisconsin solution) was discovered, which replaced the old Collins solution (with the later version Euro-Collins) and allowed the preservation of the liver outside the body for up to 12 hours.

Since 1983, liver transplantation has become an accepted therapeutic method. Pittsburgh, the city where Thomas Starzl had moved with his team, became the world capital of transplantation, a position it would remain until the early 1990s. In Europe, successful programs were built by Roy Calne at the University of Cambridge, Henri Bismuth at the Paul Brousse Hospital in Paris and Rudolf Pichlmayr at the Hanover University Hospital.

In 1989, Christoph Broelsch initiates the first successful program in Chicago with a liver fragment from an adult (usually one of the parents) to a child. After 1990, Asian surgeons (Masatoshi Makuuchi and Kyoichi Tanaka in Japan, ST Fan in Hong Kong) introduced liver transplantation from adult to adult, usually using the right hemified (whose volume represents about 65% of the liver volume).

At the beginning of the 1990s, Thomas Starzl successfully tests Tacrolimus, an immunosuppressant more effective than Ciclosporin. Using the new medicine, in 1992 he performed two xenotransplants from baboon to man, but the two patients did not survive and the method was again abandoned for a while. The main conclusion was that the two calcineurin inhibitors (Cyclosporin and Tacrolimus) effectively control acute rejection (mediated by T lymphocytes), but do not control hyperacute rejection (mediated by antibodies).

Liver transplantation was making remarkable progress based on the two types of donors: those who are "brain dead" and "living" donors (parents or relatives).

Transplantation with a fragment of liver solves pediatric patients to a great extent, especially after, thanks to Rudolf Pichlmayr and Henri Bismuth, it is proven that a fragment of liver can also be obtained by dividing the entire liver from a brain-dead donor (in which case segments 2 and 3 are transplanted to a child, and the rest of the liver, i.e. segments 1,4,5, 6, 7 and 8, to an adult). Together, the living donor (mother or father) and the split liver, covered completely most waiting lists in pediatric programs.

The living donor liver transplantation was more difficult to implement in adults, due to the risks to the donor (right hepatectomy being an operation that registered an estimated mortality of 1-1.5% in the 90s).

That's why it succeeded primarily in Asia and less so in Europe and the USA, where the legal problems in the case of donor death even led to the closure of some

transplant programs. Among the Asian programs for liver transplantation from a living donor, the greatest success was SG Lee's program from Seoul (University Center "Asan"), where the so-called dual transplantation was also introduced: when a single fragment of liver was not enough (this fragment must represent between 0.8 and 1% of the recipient's body weight), two donors were used, from which two liver fragments were taken and implanted into a single recipient.

As the experience of the transplant teams increased, the indications of liver transplantation were better defined:

- In children: biliary atresia and metabolic diseases
- In adults: Cirrhosis in terminal stages (after infection with virus B or C, alcoholic, biliary, autoimmune and, more recently, through the evolution of hepatic steatosis), acute liver failure (poisoning with poisonous mushrooms or with drugs such as paracetamol), cancer (hepatocarcinoma in the first place) and metabolic diseases (Wilson's disease, amyloidosis)

At the end of the 1990s, a new category of donors was introduced, those in so-called "circulatory death", i.e. donors with cardiac arrest who did not respond to resuscitation maneuvers but from whom, if the harvesting is done very quickly, the organs can be used for transplantation.

In Romania, liver transplantation was introduced at the "Fundeni" Clinical Institute. The first four operations (performed between 1997-1999) did not result in the survival of the patients. The first two liver transplants with survival took place in 2000 (April 15th for patient Gheorghe Penea and, respectively, May 5th for patient Gheorghe Badea), with a liver from a brain-dead donor. Currently both are alive and in very good health, 24 years after the transplant.

A very short time later, the liver transplant from a living donor was introduced (with a fragment of liver from the mother to little Timeea Balogh, aged one year at the time of the transplant). Currently, Timeea is a university graduate and mother of a 2-year-old boy.

Later, liver transplantation from a living donor to an adult was introduced, with the help of the team from the University of Essen (Christoph Broelsch, Massimo Malago).

Although the number of donors was initially reduced, the number of operations increased from year to year, reaching a maximum in 2013, with 122 liver transplant operations. It was also the year in which Professor Vladimir Hotineanu's team, partially trained at the "Fundeni" Clinical Institute, inaugurated the transplant program from the Republic of Moldova at the Republican Hospital in Chisinau. In 2014, the second transplant program was inaugurated in Bucharest, at the "Sf. Maria" Clinical Hospital in Iasi, and in 2016, the liver transplant program started at the "Sf. Spiridon" Clinical Hospital in Iasi. Finally, in 2021, the first transplants from a living donor were performed at the "Sanador" Hospital in Bucharest and the pediatric transplant program at the "Grigore Alexandrescu" Children's Hospital started (joint program with the one at the "Fundeni" Clinical Institute, where both the harvesting from the living adult donor and the splitting of the liver, in the case of split liver transplantation, are performed).

The main liver transplant program remains the one at the "Fundeni" Clinical Institute, where the range of types of operations gradually diversified, being carried out in addition to whole liver transplantation from a brain-dead donor other procedure like split liver transplantation (for an adult

and a child or for two adults), transplant from living donor to child and adult, with one or even two liver fragments (dual transplant), domino transplant.

Since 2016, the perfusion of the liver outside the body using the Liver Assist infusion machine has been introduced. The main indication of this method remains the liver from marginal donors (elderly donors, with resuscitated cardiac arrest, staying in intensive care for many days, requiring high doses of vasopressors or with a significant degree of steatosis, etc.) in which there is a risk of not function in the recipient's body ("primary non-function", a situation that requires urgent retransplantation).

Nowadays in such cases the marginal liver is installed on the machine and perfused for at least two hours, during which the aspect of the organ is monitored, the perfusion pressures in the portal vein and in the hepatic artery are evaluated and the transaminases in the perfusion liquid are measured.

After the introduction of this method, the percentage of non-primary liver functions was practically reduced to zero.

Another advance of modern surgery introduced in the program at the "Fundeni" Clinical Institute was the laparoscopic surgery for the harvesting of the liver fragment from the adult in the pediatric transplant. This first step can continue with the use of the method for the harvesting of the right hemi liver in the case of transplantation from an adult donor, an operation that is already performed in the US and Western Europe (where robotic surgery is increasingly used as an alternative to laparoscopic surgery).

The beginning of 2024 saw a revival of xenotransplantation at the international level. If in the past "donors" were chosen from primate species (chimpanzee, baboon), considered to be the closest to humans from a genetic point of view (and therefore the least exposed to rejection), nowadays there is a different approach. Advances in genetic engineering have led to the possibility of modifying the genome of animals in those places responsible for the synthesis of the blood components involved in hyperacute rejection (those that make up "the complement system"). Through the method called CRISPR (clustered regularly interspaced short palindromic repeats) - Cas9 (a protein that allows binding to the targeted DNA sequence) certain genes can be "cut out" and removed or, if necessary, replaced with others, depending on what you need. This method was considered as potentially capable of creating transgenic animals whose organs could be transplanted into humans

The animal on which the research is now focused is the pig, because its organs are very close in anatomy and function to human ones; in addition, the pig grows quickly and multiplies at a rate that allows obtaining as many organs as needed (unlike primates which, if they had proven to be suitable as organ donors for humans, would have risked disappearing very quickly as species).

The suppressed gene ("knock-out") is N-galactosyl transferase, which is involved in the synthesis of the complement system.

The American company eGenesis has managed to produce genetically modified transgenic pigs on an industrial scale so that their organs can be transplanted into humans. Starting from 2021, and especially at the beginning of 2024, several kidney, heart and liver transplants were performed in different centers in the United States. Although the initial evolution was favorable and no case of hyperacute rejection was registered, the transplanted patients did not survive in the long term, dying at intervals of weeks or months after the transplant.

Research does not stop here, however. It is hoped that, in the not too distant future, genetic engineering will obtain the "ideal" animal, whose organs can be successfully transplanted into humans. When this objective is reached, the biggest problem of organ transplantation will be solved: the insufficient number of donors compared to the large number of patients registered on the waiting lists! At that moment, each patient will be able to receive the organ he needs at the right time.

Moreover, if we are allowed to dream, this organ could be genetically modified in such a way that it is recognized as SELF by the body of the recipient. In this situation, the rejection reaction will no longer be triggered and, therefore, there will be no need for postoperative immunosuppression (which is now necessary for the rest of life in transplanted patients). It will be the moment when the golden dream of all transplantologists, IMMUNE TOLERANCE, will become reality!

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EDITORIAL

ON AN ANNIVERSARY – 145 YEARS OF HIGHER MEDICAL EDUCATION IN IAŞI

From the country's first University to the Medical-Pharmaceutical University of Iași

In his conference "The past and present status of Iași", held in the University Hall of the City on November 29, 1931, Professor Grigore T. Popa, with a remarkable and visionary sense of reality, tells a truth that, even today, should disturb simple people, academics or politicians, remaining a constant reminder: "A first-rank University in Iași means placing Iași at the forefront of Romanian culture. A decayed University in Iași means the exit of this city from the determinant factors of national culture". Fortunately, the first option has proved viable until today. The University of Iași opened on the 26th of October, 1860, and was the first modern one in the United Principalities of Moldavia and Wallachia. Within this University, the Faculty of Medicine could be inaugurated only in 1879, 1st of December. It was the result of harmonious collaboration between the Administration (*Epitropia*) of "Sf. Spiridon" Hospitals, the Society of Physicians and Naturalists (SMN), and the Michaelian Academy. The first two establishments spatially delineated a sacred equilateral triangle with the "Gate of Hope" of the University Palace included in this area, a truth that is impossible not to make people think of a deeper meaning.

Starting as a modest Faculty of Medicine of the Iași University, in 1879, it witnessed an incredible transformation, growing into an autonomous University of Medicine and Pharmacy, including four Faculties and several Colleges, bearing Professor Grigore T. Popa's name, as its most outstanding personality. Since 1999, the bust of the skilled anatomist and humanist philosopher Grigore T. Popa keeps guarding the esplanade of the Nation Square in front of the University, with the Great Union Monument, where today, among others, medicine and pharmacy students, teachers and friends walk, talk and enjoy this recreational and emotional space. Now, in 2024, on the 145th anniversary of the Medical Faculty and present day "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, we can affirm without error that the institution we serve with devotion and humility, as our brilliant predecessors did, aspired and succeeded in living up to the ideals animating the 19th century Romanian intellectuals who strove for national emancipation and for developing their own high schools and universities.

In 1873, in the House of Commons, the British prime minister Benjamin Disraeli synthetically expressed his conviction that: "A University should be a place of light, freedom and learning". Who else better than Grigore T. Popa exemplified, in Iași and in Romania, this ideal through all his activity and academic endeavor? In Iași – said Grigore T. Popa, former student and then professor of this institution – "the University has to promote science, the basis of human renewal" and a constant "reform of the spirit" for the better, thus turning into the center of enlightened knowledge and creativity.

Semper Altius! The prerequisites of Higher Medical Education in Iași

On October 26th 1860, Mihail Kogălniceanu, Prince Alexandru I. Cuza's minister of Public Instruction and Cults, claimed that: "...undoubtedly, the country needs lawyers, philosophers, doctors of justice and doctors of wisdom, but the country also needs doctors of the body, physicians". In his turn, in the session of September 16, 1860, Dr. Iacob Czihac, member of the Schools Council, read the program of the Faculty of Medicine, the Ministry of Public Instruction adopted under no. 10.976, but things could not be finalized. In fact, medical education in Iași had gained experience and already had a certain tradition. Training centers existed at least since 1757, when the establishment of the "Sf. Spiridon" Hospital, the basis of future university clinics, was officially inaugurated. In 1838, at a Congress of Naturalists in Freiburg im Breisgau, Dr. Czihac, one of the founders of the Iași Society of Physicians and Naturalists (SMN, 1830), mentioned, in his report on the education in the Principality of Moldavia, the intention to initiate a "course of surgery" in Iași. In 1842, Dr. Gheorghe Cuciureanu, one of Moldavia's protomedics and SMN's presidents, proposed the organization and functioning of a Faculty of Medicine within the "St. Spyridon" Hospital Establishments. Due to several visionary forerunners nurturing brilliant ideas, the SMN in Iași, founded in 1830, was the first modern European-type scientific society in the Romanian Countries and even in Southeast Europe. It introduced the scientific spirit to the Romanian Principalities and was the first structure to develop higher education in Romanian language, promoting the first local national academic institutions: the Michaelian Academy (1835), the University of Iași (1860) - the first in the United Principalities of Moldavia and Wallachia (1860)-, and, only later, the Faculty of Medicine (1879). On January 1st, 1887 SMN started editing the first medical journal of a Romanian academic society, the "Bulletin of the Society of Physicians and Naturalists in Iași", renamed, in 1924, "The Medical-Surgical Journal. Bulletin of the Society of Physicians and Naturalists in Iași", in order to emphasize its medical character. Printed in Romanian and in French, the journal represented the best way of scientific visibility and elevate valorization, at a European level, of the activity of the teaching staff of the young University and some of its Faculties, medicine included. Little by little it remained the forum of the Faculty of Medicine alone. Surviving until nowadays, "The Medical-Surgical Journal"/ Bulletin of the Society of Physicians and Naturalists of Iași" is a generalist periodical, appearing exclusively in English since 2012. In 2024 it reached 128 publishing years, and is on the Clarivate Analytics list, with a 0.1 impact factor in 2023. SMN motto "*Semper altius" - Always Higher* is coming more and more true, indeed.

In 1852, supported by Moldavia's Ruling Prince, Grigore Alexandru Ghica, and Moldavia's protomedic, Dr. Gh.Cuciureanu, Dr. Anastasie Fatu organised and then directed the "Gregorian Institute"- "Obstrical Art Institute" – a sort of Maternity Hospital, which integrated the first midwifery school in Moldavia, core of the future obstetric clinic. Between 1857-1859, under protomedic Ludwig Steege's mandate, Moldavian Pharmacists Guild opened a School for Pharmacy Technicians in Iași, preparing qualified personnel. By the end of 1859, Dr. Nicolae Negura, supported by Prince Alexandru I. Cuza, officially initiated, in the premises of the Michaelian Academy, a

short-lived course of surgery and pharmacy, another attempt to behind medical education in Iași and Moldavia (Moldova). However, a Faculty of Medicine could be born only almost two decades after the University of Iași was established, in 1879, and then, not as a medal awarded to Iași medicine for its contribution to the Romanian War of Independence (1877-1878), but as a contract-commitment for healing the war wounds. The courses began on December 1, 1879, when Dr. Leon Sculy held the first anatomy lesson in the headquarter of the Iași University, where the Medical Faculty owned two rooms. The former headquarter is today's administrative Palace of the University of Medicine and Pharmacy. This is how our Faculty history started, with only 14 students and two professors, Sculy Logothetidis – a skilled surgeon teaching anatomy and histology, and the renowned scientist, Petru Poni, member of the Romanian Academy, who taught chemistry, heading the Chemistry Department of the Faculty of Science. The Medical Faculty's clinical base was generously offered by "Sf. Spiridon" Hospital Administration in Iași. The beginnings of our Faculty were modest, the endowment precarious. But eventually won the desire of some enthusiasts to show the country, and those who did not believe it, that, overcoming all difficulties, Iași can assert itself in the field of medicine and pharmacy, hence in science, and not only in the field of art and culture.

The rise of the Faculty

A most significant case: The Faculty of Medicine in Iaşi entered Europe, right from its birth; it was born both in Iași and in Europe through the teachers trained in France, Germany, Austria, or Italy, integrating, from the first moment, the medical scientific spirit of Europe, here, at our home. The incomparable initiators of Romanian medical education, Leon Sculy - the first Dean of the Faculty -, Ludovic Russ senior, Aristide Peride, Ludovic Russ junior, Constantin Botez, Emanoil Riegler, Theodosie Philipescu, Gabriel Socor, Vasile Bejan, George Bogdan, Francisc Rainer, Elena Densusianu Pușcariu and others, due to their hard work and rich experience, through the suffering inherent in any struggle, placed, from its very beginning, the medical school of Iași at European and international levels. Besides, at the Iași University, lady doctors were more easily accepted and even promoted right from the dawn of the 20th century. Dr. Elena Puscariu, the first female physician to become a university professor in her country and, probably, the first woman professor of ophthalmology in the world, is just an example. Owing to the disciples of these groundbreaking professors, graduates from the young Faculty of Iași, like Ion Tănăsescu, Alexandru Brăescu, or Alexandru Moruzi, the medical school in Iași imposed itself with authority in the field of surgery, and nervous pathology, for instance. Even though the Faculty of Medicine started with 14 students, their number gradually increased up, in only 20 years, to 100 at the end of the 19th century. The number of students amplified significantly after the inauguration of the Institute of Anatomy in 1894, a building considered at that time the most modern of its kind in Southeast Europe. Today, after 130 years, this edifice, emblem of our University, renovated from the ground up, is one of the constructions that Iași is proud of. In 1912, the first wing of the new Faculty building, surrounding the central Palace, was put into use, and in 1932, the second wing. From five university clinics in 1900, it reached 21 in 1938; from

2,030 volumes existing in the library at the end of the 19th century, it reached about 22,000 in 1939. In 1913, a section of Pharmaceutical Education began to function within the University of Iași, associated to the Faculty of Medicine. In 1934, Pharmaceutical Education was concentrated in the Bucharest Faculty of Pharmacy alone; consequently, the other sections in the country ceased to exist.

Due to the great prestige acquired in the country and abroad, the Faculty's role increased substantially after the Great Union of Romania achieved in the 1st of December 1918, date after which it received students from all the Romanian provinces reunited with their mother country, but mainly from Bessarabia and Bucovina, historical parts of Moldova itself. Thus, between the two World Wars, the enrollment figure was of 1,388 students. Also during this golden interwar period of the Faculty, great personalities of Romanian medicine and culture were trained in this Faculty, such as Eugen I. Mironescu - professor of dermatology clinic, writer and important collaborator of the *"Viaţa Românească"* magazine, Gheorghe Tudoranu - professor of pathology, medical clinic and hematology, and an essayist as well, the talented publicist and writer Grigore T. Popa, the patron of our University, the one who discovered the portal pituitary system, pioneering neuroendocrinology, an acknowledged personality of both the Romanian and international academic life, man of culture - cofounder and collaborator of *"Însemnări Ieşene"*, a heroic civic spirit. Close to the students and the sick, they represented respected and loved human and professional role models and were refined persons of elevated culture.

After five decades of existence, the Faculty of Medicine in Iași was imposing itself through its high scientific and cultural tradition, through the founders of modern schools in all the disciplines and their prominent followers: Constantin Ionescu-Mihaiesti, Alexandru Slatineanu and Mihai Ciuca – bacteriology; Nicolae Leon – parasitology; Alexandru Tupa – histology and histopathology; Vasile Rascanu – physiology; Jules Nitulescu – pathophysiology; Constantin I. Parhon – neurology, endocrinology and gerontology he initially named *iliki-biology* (gr. *ηλικι:age* – age biology); Ernest Juvara, Ion Tanasescu, Nicolae Hortolomei, Vladimir Butureanu, Georghe Chipail – surgery; Oscar Franche – urology; Theodor Economu – orthopedics; Gheorghe Demetriade, Eugen I. Mironescu, Gheorghe Nastase – dermatology; Maria Franche – infectious diseases; Constantin Bacaloglu, Ion Enescu – medical clinic and cardiology; Leon Ballif – psychiatry; Emil Radu – radiology; Eugen Aburel, Gheorghe Placinteanu, Vasile Dobrovici – obstetrics and gynecology; Dumitru Cornelson – hygiene. These names are few, indeed, compared with the large number of graduates and professors who illustrated Romanian medicine. We must also mention other personalities trained on the benches of this Faculty and at the bedsides of Iași Hospitals, of whom the University has always been proud: Ion Gr. Nubert and Ion Iancu - professors of anatomy, Petre Condrea – bacteriology and inframicrobiology (virology); Constantin C. Dimitriu - medical clinic and semiology; Gheorghe Popovici – pharmacology; Maria Briese – endocrinology; Zoe Caraman – neurology; Maria Sibi – Biochemistry; Gheorghe Timosca, Mircea Rusu – stomatology, oral and maxillofacial surgery; Marcel Kapri, Traian Baran, followed by Valeriu Rusu - medical physics, renamed medical biophysics in 1948 and completed with nuclear medicine since 1963; Ion Hăulica – physiology; Carol

Stanciu – internal medicine, gastroenterology and hepatology; Eugen Târcoveanu – laparoscopic surgery; Ostin Costel Mungiu – pharmacology; Mircea Covic – genetics; Marcel Burlea - pediatrics are just a few examples. All these fields were integrated to medical education, practice and research.

From a Faculty to a complex University

In 1948, as a result of the education reform, the Faculty was transformed into the Institute of Medicine, later called Institute of Medicine and Pharmacy, which included several Faculties: General Medicine, Pediatrics, Hygiene, Pharmacy and Stomatology (Dental Medicine). Between 1956-1960, everything is concentrated in the Institute of Medicine with a single Faculty of Medicine possessing two sections: The General Medicine section and the Pediatrics section. In 1960, the Dentistry Section was re-established, and in 1961, the Pharmacy one, functioning as such until 1965, led by the dean of the Faculty of Medicine. In 1965, the Institute of Medicine changed its structure again, becoming the Institute of Medicine and Pharmacy (IMF), with three faculties, with their own deans, namely: 1.the Faculty of Medicine, integrating the Section of General Medicine and the Section of Pediatrics; 2.the Faculty of Stomatology (today: Dental Medicine); 3. the Faculty of Pharmacy.

Many of those who graduated after 1961-1970 established themselves in the Iași medical center, practicing as university teachers, heads of departments and prestigious medical practitioners in the healthcare network, illustrating the new Faculties and specialties integrated into the Institute and their rapid development.

The contribution of Iași University students over time to the progress of research and general medico-pharmaceutical activity, both in other centers of the country and abroad, should also be remembered. In the country, the creation of the Faculty of Medicine in Timişoara in 1945 was supported for a short time, by Professor Emil Hurmuzache, a reputed pediatrician from Professor Manicatide's school, followed by the future Professor Louis Turcanu; currently, the Pediatric Hospital in the Timisoara bears the latter's name; Professor Vladimir Busila helped the Department and Clinic of infectious diseases; Professor Dumitru (Luchi) Popescu developed obstetrics-gynecology, while Professor Constantin Zosin and Dr. Paula (Paulette) Zosin, illustrating Professor Ion Enescu's school, were efficiently involved in promoting internal medicine and nephrology. Equally in Timisoara, Dental Medicine benefited from Professor Nicolae Dutescu's expertise. Professor Maria Cernea, instructed with Professor Vasile Dobrovici in obstetrics-gynecology, and Professor Paul Cernea, a skilled ophthalmologist from Professor Petre Vancea's Clinic, contributed to the consolidation of the Faculty of Medicine in Craiova. In the same way, the Faculty of Medicine in Constanta has integrated teaching staff specialized in Iaşi, such as Professor Iuliana Dimofte – genetics and Professor Petru Bordei – anatomy. From Professor Ion Enescu's school of cardiology, the future professors Samuel and Olga Zoneraich established themselves in the USA where they had a successful career. In the meantime, such examples have greatly multiplied, not only in Romania, but also on an international level.

One hundred years after the founding of the school of higher medical education in Iași, in 1979, under communist rigors, the Institute of Medicine and Pharmacy Centenary was celebrated, an event with nation-

al and international participation, mainly from communist countries, which allowed to bring to attention the guiding lines of Iași medicine, its personalities, including that of Grigore T. Popa, to pay tribute to the pioneers and the tradition of this academic medical institution, so that its flame was passed on to younger generations. The first two out of the four volumes of scientific works of the event reviewed the great predecessors and the history of the departments, completing the album and the other iatrohistorical and medico-pharmaceutical syntheses edited on that very occasion. In 1979, the Iași Institute of Medicine and Pharmacy was training 3,354 students, of which 795 were students from other countries, had 450 members in the teaching staff, 46 laboratories and 38 university clinics, and a Center (Institute) for the Improvement and Specialization of Physicians and Pharmacists (IPSM). During the communist period, the University had professors of great standing, trained in the interwar period, who continued the founders' tradition, even though under undeniable threats and constraints: Ion Iancu, Vladimir Bușureanu, Alla Vâță, Petre Vancea, Martian Cotrău, Nicolae Bălan. The successful tradition went on due to several other representatives of Iași medical education: Gheorghe Chișleag, Mihai Duca, Constantin Negoiță, Petre Brânzei, Nicolae Costinescu, Emil Hurmuzache, Virgil Costea, Grigore Teodorovici, Ernest Ungureanu, Nicolae Bumbăcescu, Ioan Selmiciu, Emanoil Grigorescu, Constantin Strat, Marcel Burdea, Asia Chipail, Gheorghe Ghimicescu, Nicolae Oblu, Gheorghe Bădărau, Costache Lazăr, Mircea Chifan, Victor Strat, Gheorghe Scripcaru and others.

In 1991, our institution was transformed into the University of Medicine and Pharmacy. Since 1994 it bears the name of the reputed Professor Grigore T. Popa (1892-1948), who graduated from the Iași Faculty of Medicine. The medical school in Iași was also revived when, in 1994, to the three previous Faculties, the only Medical Bioengineering Faculty in the country was added, which brilliantly upgraded the spectrum of training for leading specialists in contemporary, highly computerized medical practice and (bio)technologies. This accomplishment owes its existence to Professor Florin Topoliceanu's dedication and visionary perseverance. Colleges, focused on complementary specializations, were in turn created over time: Dental Technique (1993), Dental Assistance and Prophylaxis, Medical Assistance, Nutrition and Dietetics, Audiology and Hearing Prosthetics, Balneophysiokinesiotherapy (Physical Therapy) and Rehabilitation (1999), Medical cosmetics and cosmetic product technology.

Over the years other numerous research centers or institutes appeared or were technically upgraded keeping pace with contemporary medical and scientific practice, not only in Romania, but also abroad: the Center for Gastroenterology and Hepatology, the Institute of Cardiology, the Institute of Psychiatry, the Endocrinology Center, Nephrology and Renal Dialysis Clinics, Center for laparoscopic surgery, Organ donation and harvesting center, Kidney transplant and Liver transplant clinics, an Algesiology center for the study of pain, together with the more classical laboratories for Radio imaging, Interventional radiology, and Nuclear medicine, an Excellence Center for Experimental Medicine (CEMEX); besides a Center for Modern Languages and Cultural Integration was set up too.

The university clinics were equipped, the educational spaces and the Central Library were updated, the dormitories for students

and residents were renovated and modernized, major repairs and consolidations were carried out in the old University buildings, which today have an elegant aspect. Students benefit from the most modern and probably the tallest educational edifice in Iași - the "Nicolae Leon" Building (Ghica-Vodă St.) - and the most modern and equipped Simulation Center (M. Kogălniceanu St.), a 7 million euros investment, bearing the name of Prof. Cristian Dragomir, a well-known surgeon in Iași. Almost all teachers specialized equally abroad; our participation in international scientific events, grants and projects substantially increased, inter-university exchanges significantly augmented from the Freiburg Program to the Bologna Project, from the Socrates Program to the Erasmus and Erasmus Plus Mobilities.

The myth of the Iași medical school is therefore a reality, it has been reshaped and regenerated in a modern academic spirit. The influx of young people and their and interest in applying for admission to the "Grigore T. Popa" University in Iași is far from a mere coincidence, since our graduates rank among the first in the residency examinations and contests.

The number of students and teaching staff continually increased. If in the academic year 2002-2003 there were 5,319 students, guided by 950 teaching staff members, in 2014, the University trained 8,687 students, of which 2,570 were students from other countries, 2,192 residents, 265 master's students and 344 doctoral students. A particular success was recorded by the French-language series. In 2019, the "Grigore T. Popa" University of Medicine and Pharmacy had four faculties and four university colleges: The Faculty of Medicine, the Faculty of Dental Medicine, the Faculty of Pharmacy, the Faculty of Medical Bioengineering, the only one in Romania since 1994. Today, with a teaching staff of 900 members, UMF prepares approximately 12,000 students in 7 specialties, 4 of which in a new field - unique in the country for the time being: medical bioengineering. Given the need for continuous training, distance education has also been implemented. University medical practice and clinical research are carried out in the 55 specialized clinics organized in 12 university clinical hospitals, in 2 university polyclinics, in the unique Cardiology Center in Moldova, as well as in the Dialysis Center. On September 5, 2017, "Grigore T. Popa" U.M.Ph. became the first medical higher education institution in Romania listed in the Times Higher Education World University Rankings. In terms of the international academic visibility indicator, U.M.Ph. in Iași ranked first in the country and in the top 500 universities in the world (428th place).

If in the decades before 1989 at the Institute of Medicine and Pharmacy in Iași, foreign students learned the Romanian language in order to complete their medical and pharmaceutical studies alongside their Romanian colleagues at the Faculty of Medicine, in the academic year 1991-1992, a first line of study was opened entirely in English, which doubled in 2013-2014. In the 2009-2010 academic year, a first line of full medical study was inaugurated in French. Since 2013-2014, two French-speaking lines, with about 75 places each, are available to students at the Iași Faculty of Medicine. Since 2009-2010, sections in foreign languages have been also introduced to the Faculty of Dental Medicine – English and French - and the Faculty of Pharmacy – English. Presently the Faculty of Medical Bioengineering – Physical Therapy and Rehabilitation Specialization equally provides undergraduate

studies and master's degree courses in English. Master degree are also released by the Faculty of Medical Bioengineering. At the end of 2014, a University Success Center *(Centre de Réussite Universitaire - CRU*) was inaugurated with the support of the Francophonie University Agency *(Agence Universitaire de la Francophonie - AUF)* - Office for Central and Eastern Europe. Presently the number of foreign students at "Grigore T. Popa" U.M.Ph. from Iași is estimated to 2,600, situating the University on the first place in Romania among similar institutions. Undoubtedly, the students' influx and their desire to enroll in the column of the "Grigore T. Popa" University in Iași is not just a happy happening.

Projects for a "Medical University City"

In 2024, after 145 years of development, we are enjoying a period of stability and constant progress: in the last 8 years, the "Nicolae Leon" building was inaugurated hosting 6 modern lecture rooms with over 700 seats (a real congress center), and a center for fundamental medical skills. The "Grigore T. Popa" University Conferences program has opened, where scientific and cultural personalities from the country and abroad are invited; the Seniors' Club (Sf. Athanasie St.) was organized along with the "I. I. Mironescu" Cultural Center; in April 2024, the "Confucius" Institute (Gh. Sîulescu St.) was inaugurated; an IT platform has been achieved for monitoring the didactic process and exams; works are in progress for the consolidation, rehabilitation and modernization of the Dentistry Polyclinic for Adults located in the Craftsmen's Insurance House (Casa de Asigurări a Meseriașilor), in its 'Vault' *(Bolta),* in front of the "Sf. Spiridon" Hospital, and offering to the Faculty of Dental Medicine modern spaces for clinical practice and students' training; the same is true for the Children's Dentistry Polyclinic (Al. Lapusneanu St.), which will provide the Faculty of Dental Medicine with spaces for clinical education and practice, equipped with state-of-the-art equipment; the former "Prof. Dumitru Bagdasar" canteen will be transformed into a modern sports center, a gym for physical education activities and for student sports competitions (Sărărie St.); the consolidation, rehabilitation and modernization of the Sports Hall - the former "1st of December" canteen is another objective to fulfill along with the renovation and modernization of the University campus infrastructure (C9 and C10 dormitories) which will improve accommodation conditions for students; the renovation of the Anatomy Institute is carried out together with the consolidation and rehabilitation of "Prof. Vladimir Bușureanu" House; the consolidation, rehabilitation and conversion of the attic room of the "1st of May" A - Student Residence; the construction of a multifunctional building, also housing a library, is part of the ongoing projects of the University which equally include a Center for Fundamental Research, consisting of an integrated platform for interdisciplinary and transdisciplinary studies, endowed with the necessary specific infrastructure allowing molecular biology, genetics, immunology analyses and so on; a Maternal-Fetal Research Center for investigations focused on the maternal-fetal health is envisaged as well; a translational medicine research center is being built close to the U.M.Ph: it is the TRANSCEND Research Center, designed to promote and consolidate excellence in interdisciplinary biomedical research; located in the Regional Institute of Oncology Iași (RIO/IRO), a "smart" hospital, TRANSCEND is financed from the

European Structural Fund; concurrently IRO develops robotic surgery using the Da Vinci Robot and aims at upgrading medical practice, education and research; recently has started the construction of the new Regional Emergency Hospital in Iași, another smart university establishment; solar panels systems for producing sustainable energy has been parallelly considered by these up-to-date strategies.

That is why in June 2024, "Grigore T. Popa" University of Medicine and Pharmacy borrowed 35.4 million euros from the European Investment Bank (EIB) through EIB's public sector loans for Central and South-Eastern Europe – intended for the rehabilitation and modernization of the University campus buildings. This transaction represents the first external loan University made and the first EIB operation within a 200-million-euro initiative meant to improve higher education in Romania. The loan granted by the EIB to the University of Medicine and Pharmacy in Iași aims at supporting renovation, modernization and (re)construction of University buildings. The University itself will invest over 70 million euros. This is the sixth agreement of the EIB with a Romanian University. A prosperous University like U.M.Ph. Iași needed such an agreement with a major international bank like EIB, enabling major investment projects. Otherwise, as the Rector pointed out, "due to legislation, Romanian Universities have no financial autonomy, and the possibility of accessing one's own funds is reduced".

Art, philosophy, cultural competence and medical humanities should eventually render higher education in this University more prone to personalized medicine and empathy, respectful of ethics and deontology applied in everyday life practice and at the patient's bedside. In accordance with this approach the International Festival of Poetry held in Iași, in May 2024, for instance, was hosted by the Museum of Medical History of our University.

"Gr. T. Popa" Publishing House developed, with over 100 books being printed annually.

The U.M.Ph. Central Medical Library has two locations (V. Alecsandri St. and in "N. Leon" Building, Ghica-Vodă St.) with several modern reading rooms; it has over 470,000 volumes, computerized materials, and ensures access to online information; it possesses specialized libraries in almost every department or discipline, 28 laboratories, IT documentation rooms; it has the Liberty online platform with 24/7 free access and an E-learning platform; it provides access to the books of the biggest medical publishing houses, access and subscriptions to most important medical journals in the world and offers students and teaching staff the possibility of consulting the latest specialty publications.

As part of its continuing medical education program, the Iași University of Medicine and Pharmacy organizes improvement and advanced specialization courses (238 proposed courses, attended by over 2415 physicians and pharmacists); it trains residents in all specialties acknowledged by the Ministry of Health (3,453 physicians, 90 from other countries). UMF includes an excellent Doctoral School, which attracts an impressive number of young people, their number constantly increasing in the fields of medicine, dentistry and pharmacy. The University has 109 doctoral supervisors, 598 doctoral students; the Doctoral School initiated 66 research projects won through national or international competitions in the last 6 years, during which time 153 doctoral theses were finished, all confirmed by CNATCDU (Na-

tional Council for the Attestation of University Titles, Diplomas and Certificates). Currently U.M.Ph. has 4 faculties, 926 teaching staff, 9,908 students, representing 75 nations, 3,947 resident physicians, 508 Ph.D. students, 359 master students and 12,697 places available in educational spaces. National and international projects enrich the University's activity maintaining it in the international scientific network.

The precious treasure of our University is the ALUMNI community, the 139 promotions of graduated physicians, along with all the pharmacists, dentists and medical bioengineers graduated from the same Alma Mater, present here, in Romania, or anywhere else in the world from Europe to the USA, Australia or Japan, have proven the nobility of the medical profession and allied sciences, and have competently cared for suffering people. Thus, they became the best ambassadors of Iași and Romania in the world.

Always a "Gate of Hope"- an Arch of Triumph

The University of Medicine and Pharmacy teaching staff always included outstanding personalities, corresponding and full members of the Romanian Academy, such as Grigore T. Popa himself (corresponding member, 1936), members of the Romanian Academy of Medical Sciences and of the Scientists' Academy of Romania. Since the 2020s "Grigore T. Popa" University awarded Doctor Honoris Causa degrees to remarkable national and international leading figures in their specialties and professions. Some of them were former students who graduated from this very Alma Mater who in the meantime gain fame in their own country or abroad. In their turn, valuable personalities of the "Grigore T. Popa" University were rewarded by similar honorary distinctions bestowed on them by Romanian and foreign Universities. Consequently, stronger cooperation relationships continue to successfully develop enabling medical and pharmaceutical education to flourish in Iași and in the whole country. In fact, after the 1990, UMF could rewire its activity to European and other global scientific networks, forging complementary opportunities for its own present and future.

Committed to scientific and ethical principles, the Masters of the Faculty founded in 1879, of the Institute of Medicine and Pharmacy established in 1948, and redimensioned as a University of Medicine and Pharmacy in 1991 - have truly and tenaciously sculpted their being, character and scientific personality in light, elevation and sacrifice, as in Elektra's myth, reigniting the column torch that enlightens us. As Professor Grigore T. Popa said, "elastic horizons must be left to the soul, which have with them the expected mirage of long distances, for which the misfortunes of the moment can be endured." The mirage worked out for our University, too. The "Gate of Hope", archway of the first modern University founded in Romania – presently "Al. I. Cuza" University -, is today the triumphal arch of our new University of Medicine and Pharmacy. Even if the Faculty of Medicine could not pass triumphantly under it on October 26th 1860, when the old pioneering institution came into being, today nothing prevents the inscription on its vault of the names of those who served, with faith and dedication, the art of medicine and medical education in Iași. The column we are evoking, under the circumstances of the 145 year anniversary of higher medical education in Iași, seems to be an endless column, like the one imagined and made by the unsurpassed Romanian sculptor Constantin Brâncuși; it

seems a humanized column comparable to the Ecorche -"the skinned man"- preserved in our Institute of Anatomy Museum, made by the same Brancusi, in which the human body perfect morphology is seen and felt as an eternal resurrection promise.

George Emil Palade and the Iași University of Medicine and Pharmacy

2024 marks 150 years since the birth of Professor Constantin I. Parhon (1874-1969), dean of our Medical Faculty (1923-1926), member of the Romanian Academy (1939), one of the founders of Romanian and international endocrinology and gerontology, specialties he promoted within the Socola School of neurology and mental diseases, he created in Iași (1912-1933). More than this, 2024 is equally the year when Romania and Iași celebrate Professor George Emil Palade, the scientist born in Iași on November 19, 1912, who in 1974, received the only medical Nobel Prize awarded to a Romanian, so far. G. E. Palade (1912-2008) honored Iași and its University of Medicine and Pharmacy by accepting the title of Doctor Honoris Causa, which this institution conferred on him on the 30th of May 1994, in the lecture hall now bearing his name. Reputed representatives of the Iași University of Medicine and Pharmacy were among those due to whom Palade received the same honorary distinction on behalf of the Romanian Scientists' Academy in 1994, as well. Palade always remembered his two master anatomists from Iași, he met, as a student, at the Faculty of Medicine in Bucharest: his doctoral thesis supervisor, Professor Francisc Rainer, and his young collaborator, Dr. Grigore T. Popa. Years later, in 1944-1945, Popa, from whom Palade confessed that he learned the accuracy of laboratory experiments, advised and helped him to pursue in-depth studies abroad. Indeed, once he arrived in the USA, Palade's glorious scientific destiny could be efficiently fulfilled. Palade welcomed in his Laboratory in the USA Romanian specialists and skilled researchers among whom some came from Iași. Tightly attached to his mother land, he constantly tried to support and encourage promising Romanian scientists. His presence in Iași and in our University certainly stimulated and ennobled us with a glow of his Nobel Prize and he still does.

Passing the flame

Crowning its 140 activity and endeavor for excellence in medical education, and still remembering the centenary of Greater Romania (1918-2018), the Royal Family of Romania offered "Grigore T. Popa" University the *Nihil Sine Deo* decoration for the important part it played in fostering a rich exchange of opinions and expertise on the latest advancements in medical bioengineering and medicine, as a whole. It is the only medical University in Romania to have won this award. In 2019, on its 140th anniversary, the country's President rewarded "Grigore T. Popa" University with the "Order of Merit for Education" in the rank of Grand Officer "for the exceptional results obtained in intellectual and human training of young generations, as well as in the process of scientific research, standing out as a model of excellence in Romanian higher education and contributing, in this way, to raising the prestige of local and national communities".

This memory recall occurs now because the need for gratitude tears is simply felt, without any sadness of the balance sheet, but also without festive explosions, trying to make it fit the pure truth which a destiny and a myth could arise from. On the 1st of December 2024, 145 years are celebrated

since the birth of the higher medical school in Iași. Remembering its evolution, we are more than convinced that we can be happy and proud to pass over the abys of time the flame of knowledge and cutting-edge science our forerunners enthusiastically once lit, promoting health and education, combating ignorance and disease. We are thankful and honored to belong to this community - a perennial belonging together of mind and soul to our Alma Mater.

Vivat Academia! Semper Altius!

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Rector of "Grigore Popa" University of Medicine and Pharmacy Iași
President of Romanian Society of Surgery,
President of the Society of Physicians and Naturalists of Iași

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EDITORIAL

ARTIFICIAL INTELLIGENCE IN SURGERY AND SURGICAL EDUCATION

Modern surgery has undergone an impressive evolution due to advancements in fundamental research, anesthesia, diagnostic tools, genetics, immunology, pharmacology, and especially artificial intelligence (AI). AI is defined as *"the study of computations that enable a machine to perceive, reason, and act."* In 1950, Alan Turing described intelligent behavior in computers as the ability to achieve human-level performance in cognitive tasks. Over the past 20 years, interest in AI in the medical field has significantly increased, leading to a true revolution and high expectations.

Modern medicine faces the challenge of acquiring, analyzing, and storing large amounts of information needed to solve complex clinical problems. The development of AI-based medical programs generally aims to formulate diagnoses, make therapeutic decisions, and predict outcomes. AI tools are designed to support the medical team in their daily tasks by processing data efficiently. This major progress has been made possible by the convergence of four key factors:

1. Large databases
2. Increased computing power
3. Deep learning algorithms, which have become the primary approach to creating intelligence at levels exceeding human capabilities, from image recognition to digital language processing
4. Significant investments

The cognitive revolution promised by AI has the potential to mitigate the shortage of doctors, particularly in fields like radiology and pathology, but it will not replace doctors in making therapeutic decisions.

Today, we can talk about **digital surgery (DS)**, which is shaping a global framework for surgical training, education, and planning. DS represents a milestone in computer-assisted surgery and the new era of automated surgery, robotic learning, and augmented environments. Digital surgery, which already exists (e.g., robotic surgery), leverages AI's power and connectivity as a crucial resource for the next generation of surgeons, particularly through machine learning on both regional and global scales.

As the **third revolution in surgery**, DS will help surgeons manage complex surgical environments, optimize team efficiency, and improve patient outcomes. It will enable healthcare teams to perform better, understand the needs of surgeons and surgical teams, and ultimately provide better patient care. Furthermore, DS will enhance hospital administration by offering a clear perspective on operating room activities, adjusting financial resources accordingly, and improving treatment efficiency while making surgical care more accessible worldwide.

By leveraging data, optimizing best practices, and refining surgical training, DS will ensure high-quality care, reduce outcome variability, and identify key factors that improve patient recovery. DS presents major opportunities, including enhanced surgical training through simulation, increased transparency in operating rooms, and the identification of factors influencing postoperative outcomes. It relies on robotic surgery, advanced instrumentation, improved visualization, connectivity, and data analysis. For DS to develop, it is essential to establish an infrastructure that seamlessly integrates robotics, AI, advanced surgical tools, and

educational programs to facilitate rapid innovation and surgical progress.

AI in Surgery: Enhancing, Not Replacing, the Human Element

AI is already integrated into all phases of surgery:

Preoperative phase: AI enhances preoperative planning by improving risk assessment and 3D modeling, allowing for better-informed decisions;

Intraoperative phase: AI aids in real-time image analysis and robotic assistance, increasing precision and minimizing complications;

Postoperative phase: AI predicts and detects complications early, personalizes recovery monitoring, and enables long-term patient follow-up.

With the rising number of surgical procedures, AI's role in surgery will continue to grow by improving precision, real-time diagnostics, customized surgical approaches, intraoperative navigation, risk prediction, and postoperative monitoring. AI has the potential to enhance patient outcomes, facilitate surgical education, and optimize surgical care.

AI supports surgeons in three main phases of their work:

1. **Preoperative planning**: AI can integrate population-level surgical data to determine patient-specific risks and expected outcomes, facilitating evidence-based treatment decisions. Deep learning techniques enable AI to recognize individual anatomical variations from medical images, helping surgeons anticipate intraoperative challenges. Sophisticated AI algorithms can process large imaging datasets to generate detailed 3D models of anatomical structures, offering interactive visualizations that enhance surgical field analysis.

2. **Intraoperative assistance**: AI-powered imaging techniques help surgeons identify anatomical structures with high precision and track instruments in real-time. AI systems assist in robotic surgeries by providing instant feedback and performing automated tasks. In colorectal cancer surgery, for instance, AI-powered tumor sensors ensure precise tumor resection, reducing positive surgical margins and improving oncological safety.

3. **Postoperative monitoring**: AI continues to assist patients beyond the operating room by enabling personalized and proactive post-surgical care. Intelligent algorithms track vital parameters, analyze data from various sources, and detect early signs of complications, allowing timely intervention. Wearable devices provide real-time recovery data, enabling healthcare teams to tailor rehabilitation plans and schedule follow-ups based on prognostic data.

From an **ethical** perspective, AI systems must undergo continuous monitoring to ensure patient safety and data privacy. Surgeons play a crucial role in decision-making with AI, requiring adequate training. As AI systems take on critical roles in healthcare-ranging from diagnostics to treatment recommendations and surgical tool control-it is vital to ensure that AI-driven decisions align with ethical standards and individual patient needs. While AI-assisted clinical decision support systems can provide precise recommendations, there is a risk of over-reliance on AI, which may lead to potential harm if AI recommendations are followed without considering patient-specific factors. Additionally, there is a concern that patient trust may erode if AI is perceived as the sole decision-maker.

AI and Surgical Education

The **benefits of AI in medical education** are numerous. AI-powered tools, such as ChatGPT, help doctors quickly and accurately respond to patient inquiries, offer personalized medical advice, and streamline administrative processes, improving patient

care efficiency. While some believe AI could replace doctors in the near future, this remains an illusion and an undesirable outcome.

One of AI's most crucial roles is in **surgical training**. The training process for surgical residents has evolved from the Halsted model (1890) of *"see one, do one, teach one"* to structured training programs.

The Rasmussen model (three-phase learning process) has proven effective:

1. **Skill-based behavior** – learning fundamental surgical gestures, understanding surgical instruments, aseptic techniques, and anatomical landmarks.

2. **Rule-based behavior** – mastering operative sequences, performing surgical procedures, and understanding surgical indications.

3. **Knowledge-based behavior** – acquiring the ability to handle critical situations, intraoperative complications, and complex surgical decisions.

The goal of surgical training is to develop professional competencies, assess theoretical knowledge, and enhance practical skills. The AI revolution is set to transform surgical education by introducing personalized, simulation-based learning experiences that redefine skill acquisition and refinement.

In **Romania**, the first **MIST-VR simulator** was introduced in Iași before 2000, significantly improving the surgical education. Today, "Grigore T. Popa" University of Medicine and Pharmacy of Iași hosts the most advanced simulation center in Romania, further enhancing surgical training. AI algorithms can assess trainees' strengths and weaknesses, tailoring training to their specific needs. AI's impact on surgical education extends beyond practice, as AI tools accelerate training by providing personalized learning experiences and objective skill assessments.

AI enhances simulation-based training by offering real-time feedback, objective evaluation tools, and tele-mentoring opportunities. AI algorithms can predict a trainee's performance and skill development, optimize training programs, facilitate communication, update curricula, and generate interactive content. Surgeons must understand AI's strengths and limitations, especially when transitioning from simulations to real-life surgeries. Future surgical training programs will incorporate AI-related knowledge and skills, requiring **multidisciplinary collaboration** among specialists to develop and implement AI in surgery.

The Ethical and Humanistic Perspective

Over **25 years of innovation**, AI models have matched human performance in certain aspects of patient care. However, this evolution has also raised challenges, including ethical dilemmas, data security concerns, and the need for appropriate regulations. Strategies for the **safe and effective integration of AI in surgery** include establishing robust safety protocols, conducting prospective clinical trials, training surgeons as AI end-users, and fostering interdisciplinary collaboration.

To **fully harness AI's potential**, continuous research, innovation, and collaboration are necessary to address technical, ethical, and organizational challenges. Aligning technological advances with patient-centered ethics and medical values will allow AI to elevate the standard of surgical care and improve outcomes worldwide. Addressing ethical, legal, and practical complexities-such as bias mitigation, patient privacy protection, AI system validation, and efficient integration into surgical practice and education-is crucial.

As some question whether **Hippocrates' Oath** remains relevant today, it is clear that this ethical code is more important than ever. Despite high-tech advancements and globalization, **the most vital aspect of sur-**

gery remains humanism and empathy-qualities no AI can replicate. If we lose this **divine gift of healing through human connection, we lose everything**, as François Rabelais once said: *"Science without conscience is the ruin of the soul."*

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EDITORIAL

GLP-1 AND GIP REVOLUTION

Obesity is a chronic, relapsing, and multifaceted condition anticipated to impact around fifty percent of the United States population by 2030. Current estimates indicate that 650 million adults and 340 million children and adolescents (ages 5-19 y.o.) worldwide are affected by obesity (1). Alongside the markedly rising incidence, obesity carries substantial medical and economic consequences for both individuals and nations. Obesity costs the U.S. $480.7 billion in healthcare and an additional $1.24 trillion in lost economic output. Various therapies have been established to facilitate weight loss, including lifestyle modifications involving nutrition, with or without exercise and/or behavioral therapy, anti-obesity drugs (AOMs), endoscopic and surgical procedures. The most common AOMs, such as glucagon-like peptide-1 receptor agonist (GLP-1 RAs) and dual GLP-1 and glucose-dependent insulinotropic polypeptide receptor agonists (GLP-1/GIP RAs) have demonstrated substantial weight loss results and are gaining popularity as therapy choices. The Food and Drug Administration (FDA) approved the use of the GLP-1 receptor agonist Liraglutide for the management of overweight and obesity in 2014, followed by Semaglutide in 2021, and the combined GLP-1/GIP receptor agonist Tirzepatide in 2023 (3).

In recent years, the field of endocrinology has witnessed a transformative shift with the emergence of GLP-1 and GIP RAs. Originally developed as antidiabetic agents, these incretin-based therapies have evolved into multi-systemic modulators with powerful effects across cardiovascular, gastrointestinal, neurologic, and metabolic domains. The predominant side effects were gastrointestinal in nature, comprising nausea (40.2%), diarrhea (20.9%), constipation (20.0%), and vomiting (16.3%). Gastrointestinal adverse events resulted in drug cessation in 6.4% of individuals who were taking Liraglutide, in contrast to 0.7% in the placebo group (4). The incidence of side events for Semaglutide was comparable amongst participants in the treatment group (89.7%) and those in the placebo group (86.4%), regardless of causality. Comparable to liraglutide, gastrointestinal side effects (e.g., nausea, diarrhea, vomiting, and constipation) were recorded (74.2% of patients receiving Semaglutide). Most of these adverse effects were deemed mild to moderate and temporary, not necessitating the cessation of medication (5). Whereas more than two-third of the patients on placebo reported at least one adverse event, 78.9-81.8 percent of participants on Tirzepatide reported at least one adverse event, regardless of whether the adverse event was caused by the medication. In many negative events were also related to the digestive system. Furthermore, many of the adverse effects were temporary, ranging from mild to moderate in severity, and mostly manifested themselves during the period when the dose of Tirzepatide was being increased. Most adverse effects manifested themselves within the first eight to sixteen weeks of using the medicine (6).

In randomized controlled trials, the occurrence of serious adverse events was

notably higher in the liraglutide group (6.2%) compared to the placebo group (5.0%) (4). Participants who administered Semaglutide reported a higher incidence of serious adverse events (9.8%) compared to those receiving placebo (6.4%) (5). Additionally, 5.1-6.3% of participants administered Tirzepatide experienced serious adverse events, compared to 6.8% in the placebo group (6). A recent study employing a randomized sample of 16 million patients revealed that the use of liraglutide and Semaglutide, compared to bupropion-naltrexone, was linked to a heightened risk of pancreatitis, bowel obstruction, and gastroparesis, while no significant association was found with biliary disease (7). Moreover, the use of GLP-1Ras is associated with a significantly reduced quality of bowel preparation, resulting in an increased necessity for repeat colonoscopies. Enhancing pre-procedural planning for outpatient colonoscopies necessitates an understanding of the cumulative effects of medications that may hinder gastric emptying. This comprehension is crucial for identifying suitable interventions and counseling approaches (8).

The cardiovascular advantages of GLP-1 receptor agonists are now well-documented. Significant trials, including LEADER (liraglutide), SUSTAIN-6 (Semaglutide), and REWIND (dulaglutide), have shown reductions in major adverse cardiovascular events (MACE), notably non-fatal stroke and cardiovascular mortality. The observed effects seem to operate independently of glucose-lowering mechanisms and are likely facilitated by a combination of weight loss, anti-inflammatory actions, blood pressure reduction, and enhancements in endothelial function (9). Recent studies indicate that dual agonists such as Tirzepatide, which targets GIP and GLP-1 receptors, demonstrate enhanced efficacy in improving cardiometabolic markers; however, long-term cardiovascular outcome data remain under development (10).

Lipid and inflammatory cell deposits in the inner layer of large and medium-sized elastic and muscular arteries are the hallmarks of atherosclerosis, a chronic inflammatory disease. Diabetes mellitus, a pro-atherogenic factor that promotes the development of atherosclerosis and/or speeds up its progression through a multifactorial process, dramatically raises the risk of cardiovascular illnesses as well as overall cardiovascular mortality. In every study, GLP-1 receptors agonist, have demonstrated significant decreases in atherosclerotic events and all-cause mortality. According to preclinical research, GLP-1RAs influence several pathways involved in the formation and advancement of plaque, contributing to the immunomodulation of atherosclerosis (11). Although they both have positive effects on the cardiovascular system, statins and GLP-1 receptor agonists function differently and have various advantages in particular domains. Statins reduce the risk of heart attacks and strokes by lowering LDL cholesterol. Contrarily, GLP-1 receptors agonists have more extensive cardiovascular effects, such as lowering major adverse cardiovascular events (MACE), enhancing the prognosis of heart failure, and possibly even lowering limb events. They are becoming more widely acknowledged for their positive effects, especially in patients with type 2 diabetes and pre-existing cardiovascular disease, even though statins have a well-established function in primary and secondary prevention of cardiovascular disease (12).

GLP-1 receptors are present in various brain regions, such as the hypothalamus, hippocampus, and cortex, which are essential for regulating appetite, cognition, and mood. Research involving animals and

early human studies indicates neuroprotective functions in models of Alzheimer's disease, Parkinson's disease, and ischemic stroke. Current trials, such as EVOKE and EVOKE+, are evaluating the effects of Semaglutide on early Alzheimer's disease. Semaglutide may exert disease-modifying and neuroprotective influence in Alzheimer's through various mechanisms, including neuroinflammatory, vascular, and other processes associated with the disease (12).

The Liraglutide, Semaglutide, and Tirzepatide (15 mg) groups had higher rates of spontaneous hypoglycemia (1.3%, 0.6%, and 1.6%, respectively) compared to the placebo groups. These events were not regarded as serious. Hypoglycemia is a potential risk that must be considered when prescribing this medication to patients receiving insulin secretagogues or insulin, as indicated by prior research (3,13).

The incidence of benign and malignant neoplasms in the Liraglutide, Semaglutide, or Tirzepatide groups was comparable to that of the placebo group. No instances of medullary thyroid cancer or C-cell hyperplasia were reported in people assigned to the liraglutide, Semaglutide, or Tirzepatide groups. No clinically meaningful elevation in blood calcitonin levels is linked with the administration of these medicines (3). Notably, a greater number of female participants in the liraglutide group, compared to the placebo group, were diagnosed with malignant and premalignant breast neoplasms. Most of these patients exhibited above-average weight decrease (4).

One review included approximately 150 cases related to potential self-injury and suicidal ideation associated with liraglutide and Semaglutide, followed the reports of depression and suicidality. The causal relationship between the medications and these incidents remains uncertain; however, analogous issues have been documented with other AOMs. Liraglutide, Semaglutide, and Tirzepatide labels contain warnings regarding the need to monitor depression or suicidal ideation, whereas other GLP-1 receptor agonists do not include this warning. Most clinical trials for GLP-1RA have excluded individuals with a history of depression or suicidal behavior. The precise reasons for this exclusion remain unclear. Prescribing these drugs requires careful monitoring and caution, especially for individuals with a history of suicidality (14).

GLP-1 and GLP-1/GIP receptor agonists have shown significant weight loss results in clinical trials as well as in real-world data. Expert opinions highlight the necessity of meticulous patient selection, collaborative decision-making, and tailored dosing to achieve an optimal balance between benefits and risks. Continued research is essential to identify and mitigate potential side effects, including effects on gastric emptying and the necessity for precautionary measures during anesthesia. The increasing use of these medications necessitates evidence-based guidelines to ensure their safe and effective application in obesity management (3).

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EDITORIAL

NON-INVASIVE VAGUS NERVE STIMULATION IN GASTROINTESTINAL DISORDERS

The vagus nerve, the primary component of the parasympathetic nervous system, plays a central role in regulating gastrointestinal sensitivity, motility, and immune responses (1). Composed of approximately 80% afferent and 20% efferent fibers, it serves as a critical communication pathway between the gut and the brain (2, 3). Afferent fibers transmit visceral information upwards from the gastrointestinal tract, including pain-related signals, thus participating actively in visceral nociception (4-6). Meanwhile, the efferent pathways influence parasympathetic preganglionic neurons in the dorsal motor nucleus and modulate sympathetic activity in the spinal cord, ultimately impacting gut motility and immune modulation (3).

This complex bidirectional network between the vagus nerve and the enteric nervous system forms the basis of the brain-gut axis, a fundamental mechanism for maintaining physiological homeostasis (1). In this context, stimulation of the vagus nerve, particularly through non-invasive transcutaneous auricular (taVNS) methods, is gaining attention for its potential therapeutic applications in gastrointestinal disorders.

Emerging evidence suggests that taVNS exerts meaningful effects across several physiological domains, including antinociception, anti-inflammation, improved motility, and restoration of intestinal barrier integrity. These mechanisms are particularly relevant in disorders marked by visceral hypersensitivity, such as irritable bowel syndrome and functional dyspepsia, now widely considered conditions of altered gut-brain interaction. Clinical studies have reported symptom relief and improved quality of life in affected individuals, reinforcing the therapeutic promise of neuromodulation in this field.

While current findings are encouraging, further investigation is needed to consolidate these observations into routine clinical practice. The modulation of vagal activity may well represent a turning point in the management of functional gastrointestinal disorders, one that addresses not just the symptoms, but the core physiological dysfunctions driving them.

The Therapeutic Potential of Vagus Nerve Stimulation in Gastrointestinal Disorders

Understanding the neurogastroenterological axis is essential in identifying innovative treatment strategies for functional and inflammatory gastrointestinal conditions. Among these, vagus nerve dysfunction has emerged as a critical pathophysiological component, especially in disorders characterized by gut-brain dysregulation and low-grade chronic inflammation (4).

Reduced vagal tone, a key marker of dysautonomia, has been consistently associated with conditions such as IBS and IBD (7, 8). Although findings related to autonomic imbalance in IBS remain somewhat inconsistent, often influenced by the predominant bowel habit subtype (9,10), the evidence suggests altered sympathetic-parasympathetic dynamics. Acute stress episodes typically provoke a sympathetic

surge, reflected in reduced heart rate variability (HRV), whereas chronic stress may perpetuate this imbalance, sustaining autonomic dysfunction over time (3,11,12). In IBS and IBD, this autonomic dysregulation often persists, potentially disrupting the brain-gut communication loop long term (13, 14).

More fundamentally, these autonomic alterations appear intertwined with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, likely mediated by disrupted communication between key limbic structures such as the prefrontal cortex and amygdala, regions central to the central autonomic network (8). Vagal tone may thus serve as both a biomarker and a mediator of pathophysiology in these conditions (3, 15).

In light of these findings, the vagus nerve emerges not merely as a passive conduit but as a strategic target for therapeutic intervention. With its extensive innervation of the gastrointestinal tract and regulatory role in inflammation, pain modulation, and motility, VNS holds considerable promise as a non-pharmacological treatment option for gastrointestinal (GI) disorders involving gut-brain axis dysfunction (4).

Vagus Nerve Stimulation: From Concept to Clinical Potential

VNS works by delivering controlled electrical impulses to the vagus nerve, modulating both neural and immune pathways (1,16,17). It is broadly categorized into invasive and non-invasive modalities. The invasive approach, already established in epilepsy, involves surgical implantation of a pulse generator and electrodes on the cervical vagus nerve (4). While effective, it carries the risks associated with any surgical intervention.

By contrast, non-invasive approaches, particularly taVNS and transcutaneous cervical VNS (tcVNS), offer a less risky alternative. taVNS specifically targets the auricular branch of the vagus nerve through surface electrodes applied to the ear concha. Unlike tcVNS, which can stimulate both afferent and efferent fibers, taVNS is limited to afferent stimulation, yet still appears effective in modulating central autonomic circuits (1).

Beyond these, percutaneous electrical nerve field stimulation (PENFS) represents another minimally invasive modality. It applies needle-like electrodes to the ear's surface, activating cranial nerves including the vagus (V, VII, IX, X) and influencing the nucleus *tractus solitarii* (nTS), a major integration hub for vagal afferents (18).

Crucially, VNS may offer a therapeutic alternative to biological therapies, particularly in inflammatory GI conditions. While biologics have revolutionized disease control, their long-term use can be hampered by adverse effects and patient reluctance to continue treatment once remission is achieved (19-22). Non-adherence remains a significant concern, with rates reported as high to 50% (23). These challenges have led many patients to explore complementary options, with neuromodulation via VNS gaining increased attention (24).

VNS has emerged as a promising neuromodulator intervention for GI disorders, particularly those characterized by chronic abdominal pain, visceral hypersensitivity, and immune dysregulation, such as IBS, functional dyspepsia (FD), and IBD (1, 4). Vagal dysfunction, commonly reflected by reduced vagal tone, is frequently observed in these conditions and has been linked to autonomic imbalance and persistent gut-brain axis disruption (7-15).

Experimental and clinical studies suggest that VNS exerts dual antinociceptive and anti-inflammatory effects by modulat-

ing both central and peripheral pathways involved in pain and immune regulation (4, 25, 26). These mechanisms include activation of the cholinergic anti-inflammatory reflex via efferent vagal fibers and stimulation of the HPA axis via afferents (25-27). Furthermore, the vagal-splenic and vagal-sympathetic pathways have been implicated in suppressing proinflammatory cytokine release, particularly tumor necrosis factor-alpha, through β2-adrenergic and α7 nicotinic acetylcholine receptor signaling (17, 28, 29).

Non-invasive modalities such as taVNS and PENFS have demonstrated symptom relief in FD and IBS, improving gastric motility, reducing abdominal pain, and enhancing quality of life (10, 30-32). Clinical response rates with taVNS reached over 80% in some trials, with sustained benefits up to 12 weeks (32). In IBD, preliminary results indicate that taVNS may induce clinical remission and reduce disease activity scores, especially in pediatric populations (10, 33, 34).

While the variability in stimulation parameters (e.g., frequency, site, duration) may influence outcomes (35), current evidence supports VNS as a viable non-pharmacological therapy for functional and inflammatory GI disorders. Its capacity to regulate autonomic tone, suppress maladaptive inflammation, and improve visceral sensory processing underscores its therapeutic potential in disorders involving gut-brain interaction.

Although preliminary evidence is promising, non-invasive VNS requires further validation through large, standardized clinical trials. IBS may in fact be more of a stress-related disorder, and non-invasive therapies such as taVNS and PENFS offer a promising option for symptom management without major side effects (36). It is essential that researchers, clinicians, and policymakers collaborate to transform this technology from an experimental curiosity to a mainstay of gastroenterological therapy. Only in this way can we offer patients a safe, effective alternative without major side effects, paving the way for holistic management of digestive disorders.

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EDITORIAL

RECURRENT RHABDOMYOLYSIS IN CHILDREN: A "RED FLAG" FOR UNDERLYING METABOLIC MYOPATHY AND THE NEED FOR NATIONAL PEDIATRIC VIGILANCE

Rhabdomyolysis is characterized by the rapid breakdown of skeletal muscle, resulting in the release of intracellular contents such as creatine kinase (CK), myoglobin, potassium, and phosphate into the circulation. This process can lead to acute kidney injury and metabolic disturbances (1).

In children, single episodes of rhabdomyolysis are usually secondary to infection, trauma, or strenuous exercise (2). However, recurrent rhabdomyolysis, defined as two or more distinct episodes, should raise suspicion for underlying metabolic myopathies, neuromuscular disorders, or genetic variants (3).

From a Romanian perspective, there are no reliable epidemiologic data on pediatric recurrent rhabdomyolysis. This gap is particularly significant given that approximately 75% of Romanian patients with rare diseases are children (4), suggesting that a notable subset may harbor unrecognized metabolic etiologies.

Pathophysiology and Triggers

Muscle injury in rhabdomyolysis may be direct (trauma), indirect (exertion, hyperthermia), or metabolic (ATP depletion leading to ion pump failure and intracellular calcium overload) (1, 5).

In pediatric populations, common triggers include: viral myositis (especially influenza B), the most frequent cause in children (5); exertional rhabdomyolysis, often seen in adolescent athletes (1); metabolic myopathies, including glycogen storage diseases, fatty acid oxidation defects, and mitochondrial disorders, which frequently present with recurrent episodes (2, 3); drugs, toxins, seizures, and electrolyte imbalances (1, 5, 6).

Why recurrent rhabdomyolysis merits attention

Acute kidney injury occurs in approximately 20-25% of pediatric rhabdomyolysis cases (6). When episodes are recurrent or severe despite minimal exertion, or when there is a family history of myopathy or malignant hyperthermia, metabolic or genetic causes should be investigated (3, 7).

Recurrent rhabdomyolysis can result from a wide range of inherited disorders, including defects in: fatty acid β-oxidation (*ACADVL*, *CPT2*, *HADHA*, *LPIN1*, *ACADM*), glycogen storage and glycolytic pathways (*PYGM*, *PGM1*, *PHKA1*, *AGL*), mitochondrial function, excitation-contraction coupling or ion channel regulation (*RYR1*, *SCN4A*), membrane-repair and structural myopathies (*DYSF*, *ANO5*, *FKRP*, *SGCA*, *SGCG*, *CAPN3*, *DMD*, *OBSCN*), and lipid and membrane homeostasis (*SMPD1*, *TANGO2*) (6-10).

Failure to recognize these underlying disorders may lead to repeated hospitalizations, preventable renal injury, and chronic kidney disease (6, 7). Conversely, early identification enables counseling, trigger avoidance, and potentially life-saving preventive strategies (5).

Diagnostic approach

A practical diagnostic approach for pe-

diatricians and nephrologists managing recurrent rhabdomyolysis should include the seven steps.

First step. *Initial Confirmation* using CK >5× the upper limit of normal or >1000 IU/L; Myoglobinuria (positive urine dipstick for blood without red cells) and the assessment of electrolytes, renal function, and urine output (1, 5).

Second step. *Evaluation of Triggers* involves review for infection, trauma, drugs, exertion, or fasting (2).

Third step. *"Red Flags"* warranting further work-up Two or more distinct episodes; No clear precipitating factor; Severe CK elevation or AKI; Early age of onset; Family history of myopathy; Persistent CK elevation between episodes (2,3).

Fourth step. *Identifying Genetic Susceptibility.* The RHABDO mnemonic helps distinguish patients whose rhabdomyolysis is disproportionate to external triggers (10) R (Recurrent episodes of exertional rhabdomyolysis), H (Hyperkalemia persisting >8 weeks after the event), A (Accustomed level of physical exercise), B (Blood CK >50× upper limit of normal), D (Drugs/medications/supplements or other factors insufficient to explain severity), O (Other family members affected or presence of exertional symptoms like cramps, myalgia).

Fifth step. *Second-Line Investigations* involves *metabolic screening* (free carnitine, acyl-carnitine profile, lactate/pyruvate, urine organic acids) and *targeted or panel-based genetic testing* (covering *ACADVL*, *ANO5*, *CPT2*, *DMD*, *DYSF*, *FKRP*, *HADHA*, *PGM1*, *LPIN1*, *PYGM*, *RYR1*, *AGL*, *CAPN3*, *CNBP*, *DMPK*, *MAGT1*, *ACADM*, *SCN4A*, *SGCA*, *SGCG*, *SMPD1*, *TANGO2*, *PHKA1*, *OBSCN*) (8-10).

Sixth step. *Management* by *a*ggressive intravenous hydration remains the cornerstone of therapy (1). Electrolyte imbalances and acidosis should be corrected, with early nephrology involvement in severe cases. Preventive measures include adequate hydration before exercise, avoidance of fasting, and education for families on early symptom recognition (3, 5).

Seventh step. *Follow-Up e*ven after recovery, CK and renal function should be monitored due to the risk of developing chronic kidney disease (7).

The Romanian context: gaps and opportunities

To date, no Romanian pediatric case series on recurrent rhabdomyolysis have been published. The limited national literature primarily focuses on adults or athletes (11). Romania continues to face challenges in metabolic screening. Newborn screening remains limited compared to other EU countries, and the nation retains the highest infant mortality rate in the Union (12). Although national infrastructure for rare diseases is improving, systematic data capture and registries are lacking (4).

These realities underscore the importance of developing a coordinated pediatric strategy, particularly in Iași, which hosts one of the country's leading medical universities and pediatric centers.

Recommendations for Romania

- Establish a national pediatric rhabdomyolysis registry, potentially under the Romanian Rare Disease Registry.
- Promote multidisciplinary collaboration among pediatric nephrology, neurology, and metabolic genetics specialists.
- Develop national guidelines for the evaluation of recurrent rhabdomyolysis, adapted from international best practices (5, 6).
- Expand access to metabolic and genetic testing across tertiary pediatric hospitals.

• Implement family education programs on early symptom recognition, hydration, and avoidance of known triggers.

In conclusion, recurrent rhabdomyolysis in children is a diagnostic "red flag" for underlying metabolic or genetic disease. Early recognition, comprehensive investigation, and preventive strategies can significantly reduce morbidity and mortality. Romania currently lacks pediatric data, registries, and structured diagnostic pathways. Establishing these resources will position national centers - particularly those in Iași - as leaders in pediatric metabolic medicine.

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EDITORIAL

REMEMBERING ALZHEIMER'S LEGACY: 110 YEARS SINCE THE PASSING OF ALOIS ALZHEIMER

Motto: "A peculiar disease of the cerebral cortex has captured my attention, one that reveals itself through profound memory loss, confusion, and a progressive erosion of the mind."

Alzheimer's disease, a devastating neurodegenerative condition, was first brought to the world's attention in 1906 by the German psychiatrist and neuropathologist Alois Alzheimer (1).

His ground-breaking work began with the study of a 51-year-old patient, Auguste Deter, who exhibited unusual symptoms of progressive memory loss, confusion, and hallucinations-an alarming decline that could not be classified within the known medical conditions of the time (2). After her passing, Alzheimer meticulously examined her brain and discovered the presence of amyloid plaques and neurofibrillary tangles, marking the first recorded case of what would later bear his name (3).

While Alzheimer's contributions were pivotal, he did not work in isolation. His mentor, Emil Kraepelin, one of the most influential psychiatrists of his era, played a crucial role in formally recognizing the disease.

In the 1910 edition of his Textbook of Psychiatry, Kraepelin classified this newly identified disorder as a distinct form of dementia, differentiating it from other types of cognitive decline that typically appeared in old age (4).

At the same time, Czech psychiatrist Oskar Fischer was independently investigating dementia and arrived at remarkably similar conclusions. In 1907, Fischer conducted a meticulous postmortem analysis of 16 elderly patients suffering from cognitive decline. He documented the accumulation of abnormal protein formations in their brains-structures he named Sphaerotrichia cerebri multiplex, which closely resemble what we now call amyloid plaques (5).

Meanwhile, Romanian neurologist Gheorghe Marinescu made crucial discoveries that predated both Alzheimer and Fischer. Collaborating with French pathologist Paul Blocq in 1892, Marinescu was among the first to describe peculiar plaque-like formations in the brain's grey matter, which he termed "nodules of neuroglial sclerosis" (6).

Each of these brilliant minds played a vital role in shaping our understanding of Alzheimer's disease. Their collective efforts laid the foundation for modern neuropathology, offering early insights into the biological mechanisms that underpin dementia. Their discoveries, once confined to the pages of medical journals, have since fueled over a century of research, bringing us closer to unravelling one of the greatest medical mysteries of our time.

Our comprehensive analysis explores the evolution of Alzheimer's disease (AD) research, highlighting the key scientific breakthroughs that have shaped our understanding of its neuropathology, clinical presentation, imaging advancements, treatments, and future directions from the early 20th century to today.

EARLY 20TH CENTURY DISCOVERIES

• 1906 – Alois Alzheimer's Landmark Case: Alzheimer detailed the case of Auguste Deter, a 51-year-old woman with severe memory loss, confusion, and hallucinations. His post-mortem analysis revealed amyloid plaques and neurofibrillary tangles, establishing the foundation for AD pathology (1).

• 1910 – Emil Kraepelin defines AD: Kraepelin, a renowned psychiatrist and mentor to Alzheimer, formally named the disease in his Textbook of Psychiatry, distinguishing it from other dementias and cementing its place in medical literature (4).

• 1907 – Oskar Fischer's Parallel Discoveries: Fischer independently described amyloid plaques in dementia patients, analyzing 16 postmortem brains and identifying protein aggregates he termed "Sphaerotrichia cerebri multiplex." Despite the significance of his findings, they were overshadowed by Alzheimer's work (5).

• 1892 – Gheorghe Marinescu's Early Observations: Marinescu and Paul Blocq were among the first researchers to describe grey matter plaques, referring to them as "nodules of neuroglial sclerosis." Their work laid the groundwork for the later recognition of amyloid pathology in AD (7).

A B C D

Fig. 1. A – Alois Alzheimer, the German psychiatrist and neuropathologist who first identified Alzheimer's disease in 1906; B – Gheorghe Marinescu, the Romanian neurologist whose early work on senile plaques contributed to the understanding of neurodegeneration; C – Emil Kraepelin, the psychiatrist who formally classified Alzheimer's disease in 1910; D – Oskar Fischer, the Czech psychiatrist who independently described amyloid plaques in dementia patients in 1907.

The recognition and understanding of Alzheimer's disease emerged through the collective efforts of distinguished researchers, whose contributions, though shaped by different times and places, converged to define one of the most complex neurodegenerative disorders (fig. 1).

MID TO LATE 20TH CENTURY PROGRESS

• 1968 – Plaques Linked to Dementia: A quantitative neuropathological study confirmed that the density of senile plaques correlated with cognitive decline, solidifying the amyloid hypothesis (8).

• 1984 – Beta-Amyloid Identified: Scientists successfully isolated and characterized beta-amyloid as the primary component of AD plaques, marking a turning point in molecular research (10).

• 1986 – Tau Protein's Role Confirmed: Researchers discovered tau protein as a key

element of neurofibrillary tangles, reinforcing the dual-pathology model of amyloid and tau accumulation in AD (10).

• The pathological hallmarks of Alzheimer's disease (AD) include the accumulation of beta-amyloid plaques and neurofibrillary tangles, which contribute to neuronal dysfunction and degeneration. Beta-amyloid plaques form due to the abnormal aggregation of amyloid-beta peptides, disrupting synaptic communication and triggering neuroinflammation. Meanwhile, tau protein abnormalities lead to the formation of neurofibrillary tangles, destabilizing the microtubule network essential for intracellular transport. The progressive accumulation of these pathological structures results in widespread neuronal loss, cortical atrophy, and cognitive decline, characteristic of AD (fig. 2).

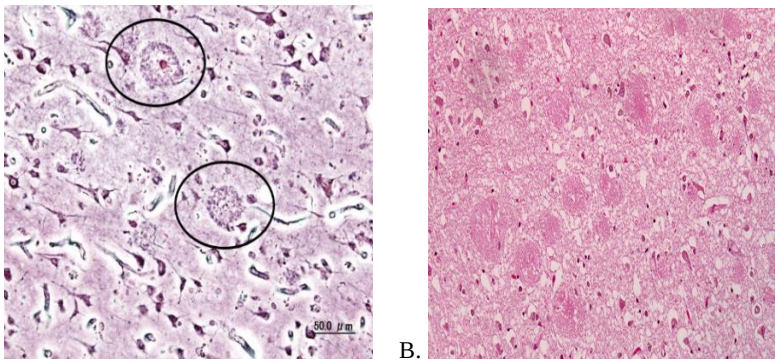

Fig. 2. A – Beta-amyloid plaques and tau pathology in Alzheimer's disease (Source: National Institutes of Health (NIH). Beta-Amyloid and Tau Pathology in Alzheimer's Disease. NIH; 2024); B – Neuronal degeneration in Alzheimer's disease, showing the widespread cellular damage caused by beta-amyloid accumulation and tau pathology (Source: Getty Images. Neuronal Degeneration in Alzheimer's Disease. Getty Images, 2024).

21ST CENTURY ADVANCEMENTS

• 2000s – Imaging Revolution: Advanced MRI and PET (fig. 3) scans transformed the ability to visualize AD-related brain changes, confirming that atrophy often begins in the medial temporal lobes, particularly in the hippocampus, before spreading to parietal and frontal regions (11).

• 2024 – Breakthrough in Blood-Based Diagnostics: A new blood test demonstrated 90% accuracy in detecting AD pathology, paving the way for early diagnosis and intervention (12).

Regional Brain Atrophy in AD

Research has mapped the progression of atrophy in AD, revealing a distinct pattern of neurodegeneration:

• Temporal Lobes: Early and severe atrophy (up to 30%) impairs sensory processing, language comprehension, and memory formation (13).

• Hippocampus: As a key structure for memory consolidation, its progressive shrinking correlates with the severity of cognitive decline (14).

• Frontal Lobes: As the disease advances, the executive function centers responsible for decision-making, planning, and social behavior deteriorate (15).

• Parietal Lobes: Spatial orientation and navigation abilities decline, contributing to disorientation, apraxia, and visuospatial deficits (16).

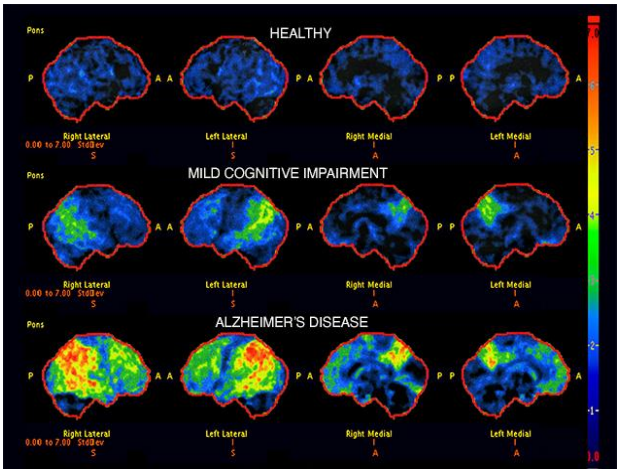

Fig. 3. PET scans illustrating progressive glucose hypometabolism from a healthy brain (top) to mild cognitive impairment (middle) and Alzheimer's disease (bottom), highlighting neurodegeneration in key brain regions (Source: Mayo Clinic. Brain scan images for diagnosis of Alzheimer's disease. Available at: https: //www.mayoclinic.org/diseases-conditions/alzheimers-disease/multimedia/img-20543518)

TREATMENT DEVELOPMENTS

Over the years, Alzheimer's disease treatments have evolved from symptomatic management with cholinesterase inhibitors in the 1990s to disease-modifying therapies targeting amyloid pathology, such as Aducanumab (2021) and Lecanemab (2023). These advancements reflect a shift toward addressing the underlying neurodegenerative mechanisms rather than just symptom relief (tab. I).

TABLE I.

Major Treatment Milestones in Alzheimer's Disease

Year	Treatment	Mechanism	Impact
1990s	Cholinesterase Inhibitors (Donepezil, Rivastigmine)	Enhances acetylcholine levels to improve cognition	Modest symptom relief in mild to moderate AD (17)
2003	Memantine	Regulates glutamate activity to prevent excitotoxicity	Approved for moderate to severe AD (18)
2021	Aducanumab	First disease-modifying therapy targeting amyloid plaques	Controversial, but approved under accelerated pathway (19, 21)
2023	Lecanemab	Anti-amyloid therapy with confirmed efficacy in slowing cognitive decline	First fully FDA-approved disease-modifying therapy (20, 21)

FUTURE PERSPECTIVES

The next frontier in AD research focuses on early detection, precision medicine, and novel therapeutic targets:

- Targeting Tau Aggregation: Therapies are being developed to prevent or reverse tau pathology, a key driver of neurodegeneration (22).
- Neuroinflammation Modulation: Investigations into microglial activation and inflammatory pathways may lead to immunotherapies that slow disease progression (23).
- Synaptic Protection Strategies: Scientists are exploring ways to enhance neuro-

plasticity and synaptic resilience, aiming to preserve cognitive function despite disease pathology (24).

In conclusion, the evolution of Alzheimer's disease (AD) research is a testament to the contributions of pioneering scientists who shaped our understanding of this devastating neurodegenerative disorder. From Alois Alzheimer's meticulous observations in 1906, which first linked cognitive decline to amyloid plaques and neurofibrillary tangles, to Emil Kraepelin's formal classification of the disease in 1910, their foundational work paved the way for modern neurology and psychiatry.

At the same time, Oskar Fischer independently described amyloid pathology in dementia patients, yet his findings were largely overshadowed by historical circumstances. Similarly, Gheorghe Marinescu's early recognition of neuroglial nodules in the brain was a key, yet underappreciated, milestone in AD research. These early scientists, each working in different parts of Europe, laid the groundwork for the neuropathological discoveries that still define AD today.

Over the past century, technological advancements have dramatically expanded our ability to study and diagnose AD. Imaging techniques like MRI and PET scans now allow for early detection, while biomarker research in cerebrospinal fluid (CSF) and blood has revolutionized diagnostic accuracy. What once required a post-mortem brain analysis can now be identified decades before symptoms appear, offering hope for earlier intervention.

Therapeutic advancements have also reflected a shift from symptomatic treatment to disease modification. The introduction of cholinesterase inhibitors in the 1990s, followed by memantine in 2003, provided temporary relief but failed to halt disease progression. More recently, Aducanumab (2021) and Lecanemab (2023) have opened a new chapter in AD treatment, focusing on amyloid clearance, though their long-term efficacy remains debated.

Despite a century of research, AD remains an incurable condition, with millions worldwide facing its relentless progression. The future of AD research lies in targeting not just amyloid and tau pathology, but also neuroinflammation, synaptic dysfunction, and genetic risk factors. As we move forward, the contributions of early pioneers like Alzheimer, Kraepelin, Fischer, and Marinescu serve as a reminder that scientific discovery is built on the perseverance of those who dare to question, observe, and innovate.

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EDITORIAL

NEUROGASTROENTEROLOGY - A NEW FIELD?

Over the past decades, Gastroenterology has undergone a profound transformation, moving beyond a purely organ-centered approach toward an integrated, multidisciplinary understanding of digestive diseases. At the heart of this evolution lies Neurogastroenterology, a field dedicated to the complex interactions between the gastrointestinal tract, the enteric nervous system, and the central nervous system. Neurogastroenterology has emerged as a distinct and essential subspecialty, dedicated to the study and management of disorders arising from dysfunctions of the gut-brain axis. In this context, the establishment and development of a Neurogastroenterology unit represents not only a scientific advancement, but also a clinical necessity for contemporary medicine.

Neurogastroenterology addresses a wide spectrum of conditions that are highly prevalent, clinically challenging, and often associated with significant patient suffering. In general population, disorders such as irritable bowel syndrome (4.1%), functional dyspepsia (7%), functional esophageal disorders (functional heartburn ~ 2-3%, globus sensation ~ 3-4%, functional chest pain ~ 1-2%), functional constipation (~11-14%), and fecal incontinence (1.5-2.5%) are now collectively classified as disorders of gut-brain interaction (1). These conditions affect millions of individuals worldwide, impose a considerable socioeconomic burden, and frequently lead to repeated medical consultations, extensive investigations, and therapeutic frustration for both patients and clinicians. Despite their prevalence, such disorders have long been marginalized, often perceived as diagnoses of exclusion or as conditions lacking a solid biological basis. The development of a dedicated Neurogastroenterology unit plays a crucial role in overcoming these misconceptions and in providing structured, evidence-based care.

A defining feature of Neurogastroenterology is its focus on the complex pathophysiological mechanisms underlying gastrointestinal symptoms. Research over recent decades has demonstrated that altered gastrointestinal motility, visceral hypersensitivity, dysregulation of central pain processing, autonomic nervous system imbalance, immune activation, and changes in the intestinal microbiota all contribute to symptom generation. These biological factors interact dynamically with psychological and social determinants, including stress, anxiety, depression, early life adversity, and coping mechanisms. A Neurogastroenterology unit provides an integrated framework in which these multifactorial mechanisms can be systematically evaluated and therapeutically targeted (1).

From a diagnostic standpoint, the Neurogastroenterology unit offers access to specialized functional investigations that are essential for accurate diagnosis and patient stratification. High-resolution esophageal and anorectal manometry, impedance-pH monitoring, gastric emptying studies, colonic transit assessments, breath tests for carbohydrate malabsorption and small intestinal bacterial overgrowth, as well as tests of anorectal sensory and motor

function, enable clinicians to move beyond symptom-based criteria toward mechanism-oriented diagnoses (2). This approach facilitates more precise phenotyping of patients, allowing clinicians to distinguish between motility disorders, sensory abnormalities, and central dysregulation. Such diagnostic precision is fundamental to the principles of personalized medicine and is associated with improved therapeutic outcomes (3).

Equally important is the therapeutic philosophy promoted within a Neurogastroenterology unit. Management strategies are inherently multimodal and individualized, reflecting the heterogeneity of underlying mechanisms.

Pharmacological treatments may include agents that modulate gastrointestinal motility, secretion, and visceral sensitivity, as well as centrally acting neuromodulators targeting pain processing and affective components of symptoms. Dietary interventions, such as low fermentable oligo-, di-, monosaccharides and polyols (FODMAP) diets or targeted nutritional modifications, play an increasingly important role. Psychological therapies, including cognitive behavioral therapy, gut-directed hypnotherapy, and stress management techniques, have demonstrated efficacy in selected patient populations. The coordinated integration of these approaches within a dedicated clinic fosters a holistic and patient-centered model of care (4).

Interdisciplinary collaboration represents another cornerstone of the Neurogastroenterology unit. Close cooperation between gastroenterologists, neurologists, psychiatrists, psychologists, dietitians, physiotherapists, and specialized nurses enhances both diagnostic accuracy and therapeutic effectiveness. Such collaboration is particularly valuable in patients with severe, refractory, or chronic symptoms, who often present with overlapping gastrointestinal and extraintestinal complaints. By addressing both somatic and psychosocial dimensions of disease, the Neurogastroenterology department contributes to improved quality of life, reduced symptom burden, and greater patient satisfaction.

Beyond its clinical impact, the Neurogastroenterology unit fulfils a vital role in medical education. Functional gastrointestinal disorders are frequently underrepresented in undergraduate and postgraduate curricula, despite their high prevalence in everyday practice. Exposure to a structured Neurogastroenterology service allows medical students and residents to develop a nuanced understanding of gut-brain interactions, diagnostic reasoning in functional disorders, and the principles of biopsychosocial medicine. This educational experience helps counteract therapeutic nihilism and fosters empathy toward patients whose symptoms may be invisible yet profoundly disabling (4). For practicing clinicians, Neurogastroenterology clinics serve as centers of excellence that promote continuing professional development and dissemination of updated clinical guidelines.

The academic value of a Neurogastroenterology clinic is further reflected in its contribution to clinical and translational research. The gut-brain axis represents one of the most dynamic areas of contemporary biomedical research, with ongoing investigations into neuroimmune signaling, microbiota-brain communication, and novel therapeutic targets. Dedicated clinics provide well-characterized patient populations, standardized diagnostic protocols, and opportunities for longitudinal follow-up, all of which are essential for high-quality research. Participation in multicenter studies and international collaborations enhances scientific visibility and positions academic institutions at the forefront of innovation in digestive health.

Within the context of academic medicine in Iași, a city with a long-standing tradition of medical scholarship and clinical excellence, the development of a Neurogastroenterology clinic carries particular significance. It aligns with the mission of tertiary care centers to address complex and unmet clinical needs while integrating education and research. Moreover, it responds to the growing demand for specialized care among patients with functional gastrointestinal disorders, who often experience delays in diagnosis and fragmented care pathways. Establishing a dedicated clinic not only improves access to expertise but also reinforces the role of the academic hospital as a reference center for the region. Academician Professor Doctor Carol Stanciu had a pivotal role in introducing functional gastrointestinal investigations in Iasi. Through his vision and dedication, modern diagnostics techniques such as motility and functional testing were improving both clinical practice and academic training (5).

In conclusion, the importance of a Neurogastroenterology unit in contemporary medical practice cannot be overstated. By providing specialized diagnostic tools, individualized and multimodal therapies, interdisciplinary collaboration, and opportunities for education and research, such clinics address a major gap in the care of patients with disorders of gut-brain interaction. Their role extends beyond symptom management, contributing to a deeper understanding of disease mechanisms and to the advancement of integrative medicine.

In academic centers such as Iași, the consolidation of Neurogastroenterology services represents both a continuation of medical tradition and a forward-looking investment in the future of patient-centered care.

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EDITORIAL

ART AND SCIENCE IN CARDIOVASCULAR DISEASE

As the progress in technology and pharmaceutical domain resulted in effective solutions for transmissible diseases such as cholera or poliomyelitis which caused millions of deaths worldwide in the past, the importance of cardiovascular disease was brought into the spotlight. Many lifesaving interventions such the percutaneous angioplasties in acute myocardial infarction led to an important impact in the survival rates increasing longevity. Furthermore, the prevention of cardiovascular disease has proven to be both effective and cost-efficient. The 2016 "European Guidelines on Cardiovascular Disease Prevention in Clinical Practice" clearly states that stopping smoking is the most cost-effective strategy for cardiovascular disease prevention (1).

Smoking is pandemic, with a worldwide prevalence of 1 billion smokers in 2011 (2). Although the rate of smoking is declining in Europe, it remains very common and is increasing in women, adolescents and the socially disadvantaged (3). A recent study performed in 187 countries to estimate the prevalence of daily smoking by age and sex and the number of cigarettes per smoker per day from 1980 to 2012 showed that the prevalence of daily tobacco smoking decreased from 41.2% in 1980 to 31.1% in 2012 for men and from 10.6% to 6.2% for women (4). The prevalence declined at a faster rate from 1996 to 2006 compared with the subsequent period, but despite the decline in prevalence, the number of daily smokers increased from 721 million in 1980 to 967 million in 2012 in the context of population growth (4).

From the economical point of view, data are also alarming. In a studied performed in 152 countries, representing 97% of world's smokers, researchers found that diseases caused by smoking accounted for 5.7% of global health expenditures in 2012, while the total economic cost of smoking was equivalent to 1.8% of global gross domestic product (5). Furthermore, smoking caused 2.1 million deaths and 13.6 million years lost to disability among adults aged 30-69 (5). In total, smoking-attributable diseases accounted for 12% of all deaths among the world's working-age population, with this proportion being highest in Europe and America where the tobacco epidemic is most advanced (5).

The statistics outlining the negative effect of tobacco consumption are plenty. A lifetime smoker has a 50% probability of dying due to smoking, and on average will lose 10 years of life (6). Smoking is responsible for 50% of all avoidable deaths in smokers, half of these due to cardiovascular disease (1), as smoking accounts for 36% of the risk attributable to a first myocardial infarction (7).

Is there any reasonable explanation for this pandemic of an apparently evidence-based proven lethal behavior? Maybe there is.

Looking back in history we see that the perception of smoking suffered important changes along the years reflecting the changing mentalities of the society, influenced by both discoveries in the medical field, political and industrial interests.

After its introduction to Europe by Christopher Columbus in1492, tobacco conquered the world. It was believed to have miraculous powers as suggested by Robert Burton in his "The Anatomy of Melancholy" (1621): "Tobacco, divine, rare, superexcellent tobacco, which goes far beyond all panaceas, potable gold and philosopher's stone, a sovereign remedy to all diseases" (8). Books like "Panacea or the Universal Medicine, being Discovery of the Wonderful virtues of tobacco taken in Pipe with its Operation and use both in Physick and Chyrurgery" (1624) or "Tabacologia" describe various positive effects of tobacco. It was the "holy herb" of the mid-sixteenth century and the penicillin of the seventeenth century (8). The first to oppose the use of tobacco was King James I of England who declared it to be immoral and unhealthy in his "Counterblaste to Tobacco" from1604 while in 1956 Auerbach documented the cardiopulmonary disorders and malignancies that resulted from the use of tobacco (8).

As the perception of smoking changed in society along the history so did the connotation attached to the artistic representation of smoking in art. Following the artistic representation of smoking we can indirectly track the evolution of society`s perception toward a major cardiovascular risk factor.

In his chapter "Symbol and Images: Smoking in Art since the Seventeenth Century" from the "Smoke. A Global History of Smoking", Benno Tempel provides a brilliant illustration of the multiples changes in meaning associated with the artistic representation of smoking (9). In the seventeenth century the representation of smoking in art was associated with lower class society, drunkenness and social deviance (10) as depicted by Dutch painters such as Gerard ter Borch and the Flemish artist David Teniers the Younger. In the eighteenth century, it depicted eroticism and pleasure as in the artworks by Antonia Guardi, Joseph Vernet and Jean Etienne Liotard depicting harem women smoking to emphasize their erotic passivity and boredom (9). In the nineteenth century, smoking pointed out social differences as it was exclusively the privilege of men (9). During the Romantic period smoking became tightly related to intellectualism, literature and creation as illustrated by the Edouard Manet's "Stephane Mallarme". The Impressionists painted smoking scenes as a part of the everyday life, with no additional meanings. Smoking regained the symbolic power in the postimpressionist era when artists like Edvard Munch used smoking to symbolize psychological problems as in his "Self-Portrait". In the 20th century smoking in art exhibited a wide variety of connotations ranging from social and economic status to the decrease of smoking attractiveness which was captured by artists like Mel Ramos and Tom Wesselmann who exploited the clichés of sex and cigarettes (9).

The representation of smoking in art had a great influence on the smoking phenomena in women. While initially smoking was inacceptable for women, the illustration of female smoking denoting prostitutes as painted by Henri Toulouse-Lautrec and Vincent van Gogh, after 1900 it gradually became a symbol of charm, the representation of women smoking in art making it a more social acceptable habit (9).

Wilson and colleagues (11) exemplified the important symbolic power of the representation of smoking in art along the years in their work "Might artworks from online-

museum collections contribute to medical education?" In January 2014, they identified artworks with tobacco smoking content by analyzing the Google Cultural Institute's "Art Project" which comprised 309 art collections from 49 countries, namely 55,004 artworks by 8654 named artists (11). There were 356 artworks with smoking content spread over five centuries: 1600s (5.9%), 1700s (6.7%), 1800s (43.3%), 1900s (37.1%), and 2000s (7.0%). Paintings (at 66.0%), predominated over other forms such as photographs (18.8%) and drawings (10.7%) (11). They illustrated the different meanings of tobacco smoking by selecting 10 representative artworks. Smoking in art was attached to a wide variety of meanings ranging from immoral behaviors, drunkenness, sexual licentiousness, reflected the epidemic of female smoking, its association with alcohol, sensuality, sexuality, nonconformity, rebelliousness, mental disorders or glamour (11).

Another very interesting study performed by Wilson and colleagues (12) analyzed the artworks produced between 1950-1999 and exhibited in The National Portrait Gallery in London finding 53 portrayed smoking by identifiable individuals (5%) out of the 1063 artworks of the collection. They found that the rate of portrayal was highest in the 1950s (10%) and 1960s (11%) and declined sharply thereafter, smoking virtually disappearing from portraiture in the 1990s (12). As expected, men were more often represented than women with predominant occupations of writers, painters, artists, and politicians while there were no portrayals of smoking among scientists, engineers, mathematicians, astronomers, inventors, and doctors (12). Cigarettes were the dominant form of tobacco product (64% of portrayals), followed by pipes (26%), and cigars (9%) (12).

Apart from the awareness induced by the evidence based medical effects of tobacco, the industry of media and entertainment had a great importance in the history of smoking, as tobacco use in movies and television is a subtle and powerful form of promotion (13).

The article "How the tobacco industry built its relationship with Hollywood" (13) brings into the spotlight secret internal tobacco industry documents. The declarations of important figures of the tobacco industry and correspondence with the movie industry help us understand the continuous siege we are intentionally exposed to increase the tobacco consumption.

As early as 1792, the tobacco industry realized that product placement in movies is "better than any commercial that has been run on television or any magazine, because the audience is totally unaware of any sponsor involvement" (13, 14). The tremendous power of smoking on screen is brilliantly exposed in a Philip Morris marketing plan from 1989: "We believe that most of the strong, positive images for cigarettes and smoking are created by cinema and television. We have seen the heroes smoking in "Wall Street", "Crocodile Dundee", and "Roger Rabbit". Mickey Rourke, Mel Gibson and Goldie Hawn are forever seen, both on and off the screen, with a lighted cigarette. It is reasonable to assume that films and personalities have more influence on consumers than a static poster of the letters from a B&H pack hung on a washing line under a dark and stormy sky. If branded cigarette advertising is to take full advantage of these images, it should do more than simply achieve package recognition-it has to feed off and ex-

ploit the image source" (13, 15). Moreover, documents from 1980 reveal that the tobacco industry required specific criteria for tobacco product placement in a film like "positive exposure", that the stars are people "with whom we would want the product associated", and if "we could work the product into the script" (13,16).

The article (13) explains in detail and exemplifies with concrete situations how tobacco industry understood the value of placing and encouraging tobacco use in films. The strategies included placing products and tobacco signage in positive situations on screen, while keeping brands away from negative situations, writing favorable articles related to smoking, encouraging professional photographers to take pictures of actors smoking specific brands, paying and even offering free cigarettes to producers and famous celebrities to use a certain brand in real life and on the screen. It also specifies movies where smoking-related products were used - "Superman II", "The Jazz Singer", "Backroads", "Cannonball Run", "Pennies from Heaven", "Blowout", "Falcon's Crest", "Dallas", "Knot's Landing", "Grease", "Rocky II", "Airplane", "Little Shop of Horrors", "Crocodile Dundee", "Die Hard", "Who Framed Roger Rabbit", and "Field of Dreams (13, 17).

The intense activity of the tobacco industry reflected in the prevalence of smoking scenes in the movies. A random sample of top-grossing films from 1950 through 2002 indicated that the amount of smoking decreased from an average of 10.7 events per hour in 1950 to a low of 4.9 events per hour in 1980-1982 and then increased rapidly to 10.9 events per hour in 2002 (18).

Researchers pointed out that movies rarely depict the negative health outcomes related to smoking and contribute to increased perceptions of smoking prevalence and association with benefit such as glamour, independence, rebelliousness, relaxation, stress relief, romance, socializing, celebrating, pensive thinking, and confiding in others (18).

In an analysis of how smokers were depicted in 100 popular films from 1940 to 1989 McIntosh and colleagues found that smokers were depicted as more romantically, more sexually active and more intelligent than nonsmokers showing that Hollywood's depiction of smoking tends to ignore the negative consequences while depicting smokers a bit more positively than nonsmokers (19).

Under these circumstances of movies depicting an increased prevalence and benefits of smoking, the impact of movie industry upon youth became extensively studied.

In 2002 Sargent and colleagues provided empirical evidence that viewing movie depictions of tobacco use is associated with higher receptivity to smoking prior to trying the behavior (20).

Charlesworth and colleagues concluded in 2005 that smoking in the movies leads adolescents to hold more protobacco attitudes and beliefs, which is consistent with the observed dose-response relationship between exposure to smoking in the movies and initiation of adolescent smoking (18). The images of smoking in movies both normalize the behavior and downplay the negative health effects associated with smoking, encouraging more tolerant, neutral, or nonchalant attitudes about smoking, establishing the perception that smoking is normal, prevalent, and even desirable in society, especially among adults (18). Although teens generally acknowledge the long-term health risks associated with

smoking, they immediately experience the perceived short-term benefits of smoking, such as looking tough or sexy or fitting in with their peers, which reinforces and motivates adolescent smoking (18).

It is argued that according to a population-attributable risk calculation movies account for almost 390 000 new adolescent regular smokers in the United States annually (21) which is nearly equal to the 400 000 (22) active smokers that die every year.

In 2016, the first meta-analysis of longitudinal studies of the association between exposure to smoking imagery in movies among young people and the risk of becoming a smoker show that the most exposed young people are more than 40% more likely to become smokers than the least exposed (23).

There is a tight connection between art and science in the society's perception of smoking. The artistic representation of smoking is associated with a wide variety of connotations along the centuries ranging from poverty, decadence, elegance, intellectuality, romance, to destruction and death. The movie on the other hand, mainly due to the tobacco industry interests, tends to associate smoking with favorable stereotypes while omitting it's the negative effects with deleterious impact on the youth.

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ROLE OF THE REHABILITATION HOSPITAL IN THE SOCIAL AND ECONOMIC INTEGRATION OF PERSONS WITH DISABILITIES

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ROLE OF THE REHABILITATION HOSPITAL IN THE SOCIAL AND ECONOMIC INTEGRATION OF PERSONS WITH DISABILITIES (Abstract): **Aim**: The perceptions towards disability and the disability legislation has changed over the last years. However, for the persons with disabilities and their families a clear path to follow to manage their conditions and successfully integrate in the economic and social life has not been established, yet. **Material and methods**: Analysis of the status of disability practice in Europe and in our country, and an attempt to establish the role of the rehabilitation doctor and regional rehabilitation hospital in the long-time management of these patients. **Results**: The largest share of a hospital income is based on contracts with the National Health Insurance Agency, so all rehabilitation procedures conducted during hospitalization must fit into standardized protocols developed by expert committees. The same is true for the outpatient rehabilitation. **Conclusions**: Many aspects would require improvement in our country and it is the duty of the rehabilitation doctor to ensure personalized care for all patients. Therefore, there is a need for some changes, starting with attracting collateral funding, implementation of national healthcare programs and establishment of non-governmental agencies. **Keywords:** DISABILITY, REHABILITATION HOSPITAL, SOCIAL REINTEGRATION.

Disability is a generic theme, a so-called "problem" recognized worldwide. However, it is socially problematic to define clearly and to establish concrete terminology in this relatively controversial area.

Regarding the antithesis disability-ability, we subjectively establish a degree of normality of the population, to establish later, subjectively, the disability (1).

Disability should not be seen and should not be an impediment to success - neither for the patient nor his/her family, nor for the "auxiliary" people involved in this issue.

Reactions to disability have changed over time, largely because people with disabilities started to unite in associations and non-governmental organizations (NGOs), which has led to various national and international initiatives to support them (2, 3, 4, 5).

Adopting and organizing a quality man-

agement system centered on personalized healthcare should be a strategic decision of each hospital. The design and implementation of such a system is primarily the duty of the manager, being influenced in this respect by specific needs and objectives, the services he provides and which he intends to implement in the future, and by the size, structure and profile of the unit he is leading (6).

Patients with disabilities must be effectively integrated socio-economically to contribute within the limits allowed by the type and degree of disability to the country economic growth. Thus, disability costs will be more easily borne by both patients and family, and by the society (7, 8, 9).

Because a great part of the rehabilitation and integration of people with disabilities is realized in the community it is our duty as doctors to ensure the continuity of this rehabilitation by establishing good collaborative relationships with the state institutions and various NGOs that manage these patients, to facilitate their access to continuous rehabilitation services, avoiding bureaucratic obstacles (8, 9).

MATERIAL AND METHODS

We evaluated the role of a rehabilitation hospital, the Botosani "Sf. Gheorghe" Rehabilitation Hospital, in the social and economic integration of persons with disabilities.

Several management, quality-of-care and financial indicators, the implementation of social policies in the Botosani Rehabilitation Hospital and Botosani county, as well as the status of management of the people with disabilities nationwide were assessed.

The statistical analysis was performed using *SPSS 18.0* software (SPSS Inc., Chicago, IL, USA).

We also wrote a series of proposals and plans management of these people, to fulfil objective needs and increase their active participation in the social and economic life.

Medical services are provided by two clinics of Rehabilitation, Physical Medicine and Balneology (RMFB) with separate compartments for neurological, cardiovascular and pulmonary rehabilitation, a department of rheumatology, and one of geriatrics-gerontology, totaling 195 beds. The clinic also has a 25-bed short-stay unit and 7 outpatient consulting rooms: internal medicine, ENT, rheumatology, neurology, rehabilitation, physical medicine and balneology, geriatrics and gerontology, ophthalmology. Medical rehabilitation services are provided in the treatment base (10).

The hospital also has a medical laboratory, a functional exploration laboratory and a medical radiology and imaging laboratory.

Within the hospital, until 2014, the National Program for Active Monitoring of People with Disabilities was functioning.

Botoşani County is in North-east part of Romania, with a total area of 4,986 km^2. The administrative-territorial organization of Botosani County in municipalities, towns, communes and villages has the following proportions: 2 municipalities, 5 towns, 71 communes and 333 villages. Botosani municipality, located in northeastern Romania, is the largest town and the capital of Botosani County; it has a population of about 115,000 inhabitants (11).

The total population of the county is 397,151 inhabitants (2015), distributed as follows (12): 196,905 (49.6%) male inhabitants and 200,246 (50.4%) female inhabitants; 161,518 (40.7%) urban population

and 235,633 (59.3%) rural population.

Age-group distribution: children and students (0-19 years): 98,324; active age population (20-64 years): 226,648; elderly (≥ 65 years): 72,179 inhabitants (11).

The distribution of the hospital staff is as follows (11): total jobs approved on 31 December 2016: 206, of which: 3 for the Steering Committee, 22 doctors, 8 health professionals with higher education, 92 nurses, 51 auxiliary staff, 12 technical, economic and socio-administrative staff and 18 workers (11).

The distribution of hospital beds over the different units is as follows (6): Department of Rehabilitation, Physical Medicine and Balneology I- 90 beds, of which 34 beds for Neurological Rehabilitation Department, 56 beds for the Department of Rehabilitation, Physical Medicine and Balneology, 15 beds for Rheumatology Department and 24 beds for Geriatrics and Gerontology Department. The Department of Rehabilitation, Physical Medicine and Balneology II has 66 beds, of which 26 beds for Cardiovascular rehabilitation, 12 beds for pulmonary rehabilitation, 22 beds for Rehabilitation, Physical Medicine and Balneology and 25 beds for the short-stay unit (6).

RESULTS

The number of patients discharged in 2016 was 4961 (fig. 1), of which 2,442 (49,2%) from the Department of Rehabilitation, Physical Medicine and Balneology I, 1711 (34,48%) from the Department of Rehabilitation, Physical Medicine and Balneology II, 313 (6,3%) from the Rheumatology Department and 495 (9,97%) from the Geriatrics and Gerontology Department (11).

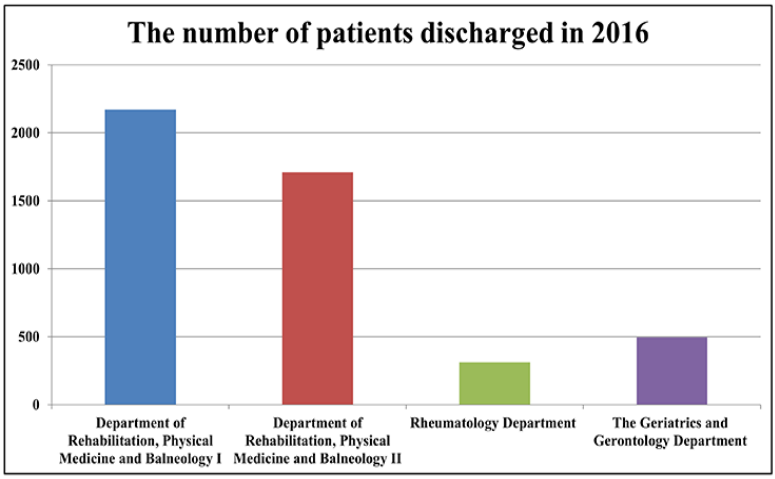


Fig. 1. The number of patients discharged in 2016

Assessment of the economic and financial situation of the hospital

From table I, the highest share of the hospital income is represented by contracts with the National Health Insurance House (CNAS) (more than 90% over the whole analysis period). At the same time, it is noticed that the percentage of income from other sources, i.e. hospital own incomes, is decreasing (from 5.62% in 2014 to 4.26%

in 2016).

Analyzing the expenditures over the period 2014-2016 (tab. II), it is noticed that the largest share of the total expenditures is for personnel expenditures. At the same time, the percentage of these expenditures is increasing, which is explained by the fact that, starting with 2015, the salaries of employees increased because of Ordinances no 35/2015, 20/2016, 43/2016 and Law no 250/2016.

TABLE I

Income structure by funding sources (years 2014-2016) (RON) (12)

	Funding source	2014	2015	2016
A	**CNAS contracts**	**8,835,418 (92.4%)**	**8,991,405 (92.55%)**	**11,241,973 (91.06%)**
1.	Continuous hospitalization	8,195,449	8,034,848	10,142,753
2.	Short stay unit	161,155	256,212	273,793
3.	Laboratory	152,774	303,618	385,572
4.	Outpatient care	135,134	133,979	175,551
5.	Rehabilitation base	190,906	262,748	264,304
B	**Public Health Department contract**	**222,512**	**265,032**	**367,506**
C	**Hospital own income**	**496,662 (5.62%)**	**447,308 (4.60%)**	**527,039 (4.26%)**
D	**Income from concessions and rentals**	**4,500**	**10,211**	**6,146**
E	**Interests**	**551**	**929**	**0**
F	**FNASS subventions for salary raises**	**0**	**0**	**202,184**
	Total Income	**9,560,079**	**9,714,886**	**12,344,848**

TABLE II

Expenditures (years 2014-2016) (RON) (12)

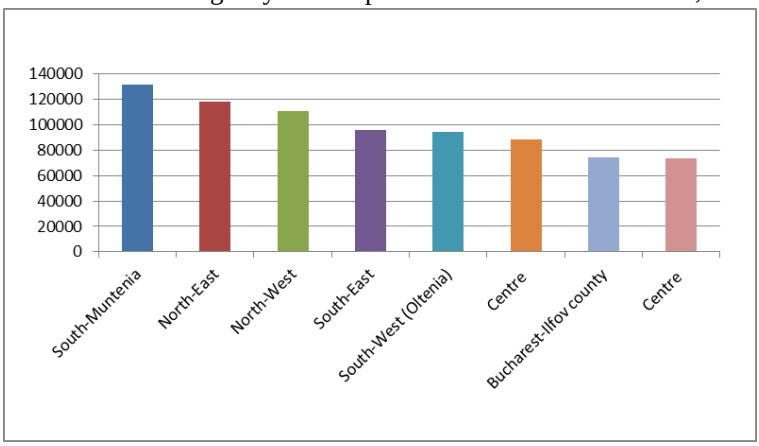
Indicator	2014	2015	2016
Personnel expenditures	6,154,821 (68.76%)	6,882,570 (67.49%)	8,460,151 (71.36%)
Goods and services	1,724,772 (19.26%)	2,248.384 (22.04%)	2,285,293 (19.27%)
Drugs	462,798 (7.51%)	468,021 (6.80%)	478,509 (5.65%)
Other expenditures - scholarships	45.184	46,975	59,884
Capital expenditures	563,538	551,685	571,819
Total	**8,951,113**	**10,197,635**	**11,855,656**

The implementation of social policies for people with disabilities

According to the World Health Organization Report (2), the prevalence of people with disabilities varies with age and disability; there is a slightly higher percentage of people with both severe and moderate disabilities in Europe compared with the rest of the world.

On December 31, 2016, the total number of adults with disabilities in Romania was of 786,546, of which 768,456 (97.7%) in the care of families (not institutionalized) and 18,090 (2.3%) institutionalized (12).

TABLE III
Total number of persons with disability in Romania
(Source: National Agency for People with Disabilities – ANPD, 2016)



Regarding the regional distribution, according to the National Agency for People with Disabilities (ANPD), the number of people with disabilities in the Northeast Romania is 118,304 (tab. III). According to ANPD, this region is ranked second at national level in terms of the number of people with disabilities (12).

The national distribution places Botosani County under the average of 18727.28 (total number of disabled persons/total number of counties), with an effective number of persons with disabilities of 13577. Most adults have a physical disability, followed by people with a somatic and mental disability (11).

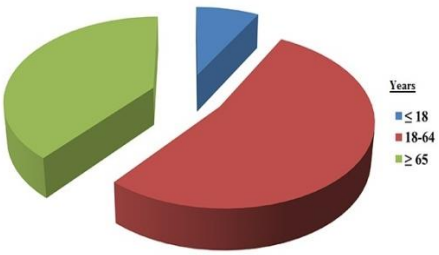


Fig. 2. Age-group distribution of people with disabilities

Age-group distribution reveals that the highest percentage of people with disabilities is in working age age-group (18-64 years) - 53%, followed by the age group over 65 years- 39% (11) (fig. 2).

DISCUSSION

Through this paper it is desirable to promote patient-centered and disability-based healthcare using the medical-social support hexadecimal scheme, and to promote personalized healthcare.

It is also important to seek and attract short, medium and long-term funding sources to be able to provide long-term rehabilitation health care and to make partnerships with governmental institutions and non-governmental organizations.

An important part in these actions is promoting them in the media by organizing interdisciplinary events at the hospital level with the involvement of social workers in partnerships: doctors, nurses, social workers, occupational therapists, psychologists, priests, organizing and coordinating support groups for patients with disabilities, in partnership with social assistance services at the

level of local authorities, organizing and coordinating programs and projects at local level for the socio-economic and professional reintegration of people with disabilities.

Designing and printing informative materials (flyers, brochures) and promoting existing ones (sites, leaflets, brochures) for patients, family, can be useful to properly orientate to social assistance services.

The rehabilitation physician's duty is to increase the quality of life of patients with a certain degree of disability; hence the obligation to identify patients at risk (elderly, disadvantaged, vulnerable or non-functional).

Development of programs for the continuous rehabilitation of people with disabilities by organizing thematic meetings with the participation of patients, the family, as well as the medical staff involved in rehabilitation: specialized doctors, nurses, social workers, occupational therapists, psychologists and collaborations and partnerships with companies that sell or distribute healthcare devices at home; good cooperation with the National Health Insurance Agency and the county branches can be also useful in order to provide financial assistance to the patient for the purchase and use of these types of devices.

Another useful action would be to establish a loco-regional database of patients with disabilities to properly track their everyday work for socio-economic-professional integration.

In the end, an important step would be the establishment of a non-governmental association / non-profit foundation to acquire an infrastructure of medical devices and home care facilities available on loan for patients with a certain degree of temporary disability (e.g., chairs or cradles, crutches, walking sticks, prostheses, prostheses or non-personalized orthotics, special mattresses, mobilization or transfer devices etc.).

CONCLUSIONS

This study shows that establishing protocols for admission and treatment during hospitalization is strictly necessary in the Botosani Rehabilitation Hospital.

Community social rehabilitation and social reintegration assistance must start in the hospital.

During the hospitalization, it is necessary to have a complex interdisciplinary team (psychologist, occupational therapist, social worker etc.).

Post-acute cases with moderate and low disability levels will be analyzed through diagnostic protocols and rehabilitation therapy to be able to follow the outpatient treatment.

Social cases will be discharged after analysis with the region's NGOs.

Home treatment will be reviewed together with local specialist companies that can ensure proper care for the patient.

The readmission of aggravated chronic cases requiring hospital admission will be assessed based on specific protocols for each pathology.

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NEWS

ROLE OF AML SPECIFIC CYTOTOXIC ANTIBODIES IN GVL RESPONSE

It is well established that most acute myeloid leukemia (AML) patients can obtain remission only by means of allogenic hematopoietic stem cell transplant(HSCT). This process induces a graft versus leukemia immune response (GvL) mediated by T cells and NK cells. However, there is not much data showcasing the contribution of B lymphocytes, so a study was conducted that focused on B cells producing monoclonal antibodies against antigens expressed on the cell surface of leukemic cells but not on normal hematopoietic and non-hematopoietic cells. It is interesting that a number of these donor-derived antibodies recognized the U5 snRNP200 complex, a spliceosome component normally found inside cells. In AML however, the U5 snRNP200 complex is exposed on the surface membrane of leukemic blasts and can promote apoptosis in a FcR dependent way in the absence of cytotoxic leukocytes or complement. This potentially paves the way for novel tumor growth inhibitory therapy (AML-specific cytotoxic antibodies in patients with durable graft versus leukemia responses Marijn A. Gillissen, Martijn Kedde, Greta de Jong, et al., *Blood* 2017: blood-2017-02-768762; doi: https://doi.org/10.1182/blood-2017-02-768762i).

V. A. Cianga

CHILDREN BORN SMALL FOR GESTATIONAL AGE, FIVE YEARS EXPERIENCE WITH GROWTH HORMONE THERAPY

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CHILDREN BORN SMALL FOR GESTATIONAL AGE, FIVE YEARS EXPERIENCE WITH GROWTH HORMONE THERAPY(Abstract) **Aim:** Recombinant Human Growth Hormone (rhGH) is an effective treatment for short children born small for gestational age (SGA) who fail to demonstrate catch-up growth by 2-4 years of age. These children usually don't have classical GH deficiency, but either low GH secretion or reduced sensitivity to GH. The goals of therapy are to achieve a normal height in early childhood and an adult height within the normal target range. We assessed the efficacy and safety profile in the first 5 years of rhGH treatment in ten SGA children. **Material and methods:** The study enrolled 10 SGA children (6 boys, 4 girls). All patients were given a mean dose of 0.35mg/kg/wk. and followed for a period of minimum 5 years (mean 5.68 yrs.). **Results:** The mean height expressed in standard deviations (SD) raised from -2.43 at diagnosis to +0.28 after 5 years. In the first 5 years of therapy there were no cases of diabetes mellitus or impaired glucose tolerance, 2 (20%) patients presented impaired fasting glucose, 1 (10%) patient developed hypothyroidism and 4 (40%) patients presented transitory subclinical hypothyroidism. Mean IGF-1 values were higher, but not exceeding +2DS. Conclusions: GH therapy is reasonably safe and effective in increasing linear growth in children born SGA who fail to have catch-up growth. Maximum height velocity was registered in the first year of treatment, 11.76 cm/year and declined in time. Adverse events due to GH treatment were no more common in the SGA population than in GH-deficient children. **Keywords:** SMALL FOR GESTATIONAL AGE, RECOMBINANT HUMAN GROWTH HORMONE.

Many children with short stature do show compromised adult height relative to mid parental height for age, sex, and population group (1). When children with short stature have been born with a low weight and/or length, they are classified as short associated with being small for gestational age (SGA). International Societies of Pediatric Endocrinology and the Growth Hormone Research Society, as well as the International Small for Gestational Age Advisory Board, recommended that the term refer to infants whose birth weights and/or lengths are at least two standard deviation

(SD) units less than the mean for gestational age (1,2). According to this definition, approximately 3%-10% of newborns are considered SGA. While most of these infants undergo catch-up growth, 10%-15% remain small for their age at the age of 2 years. In addition to short stature, SGA infants may be predisposed to altered body composition and (in adult life) hypertension and cardiovascular disease (1, 3). Data from epidemiological studies indicate that children born SGA are also at increased risk of insulin resistance and type 2 diabetes in adulthood (1). There is now evidence for an increased prevalence of metabolic syndrome among adults who were born SGA (4). Important is that their height, weight, and body mass index are closely monitored to prevent excessive weight gain, as children born SGA who catch up in weight and height are at a higher risk than those without catch-up (5). In summary, SGA is associated not only with shorter than average height, but also with potential health disadvantages, such as reduced body fat and a lack of appetite as compared with individuals who were not born SGA; there is also an increased prevalence of metabolic disorders in later life among patients born SGA as compared with those born AGA. Being born SGA is associated with lower cognitive ability in mathematics and reading comprehension and with more emotional, conduct, and attention deficit hyperactivity disorders. A relatively recent study (6) showed low values of psychomotor development and IQ in 60 children born with intrauterine growth restriction (IUGR) aged between 3 months and 16 years. The Wechsler Intelligence Scale for Children scores showed a slight worsening. The impact on the IQ seems to be inherent to the condition of being small at birth and not to the growth rate. In healthy people, Growth hormone (GH) and Insulin-like growth factor (IGF) interact to regulate growth. In utero, IGF-I, IGF-II and insulin are the key factors in regulating fetal growth, with GH having little effect. Postnatally, the contribution of IGF-I becomes more important than that of IGF-II, and neonates with growth restriction have lower IGF-I levels than neonates with a normal growth. Importantly, it has been reported that a high endogenous GH secretion significantly increases the IGF-I levels in short GH-sufficient neonates born SGA at 3 days postnatally as compared with AGA controls (7). This is evidence for a functionally activated GH-IGF-I axis in SGA neonates (8). Approximately 60% of the children with a persistent short stature who were born SGA have an abnormal GH secretion and reduced IGF levels (9). Although children with a short stature who were born SGA frequently have GH levels lower than normal, most are not usually classified as being GH deficient by recognized criteria. The status of the GH-IGF axis at birth or in early postnatal life is not predictive of later growth, and therefore hormone measurements in the SGA infant or child are not indicated in routine care. Early evaluation of short children born SGA is recommended, and those under 2 years of age with a current length below −2.5 SD should be referred for evaluation. Over the last 30 years, several recombinant human growth hormones (rhGH, Somatotropin) products have been approved for the treatment of children with growth disorders associated with short stature, and multiple studies, both observational and randomized, have established the efficacy and safety of rhGH products. 10% of SGA babies fail to show catch-up growth despite

good nutrition after birth. They continue to grow at a percentile that is lower than - 2 SD and their target height percentile. SGA babies with birth length more than -2 SD below the mean have a 7 times higher risk of having short final height, and those with a birth weight more than -2 SD below mean have a 5 times higher risk, indicating that birth length has greater influence on final height than the weight. Of the infants who do not show catch-up growth, 50% end up as short adults. They constitute 14 - 22% of short adults (10). Recombinant human Growth Hormone has been used to help short SGA children achieve catch-up growth. In July 2001, GH was approved by the US Food and Drug Administration for the long-term treatment of growth failure in children who are born SGA and do not achieve catch-up growth by 2 years.

MATERIAL AND METHODS

Observational study on ten pre-pubertal children (Tanner stage I) born SGA with growth disturbances defined as current height standard deviation score (HSDS) \-2.5 (and parental adjusted SDS \-1) for chronological age and sex (tab. I). Patients were treated with rhGH 0.035 mg/kg/day by subcutaneous injection in the evening. The objectives include an evaluation of efficacy through changes in height parameters; measurement of rhGH-induced serum markers IGF-I; incidence and adverse events (AEs).

TABLE I.

Data from the first 5 years of rhGH therapy

Parameter	Baseline	1 year	2 yrs.	3 yrs.	4 yrs.	5 yrs.
Chronological age (yrs.)	6.29	7.29	8.29	9.29	10.29	11.29
Bone age (yrs.)	4.05	4.75	5.56	7.15	9.3	11.05
Mean IGF-1 values (ng/ml and SD)	77.5 (+0.24)	305.38 (+1.49)	258.71 (+1.54)	329.85 (+1.26)	412.44 (+1.47)	476.55 (+1.85)
Height (SD)	-2.43	-1.37	-0.91	-0.68	-0.43	0.28
Growth velocity (cm/yrs.)	-	11.76	9.24	8.16	7.68	6.24

Another objective was to evaluate the long-term effect of rhGH treatment on the development of diabetes mellitus in short children born SGA. Height was measured in cm and HV calculated. HSDS and HV were assessed at baseline and at 6-month intervals. To determine bone age, X-rays of the left hand and wrist were obtained at baseline and every year thereafter. IGF- was measured at screening and at every 6 months thereafter. Fasting plasma glucose, 2-h OGTT, HbA1c were measured at baseline, and annually thereafter. All AEs were recorded at each visit and their incidence was reported. Physical examinations and vital signs were performed at each visit. Hematology, blood chemistry, thyroid function tests - free thyroxine (FT4) and thyroid stimulating hormone (TSH) levels-, lipids were assessed at baseline, at every 6 months. Paired t tests were used to calculate p values, with statistical significance defined as $p<0.05$.

RESULTS

Body height increased steadily throughout treatment period; the mean height standard deviation score improved by 2.71, from -

2.43 at baseline to +0.28 at 5 years of therapy; the changes in HSDS decreased with time (fig. 1). Mean height velocity was maximum in the first year (11.76 cm/year), decreasing in the second (9.24 cm/year), third (8.16 cm/year), fourth (7.68 cm/year) and fifth year of treatment (6.24 cm/year). Growth velocity decreased slightly but remained higher than at baseline ($p<0.0001$) (fig. 2).

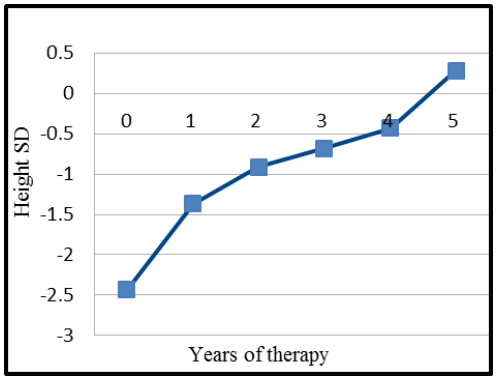

Fig. 1. Height SD during first 5 years of rhGH therapy

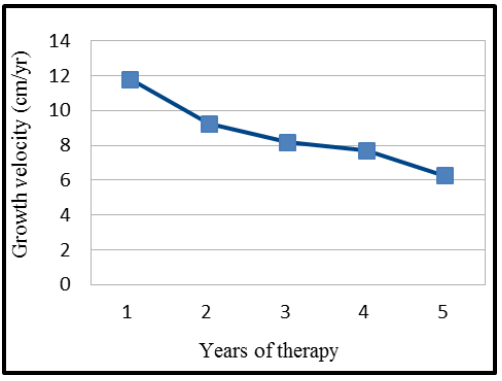

Fig. 2. Growth rate during the first 5 years of rhGH treatment

Chronological age at therapy initiation varied between 4 and 12 years (mean 6.29 yrs.). Bone age at diagnosis was late by a mean value of 2.24 years, decreasing after 5 years of treatment at a mean value of 0.24 years (fig. 3). IGF-I serum level indicated that at baseline most patients had low (\-2 SD) IGF-I levels. During the treatment IGF1 has normalized and even has risen above normal. Mean IGF-1 values were higher than normal range after five years, but not exceeding +2DS (fig. 4).

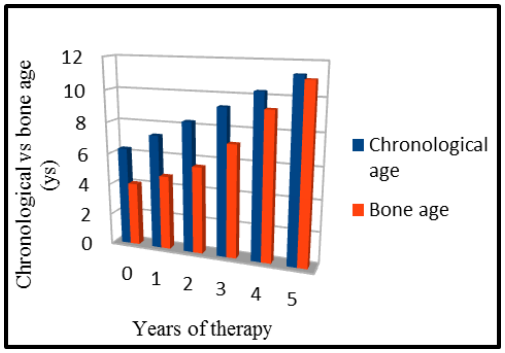

Fig. 3. Evolution of bone age during first 5 years of therapy

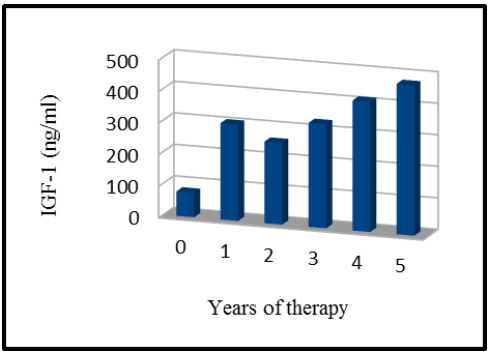

Fig. 4. IGF-1 values during first 5 years of treatment

In the first 5 years of therapy there were no cases of diabetes mellitus or impaired glucose tolerance (glucose between 140-200 mg/dl at OGTT; normal values of HbA1c). Two (20%) patients presented impaired fasting glucose (fasting glucose

between 100-126 mg/dl). One (10%) patient developed hypothyroidism. Four (40%) patients presented transitory subclinical hypothyroidism (elevated TSH, normal FT4 values, no clinical signs) (fig. 5).

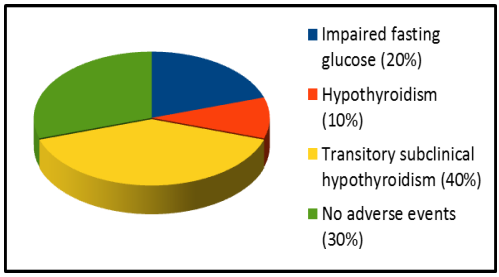

Fig. 5. Adverse events during first 5 years of rhGH treatment

DISCUSSION

Early initiation of rhGH therapy has as result the complete recovery of statural deficit in 5 years, according to growth prognosis calculated by parental heights. Growth response to GH treatment of non-GH deficient SGA children was done by Zegher et al (11). The analysis revealed that GH was an effective treatment for normalizing short stature in non-GH deficient SGA children during early childhood and puberty. According to consensus guidelines, a change in HVSDS of more than +0.5 within the first year of GH treatment is a growth response (1). In a large international cohort of children born SGA, HVSDS had to be at least +1 after the first year of treatment for patients to be eligible to continue with therapy; therefore, all patients regarded as responders in the present study achieved a growth response as defined in the consensus guidelines (3). Analysis of data from KIGS (Kabi-Pharmacia International Growth Study database) (12), which included data from 613 children born SGA who failed to manifest spontaneous catch-up growth, revealed that the age at which GH therapy was started was second only to dosage as the most important predictor for response. During the 1st year of therapy, the response to GH correlated negatively with age at initiation of treatment. The treatment response during the 2nd year was best predicted by a three-parameter model: HV during the 1st year of treatment, age at the start of treatment, and rhGH dose. The study confirmed the superior efficacy of beginning therapy earlier and with higher doses of rhGH treatment as compared with delaying therapy or using lower doses (3, 4, 13). An independent, international SGA Advisory Board (2) and the 2006 consensus meeting of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society (1) both developed a consensus for management of short children who were born SGA. Therapy was recommended for short children born SGA who do not manifest spontaneous catch-up growth by the age of 2-4 years. It was concluded that therapy with GH is effective and safe. The International SGA Advisory Board (4) recommended the initiation of rhGH therapy at 0.48 mg/kg/week; Long-term continuous treatment is effective, even at a dose of 25 µg/kg/day. Bone age for skeletal maturation should be done, although it is not a good predictor of adult height. Our study limitations, like many others, include the lack of a control group and the age of patients when rhGH treatment was initiated. However, the clear treatment benefits observed in most patients' deficiency.

CONCLUSIONS

In our study GH therapy was reasonably safe and effective in increasing linear growth in children born SGA who fail to have catch-up growth. Maximum height velocity was registered in the first year of

treatment, 11.76 cm/year and declined in time. No severe adverse events were registered. No malignancies were observed to date.

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EDITORIAL

SURGICAL ONCOLOGY - THE MULTIDISCIPLINARY TEAM (MDT) IMPORTANCE

"Surgery has undergone many great transformations during the last fifty years, and many are to be thanked for their contributions – yet when we think of how many remain to be made, it should rather stimulate our inventiveness than fuel our vanity".

Sir Percival Pott, 1714-1788, Surgeon,
St. Bartholomew's Hospital, London, UK

Cancer is in our days a multidimensional and multifactorial reality. Hanahan and Weinberg in their paper called "*The Hallmarks of Cancer*" showed that to progress and to become a threatening disease, the tumor cells need to relate to different genetic and molecular events (1).

All over the world, cancer is the second leading cause of death after cardiovascular diseases, and a substantial amount of resources are allocated to provide patient care, rehabilitation, education and cancer research. A rapid development of Oncology is associated with a new specialties evolution.

Oncological surgery means specific technique and approach to tumorous conditions. It is technique adapted to, and based upon our knowledge, of tumor biology.

Surgical oncology covers a much wider field than oncological surgery: the role of the surgeon in the tumor treatment. Apart from the surgical act, the activities are basic research, prevention and many other fields (2). Surgery is the oldest oncological discipline. The mantra of the cancer surgeon was that increasingly radical surgery, would enhance cure rates. Preservation of form, function, and quality of life, without compromising survival, is the new mantra. Today's surgeons, no longer the uneducated technicians of history, are highly trained medical professionals and together with oncologists, radiologists, scientists, anesthetists and nurses, have made cancer surgeries routine and save (3).

From a historical perspective, surgical approaches to cancer existed far before the discipline now known as "Surgical Oncology". The surgical treatment of cancer originated as an approach focused on radical and debilitating tumor resections and extended lymphadenectomies. These "bigger is better" procedures, are focused on removing all loco-regional tissues, including those with any potential local extension. Much has changed over the last several decades that has made surgical oncology an academic and evidence-based specialty. What has led to modern surgical oncology, being the field it is today, is the development of large prospective databases, the design and participation in randomized controlled trials, and the translational research, that has ushered specialized and customized patient cancer therapy effective (4). Surgical oncology is more of a cognitive, than a technical surgical specialty. Apart from a small cluster of index operations, such as regional pancreatectomy, limb salvage and retroperitoneal sarcoma surgery, isolated limb perfusion, and

multisegment liver resection, most of the surgical procedures that are performed by surgical oncologists, are like those performed by a surgeon not oncologically trained**.** What frequently differentiates these two types of surgeons is not merely knowledge about how to do a specific operation, but an awareness of how and when to do that operation; that is, the cognitive knowledge of contemporary multimodality cancer care (5). Surgical Oncology is a consumptive surgery that requires new technologies and high human resources. In a study where the oncologic surgeons were surveyed, almost 94% of the persons, included in the study had experienced one occupational symptom, within the past 6 weeks and 28% reported having an occupational injury at some time, during their career. The surgeons manifested nonspecific symptoms such as fatigue, general physical discomfort and the cervical spine pain (38%) that were associated with prolonged operations concluding that is necessary to improve human engineering in the operating room as an important strategy for reducing occupational injury (6).

The unique difficulty for the surgical oncologist comes with understanding the balance between surgical and non-surgical therapies for solid tumors. A practitioner must to be familiarized with high variety of treatment modalities and should be able to select the right patients for surgery at the right point in the evolution of the disease. Beside that un optimal surgical treatment should be given to a high level of proficiency, that will ensure the best clinical outcome for the patient (7). In our days, when talking about solid tumors, surgical resection represents the most important possibility of treatment, giving the only hope of cure for patients in most cancer. Over the past several decades the field of Surgical Oncology has become much more defined, resulting in important contributions to the diagnosis and management of a wide variety of cancers. When searching on the Medline data base references to 'oncology' from 100,000 results more than 15% contain the additional reference term 'surgery' (8). Fundamental research in oncology, also being a major task, a continuous challenge for the surgical oncologists, likewise means intensive contacts with other specialties; multidisciplinarity is essential (fig. 1).

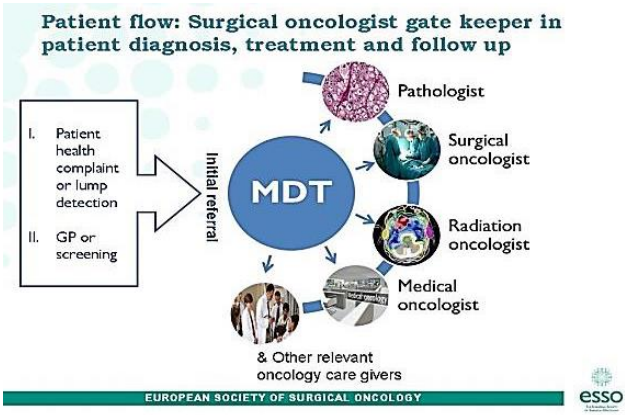

Fig. 1. MDT- multidisciplinary team (https://worldspanmedia.s3.amazonaws.com/media/esso/gallery/page-content-items/mission-vision.jpg)

With the increasing incidence of cancer and growing complexity of cancer care, multidisciplinary teams have the potential of improving the quality of life and increasing survival of cancer patients. Although there are several challenges to the success of the multidisciplinary teams, continuous commitment and support will ensure that multidisciplinary teams achieve its full potential in delivering effective and efficient, cancer care. Controversies, which are inevitable within a team that is striving to reach decisions concerning complex situations, thus require a variety of approaches for dealing with them when they occur (9).

Multidisciplinary team meetings are also known as tumor boards, multidisciplinary cancer conferences, multidisciplinary case reviews, or multidisciplinary clinics, in different health care systems. These different terms may represent the variations in the organizational structure, membership, approach, focus, and the decision processes of these meetings (10). Multidisciplinary team model in cancer care was introduced and endorsed to ensure that care delivery is consistent with the best available evidence. Over the last few years, regular meetings have become a standard practice in oncology and gained the status of the key decision-making forum for patient management. Every cancer case must to be discussed at a multidisciplinary conference where specialists involved in diagnosis and management are present. The role is to provide diagnostic, staging, and treatment-related elements that will be represented by the personalized therapeutic plan. A fully trained general surgeon is considered to have sufficient technical skills associated with knowledge and experience to approach the solid tumor and to provide the best medical care to the patient. Simulation and virtual simulation are very important steps to enhance technical skills and to improve operating room performance (11).

Performance in surgery depends on non-technical skills such as decision making, patient factors and technical skills of the healthcare team. Technical factors that affect decision making include the information about patients, robust CT and video-conferencing equipment, a minimum dataset with expert review of radiological and pathological information, implementation and recording of the MDTs decision. Non-technical factors with an impact on decision making include consensus on decisions and communication with patients and primary care, open discussion, attendance of team members at meetings, leadership and teamwork. To raise the quality of care for patients with cancer, a strong decision making based on a factors optimization is required (12).

The advantage of a MDT, is that the patient could benefit of different specialties consultation during one single visit. Between 4 and 45% of the patients discussed at the MDT meeting occurred changes in diagnostic. The results after a MDT case evaluation showed changes in staging and diagnosis, initial management plan of the patient, higher rates of treatment and a shorter time to starting the treatment after diagnosis, a better survival and accordance with international guidelines. Still is only a little evidence that show the impact of MDT over the clinical outcome of the patients (13).

Multidisciplinary meetings (MDMs) make sure that comprehensive treatment plans for patients with cancer are made. Although many doctors attend MDMs and contribute to the decision-making process, only a few will become involved in a patient's care after the team meeting. An issue that is debated all over the medical filed is represented

by the legal aspect of these meetings. If a patient wished to recover damages, all doctors present at the meeting would be personally accountable for decisions related to their area of expertise ant that is why doctors should be aware of the legal implications of their participation meetings (14).

The MDT activities are confronting with some important issue that many times are difficult to be corrected such as: non-uniform access to specialist care, frequent reporting of inadequacies in cancer services, disjointed referral system, large variations in frequency of individual treatments used, caseload for doctors treating cancer, and patient survival. Resources like staff time, founding, evidence of MDT effectiveness, communication, administrative support, clinical trials and clinical outcome had to be continuously evaluated to ensure a good efficiency of the MDMs (15).

Cancer treatment nowadays needs a multimodal approach, based on a MDT activity. That means, neoadjuvant treatment, surgery and where it seems to be necessary even an adjuvant treatment. It is not an option to stay still. The major advances in radiation and medical oncology of the past few decades have changed the face of cancer treatment forever. Tumors previously thought unresectable with curative intent, can now be brought to potentially curable surgical resection using neoadjuvant strategies. Adjuvant radiotherapy following a conservative surgical procedure allows the patient to be spared from the morbidities associated with radical surgery or radical radiotherapy, where higher doses of radiation are administered. It is likely that the use of adjuvant radiotherapy will increase in the future, together with an increase in usage of chemotherapy and/or novel biological agents, especially within the context of multimodality management of cancer. In this scenario, the place of multi-disciplinary approach is especially important, with input from specialists such as oncologists (surgical and non-surgical), radiologists and pathologists at the outset, so that the optimal management decision is made before embarking on treatment (16). With advances in the basic sciences, newer therapies have emerged in the treatment of cancer. One of the key developments was the use of antibody therapy. Chemotherapy treatments for cancer have advanced considerably in the past several decades. In addition, supportive measures have also improved significantly, and chemotherapy today is much better tolerated than it was only a decade ago. There are more treatment options, more effective therapies, and new ways of delivering drugs preferentially to the tumor.

Surgeons with oncological practices need to bring their personal drive, dedication and intellectual rigor to the cancer trials and research teams. By integrating into local, regional, national and international organizations, surgical oncologists will continue to enhance the quality and success of cancer trials and studies that change clinical practice and benefit individual patients and populations (17).

The future of surgical oncology is not simply to remove more body parts, but to better select the best treatment for each individual patient.

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TOPICALITIES IN THE SUDDEN UNEXPECTED DEATH SYNDROME IN INFANTS

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TOPICALITIES IN THE SUDDEN UNEXPECTED DEATH SYNDROME IN INFANTS (Abstract): Sudden Infant Death Syndrome (SIDS) represents the third cause in postnatal mortality. The pathogenesis is multifactorial. SIDS victims can present sign of preexisting chronic asphyxia, persistent increase in dendritic spine and delayed maturation of synapses in medullary respiratory centers, if a decreased reactivity of 5 hydroxytryptamine 1A (5-HT1A) and 5-HT2A in the dorsal nucleus of the vagus, solitary nucleus and ventrolateral medulla. SIDS is an exclusion diagnosis, so that inexplicable SIDS is the accepted term. The objective of this report is to present current data about the pathogenesis oh this syndrome and the medico-legal measures applied in preventive and curative aim. The "face-up" sleeping position has cut in half the SIDS frequency. In conclusion, the infant sleep studies represent an important line for the future research to provide sufficient explanation of the sudden death in these infants. **Keywords**: SUDDEN DEATH, PATHOGENESIS, CLINICAL EVENTS, MEDICO-LEGAL MEASURES.

The sudden infant death syndrome (SIDS) is the third cause of postneonatal (1 month to1 year) mortality in industrialized countries (1). The phrase unexpected infant death syndrome (UIDS) is preferred, as it includes all cases of SIDS, defined as the brutal death of a child under 1 year, installed in full health and unexplainable even after the postmortem investigations, including a full and scientific necropsy, extended complementary examinations, full anamnesis and clinical examination and a detailed inquiry at the tragedy scene. SIDS is an exclusion diagnosis, as a series of non-SIDS causes (18.6%) were identified within the UIDS, such as: infections (7.2%), congenital cardiopathy (2.7%), child abuse (2.5%), genetic and metabolic diseases (2.1%) (2). In consequence, SIDS is classified in explainable SIDS (UIDS) and unexplainable SIDS. The SIDS incidence varies between 1.5-2‰ live births in 1994 and 0.45‰ live births in 1999, representing 15% of the pediatric deaths (3). The maximum frequency is recorded between the ages of 2 and 4 months and during the winter months; in 53% of the cases, the infant with SIDS sleeps in the same bed with at least 2 other persons and associates a low social-economic status (4).

ETIOPATHOGENY

Due to the definition from 1970 given by Beckwith (2), SIDS was considered as a causeless death. Subsequently it has been observed that not all the cases of sudden death were normal from the anatomopathological point of view, but they associated brain abnormalities original from the intrauterine life, which can determine sudden death in a vulnerable period after birth. In 1972 the hypothesis of the triple risk in SIDS was released, including: general vulnerability, the risk specific to the age and precipitation factors (5). The most important hypotheses in SIDS are the respiratory one (apnea), cardiac one (arrhythmia) or visceral dyskinesia (glottal spasm and/or gastro-esophageal reflux) (6). It has been demonstrated that the autonomous system (respiratory, cardiovascular, upper digestive) participates in all these hypotheses, even if the arrhythmogenic cardiac dyskinesia, as the leading cardiac system is under strictly autonomous control (7).

The subsequent ethipathogenic concepts assert multifactorial involvement in the SIDS, as follows: genetic factors, infant risk factors, maternal risk factors and environmental factors.

The *genetic factors* are demonstrated through the new discoveries in the field of molecular genetics and the identification of genetic polymorphism of the victims of sudden death. Almost two thirds of the cases have signs of preexisting chronic asphyxia and higher expression of the vascular endothelium growth factor (VEGF) in the cephalorachidian fluid. Brain abnormalities in infants with SIDS consist of the significant increase of the denditric extensions and late maturation of synapses in the medullary respiratory centers, as well as the decrease in the immunoreactivity of the receptors of 5 hydroxytryptamine 1A (5HT2A in the vagus nerve, of the solitary nucleus and of the ventrolateral medulla, and increases were discovered in the brain periductal grey matter, along with gliosis (8).

The hypoplasia of the medullary arcuate nucleus, an important cardiorespiratory center was discovered in over 50% of the SIDS cases. This can associate with alterations of other structures of the brain stem, such as hypoplasia of the Kolliker parabrachial area and of the reticular formations of the respiratory system. Rarely cases of SIDS were reported in relation to the neuronal hypoplasia and immaturity of the hypoglossal nucleus responsible for the deglutition alteration (9).

The postmortem genetic studies discovered genetic differences including defects of the sodium channels, serotonin-transporters and abnormalities of the genes of the autonomous nervous system, the C4 complements and IL-10. The long QT syndrome is associated with the sodium channels gene (SNC 5A) defect. Over 100 mutations were identified, of which over 30 are associated with the prolonged QT syndrome (10).

Molecular genetic studies in the SIDS victims identified mutations from the early embryological development of the autonomous nervous system. The relevant genes are MASH 1 (mammalian achaetescute homolog 1), BMP 2 (bone morphogenetic protein 2), PHOX 2b (paired-like homeobox 2b), RET (rearranged transfection factor), ECE 1 (endothelin converting enzyme 1), EDN 1 (endothelin 1), TCLR 3 (T cell leukemia homeobox) and EN (engrailed 1). The mutations of these genes can affect the cardiorespiratory control, but up to the present the genotypic differences between the SIDS victims cannot be related

to any type of defined clinical phenotype, putting to the issue the interaction between the genes and the environmental risk factors (11).

The risk factors for SIDS specific to the infant are the male gender, age between 4 and 6 months, low weight upon birth and prematurity.

The maternal risk factors for SIDS are age under 19 years, active smoking, which doubles the risk for sudden death, the use of illicit drugs, short time between births and limited or delayed prenatal cares (12).

The environmental risk factors for SIDS are the ventral position during sleep, passive smoking, high temperature, humidity, the cold season, sleeping in the same bed with someone else and poverty (13).

FORENSIC MEDICAL MEASURES IN SIDS

The prior clinical events are determined by the synergy of the changes in the respiratory rhythm on the heart rhythm. Originally, 2-3 days before the fatal event, the infant has tachycardia, profuse sweats, and subsequently bradycardia and low blood pressure. These elements suggest an abnormal sympathetic and parasympathetic activity secondary to the dysfunction of the respiratory rhythm on the heart rate, like the state of shock, when sympathetic excitation (profuse sweats, tachycardia, increase in the blood pressure) happens in the compensatory stage, and sympathetic inhibition (bradycardia, low blood pressure) happens in the decompensating stage, resulting in the loss of tissue perfusion and death. Abnormalities could emerge in the brain structures controlling the variations of the respiratory rhythm, heart rate and the blood pressure values during sleep (cerebellum, medullar central surface, Raphe caudal nucleus, the inferior olive and the fastigial nucleus of the cerebellum). It can be speculated that SIDS is the result of a decrease in the uncompensated blood pressure, because of cerebellum lesion (14).

The forensic medical measures in SIDS are different if applied in curative or preventive purpose.

At the site of a SIDS case firm administrative and medical measures are imposed, which in certain countries are enacted through circular letters of the Ministry of Public Healthcare, mentioning: the direct phone line with the reference regional centers, the manner of urgent transportation of the body of the deceased infant by some medical team or competent personnel in a vehicle authorized by the police to the morgue. The body is transported to the forensics department, after which the police will carry out an investigation, if there are suspicious circumstances of the decease, the clinical examination reveals abuse signs, the refusal of an autopsy, decease after 9 months of age, and if there have been any other deceases of family members in suspicious circumstances.

The main purpose of the medical actions is the detection of the cause of death, possibly in 50-75% of the cases = explainable sudden death (UIDS) and the determination of the risk in siblings, to answer the parents' question about what would happen to their other children. The etiological medical investigation includes a full physical examination of the deceased infant (weight, height, perimeters, tegument aspect, liver, spleen), X-rays (thorax, skeleton, cranium), biological sampling (intracardiac puncture – blood, suprapubic puncture – urine, lumbar puncture – CRL) for the bacteriological, virological, biochemical and genetic examinations. The anatomopathological

examination will perform numerous histological sections in all organs (15).

The SIDS relation with the risk of repetition in siblings is like the risk in the general population, the sporadic cases being more frequent than the family ones. Nevertheless, there are families with high risk of repetition of the sudden death, such as: several sudden deaths in the genealogical tree, sudden death after 1 year, the metabolic disease, hereditary vagus hyperreactivity, precarious social-economic conditions, abuse and bronchomalacia in autopsy. The major indications of exploration of the collaterals of a suddenly deceased infant, by means of a 48-hour hospitalization, are the following: clinical signs of gastro-esophageal reflux (GER), uncontrolled fear, family vagus field and congenital prolonged QT syndrome. The twin of a suddenly deceased infant runs a risk 22 times higher for SIDS because of identical infectious and environmental factors, rather than because of genetic factors. Consequently, the twin will be hospitalized urgently with his/her mother, for a clinical examination and paraclinical explorations to exclude infections, GER, vagus hypertonia and congenital prolonged QT syndrome (16).

The prevention of SIDS consists in the elaboration of guides comprising recommendations for a risk-free sleep of infants. The recommended position for sleep in infants is the supine position ("lying on the back face upward"), in a crib with a firm mattress, no pillow or cover, in a room with the temperature between 18 and 20° C. The result of this measure is the decrease in the SIDS frequency by 50% (17).

CONCLUSION

SIDS is a complex multifactorial entity, whose frequency can be decreased by adopting preventive measures for the infant's sleep. Of the multitude of the physio pathological mechanisms involved, the study of the infant's sleep if a research line for the future, to find out why some infants die in their sleep, as a functional respiratory and cardiac response to different stimuli in the external environment.

It is of great need to pay attention to the environmental risks factors and the maternal factors as well. Even though no matter how much you prevent it, SIDS still appears to infants, in many cases, the smoking, the drug addiction or alcohol consuming during the pregnancy or after birth can lead to SIDS.

Also, in addition, we consider that infants decease cases should be highly supported and funded by with the great help from pediatricians and other medical specialists.

To get close to the factors and mechanisms of SIDS, and to break down a vicious and pathophysiological circle that also happen to affect infant's siblings.

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NEW DRUGS FOR LOWERING LDL-CHOLESTEROL

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NEW DRUGS FOR LOWERING LDL-CHOLESTEROL (Abstract): LDL-Cholesterol (LDL-C) is a well-known risk factor for cardiovascular disease. Although statins are the mainstream treatment for lowering LDL-C level, additional LDL-lowering therapies are needed to reduce residual cardiovascular risk, especially in patients at very high risk, or with hereditary lipid disorders or statin intolerance. The proprotein convertase subtilisin/kexin type 9 (PCSK9) is a key regulator for LDL-Receptor activity and an attractive target for the treatment of hypercholesterolaemia. From its discovery in 2003, several therapeutic approaches to the inhibition of PCSK9 have been proposed. Monoclonal antibodies that bind to PCSK9 received marketing approval in 2015 (alirocumab and evolucumab) or are being evaluated in phase III trials (bococizumab). Many other molecules are in preclinical studies, phase I or II clinical trials. Another point of interest carefully investigated is the cardiovascular benefit of reducing LDL-C using these new molecules. High hopes are invested in them. **Keywords:** LDL-CHOLESTEROL, PCSK9 INHIBITORS, ALIROCUMAB, EVOLOCUMAB.

Low-density lipoprotein (LDL) is removed from the circulation mainly by hepatic uptake. LDL binds to the LDL receptor (LDLR) and the LDL-LDLR complex is internalized into clathrin-coated vesicles by endocytosis. In the endosomes, LDL is separated from its receptor. Further, LDL is degraded in the lysosomes and the LDLR is recycled for later reuse.

The proprotein convertase subtilisin/kexin type 9 (PCSK9) was identified in 2003 by Seidah et al. (1) and is a key regulator for LDLR activity. The major function of PCSK9 is the degradation of the LDLR (2). Secreted PCSK9 binds to the LDLR. When LDL-LDLR complex is formed, the PCSK9 is internalized together with it. The binding of PCSK9 to LDLR induces a change in LDLR conformation, avoiding normal recycling of LDLR to the plasma membrane and enhancing the LDLR lysosomal degradation (3). Consequently, it reduces the number of LDLR on the cell surface and the liver uptake of LDL, thereby increasing the plasma levels of LDL-C. As high LDL-C levels have consistently been associated with an increased risk of coronary heart disease,

PCSK9 is an attractive target for the treatment of hypercholesterolaemia. Recent data suggest that its involvement in lipid metabolism is even greater, PCSK9 having a role in triglyceride metabolism and triglyceride accumulation in visceral adipose tissue (4).

Several therapeutic approaches to the inhibition of PCSK9 have been proposed.

MONOCLONAL ANTIBODIES

The most successful approach was the monoclonal antibodies that bind to PCSK9 near the catalytic domain, interact with LDLR and hence inhibit the function of PCSK9.

Alirocumab (Praluent) and evolocumab (Repatha) are fully human monoclonal antibodies that specifically bind to PCSK9. In 2015 they received marketing approval from European Medicines Agency and the U.S. Food and Drug Administration. Bococizumab is a humanized monoclonal antibody that specifically binds to PCSK9 that is currently in phase 3 clinical trials.

Alirocumab and evolocumab are indicated as an adjunct to diet and maximally tolerated statin therapy for the treatment of adults with heterozygous familial hypercholesterolemia (HeFH) or clinical atherosclerotic cardiovascular disease, who require additional lowering of LDL-C. Evolocumab is also indicated as an adjunct to diet and other LDL-lowering therapies for the treatment of patients with homozygous familial hypercholesterolemia (HoFH) who require additional lowering of LDL-C.

The ODYSSEY Programme was designed to assess the efficiency and safety of alirocumab in a broad spectrum of patient populations. When alirocumab was compared with ezetimibe in patients at moderate to high cardiovascular risk with statin intolerance due to muscle symptoms, it was found that alirocumab reduced mean LDL-C by 45.0% *vs* 14.6% with ezetimibe ($p<0.0001$), with fewer skeletal-muscle adverse events *vs* atorvastatin (5).

When alirocumab was added to atorvastatin, it provided significantly greater LDL-C reductions *vs* adding ezetimibe, doubling atorvastatin dose, or switching to rosuvastatin and enabled greater LDL-C goal achievement (6). ODYSSEY Combo II compared the efficacy and safety of alirocumab *vs* ezetimibe, as add-on therapy to maximally tolerated statin therapy in high cardiovascular risk patients with inadequately controlled hypercholesterolaemia and again, alirocumab achieved significantly greater reductions in LDL-C compared with ezetimibe, with a similar safety profile (7).

ODYSSEY FH I and FH II assessed the long-term alirocumab treatment in patients with HeFH and inadequate LDL-C control on maximally tolerated lipid-lowering therapy. Patients without a history of cardiovascular events and those who had suffered a myocardial infarction or ischemic stroke were eligible to enroll in the study if their LDL-C levels were not at goal according to current guidelines for primary or secondary prevention. Patients received stable high-dose statin therapy with or without other lipid-lowering therapy (fenofibrate, ezetimibe). The study demonstrated that alirocumab reduced LDL-C levels from baseline by 57.9% (FH I) and 51.4% (FH II) *vs* placebo. Alirocumab was generally well tolerated, with consistent LDL-C reductions observed throughout the study. By doubling the antibody dose when LDL-C goal was not achieved on the starting dose, some 59–68% of patients achieved an LDL-C goal of <70 mg/dL (8). Alirocumab also reduced apolipoprotein B levels (by 54%, $p<0.0001$) and plasma Lp(a) levels

(by 20.5–30.3%) (9).

A major study, the ODYSSEY Long Term Trial, enrolled 2341 high-risk patients with HeFH, known coronary artery disease or coronary artery disease risk equivalent, in a randomized, double-blind, placebo-controlled trial. Eligible patients had LDL-C levels above 70mg/dL and were currently taking either high-dose statins or maximum tolerated dosages. Other lipid lowering therapies were also allowed. A post-hoc analysis of cardiovascular events that included a composite of death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke or unstable angina requiring hospitalization showed lower rates with alirocumab. Heart failure requiring hospitalization and ischemia-driven revascularization were not included as cardiovascular events in this post hoc analysis. When these events were included, the difference between groups was no longer significant (10). Therefore, a hope for an enlightening response is expected from ODYSSEY Outcomes (currently in the period of enrollment), an ongoing trial that is intended to provide an assessment of the cardiovascular benefit of alirocumab in approximately 18,000 patients over a period of 5 years.

The PROFICIO Programme was designed to assess the efficiency and safety of evolocumab. Comparing evolocumab with ezetimibe in statin intolerant patients due to muscle symptoms, it was found that evolocumab reduced LDL-C from baseline by 55% to 56% (corresponding to treatment differences: 140 mg sc q2weeks = Q2W dose, respectively 420 mg sc once monthly = QM dose) *vs* ezetimibe, with low incidence of muscle adverse events (12). Evolocumab also reduced Lp(a) levels by 22% to 27% compared to 1.7 - 5.8% in ezetemibe-treated patients. Of the evolocumab-treated patients at high risk, more than 75% achieved LDL-C <100 mg/dl compared with less than 10% of ezetimibe-treated patients.

When evolocumab was added to a guideline-recommended lipid-lowering therapy (10 mg atorvastatin, 80 mg atorvastatin with or without 10 mg ezetimibe, according to the patient's ATP III defined cardiovascular risk), percent reductions in LDL-C in the evolocumab group *vs* placebo were 48.5% in the group receiving atorvastatin 80 mg plus ezetimibe, 56.8% in the group receiving atorvastatin 80 mg and 61.6% in the group receiving atorvastatin 10 mg (13). The LDL-C mean reduction in evolocumab group *vs* placebo was 57%. There were also significant reductions from baseline in apolipoprotein B, Lp(a) and triglycerides, as well as 5.4% increases in HDL-C and 3.0% in apoA1. In patients undergoing moderate or high intensity statin therapies, the favorable effect of evolucumab *vs* placebo or ezetimibe in lowering uncontroled LDL-C levels was once again proven without a doubt (14).

RUTHERFORD-2 Study assessed the safety and efficacy of evolocumab in patients with HeFH and inadequate LDL-C control on maximally tolerated lipid-lowering therapy. Evolocumab achieved mean percent reductions of LDL-C of 60.2% (Q2W dose) and 65.6% (QM dose), compared to placebo ($p < 0.0001$). LDL-C <70 mg/dl was achieved by 68% of patients in the evolocumab Q2W group and by 63% in the evolocumab QM group, compared to only 2% in each of the placebo groups. Patients with receptor-negative mutations had a similar response to treatment compared to those with receptor-defective mutations or those with mutations in apolipoprotein B (15). TESLA study was conducted in pa-

tients with HoFH receiving stable background lipid-lowering treatment and not on apheresis. Evolocumab significantly reduced LDL-C by 30.9%. Patients with defective receptor mutation had the best response to treatment and had significantly higher reduction in LDL-C compared to those with single LDLR negative mutation (16).

Patients enrolled into a longer-term extension trial were re-randomized to the evolocumab plus standard therapy *vs* standard therapy alone without placebo control. 70% of patients were receiving background statin therapy. LDL-C was reduced by 61% to a mean of 48 mg/dL. Approximately 80% of patients had other cardiovascular risk factors including hypertension, diabetes, metabolic syndrome, current cigarette use, family history of premature coronary artery disease or familial hypercholesterolemia. The cardiovascular events (death, myocardial infarction, unstable angina requiring hospitalization, coronary revascularization, stroke, transient ischemic attack and heart failure requiring hospitalization) occurred in a 1% of the evolocumab group *vs* 2% of the standard therapy group (17). The FOURIER Study is ongoing to confirm this finding and to determine if adding a PCSK9 inhibitor to standard statin therapy with will further decrease cardiovascular events over long-term follow-up.

Very little data is available on efficacy and safety of bococizumab. In a phase 2b dose-ranging study of subjects with hypercholesterolemia on stable doses of statin, compared with placebo, bococizumab significantly reduced LDL-C levels across all doses (18).

GENE INHIBITORS

Several other agents inhibit the gene responsible for the synthesis of the PCSK9 protein. Antisense oligonucleotides looked promising after the initial preclinical studies, but two phase I trials were terminated and development of the drugs had not been continued. ALN-PCS is a small interfering RNA that inhibits PCSK9 synthesis. ALN-PCS02 was successfully tested in a phase I trial on healthy volunteers with raised cholesterol who were not on lipid-lowering treatment (19).

MIMETIC PEPTIDES

The catalytic domain of mature PCSK9 binds to the first epidermal growth factor-like repeat A domain (EGF-A) of the LDLR, while the C-terminal domain binds to cell surface proteins, including annexin A2 (20). In order to induce its internalization and degradation, PCSK9 binds to EGF-A domain of LDLR. A mimetic peptide, which mimics the actions of EGF-A, was demonstrated to competitively inhibit PCSK9-mediated degradation of LDLR in HepG2 cells. Mimetic peptides currently being investigated are: EGF-AB peptide fragment, LDLR (H306Y) subfragment and LDLR DNA.

SMALL MOLECULE INHIBITORS AND ADNECTINS

Small molecule inhibitors and adnectins targeting circulating PCSK9 are currently being developed. Orally administered small-molecule inhibitors may act by altering the sequence of PCSK9 auto-catalytic intracellular processing, PCSK9 secretion or LDLR interaction. SX-PCK9 and TBD are in preclinical studies (21). Adnectins are genetically engineered target-binding proteins, similar to monoclonal antibodies, including binding to targets with similar affinity and specificity, but differ in terms of sequence and lack of

disulphide bonds in their single-domain structure (22). The adnectin BMS-962476 has recently completed its phase 1 clinical trial and demonstrated good tolerability.

CONCLUSIONS

Multiple large studies have declared statins as the mainstream treatment for lowering LDL-C level, but additional therapies are certainly needed in order to reduce residual CV risk, especially in patients at very high risk, or with hereditary lipid disorders or statin intolerance. As we highlighted in this brief material, the addition of alirocumab or evolocumab to the existing lipid-lowering therapy has resulted in a significant, consistent and sustained reduction in LDL-C levels in patients with varying levels of CV risk. Even if at the moment the cardiovascular benefit of using these new drugs is still under investigation, high hopes are invested in them.

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NEWS

CARDIOVASCULAR RISK FACTORS IN PATIENTS WITH IDIOPATHIC INFLAMMATORY MYOPATHIES

Cardiovascular (CV) risk constitutes a major concern in patients with dermatomyositis and polymyositis. A multicentric Danish study evaluated cardiovascular risk factors and coronary artery calcification (CAC) in 76 patients with idiopathic inflammatory myopathies (IIM) and a control group formed of 48 sex- and age-matched healthy subjects. Obesity was more frequent among IIM patients than in healthy individuals (33% in patients as opposed to 11% healthy individuals, $p<0.05$). Waist/hip ratios and serum tryglycerides also had higher values in patients with IIM compared to healthy controls ($p<0.05$). Arterial hypertension and diabetes mellitus were more prevalent in IIM patients ($p<0.05$). The extent of coronary artery calcification was estimated using the Agatston score which showed higher mean values in patients compared to controls but results did not reach statistical significance. It was shown that glucocorticoid therapy, higher age at the moment of diagnosis, high levels of glycated hemoglobin (HbA1c) and smoking status correlate with higher coronary artery calcification scores in patients ($p<0.05$). However, C-reactive protein levels did not asociate with a greater risk of CAC in the study group. Although not elucidating the mechanism behind increased CV mortality in IIM, the study brings to attention a series of important modifiable CV risk factors in patients with IIM. (Diederichsen L.P. et al. Traditional cardiovascular risk factors and coronary artery calcification in adults with polymyositis and dermatomyositis: a Danish multicenter study. *Arthritis care & research* 05.2015, doi : 10.1002/acr.22520. 67: 6 : 848-854).

Elena Rezuș

EATING BEHAVIOR IN RESPONSE TO ACUTE STRESS

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EATING BEHAVIOR IN RESPONSE TO ACUTE STRESS (Abstract): Obesity is a medical and social problem with a dramatically increasing prevalence. It is important to take action since childhood to prevent and treat obesity and metabolic syndrome. Infantile obesity affects all body systems starting in childhood and continuing to adulthood. Understanding the impact of stressors on weight status may be especially important for preventing obesity. The relationship between stress, eating behavior and obesity is not fully understood. However, there is evidence that stress causes disorders in hypothalamic-pituitary-adrenal (HPA) axis, system that regulates both stress and feeding responses. Also, the response is different depending on the type of stressors. Chronic stress, especially when people live in a palatable food environment, induces HPA stimulation, excess glucocorticoids, insulin resistance, which lead to inhibition of lipid mobilization, accumulation of triglyceride and retention of abdominal fat. **Keywords**: EATING BEHAVIOR, FOOD INTAKE, ACUTE STRESS, CORTISOL, OBESITY.

In modern society, stress is everywhere and affects people of different ages, including children. It is difficult to define stress because it is so different for each of us. We can say that it is the generalized, non-specific response of the body to any demand for change. Stress can trigger many diseases, including obesity and other eating disorders (1).

Obesity is a heterogeneous disease that is difficult to treat (2,3). It is associated with risk factors for cardiovascular diseases and accelerated atherosclerotic processes, including hypertension, atherogenic dyslipidemia, atherosclerosis and structural and functional cardiac changes (1). The association of stress with obesity was suggested by Cushing's syndrome. Patients with this syndrome have upper body obesity, high levels of cortisol, atherosclerosis, glucose intolerance and hypertension (3).

Stress can be acute, short term or chronic, long-term. Stressors can be external (such as cold exposure, hunger, social stressors) or of internal cause (sleep deprivation) (4). When stress occurs, mechanisms are activated to maintain homeostasis. Homeostasis is the property of a system in which the variables are regulated so that internal conditions to remain stable and relatively constant. Homeostasis implies remaining stable by being the same, while allostasis is achieving stability through change. Allostasis is associated with

changes in food choice, appetite, energy storage and mobilization (4). This implies alteration in HPA axis hormones, autonomic nervous system, cytokines or a number of other systems. There is a close interaction between stress, allostasis, energy balance and eating patterns. Stress can influence human eating behavior in a bidirectional way (4).

Stress response. Allostasis is maintained in stress conditions by adaptive reactions originating in the central nervous system and in the periphery. Acute stress induces a fight-or-flight reaction with behavioral, autonomic and endocrinological changes. Heart rate and blood pressure are increased and energy spent on digestion and reproduction is minimized. Energy is saved for vital organs: brain, heart and muscle tissue to save life. So, in acute stress appetite and food intake are suppressed. However, there are situations of weight gain under stress. What determines direction of eating is not explained yet but HPA axis has an important role in both situation (4).

Stress response has two major components: the HPA axis and the autonomic nervous system (ANS) which encompasses the sympathetic and parasympathetic nervous system (fig. 1). Stress response depends on intensity, duration and type of stressor (5).

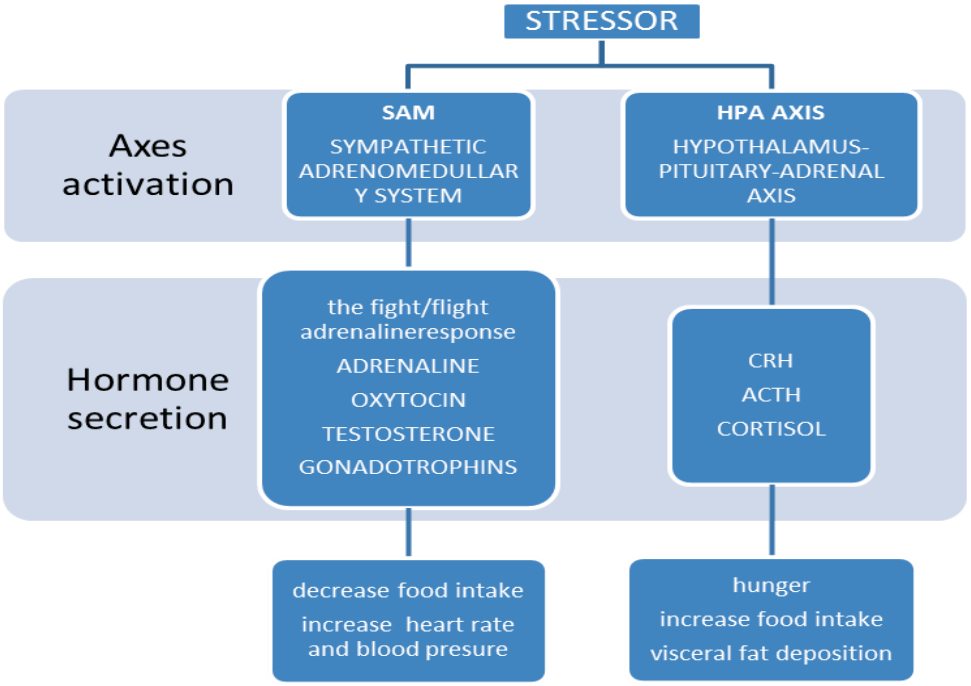

Fig. 1. Stress response (modified after Torres et al.) with two major components: SAM and HPA axis

There are two antithetical responses to stress according to the stressor type:

- ***Challenge stressor,*** a demanding but controllable situation that activates the sympathetic-adrenomedullary (SAM) system, with fight/flight response, which shuts

down digestion (6). For this type of stressor, the body has the resources to cope with.

• ***Threat stressor,*** for which the body does not have the resources to cope with or social stress (public embarrassment or failure, feeling defeated) activates HPA axis with cortisol release. Neurons of the paraventricular nucleus of the hypothalamus initiate stress response by corticotropin-releasing hormone (CRH) which modulates HPA axis. CRH stimulates the secretion of ACTH from the anterior pituitary. ACTH acts on the *zona fasciculata* of the adrenal cortex increasing cortisol secretion. Circulating cortisol has a negative feedback to the brain, limiting cortisol secretion, protecting the organism from prolonged cortisol exposure in chronic stress (6). It is known that cortisol stimulates hunger and feeding and visceral fat deposition. Glucocorticoids increase insulin secretion but has influence on orexigenic neuropeptide (NPY). Immune cells or adipocytes produces cytokines that may stimulate the HPA axis.

Eating behavior as a consequence of body response to acute stress.

To study the relationship between stress and eating behavior it is important to take into account the different types of stress. Stress can be acute (short term stressors) or chronic. Stressors can be physical (extreme temperatures, elevated sound levels, over-illumination, surgery, trauma), physiological (pain, hemorrhagic shock, intense exercise), chemical (acid-base imbalance, hypoxia), psychological or emotional (anxiety, fear, sorrow) and social (low social status, giving a speech, meeting new people, discrimination) (3).

Animal research. In animal studies all variables (type and intensity of the stressors, food offered) can be well controlled. Acute stress can be induced using stressors such as cold exposure, tail pinch, hunger, physical restraint, inescapable foot shock, exposure to a socially dominant member of the same species (6,7). Rats can be used for studying the effects of acute stress in a controlled laboratory setting with accurate evaluation of food intake. Stressors of different intensity can be used (6,7). Also, another factor is the type of food offered: commercial food for rodents (rat chow) or highly palatable food (sweetened milk). In rats, stress can lead in some cases to increases but mainly to decreases in food intake (6,7). The severity of stress is also very important. A severe stress is associated with a decrease in food intake. Moderate and mild stressors also lead to a decrease in food intake but increase the ingestion of highly palatable food (5,6,7).

According to the intensity and type of stressors the effect was either a decrease in food intake in rats exposed to acute physical stress (ex. pinch tail), or increased food intake after exposure to social stress (exposure to a socially dominant member of the same species, resident-intruder paradigm or social defeat), especially when offered a calorically dense diet (6). The last situation reproduces the modern society with numerous stressors, especially social stressors, and exposure to energy dense, highly palatable food.

Most studies highlight that stress reduces food intake in rodents, unless palatable food is given during the stress period. Also, administration of glucocorticoids increases food intake. Adrenalectomy reduces food intake, while subsequent corticosterone replacement normalizes it (3).

Human studies. Human studies confirm ingestion of palatable foods in response to stressful situations. Association between stress, comfort food and obesity is

complex. In humans it is difficult to differentiate the emotions because it could be intricate (6). Human studies show that stress decreases food intake in 30% of cases while most of subjects, 70%, increase their food intake with preference for palatable food (7,8). In modern society, a palatable food environment, it is more likely for people to increase food intake in stressful periods. Also, they will choose high calorie foods to cope with stress.

Stress reactivity has been studied in people with eating disorders (bulimia, anorexia and binge eating disorder - BED). Subjects with BED had either greater basal cortisol or greater cortisol reactivity (6,9).

Stress can be induced in laboratory conditions with the Trier Social Stress Test (TSST). After inducing stress with TSST subjects with high cortisol levels were likely to eat more calories, especially calorically dense food. On the control day there were no difference between high and low cortisol reactor groups (6). Another study compared a similar threat stressor to a positive challenge stressor (identical tasks but with positive feedback from the audience). The "threat" condition stimulated greater food intake, particularly of highly palatable food than the "challenge" condition (10,11). The relationship between cortisol and food intake in humans may also involve the effects of glucocorticoids on Neuropeptide Y (NPY), CRH, leptin as well as opioid and endocannabinoid signaling (12,13). Through multiple mechanisms, excess glucocorticoids determine insulin resistance. Even though cortisol causes hyperinsulinemia, this high insulin level does not affect plasma glucose concentration because of insulin resistance.

Stressors stimulate HPA axis and interfere with the reward system. Under stress conditions the mesolimbic dopaminergic system is activated and increased CRF secretion is stimulated. Food and drugs of abuse can stimulate similar pathways in the nervous system, including the dopaminergic and opioidergic systems. As well as drugs, hyper palatable foods alter CRF, GC and noradrenergic activity and increase sensitization of reward pathways which influence preference for addictive substance and hyper palatable foods and increases craving and intake. Repeated stimulation of the dopaminergic reward pathways may trigger neurobiological adaptions that may promote progressively compulsive behavior (4, 16)

Repeated stimulation of the reward pathways through highly palatable food may lead to neurobiological adaptations that determine overeating characterized by frequent drive to initiate eating. Opioid release increases palatable food intake and palatable food sustains opioid release. When stress becomes chronic, highly palatable food may appear to be "addictive" because the body is using food for coping with stress (4, 13, 14)

CONCLUSIONS

Our analysis of the literature studies revealed that the impact of stress depends on the intensity and type of stressors: acute or chronic. It involves multiple pathways, molecules and receptors. An important role is played by HPA axis which interferes with the reward systems. Repeated stimulation of the reward pathways through highly palatable food leads to overeating because of the neurobiological adaptations. Also, palatable food can lead to opioid release which in its turn increases the palatable food intake. Palatable foods can activate the brain reward system, so repeated stimu-

lation of the reward pathways through highly palatable food may lead to overeating with frequent drive to initiate eating. Moreover, the exogenous factors (nutritional) have a pro-obesity action by increasing the caloric intake determining energy imbalance. Additional studies are needed to understand the complex relationship between stress, alterations in HPA activity and obesity because in humans it is difficult to differentiate the emotions because it could be intricate.

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ADHD (ATTENTION DEFFICIT HYPERACTIVITY DISORDER) – A TROUBLING ENTITY, SOMETIMES PERPETUATING DURING ADULT LIFE

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ADHD (ATTENTION DEFFICIT HYPERACTIVITY DISORDER) – A TROUBLING ENTITY, SOMETIMES PERPETUATING DURING ADULT LIFE (Abstract): Attention deficit hyperactivity disorder (ADHD) is considered a neurologic development disorder resulting in impairment of attention and inhibitory control, manifested as attention deficit, hyperactivity, impulsiveness; symptoms should develop between age six and twelve and have to persist for more than six months. Approximately 30-50 % of the diagnosed cases are manifesting the disorder during adulthood and 2.5-5 % of the adults are suffering of ADHD. Genetics are important factors in ADHD, being involved in 75 % of the cases, as well in the persistence of ADHD during adult life. Three subtypes of ADHD are described – one in which is predominating the attention deficit, one with predominant hyperactivity and impulsiveness and a third combined subtype. Diagnosis criteria in ADHD are established by the American Psychiatric Association (DSM criteria) and by World Health Organization. Differential diagnosis is mainly considering bipolar disorder and borderline personality disorder. Management of ADHD is including behavioral therapies and medication, alone or combined. Stimulant medications such as amphetamine represent the therapy of choice, being effective in 80 % of the cases. New data are underlying the need for following up of the cases during adulthood, since the risk for development of psychiatric conditions such as depression, anxiety, as well as the suicidal behavior is higher than in the general population. **Keywords**: ADHD/ATTENTION DEFICIT HYPERACTIVITY DISORDER, HYPERACTIVITY, IMPULSIVENESS.

DEFFINITION, GENERAL FEATURES

Attention deficit hyperactivity disorder (or hyperkinetic disorder) is considered a neurologic developmental disorder in which are present problems of executive functions, like attention control and inhibitory control which are generating attention deficits, hyperactivity or inappropriate impulsiveness (related to age) (1,2).

Diagnosis is established only when symptoms are lasting for more than six months and are appearing between age six to twelve.

In 2013 World Health Organization estimated 39 million people affected by the disorder; 3-5 % of children are affected. ADHD is three times more frequent in boys

than in girls. Around 30-50 % of people diagnosed during childhood show symptoms as adults and 2.5-5 % of the adults have the disorder (3).

The disorder was first clearly described by George Still in 1902. Others terms used for ADHD are minimal brain dysfunction or minimal brain damage (5).

ADHD is divided into three subtypes: a form with predominant attention deficit, one with predominant hyperactive-impulsive behavior and one combined (4).

MAIN ETIOLOGIC AND PATHOPHYSIOLOGIC FEATURES

Even if the cause of ADHD is not known, the disorder is believed to be the result of interactions between genetic and environmental factors (5). Twins studies demonstrated that the disorder is often inherited, genetics being involved in 75 % of the cases. Genetic factors seem to be also involved in the persistence of ADHD during adulthood. Genes involved are mainly regulating dopamine neurotransmission (DAT, DRD_4, DRD_5, $TAAR_1$, MAOA, COMT and DBH) (6,7).

From the evolutionary perspective, hyperactivity should have been beneficial in situation of risk exposure, like exploring new areas, thus should have been benefic for the community even if harmful for the individual (8).

A reduction in the volume of certain brain structures was observed in children with ADHD, as well as a proportionally important decrease in the volume of the left-sided prefrontal cortex. Posterior parietal cortex is also thinner in ADHD subjects (9).

Recent models of ADHD are demonstrating that the disease is associated with dysfunction in central neurotransmitter systems, especially those involving dopamine and norepinephrine. Dopamine and norepinephrine pathways which are projecting into the prefrontal cortex and striatum are responsible for the modulation of the executive functions (cognitive control of behavior), motivation, reward perception and motor function; these pathways play an important role in the pathophysiology of ADHD (10).

DIAGNOSIS

Diagnosis is established on person's childhood behavior and mental development, excluding effects of drugs, medications or psychiatric conditions, which otherwise may explain the symptoms. It may be regarded as the extreme of one or more common human traits. The diagnosis is not excluded or confirmed by response to specific medications. Imaging studies of the brain are not relevant for diagnostic purposes (11).

ADHD is classified among disruptive behavior disorders, along with oppositional defiant disorder, conduct disorder and antisocial personality disorder (12).

According to the American Psychiatric Association (DSM criteria) are differentiated three subtypes of ADHD: one subtype is mostly characterized by inattentiveness (easily distracted, forgetful, daydreamer, with concentration and completing tasks difficulties). The predominant hyperactive-impulsive type is manifesting as restless, hyperactive, with difficulties in waiting and remaining seated, immature conduct; the third type is a combination of the first two subtypes, or the combined type (13).

Inattention symptoms include: being easily distracted, missing details, switching frequently from one activity to another; difficulty in maintaining focused on one

task; not seeming to listen when spoken to; daydreaming, getting easily confused and moving slowly, showing difficulties in processing information as quickly and accurately as others; difficulty in focusing attention on organizing and completing a task or learning new data; becoming bored with a task after a few minutes (13).

Hyperactivity is characterized by fidget and squirm, talking continuously, trouble to sit during dinner, school or doing homework; the subject is all the time in motion and has difficulties in achieving quiet activities. These symptoms of hyperactivity tend to fade away with age and in teens and adults with ADHD they become an inner restlessness (14).

Impulsivity is revealed by symptoms such as: impatience, inappropriate comments, acting with no regard for consequences, difficulty in waiting (as their turns in games) and often interrupting others talking or activities.

Diagnosis is established in the presence of six to nine long term symptoms of inattention, hyperactivity-impulsivity or both, lasting for at least six months (15).

Symptoms should appear at the age of six to twelve, in more than one situation, like for example at home and at school, and they must cause social, educational or work related problems (14).

Differential diagnosis must take into consideration the bipolar disorder and borderline personality disorder. Symptoms of anxiety disorder, antisocial personality disorder and mental retardation may sometimes overlap with ADHD or may occur at the same time with ADHD. Diseases which symptoms may mimic ADHD are especially hyperthyroidism, seizure disorder, lead intoxication, sleep apnea and drug interactions (16). Primary sleep troubles may cause attention deficit, while ADHD may alter sleep quality; sleep problems must be treated in children with ADHD.

THERAPEUTIC OPTIONS

Management of ADHD is including behavioral therapies and medication, alone or in combination.

Behavioral therapies are recommended mainly in those cases with mild symptoms and in preschool aged. They include behavior therapy, cognitive behavioral therapy, interpersonal therapy, family therapy, school-based interventions, neuro-feedback (12).

An important factor in reducing psychological problems (such as major depression, school failure, criminality, drug use disorders) is the friendship with persons who are not involved in delinquent activities (17, 18).

Stimulant medications such as amphetamine are the therapy of choice being effective in approximately 80 % of cases.

Alternatives include bupropionum, guanfacine, clonidine, atomoxetine. Stimulant medication is also improving the academic performance (19).

Overdose of stimulants is associated with symptoms of mania; very rare (0.1 %) such symptoms may appear at therapeutic doses in the first weeks of treatment with amphetamine or methyl-phenidate. Use of stimulants in doses above the therapeutic range is associated with addiction and dependence (18,19).

PROGNOSIS

Proportion of children showing criteria for ADHD drop at half in the three years following the diagnosis, whether if treated or not. ADHD is persisting into adulthood in approximately 30-50 % of cases.

Generally, ADHD individuals are developing coping mechanism as they become mature, thus attempting to compensate their symptoms. However, higher risk for depression, anxiety and suicidal attempt is stressing the importance of surveillance and appropriate interventions in adults with ADHD (11, 16).

Controversies in what concerns ADHD are ranging from the opinion that ADHD should be viewed as situated in the normal range of behavior to viewing it as a genetic condition. Most specialists are considering it a medical condition, at least in the cases with severe symptoms (20).

CONCLUSIONS

In what concerns ADHD diagnosis, we notice that while the mild symptoms do not need to be diagnosed, most severe cases must be treated and followed-up since the disorder is interfering with social and educational activities, as well as with work in these subjects.

Recent data demonstrated that persistence of ADHD during adulthood (2.5-5 % of the adults) is increasing the risk for development of psychiatric conditions (depression, anxiety, suicidal behavior) which recommend the surveillance and adequate therapy in these cases.

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NEWS

TIMING OF ESOPHAGECTOMY IN MULTIMODAL THERAPY OF ESOPHAGEAL CANCER

Neoadjuvant radiochemotherapy [n(R)CT] has become the standard of care in the multimodal therapy concept for patients with locally advanced esophageal cancer. The question is whether the length of the interval between completion of n(R)CT and surgery can affect the postoperative outcome, tumor response and long-term survival. In a study of 106 patients with adenocarcinoma and squamous cell carcinoma of the esophagus, treated between 2006 and 2013, on the basis of the median time interval to surgery, patients were divided into two groups [group A $\leq$ 40 days (n = 54) and group B> 40 days (n = 52)] and compared concerning demographic data, preoperative risk scores, morbidity, outcome, tumor response and long-term survival. Group A showed a trend towards a higher mortality risk as preoperatively assessed by the physiological and operative severity score for the enumeration of mortality and morbidity in esophagogastric surgery patients (O-POSSUM) (p = 0.064) and group B showed a trend towards a higher rate of complete responders (p = 0.097). So, concerning perioperative morbidity and mortality, delayed surgery after n(R)CT shows no benefit for the patient's outcome; however, the rate of complete tumor response is higher in patients with a time interval of more than 40 days, although this does not influence long-term survival or recurrence rates. (Müller A.–K. et al. Timing of esophagectomy in multimodal therapy of esophageal cancer : Impact of time interval between neoadjuvant therapy and surgery on outcome and response. *Der Chirurg* 09.2015, doi: 10.1007/s00104-014-2916-6. 86 : 9 : 874-880).

Roxana Gherasim

DEPRESSION - A FELLOW TRAVELER WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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DEPRESSION - A FELLOW TRAVELER WITH SYSTEMIC LUPUS ERYTHEMATOSUS (Abstract): Systemic lupus erythematosus (SLE) is a chronic multisystem inflammatory disorder that occurs primarily in women of childbearing age, immunologic abnormalities being a prominent feature of the disease. Psychiatric disorders frequently coexist, depression being the most common mood disorder in neuropsychiatric lupus. This literature review was performed through searching MEDLINE database for full-text English-language articles - original research, systematic review and updates published in the last five years (2010-2015), using the keywords "depression and systemic lupus erythematosus". The main outcomes identified were prevalence and predictors of depression in various cultural and ethnic groups, depression-related clinical issues (suicidal ideation, cognitive impairment, altered body image, sleep and sexual disturbances, influence of SLE treatment), and influence on quality of life. A multidisciplinary approach that takes into account the polymorphism and individual variability of the SLE clinical manifestations helps to improve early detection of depression, which is responsible for the increased risk of comorbidities, suicidal attempts, decreased treatment adherence, and impaired quality of life. Physicians across all specialties involved in the care for lupus patients should be aware of the major prevalence of this condition, while helping patients to cope with their disabling disease. **Keywords**: DEPRESSION, SYSTEMIC LUPUS ERYTHEMATOSUS, NEUROPSYCHIATRIC LUPUS.

Systemic lupus erythematosus (SLE) is a chronic multisystem inflammatory disorder that occurs primarily in women of childbearing age, immunologic abnormalities being a prominent feature of the disease. Diagnosis is based on clinical and immunological criteria as defined by the 1997 American College of Rheumatology (ACR) and the 2012 Systemic Lupus International Collaborating Clinics (SLICC) criteria (1).

The course of the disease is chronic, relapsing, and unpredictable, with flares and remissions that may last for years. Medication used to treat SLE depends on the severity of the disease and clinical manifestations, including biologic and nonbiologic disease-modifying antirheumatic drugs (DMARDs),

corticosteroids and nonsteroidal anti-inflammatory drugs, and antimalarials (2).

In chronic somatic diseases, like SLE, psychiatric disorders frequently coexist, patients having increased level of worry and concern about their condition, the psychological disturbances negatively affecting their emotional status and quality of life and leading eventually to increased risk for disability and death (3, 4). Patients with lupus suffering from one or more neuropsychiatric symptoms, such as cognitive impairment, anxiety, depression, mood disorder, psychosis, represent a clinical category named "neuropsychiatric lupus" (NPL), which includes a broad spectrum of conditions, 12 central nervous system and 7 peripheral nervous system – related, according to the 1999 American College of Rheumatology recommendations (5). These can result in increased severity, refractoriness to medical treatment and marked functional impairment (6).

DEPRESSION AS PART OF THE NEUROPSYCHIATRIC LUPUS

Understanding of neuropsychiatric lupus is still limited, but several pathogenic pathways were identified, some of them related to depression (5, 7) (tab. I).

TABLE I

Pathogenic pathways in neuropsychiatric lupus (according to Kivity at al., 2015) (5)

Pathogenic pathway	Clinical relevance
Vasculopathy	small vessel thrombotic-vasculopathy in brains of NPL patients
Autoantibodies	➢ anti-ribosomal-P - lead to neuronal apoptosis ➢ anti-DNA/NR2 - neuronal apoptosis, hippocampal neuron damage coupled with memory loss ➢ anti-DNA 16-6 idiotype - correlate with disease activity, cause histological changes in the hippocampus and amygdale, affecting behavioral and cognitive functions in mice ➢ anti-phospholipid/anticardiolipin - activation of coagulation pathways, predisposing to focal neurological manifestations (stroke, transverse myelitis, chorea), seizures, migraine, and cognitive impairments ➢ anti-GABA - high levels in cerebrospinal fluid
Blood brain barrier disruption	enable auto-antibodies to penetrate the brain and cause their pathogenic effects, further inducing the production of pro-inflammatory cytokines (anti-ribosomal-P, anti-NR2 antibodies)
Cytokines	IL-2, IL-10, interferon (IFN)-α, IFN-γ elevated in serum and cerebrospinal fluid

Depression is the most common mood disorder in neuropsychiatric lupus, having a lifetime prevalence of up to 65% in SLE patients. An association of depression and specific antibodies directed at ribosomal-P, N-methyl-D-aspartate (NMDA) receptor, and other neuronal epitopes has been suggested (5, 8).

Symptoms of clinical depression in lupus are lack of energy, diminished sexual appetite, sleep disturbances, modified eating pattern, and fatigue (3, 9) that could be attributed to both conditions – SLE and depression. Sometimes clinical depression is a symptom of neuropsychiatric lupus, as a result of the complex interplay of biochemical abnormalities affecting the brain, but other times depression comes later in the course of disease, as a result of patient response to the burden of disease, social

consequences, and symptoms severity, leading to feelings of sadness, helplessness and rejection, both instances being part of a comprehensive approach to depression in SLE (10).

Physicians usually use the SLE Disease Activity Index (SLEDAI) to determine the level of disease activity and weather the flare is mild, moderate, or severe; but depression is not one of the specific symptoms on SLEDAI, needing other type of workup to determine it, and that might be a reason for overlooking this diagnosis and related-issues, even though they have a great impact on quality of life and the course of disease (11, 12). The importance of early identification of neuropsychiatric involvement in SLE, when symptoms could be mild, required the development and validation of simple clinical screening tools, such as physician-administered or self-reported standardized questionnaires. This assessment is challenging and there is need for validation in large cohort studies, cross-culturally adapted (6, 13).

This literature review was performed through searching MEDLINE database for full-text English-language articles - original research, systematic review and updates, published in the last five years (2010-2015), using the keywords "depression and systemic lupus erythematosus". The research initially identified 429 articles and excluded studies on topics involving cutaneous lupus erythematosus without systemic involvement, drug-induced lupus erythematosus, and pediatric age; a number of 30 articles were retained. The main outcomes related to depression in SLE patients were prevalence and predictors of depression in various cultural and ethnic groups, clinical issues depression-related (suicidal ideation, cognitive impairment, altered body image, sleep and sexual disturbances, influence of SLE treatment), influence on quality of life.

DEPRESSION IN LUPUS – PREVALENCE DATA AND PREDICTORS AROUND THE WORLD

There are many studies conducted all over the world to help identifying the symptoms and particular appearance of depression related to local culture and religious beliefs. In some geographical areas, depression may be underrated and patients do not receive the proper treatment.

In a recent study on European population, the prevalence of depression in SLE subjects is 16.6% compared to 6.7% in the general population ($P < 0.001$), as resulted from the ODIN study (Outcome in Depression International Network) and it is not related to disease activity or organ damage (14).

In Asia, a Chinese cross-sectional study (15) investigated the relationship between disease parameters, quality of life, and psychological status in patients with SLE. It included 170 patients with SLE and 210 healthy individuals matched for age, gender, education, and work status. This study revealed that 20.3% of SLE patients were diagnosed with anxiety and 32.9% with depression, significantly higher than healthy people. Severe disease status, higher disease activity and lower quality of life significantly correlated with depression and anxiety in the Chinese SLE patients. Risk factors included poorer coping styles, ongoing life events, exposure to stress, and low social support. Impaired mental health and pain were the most powerful predictors of anxiety and depression in this study. A cross-sectional study conducted in Thailand on 62 SLE patients identified the presence

of depression in 45.2% and anxiety in 37.1% of the patients. It also concluded that higher anxiety score (HAM-A score) and younger age were significant predictors of depression in SLE (16). In addition, a retrospective study in Saudi Arabia found 15.2% SLE patients with depression and 17.4% with signs of psychosis (17).

In South America, a Chilean study examined 83 patients with SLE for common mental disorders, psychological sufferance, disease activity (SLEDAI), and biological determinants. A major depressive episode was the most frequent diagnosis (21.7%), suicidal ideation was present in 9.6%, but there was no association with disease activity and anti-ribosomal P antibodies (18).

A major longitudinal study enrolling 1609 North-American SLE patients in the Hopkins Lupus Cohort aimed at identifying the predictors of depression in lupus. The trial demonstrated an incidence of depression of 29.7 episodes per 1000 person-years and found several independent predictors of depression in lupus: recent diagnosis, non-Asian ethnicity, myelitis, cutaneous activity, disability, and higher-dose prednisone (>20 mg daily) (19). In another cross-sectional study that used structured clinical interview techniques for diagnosing depression, major depressive disorder was the most prevalent disorder (17%), followed by minor depression (6%) and dysthymia (4%) (20).

DEPRESSION-RELATED CLINICAL ISSUES

Sleep and sexual disturbances. Sleep (insomnia, hypersomnia) and sexual disturbances are common in patients coping with chronic disease, lupus included. An Italian clinical trial compared 81 female SLE patients with 53 hypertensive female patients as controls regarding the sleep pattern. The collected data showed a 2.5 times higher probability of poor sleep quality in SLE patients compared to the hypertensive ones. Depressive symptoms were significantly associated with sleep disturbances in SLE, but not with the activity or duration of the disease (21). These findings suggest that depression might be responsible for poor sleep quality in SLE.

Sexual function in women with SLE was analyzed in a cross-sectional, case-control study including 65 lupus patients and 55 healthy subjects. Women with SLE reported significantly impaired sexual function, associated with somatization, obsessive-compulsive behavior, depression and paranoid ideation; multivariate analysis indicated that depression, vitality and Positive Score Discomfort Index (PSDI) were significantly associated with low sexual function, eventually affecting the quality of life (22).

Suicidal ideation. It is of major importance to evaluate the patients diagnosed with depression and SLE for suicidal ideation, in order to prevent fatal events. A study conducted in Iran (23), which included 85 SLE patients, concluded that 60% of the patients had depression, the most common depressive symptoms being fatigue (88%), irritability (82%), sadness (77%), and somatic preoccupation (76%). The least common symptoms of depression were weight loss (34%), low energy level (28%), and suicidal ideation (10%). In a larger Chinese study, including 285 patients, 34% had current suicidal ideation. The individual risk factors found by the researchers were religious beliefs, financial problems, duration of SLE, poor family functioning, and negative coping style (24).

Cognitive impairment associated to depression. Patients presenting with de-

pression and SLE have to be screened for cognitive impairment because they seem to be more exposed (9) and it may have a deleterious influence on the evolution of the disease and course of treatment. In addition, it seems that SLE patients with cognitive dysfunction have higher titers of auto-antibodies also linked to depression, such as anti-ribosomal-P, anti-N-methyl-D-aspartate (NMDA) glutamate receptor (5, 8). It is known that cognitive impairment is present in 80% of SLE patients ten years after diagnosis, but it could exists even in early stages, related to depression. A multicenter cohort study included 111 patients newly diagnosed with SLE who underwent depression and cognitive function testing, the results showing that depressed lupus patients had poorer cognitive performance. Treatment for depression might improve cognitive functioning in these patients, but further studies are needed (25).

Body image. Body image is also affected in chronic disabling disease with significant cutaneous and musculoskeletal involvement like lupus. Studies showed that body image quality of life inventory (BIQLI) scores were significantly worse in SLE patients than in non-SLE patients, being correlated with self-reported depression and overall damage (26). Education, cognitive behavioral therapy, and cosmetic training lead to improved measures of body image, psychological well-being, and quality of life, showing that altered body image is modifiable in SLE (27).

Associated clinical conditions in SLE patients with depression. From the clinical picture of SLE, lupus arthritis seems to be related to depression. A cohort study of 127 lupus patients identified moderate or severe depressive symptoms in 41.7% of patients, the most significant variable associated with these symptoms being pain from lupus arthritis (28). A Spanish study including 84 SLE patients aimed to determine the possible relationship between fibromyalgia and psychiatric disorders in SLE. First of all, it identified fibromyalgia in 35.7% of the SLE patients, depressive symptoms in 19% and anxiety in 35.7%. The authors concluded that there is a high prevalence of fibromyalgia in SLE patients and a strong association with depressive and anxiety symptoms ($P<0.001$), but little or no impact of disease activity on psychiatric symptoms (29).

Influence of immune response. In this respect, there are conflicting results: some trials revealed that cerebro-reactive autoantibodies present in the cerebrospinal fluid (anti-N-methyl-D-aspartate and anti-ribosomal P) can cause significant damage to brain neurons (including the amygdala), potentially leading to depressive symptoms (5, 8) while others did not demonstrate a significant association between anti-ribosomal P antibodies and depression in SLE (18). The potential role of pro-inflammatory cytokines in developing depression and poorer quality of life is still unclear. A case-control study conducted in Singapore revealed that higher serum tumor necrosis factor-alpha levels are significantly associated with more severe depressive symptoms and decreased quality of life as assessed by SF-36, in patients with SLE (30).

Influence of SLE treatment. Medication is critically important in the therapeutic approach of SLE but many drugs have side effects that include depression. Glucocorticoids are widely used but they display severe side effects after prolonged use, such as hypertension, dyslipidemia, diabetes, behavioral changes, immunosuppression, memory impairment, mood disturb-

ances, gastrointestinal bleeding, glaucoma and cataract, osteoporosis.

It is necessary to strictly asses the patients prior to prescribing this drug, especially in larger doses (> 20 mg daily), in order to maintain the lowest necessary dose to achieve therapeutic effects, even though it is not clearly defined that corticosteroids are exclusively responsible for the appearance of major depressive disorder in patients with SLE. One study on 57 female patients with SLE found that of these up to 80% were treated with at least one corticosteroid and a large number of them presented emotional disturbances, such as depressive state, that coincided with the prolonged use (31) while another prospective longitudinal study (32) highlighted the importance of psychiatric examination prior to prescribing corticosteroid therapy because of the early onset of depression and anxiety during the first week of treatment. Risk factors were higher doses and prior history of psychiatric disorders, underlying the need to identify patients at risk, because up to 22% of the patients with depression and corticosteroid treatment develop a severe form of disease, with suicide attempts (33). So far, there is no particular medication that had been shown specific for treating depression associated with SLE.

Quality of life in patients with SLE and depression. Systemic lupus erythematosus is a long-term complex disorder also affecting social functioning. When assessing patients with SLE it is important to take into account the influence of depression on patients' quality of life. In patients in the PATROL study (Patients Reported Outcomes in Lupus), namely 125 Caucasian and Hispanic lupus patients, quality of life assessment was done using the Short Form-36 (SF-36) questionnaire. Depression, and not disease activity as measured by SLEDAI, significantly correlated ($p<0.0001$) with the majority of SF-36 domains, and appeared to have a major impact on quality of life in both ethnic groups with SLE (33).

Major contributors to decreased quality of life in lupus include fatigue, fibromyalgia, depression, cognitive dysfunction, altered body image, sleep and sexual dysfunction, but these are not related to disease activity (22, 26, 34). A meta-analysis including six randomized controlled trials comprising 394 participants and conducted by Chinese researchers concluded that psychological interventions (cognitive-behavioral therapy) significantly reduced the degree of depression (standard mean difference = −0.44, 95% [CI]: −0.78–0.10; P = 0.01), improving the quality of life, even if there was no effect on pain, disease activity, fatigue or mental health (4).

In the absence of large epidemiological studies and SLE databases there are multiple areas of "unmet need" in these patients, including firstly delayed diagnosis, depression/anxiety symptoms, sleep disturbances, and poorer quality of life, as a recent Australian systematic review has shown (35).

CONCLUSIONS

Given the polymorphism and individual variability of the SLE clinical manifestations we believe that the multidisciplinary approach could help improve early detection of depression, which is responsible for the increased risk of comorbidities, suicidal attempts, decreased treatment adherence, and impaired quality of life. Based on the available research data, physicians across all specialties involved in caring lupus patients should be aware of the major prevalence of

this condition, while helping patients to cope with their disabling disease.

Patients with systemic lupus erythematosus should be permanently encouraged, right from the time of diagnosis, to build-up a personal strategy of defense against depression, through physical activity, psychological counseling, cognitive behavioral therapy, and social exercise, in addition to the pharmacological approach when needed.

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ORTHOREXIA NERVOSA: THE UNHEALTHY OBSSESION FOR HEALTHY FOOD

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ORTHOREXIA NERVOSA: THE UNHEALTHY OBSESSION FOR HEALTHY FOOD (Abstract): The aim of this paper is to inform about a form of food extremism which has been named orthorexia (right feeding). Although orthorexia nervosa has the clinical criteria for being structured as a unique, distinctive disease, with physical, psychological and social consequences, it is not yet present in the recent psychiatric classifications. There are still controversies whether it can be included in the eating or in the obsessive-compulsive disorders. Orthorexia represents a pathological obsession for healthy eating characterized by qualitative food restrictions, strict avoidance of foods considered "impure" and ritualized ways of eating. Orthorectics create a world of their own in which they are in control of the safety and purity of the foods they eat, searching for spirituality in the kitchen and feeling superior due to their choices, even if the results are the deterioration of health and social isolation. There is still need for population studies, based on valid and unanimously recognized criteria, in order to have the complete picture of this new pathology that has both medical and social origin. **Key words:** ORTHOREXIA, ORTHOREXIA NERVOSA, EATING DISORDERS.

The 21st century can be called the century of consumption. Food, especially low nutritional value food, is available at reasonable prices. The abundance of foods and technologies to produce food, the accumulation of precise data about the physiological effects of foods, and discussion over the mono-cultural traditional food practices, all lead to a crisis in food model. The result is an eating behavior characterized by anxiety and ambiguity that evolves on one hand to the glorification of traditional foods that carry symbols and on the other hand to a modern diet founded on an individual genetics invalidated by studies, but extrapolated to everybody (1).

Obesity is the calamity of the century. The prevalence of obesity is rising rapidly with fatal consequences for health, through suffering and costs. In the same time, we face the expectations of being healthy and normal weight in order to conform to the ideals of the society. The epidemic of obesity has led to a cultural change that stresses the importance of healthy behaviors in order to achieve the desired weight. Many persons are tempted to change their lifestyle, by adhering to different diets, exercising and managing stress (2). One might argue that this change that focuses on a healthy lifestyle is a positive result of the obesity epidemics. Through a variety of sources, the population becomes exposed to a series of information about ways to im-

prove their diet. It becomes obvious that different types of food products are associated by some persons with health (3). The inflation of legislation to regalement and manage dietary risks generates a perverted effect, increasing the imaginary dangers. The rational of the consumer is generally binary (with risk or without risk) and non-probabilistic. The psychological impact of legislation of risk is stronger than the risk itself and brings a bonus of power to self-control in order to obtain the desired hygiene or at least the illusion of food security. However, in recent years, evidence has emerged suggesting that a subset of the population takes healthy eating too far, towards food "extremism", with negative physical, psychological and social consequences (4). These are the orthorectics.

The definition of orthorexia. The term orthorexia means rightful eating, being a combination of the Greek words "orthos" = right, correct and "orexis" = appetite.

Orthorexia is not a weight loss diet, but a pathological fixation for food perfection. Orthorectics are not just obsessed with rightful eating, but have also a specific attitude regarding food, the cooking of food and avoidance of certain foods that they consider bad for their health. The quality of the products they consume is more important than personal values, carrier related plans or social relationships. The desire to eat healthy foods is not an abnormal behavior in itself, but it is defined as orthorexia nervosa (ON) only when it makes the person give up their normal lifestyle. ON can be seen as a negative behaviour when optimal nutrition is related to anxiety and concern regarding health and the quality of foods. Orthorectics install a series of constrains which affect the nature, the terms and the rhythm of nutrition (5).

Initially conceptualized by Bratman in 1997 (6), the term ON was invented to describe an "unhealthy fixation to eat healthy foods" (7). Although Bratman claims that ON is an unique type of eating disorder, in which factors like taste or pleasure are less important, and health and nutritional value are more important and which focuses on the quality of foods, rather than the quantity, other researchers indicate that this uniqueness cannot be demonstrated (8,9).

It is not fully investigated how dangerous the clinical disorders of ON are to the individual, but, based on personal experience, Bratman claims that ON is a disorder with negative physical, psychological and social effects which escalade with time, in an orthorectic society (10).

The physical consequences attributed/associated with ON. The negative physical consequences of ON result from the fact that strict diets exclude groups of important foods. ON frequently leads to avoidance of previously preferred foods, thus reducing the number of foods consumed. People with ON exclude from their diets everything that is considered "impure" or imperfect in any way. This eating style has serious consequences through caloric deficit and lack of vitamins and minerals (11). Ironically, the quest for dietary perfection can lead to anemia, osteopenia and other medical consequences (12). In the desire to add years to life, orthorectics make life meaningless. This focus becomes an obsession, self-discipline becomes self-punishment and the effort could turn into addiction.

The psychological consequences attributed/associated with ON. People with ON can dedicate a long time to planning, organizing, buying and preparing unpollut-

ed and healthy food. They can feel the need to "punish" themselves with more and more drastic food restrictions when they disobey the self-imposed rules. Some people may feel that adhering to a perfect diet help them reach a feeling of personal purity and perfection. After a while, the strict control over food leads to a feeling of relief, energy and power. Control over personal eating habits is viewed as rewarding, which only strengthen their beliefs (13). One can detect some pseudo-spiritual connotations with eating pure foods, religious elements and specific rituals in the kitchen, regarding cooking (12). People with ON describe their symptoms as an overwhelming, obsessive physical and psychological desire to feel pure, natural and healthy, which begins to overcome other pleasant aspects of life (4). "Healthy" eating becomes an (unhealthy) addiction and "pure" eating is the only way to be healthy, even if the cost is losing other opportunities of life and just the illusion of total safety and the desire for complete control.

The social consequences attributed/ associated with ON. People who suffer from ON are often socially isolated, as a consequence of their lifestyle, avoiding groups where their food choices could be questioned or contested. They may feel the need to bring to dinners their own food, which fulfill their idealized nutritional standard and for which they spent their whole time searching and preparing. These persons are characterized by discipline and a sense of moral superiority, of extreme pride over those who eat incorrectly, which only increases their social isolation from all those who do not understand the fact that food could be ritualized (4,6). People who think that only their diets are acceptable go to extreme length to convince those around them who do not adhere to this new „religion" or who do not show devotion to healthy food.

Through these three types of consequences (physical, psychological and social), Bratman suggests that ON is a distinctive psychiatric disorder. As research progressed, the possibility was raised that ON is a version of an eating disorder or an anxiety disorder (9).

ON as an eating disorder. Eating disorders are psychiatric disorders defined as abnormal eating habits which could include either insufficient or excessive food intake, detrimental to the physical and mental health of an individual. Anorexia nervosa (AN) and Bulimia nervosa (BN) are the most common specific forms of eating disorders, but included in the classification are also Binge Eating Disorder (BED) and Eating Disorder Not Otherwise Specified (EDNOS).

Bratman himself shows some resembles to AN. Like anorectics, people with ON are so focused on control over food that their life unbalances by losing contact with previous food habits and the perspective of having to change the new eating behavior. ON and AN are considered to have in common a genetic predisposition for perfection, high levels of anxiety and a need to control the environment (14). Orthorectics often prefer to starve than to eat foods they consider "impure".

However, one should notice that, despite the shallow characteristics which could indicate some overlap, diagnosis of AN implies other supplemental important criteria, like reduced weight, compensatory behaviors and amenorrhea. As a result, some researchers (13, 15) suggested that, rather than being classified as an Eating Disorder, ON could be better considered a

risk factor for future eating disorders.

However, Bratman has observed two main differences between ON and eating disorders. First of all, in diseases like AN or BN, the disorders in eating are expressed quantitatively, whereas in ON, they are expressed qualitatively. More recent research indicate that this is not always true, as some persons with eating disorders have individualized rules about the quality of the foods they consume (8, 11). Bratman has claimed another difference between ON and eating disorders, which focuses on motivation. More precisely, he claimed that, unlike AN, where the goal is weight loss, people with ON are lead by the desire to obtain a feeling of perfection and personal purity (6). However, recent studies suggest that these motivations, especially those to reach perfection, are also present in people with AN (14, 16).

To the original arguments of Bratman, results of recent studies show that there are differences regarding the prevalence of ON in men and women. Both AN and BN are more frequent in women (17), but authors raise the possibility that ON is more frequent in men (7, 18, 19). However, one must notice that research about BED show the same results as for ON regarding the higher frequency in men (20).

ON as an anxiety disorder. Other arguments claim that ON could be better conceptualised as an anxiety disorder, especially as a variance of obsessive-compulsive disorder (OCD).

An obsession is an irrational thought which appears repeatedly. A compulsion is a certain behavior that a person does repeatedly with the aim of protecting themselves against an obsession. Obsessive-compulsive neurosis is one type of anxiety disorder in which patients realize that their obsessions are irrational but worry that they can become reality and face them by completing ritualized actions (compulsions). Ritualized behavior can reduce anxiety, distress of a person, but by no means can it bring any satisfaction or pleasure. Reducing risk through control increases fear of risk.

Bratman (6) describes as characteristics of ON the obsessive adherence to a strict diet, careful selection and examination of all foods in regards to their content, origin, presence of artificial ingredients/additives, preservatives, and others, detailing and engagement in extremely careful planning of meals, confrontation with guilt if there is deviation from self-imposed food restrictions. The ways of preparing food, the tools used in the kitchen and other instruments used are part of the obsessive ritual. The overlapping point between ON and OCD are anxiety and perfectionism. From this perspective, personal food restrictions in ON are meant to reduce anxiety related to food, which is determined by the current cultural focus on establishing models for healthy living (21). Obviously, in this moment, there is need for further studies in order to determine if ON is truly a distinctive disorder or it is better characterised as an OCD. In short, the exact nature of ON is unclear. It could also be argued that ON is not a psychological disturbance, but a tendency in society. Unfortunately, too many physicians recommend dietary restrictions for all sorts of diseases (asthma, allergies, irritable bowel disease) in order to eliminate certain “irritants” from the dietary plan. This could be a potential step towards developing food fixations (22).

The diagnosis of ON. Steve Bratman has proposed a test which includes 12 questions to detect orthorectics. In his opinion,

which is not generated by an empirical research, the diagnosis of ON would be put when a person: spends more than 3 hours per day thinking about and preparing healthy food**;** feels superior to those with differing eating habits**;** follows a particular self imposed dietary regimen rigidly and engages in compensatory restriction to make up for any dietary indiscretions**;** attaches self-esteem to adherence to the self imposed diet (feeling of self-satisfaction when complying with the self imposed dietary regimen)**;** makes consumption of healthy diet the central focus of life, at the expense of other personal values, relationships, previously enjoyed activities, and sometimes, ironically, physical health**;** makes nutritional value of meal more important than the pleasure of eating it.

Domini et al (23) have validated a questionnaire for the diagnosis of ON. It is known as the ORTO-15 questionnaire and has 15 multiple choice questions (with answer choices: always, often, sometimes, never) which evaluate the prevalence of ON. A score of 1 is given to the answer that indicates orthorexia, where as a score of 4 is given to the answer indicating "health". The sum of the scores represents the final score. Donnini et al proposed a cut-off score of 40 for ORTO-15 test. A score below 40 indicates that the person has orthorexic tendency. In this way, the Italian researchers reported a prevalence of ON of 57.6%. If the cut-off value was set at 35, the prevalence droped to 21% (24). In 2015, the questionnaire was validated also for adolescents (25).

The application of the procedure developed by Robins and Guze in 1970 (26) to the diagnosis framing of ON in comparison to other eating or anxiety disorders shows the gaps in research. The few existing studies focus entirely on the first step of determining diagnosis validation (clinical description), whereas the diagnosis construction of ON has to demonstrate other four criteria: Laboratory findings; Delimitation from other disorders; Follow-up studies; Family studies.

The majorities of studies focus on very small study samples like homogeneous groups of medical students, nutrition and dietetics students or performance artists, and lack experimental rigors. There is a lack of systematic investigation and thus the studies are not comparable. In 2013, Varga et al found in literature only 11 studies regarding ON (27). Even in this small number of studies, the applied criteria and the results regarding the prevalence of ON were extremely variable. The average prevalence of orthorexia was 6.9% for the general population and 35-57.8% for high risk groups (health professionals, artists). Risk factors include obsessive-compulsive characteristics, previous food related disorders and socio-economic status (18, 19, 21, 28-32).

Current practices suggest that, in order to have success in treating ON, one should combine psycho-education, cognitive behavior therapy and sometimes medication (5, 12, 22).

CONCLUSIONS

The validated diagnosis criteria for ON are somehow controversy (33,34), because ON has not been officially recognized as a distinctive disorder by the American Psychiatric Association in Diagnostic and Statistical Manual 5th edition (DSM-5), or by the World Health Organization's International Classification of Diseases (ICD-11). There is need for the development of supplementary psychometric tests in order to be sure that future research will be im-

proved by using reliable and validated evaluation instruments. Establishing prevalence rate, determining psycho-social risk factors and psycho-pathological co morbidities will create efficient therapeutic interventions (35).

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VITAMIN D DEFICIENCY AND THE CLINICAL CONSEQUENCES

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VITAMIN D DEFICIENCY AND THE CLINICAL CONSEQUENCES (Abstract): Vitamin D is important for good health, growth and strong bones. Vitamin D is mostly made in the skin by exposure to sunlight. Most foods contain very little vitamin D naturally, though some are fortified with added vitamin D. Hypovitaminosis D is associated with cardiovascular disease, the metabolic syndrome, type 2 diabetes mellitus, cancer as well as with increased mortality. Further, Vitamin D deficiency is related to depression and impaired cognitive function. Increasing age and elevated body fat mass contribute to an increased risk of Vitamin D deficiency. A mild lack of vitamin D may not cause symptoms but can cause tiredness and general aches and pains. A more severe lack can cause serious problems such as rickets (in children) and osteomalacia in adults). During menopause, the decline of estrogens results in increased bone turnover, a decrease in bone mineral density and elevated fracture risk. Treatment is with vitamin D supplements. Some people are more at risk of vitamin D deficiency, and so are recommended to take vitamin D supplements routinely. These include all pregnant and breastfeeding women, all infants (babies) and young children aged 6 months to 5 years, people aged 65 and over, and people who are not exposed to much sun. There are precise recommendations regarding a sufficient Vitamin D intake in order to prevent bone loss in peri- and postmenopausal women. It is also recommend routine supplements for certain people with darker skin, and for people with certain gut, liver or kidney diseases. **Keywords:** VITAMIN D DEFICIENCY, RICKETS, OSTEMALACIA, VITAMIN D SUPPLEMENTATION.

Vitamin D has been well-known for its function in maintaining calcium and phosphorus homeostasis and promoting bone mineralization. In the past few years, there has been a proliferation of publications on the effects of vitamin D and/or calcium on skeletal and nonskeletal health. In 2011 at Annual Meeting and Clinical Congress of the American Association of Clinical Endocrinologists Professor Paul D. Miller(1) presented a manuscript which represents a systematic review of data from the search engines of PubMed, Medscape, and Scopus. The search words used were "vitamin D", "calcium", "cardiovascular events", "cardiovascular mortality", "all-cause mortality", "vascular calcification", "chronic kidney disease", "renal stones", and "hyper-calciuria". This presentation have followed of many other that they have tried to give an answer to what extent the level of vitamin D is responsible for bone patholo-

gy as well as and other chronic disease. Another question has been which is optimum level of vitamin D to prevent these diseases. Most of the evidence is derived from case-control and cohort population studies. Minimal data are derived from prospective, double-blind, placebo-controlled studies. The evidence is, therefore, limited by the nature of the study designs.

VITAMIN D. METABOLISM AND BIOLOGICAL FUNCTIONS

Humans obtain vitamin D through dietary intake and exposure to sunlight. In this regard, even with the most intense and prolonged sunlight exposure, the maximum level of vitamin D that can be achieved is approximately 60 ng/mL. Cutaneous vitamin D3 production is influenced by skin pigmentation, sunscreen use, time of day, season, latitude, altitude, and air pollution(2) .Very few foods naturally contain vitamin D.

The two major biologically inert precursors of vitamin D are vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol) . Vitamin D3 is formed when 7-dehydrocholesterol in the skin is exposed to solar ultraviolet B, and then converted to previtamin D3. Excess UVB rays transform previtamin D3 into biologically inactive metabolites, tachysterol and lumisterol. Vitamin D2 is plant derived, produced exogenously by irradiation of ergosterol, and enters the circulation through diet. Both vitamin D precursors resulting from exposure to the sunshine and the diet are converted to 25-hydroxyvitamin D [25(OH)D] (calcidiol) when they enter the liver. 25(OH)D is the major circulating form of vitamin D. In order to be biologically active, additional hydroxylation in the kidneys is needed to form active 1,25-dihydroxyvitamin D [1,25(OH)2D] (calcitriol)=Hormone D(3). The 1,25(OH)2D performs many of its biologic functions by regulating gene transcription through a nuclear high-affinity vitamin D receptor (VDR)(4).This active metabolite of vitamin D binds to the nuclear VDR, which binds retinoic acid X receptor to form a heterodimeric complex that binds to specific nucleotide sequences in the DNA known as vitamin D response elements. There are estimated to be 200 to 2000 genes that have vitamin D response elements or that are influenced indirectly, possibly by epigenetics, to control a multitude of genes across the genome(4).It is universally accepted that the circulating level of 25-hydroxyvitamin D should be used as an indicator of vitamin D status due to its ease of measurement, long half-life in circulation (approximately 2 or 3 weeks), and the correlation of its level with clinical disease states . Vitamin D plays an important role in maintaining an adequate level of serum calcium and phosphorus. Without vitamin D, only 10 to 15% of dietary calcium and about 60% of phosphorus is absorbed (5). Therefore vitamin D has a great effect in forming and maintaining strong bones. It has also recently been found that vitamin D receptors exist in a variety of cells thus it has a biological effect on more than mineral metabolism (6). The 1,25(OH)2D interacts with its VDR in the small intestine to increase the efficiency of intestinal calcium absorption from approximately 10% to 15% up to 30% to 40% and intestinal phosphorus absorption from approximately 60% to 80%(7). It also interacts with VDR in osteoblasts to stimulate a receptor activator of nuclear factor kB ligand, which, in turn, interacts with receptor activator of nuclear

factor kB on immature preosteoclasts, stimulating them to become mature bone-resorbing osteoclasts (8). The mature osteoclast removes calcium and phosphorus from the bone to maintain blood calcium and phosphorus levels. In the kidneys, 1,25(OH)2D stimulates calcium reabsorption from the glomerular filtrate(7). The VDR is present in most tissues and cells in the body. Many of these organs and cells, including the brain, vascular smooth muscle, prostate, breast, and macrophages, not only have a VDR but also have the capacity to produce 1,25(OH)2D(7). This production probably depends on the availability of circulating 25(OH)D, indicating the biological importance of sufficient blood levels of this vitamin D metabolite(8).

VITAMIN D DEFICIENCY

Vitamin D deficiency has been recognized as a pandemic phenomenon with a variety of health consequences (2). Low vitamin D status has been associated with an increased risk of type 1 diabetes mellitus, cardiovascular disease, certain cancers, cognitive decline, depression, pregnancy complications, autoimmunity and allergy (3).

The US Endocrine Society recommended that vitamin D deficiency be defined as a 25(OH)D level of 20 ng/mL or less, vitamin D insufficiency as 21 to 29 ng/mL, and vitamin D sufficiency as 30 ng/mL or greater for children and adults. It suggested that maintenance of a 25(OH)D level of 40 to 60 ng/mL is ideal, and that up to 100 ng/mL is safe (9). Recent recommendations of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO)(10) for the optimal management of elderly and postmenopausal women regarding vitamin D supplementation have also indicated that patients with serum 25(OH)D levels less than 20 ng/mL have increased bone turnover, bone loss, and, possibly, mineralization defects compared with patients with serum 25(OH)D levels of 20 ng/mL or greater. Similar relationships have been reported for fragilty, nonvertebral and hip fracture, and all-cause mortality, with poorer outcomes at less than 20 ng/mL. Thus, ESCEO recommended that 20 ng/mL be the minimal serum 25(OH)D concentration at the population level and in patients with osteoporosis to ensure optimal bone health. Also, in fragile elderly individuals who are at elevated risk for falls and fractures, ESCEO recommended a minimal serum 25(OH)D level of 30 ng/mL for the greatest effect on fracture(10).

CAUSES OF VITAMIN D DEFICIENCY

There are many causes of vitamin D deficiency. Generally, they can be divided into two groups: UVB-related deficiency and medical/physical condition-related deficiency.

UVB-related deficiency. *The elderly*, due to the decreased presence of skin 7-dehydrocholesterol which is the precursor for UVB mediated synthesis of vitamin D, are particularly at risk of vitamin D deficiency. Moreover, reduced mobility or institutionalization that discourages sun exposure, reduced renal production of 1,25-dihydroxyvitamin D as well as decreased intake of fortified foods pose great difficulties in vitamin D formation in body. People with *dark skin* have great amounts of melanin in their epidermis. Melanin competes with 7-dehydrocholesterol for absorption of UVB photons. Therefore, people of color are less efficient in producing vitamin D than are whites. It has been established that

the ozone layer can absorb UVB. Zenith angle decides the thickness of ozone layer which sunlight needs to penetrate and it is dependent on factors such as *time of day, season of the year, and latitude.* Thus those factors have great effects on vitamin D production (11). *Sunscreens can* efficiently absorb UVB radiation. This dramatically prevents the interaction of UVB with 7-dehydrocholesterol, the process of previtamin D3 generation.

Medical / physical condition - related deficiency. As a fat-soluble vitamin, vitamin D requires the presence of dietary fat in the gut for absorption. Certain pathological conditions, such as Crohn's disease, cystic fibrosis , celiac disease, surgical removal of part of the stomach or intestines are associated with *fat malabsorption* and thus may lead to vitamin D deficiency. It is well recognized that long-term use of some *antiepileptic drugs*, including phenobarbital, phenytoin, and carbamazepine and the antimicrobial agent rifampicin (RIF) can result in osteomalacia. *Chronic kidney disease* such as patients with stage 4 or 5 chronic kidney disease, as well as those requiring dialysis, leads to an inability to make sufficient 1,25-dihydroxyvitamin D which has a direct effect in inhibiting parathyroid hormones expression. *Obese people* are prone to be vitamin D deficient since they have lower 25 hydroxyvitamin D levels . A number of studies proved that the vitamin D3 precursor 7-dehydrocholesterol levels in the skin of obese people were not significantly different from non-obese people. One explanation was that the subcutaneous fat, which is known to store vitamin D, sequestered more of the cutaneous synthesized vitamin D, which results in less release of vitamin D from the skin into the circulation in the obese subject than non-obese subject (12).

VITAMIN D INADEQUACY- EPIDEMIOLOGY

Prevalence of vitamin D deficiency in adolescents and adults. It has been estimated that 20% to 80% of US, Canadian, and European men and women are vitamin D deficient. The prevalence of serum 25(OH)D levels less than 20 ng/mL was almost one-third of the US population (32%). In the Healthy Lifestyle in Europe by Nutrition in Adolescence study, 25(OH)D levels less than 30 ng/mL were reported to be approximately 80% in adolescents from the 9 European countries. In a study on the vitamin D status of Australian adults, vitamin D deficiency (25[OH]D <20 ng/mL) was 31% (22% in men and 39% in women); 73% had 25(OH)D levels less than 30 ng/mL(13,14).

Vitamin D status during pregnancy, birth and childhood. Maternal vitamin D deficiency predisposes to low vitamin D stores in the newborn and increases infantile rickets because the mother is the only source of vitamin D during pregnancy. The prevalence of vitamin D deficiency and insufficiency during pregnancy is of special concern and ranges from 8% to 100%, depending on the country of residence and the definitions of vitamin D deficiency and insufficiency. In the United States, vitamin D deficiency and insufficiency is estimated to be 27% to 91% in pregnant women. This rate is estimated to be 36% to 65% in Canada, 45% to 98% in Asia, 70% to 100% in Europe, and 25% to 65% in Australia and New Zealand. In the United States, it is estimated that 50% of children aged 1 to 5 years and 70% of children aged 6 to 11 years are vitamin D deficient or insufficient (12,13).

VITAMIN D INTAKE IN DIFFERENT CONDITIONS

Musculoskeletal consequences of vitamin D deficiency. The skeletal consequences of 25(OH)D insufficiency include *secondary hyperparathyroidism*, increased bone turnover and bone loss, a decrease in BMD due to the increased bone resorption by PTH as well as a mineralization defect causing *osteomalacia*, and increased risk of low trauma fractures(13). The most common etiology of *rickets*, historically and presently, is vitamin D deficiency. Children begin to manifest classic clinical signs of rickets between 6 months and 1.5 years that include rachitic rosary, widened epiphyseal plates at the end of long bones, and bowing deformities of the legs. Proximal muscle weakness is a prominent clinical feature of vitamin D deficiency (13).

Osteoporosis. Approximately 33% of women aged between 60 to 70 and 66% of those over 80 have osteoporosis . Vitamin D deficiency is associated with the marked suppression in intestinal Ca absorption and the impairment of Ca balance, which results in low bone mineral content and density. Reduced bone mineral density (BMD) increases the risk of fractures, which significantly contributes to morbidity and mortality of older persons (15). Most evidence suggests that adequate calcium and vitamin D intake along with exercise during childhood will maximize bone mineral content that can be sustained in young and middle aged adults as long as they also have a healthy lifestyle, adequate calcium intake, and a healthy vitamin D status. Accruing maximum bone mineral content during childhood, and maintaining peak bone mineral density in young and middle-aged adults, will likely reduce the risk of fracture later in life, when there is a disruption in bone remodeling due to menopause and aging.

Nonskeletal consequences of vitamin D deficiency. A large number of trials have been conducted over the past two decades into the effects of vitamin D on disease management and prevention. Observational studies have found a decreased risk of many disorders, including certain types of cancer, mental disorders, infectious disease, cardiovascular disease, type 2 diabetes mellitus, and autoimmune disorders, associated with serum 25(OH)D levels greater than 28 to 32 ng/mL(7). It has, therefore, been argued that 25(OH)D levels should be in the range of 28 to 40 ng/mL to maximize these nonskeletal benefits (13).

Autoimmune diseases. Vitamin D has been defined as a natural immune modulator. Epidemiologic, genetic, and basic science studies indicate a potential role of vitamin in the pathogenesis of certain systemic and organ specific autoimmune diseases, such as type 1 diabetes mellitus, multiple sclerosis, rheumatoid arthritis, and Crohn disease. *Multiple sclerosis (MS).* Evidence continues to accumulate supporting a protective role for vitamin D in its risk and progression. Notable recent findings are that high 25(OH)D levels at the time of a first demyelinating event predicts a lower MS risk and a decreased risk of them in offspring whose mothers had high 25(OH)D levels. High doses of vitamin D3 (up to 280,000 IU/ wk for 6 weeks) have been used safely in patients with MS(13). The progression and activity of MS were not affected, but the number of gadolinium-enhancing lesions per patient assessed by nuclear magnetic brain scan was significantly reduced. *Rheumatoid Arthritis (RA)* is an autoimmune disorder of unknown etiology in which both genetic and nonge-

netic factors contribute to disease susceptibility. Studies showed that when confronted by an inappropriate and overly exuberant immune response, vitamin D may act in a paracrine manner to decrease T cell responsiveness through the inhibition of cellular proliferation and reduction in lymphokine production (12). Vitamin D deficiency is prevalent in *Crohn's Disease*. It is an inflammatory bowel disease which often leads to osteopenia and osteoporosis due to malabsorption of vitamin D.

Cancers. Association studies have related higher serum levels of 25(OH)D to reduced incidence of many types of cancers. It has been hypothesized that the local conversion of 25(OH)D to 1,25(OH)2D in healthy cells in the colon, breast, and prostate can help prevent malignancy by inducing cellular maturation, inducing apoptosis, and inhibiting angiogenesis while enhancing the expression of genes including P21 and P27 to control cellular proliferation (4,7). A reanalysis of the WHI concluded that in 15,646 women (43%) who were not taking personal calcium or vitamin D supplements at randomization, the calcium and vitamin D intervention significantly decreased the risk of total, breast, and invasive breast cancers by 14% to 20% and the risk of colorectal cancer by 17% (16). Higher dietary intake of vitamin D had a progressively lower risk of pancreatic cancer compared with those who had the lowest intake.

Cardiovascular disorders. Observational studies in humans found that 25(OH)D and 1,25(OH)2D levels are inversely related to coronary artery calcifications and are lower in patients with myocardial infarction(17). An *in vitro* study suggested that low 25(OH)D levels influence the activity/expression of macrophages and lymphocytes in atherosclerotic plaques, thus promoting chronic inflammation in the artery wall. A short course of treatment with vitamin D (4000 IU for 5 days) effectively attenuated the increase in circulating levels of inflammatory cytokines after an acute coronary event. These findings provide support for the anti-inflammatory effects of vitamin D on the vascular system and suggest mechanisms that mediate some of its cardioprotective properties. Observational studies indicated that a serum 25(OH)D level less than 30 ng/mL was strongly associated with hypertension and metabolic syndrome. This effect is thought to be partly mediated through regulation of the renin-angiotensin aldosterone axis.

Diabetes mellitus and metabolic syndrome. Children with vitamin D deficiency or insufficiency had a 2.5-fold higher risk of an elevated blood glucose level, a 2.4-fold increased risk of elevated blood pressure, and a 4-fold increased risk of metabolic syndrome, a prelude to type 2 diabetes. A meta-analysis of 11 prospective studies provided the association between circulating 25(OH)D levels and type 2 diabetes. It suggested a strong inverse association between serum 25(OH)D concentration and incidence of type 2 diabetes, but no evidence of a beneficial effect of vitamin D supplementation on type 2 diabetes (7).

Respiratory tract diseases and wheezing disorders. Children with rickets were at higher risk for upper respiratory tract infections and for dying of them (13). Macrophages have a VDR, and when they ingest an infectious agent, such as tuberculosis bacillus, the toll-like receptors are activated, resulting in signal transduction to increase the expression of VDR and

CYP27B1(7). High 25(OH)D levels during maternity were associated with a decrease in childhood wheezing by nearly 50% compared with low maternal 25(OH)D levels. Newborns with 25(OH)D levels less than 10 ng/mL were twice as likely to develop respiratory tract infections compared with those with levels of 30 ng/mL or greater.

Neurologic disorders. The brain has a VDR and has the ability to produce 1,25(OH)2D3. *In vivo* mouse studies found that *in utero* hypovitaminosis D impairs brain development and leads to persistent changes in the adult brain. There is minimal evidence for an association of low maternal vitamin D status with risk of autism. The 1,25(OH)2D3 seems to have a neuroprotective role, inducing remyelination by endogenous progenitor cells and stimulation of amyloid-b clearance by macrophages of patients with Alzheimer disease.

Mortality. Vitamin D deficiency is associated with an increased risk of total mortality. Most, but not all, studies documented increased mortality rates in patients with low 25(OH)D concentrations. In a recent meta-analysis with 70,528 randomized participants (86.8% females) with a median age of 70 years, vitamin D supplementation with or without calcium reduced mortality by 7%. However, vitamin D supplementation alone did not affect mortality, but risk of death was reduced if vitamin D was given with calcium (12, 13).

TREATMENT AND PREVENTION OF VITAMIN D DEFICIENCY

The source of vitamin D in humans is sun exposure. Although excessive exposure to sunlight increases the risk of non melanoma skin cancer, which is easy to detect and easy to treat. Time of day during *sun exposure*, season, latitude, and degree of skin pigmentation dictate how much vitamin D3 is produced during sun exposure. For a white person, if 30 minutes of June noontime sun would cause a mild sunburn, then 10 to 15 minutes of exposure followed by good sun protection should be sufficient to produce adequate vitamin D. Very few *foods* naturally contain vitamin D. *Foods fortified* with vitamin D usually contain 100 IU per serving. Food fortification with vitamin D (especially in milk) or some cereals, juices, other dairy products, some margarines, bread are effective in significantly increasing 25(OH)D levels in the population (18,19). *Vitamin D Supplementation***.** It is recommended 600 to 800 IU daily should meet the requirements to optimize bone health in most of the population, higher vitamin D intakes (1000-2000 IU) are needed to reach and maintain 25(OH)D levels greater than 30ng/mL (20). An effective strategy to treat vitamin D deficiency and insufficiency in children and adults is to give them 50,000 IU of vitamin D2 once a week for 6 and 8 weeks, respectively (20).

To prevent recurrence of vitamin D deficiency in children, administration of 600 to 1000 IU/d is effective. For adults, to prevent recurrence of vitamin D deficiency, administration of 50,000 IU of vitamin D2 every 2 weeks is effective (19,20).

Pregnancy and lactation. The 25(OH)D passes from the placenta into the bloodstream of the fetus. Because the half-life for 25(OH)D is approximately 2 to 3 weeks, the infant can remain vitamin D sufficient for several weeks after birth, as long as the mother is vitamin D sufficient. Maternal supplementation with 2000 and 4000 IU/d of vitamin D during pregnancy

improved the maternal/neonatal vitamin D status. Human breast milk and unfortified cow's milk have little vitamin D (21). Only after lactating women were given 4000 to 6000 IU/d of vitamin D was enough vitamin D transferred in breast milk to satisfy the infant's requirement (21).

Vitamin D supplementation in special conditions. Because body fat can sequester vitamin D, it is now recognized that children and adults who are obese require 2 to 5 times more vitamin D to treat and prevent vitamin D deficiency. Patients taking antiseizure medications, AIDS medications, and glucocorticoids often require more vitamin D to satisfy their requirements. *Type of Vitamin D Supplementation*: Either vitamin D2 or vitamin D3 can be used for vitamin D supplementation, although there is controversy regarding vitamin D3 vs vitamin D2 for achieving and maintaining higher serum 25(OH)D levels. Although a recent meta analysis indicated that vitaminD3 is more efficacious than is vitamin D2 (7).

Safety and intoxication. Vitamin D intoxication is characterized by hypercalcemia, hypercalciuria, and hyperphosphatemia, which, in turn, are responsible for soft-tissue and vascular calcifications and nephrolithiasis in the long term. Serum 25(OH)D levels are usually markedly elevated (>150 ng/ mL) in individuals with vitamin D intoxication. Daily doses of vitamin D3 up to 10,000 IU were safe in healthy males, and there was no evidence of hypercalcemia or hypercalciuria for 5 months (22). Higher doses of vitamin D (up to 40,000 IU/d) are still safe provided that a serum 25(OH)D concentration of 200 ng/mL is not exceeded.

CONCLUSIONS

A sufficient Vitamin D status might be beneficial regarding bone, cardiovascular and overall health. Vitamin D deficiency is a common underdiagnosed condition that has received increasing attention in the world. Vitamin D deficiency during pregnancy may adversely influence placental development and fetal programming. An effective strategy to prevent vitamin D deficiency and insufficiency is to obtain sun exposure, ingest foods that contain vitamin D, and take a vitamin D supplement to increasing serum 25(OH)D levels above 30 ng/mL.

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ELECTROCARDIOGRAPHIC CHANGES IN THE MOST FREQUENT ENDOCRINE DISORDERS ASSOCIATED WITH CARDIOVASCULAR DISEASES. REVIEW OF THE LITERATURE

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ELECTROCARDIOGRAPHIC CHANGES IN ENDOCRINE DISORDERS (Abstract): Cardiovascular abnormalities associated with endocrine diseases are often frequent and due to complex relationships between endocrine glands (with internal secretion) and cardiovascular system (heart and vessels). Certain hormones secreted by the endocrine glands (particularly the thyroid and pituitary gland) excesses or deficiencies, are involved in morphogenesis, growth processes and activity regulation of cardiovascular system, most often in connection with the autonomic nervous system. There are also a lot of electrocardiographic changes caused by hormonal disorders that requires differential diagnosis and represents the source of erroneous diagnosis. Endocrine pathology occurred later than a heart disease, may worse heart function. Ignoring the cardiovascular events that may occur in the evolution of endocrine diseases, may induce increased mortality due to cardiovascular complications. **Keywords:** ENDOCRINE DISEASES, ELECTROCARDIOGRAM, RHYTHM TROUBLES.

Endocrine pathology offers a large broadband of multiple interference and implications in cardiovascular pathology. These connexions are supported by complex relationships between endocrine glands (with internal secretion) and cardiovascular system (heart and vessels). It is well known that certain hormones secreted by the endocrine glands (particularly the thyroid and pituitary gland) are involved in morphogenesis, growth processes and activity regulation of cardiovascular system, most often in connection with the autonomic nervous system (1, 2).

This complex relationship between endocrine and cardiovascular pathology is not new.

In 1835, Robert Graves (one whose name will carry disease - Basedow Graves) described...*"Three cases of prolonged violent palpitations in women with thyrotoxicosis..."*. Twenty years later, in 1855, Thomas Addison described in a highly suggestive phrase (which later would clearly outline the clinical picture of the disease)... *"patients with small adrenal capsule have weak and compressible pulse and as a progress of the disease, the body loses weight, pulse becomes smaller and weaker, and the patient, after a long time and grad-*

ually falls off... ".

Therefore since the mid-nineteenth century were known or suspected effects of thyroid hormone and adrenal gland diseases of the cardiovascular system (2).

The problem is up-dated now. A meta-analysis published in 2007 in the European Journal of Endocrinology emphasized that thyroid hormone excess would increase cardiovascular mortality of about 1.7 times. Another study published in 2011 confirmed the same fact, speaking about increasing cardiovascular mortality with 20% in persons with hyperthyroidism.

The interrelations between endocrine and cardiovascular pathology are important due to several reasons: 1. The cardiovascular symptoms may mask the endocrine disease. This is the major source of confusion and diagnoses errors 2. After diagnosis, the endocrine disease will require specific treatment. 3.Ignoring the cardiovascular events that may occur in the evolution of endocrine diseases, may induce increased mortality due to cardiovascular complications. Endocrine pathology occurred later than a heart disease, may worse heart function. There are also a lot of electrocardiographic changes caused by hormonal disorders that requires differential diagnosis and represents the source of erroneous diagnosis (1, 3, 6, 20).

Pituitary gland pathology

The excess of growth hormone (GH) also has implications in cardiovascular disease. Acromegalic cardiomyopathy is the most severe form of cardiac damage. The main clinical aspects are: arterial hypertension and early atherosclerotic signs induced by acromegalic cardiomyopathy.

On ECG the main abnormalities are: left axis deviation, left ventricular hypertrophy with T-wave inversion, septal Q-waves, ST-T wave depression, abnormal QT dispersion and conduction defects that occurr in 50% of patients, left bundle-branch block, atrial and especially ventricular arrhythmias (induced by cardiac dilation). These changes are nonspecific. Moreover, sick sinus syndrome may be associated with acromegalic cardiomyopathy (1, 3, 4, 5, 7, 9, 10, 18).

Thyroid gland pathology

Thyroid hormones (thyrotoxicosis) excess induces both cardiac and vascular effects. The thyroid hormones activity may be performed through cardiac specific receptors or mediated by the sympathetic nervous system (which is over stimulated). The excess of thyroid hormones increases the metabolic needs of the tissues (high demand of oxygen in the peripheral tissues) (1, 2, 3, 21).

The main cardiac effects of excessive thyroid hormone are: increased contractility, automaticity and atrial excitability (with consequent risk of supraventricular arrhythmias) (2, 3, 19).

The vascular effects of thyroid hormones are: vasodilatation in skin and muscle and decreased peripheral vascular resistance (2, 3).

Cardiac manifestations of hyperthyroidism may be functional (as hyperkinetic syndrome) and organic (cardio thyreosis) (21).

Hyperkinetic syndrome - often brings the patient to the emergency room. The most common electrocardiographic abnormalities are: sinus tachycardia, hyper voltage, normal morphology of QRS complexes. Each of these entities has clinical and therapeutic particularities. Hyperthyroidism is considered an aggravating and precipitating factor of heart ischemia and heart failure.

As main characteristics:

- Atrial fibrillation from cardiothyreosis is first repetitive and then becomes permanent, with fast ventricular rate and resistance to digital treatment. The sinusal rhythm may be recovered only after achieving the euthyroidian status. Despite high ventricular rate, atrial fibrillation from cardio thyreosis is rarely followed by hemodynamic instability;

- Pectoral angina occurs frequently in patients with hyperthyroidism (hyperthyroidism aggravates or induces angina episodes) ; in some cases thyroid hormones may induce vasospasm in normal coronary artery. However, angina occurs in patients with subclinical coronary heart disease (coronary bed being affected by atherosclerosis, including microvascular injury);

- Hypothyroidism occurs due to insufficient synthesis, transport and / or reception of thyroid hormones. It's cardiovascular effects are mainly the consequence of decreasing metabolic needs of myocardial or peripheral tissues (vascular permeability and capillary growth);

- Electrocardiographic abnormality in hypothyroidism (for differential diagnosis in the clinical context) includes: sinus bradycardia, micro voltage, QT prolongation, conduction disturbances, changes in repolarization phase, T wave flattening (1, 2, 3, 5, 6, 9, 10, 11, 12, 17).

Parathyroid diseases

Hyperparathyroidism (excessive secretion of parathyroid hormone), have the following consequences: hypercalcemia, hypercalciuria, hypokalemia, and hyperphosphaturia. The calcium ion increases myocardial contractility and peripheral vascular resistance and induces nephrocalcinosis. Cardiovascular manifestations of hyperparathyroidism are very polymorphic: arterial hypertension, cardiac calcifications (may induce the occurrence of arrhythmias), accelerated atherosclerosis (pectoral angina, myocardial infarction) (23).

The characteristic ECG modification oh hypercalcemia is QT interval shortening but it may induce also severe conduction disturbances: total AV block and sinus arrest, ventricular arrhythmias (PVCs, ventricular tachycardia, ventricular fibrillation) and promotes digitalis intoxication complications.

Hypocalcemia from hypoparathyroidism induces electrocardiographic abnormalities, consisting of: QT prolongation (may induce malignant ventricular arrhythmias) and changes in repolarization phase (ST) without stretching T-wave. Rarely, hypocalcemia may induce dilated cardiomyopathy which becomes reversible once with serum calcium values control (1, 2, 3, 5, 6, 16).

Adrenal diseases

Their correlation with cardiovascular pathology is quite complex, due to the effects of both mineralocorticoid and glucocorticoid hormones.

Hypercortisolism (excessive production of glucocorticoid hormone- dependent or independent of ACTH) has as main cardiac manifestations: moderate arterial hypertension, accelerated atherosclerosis (at coronary level may produce angina attacks). The main ECG abnormalities is secondary left ventricular hypertrophy (related to arterial hypertension or coronary atherosclerosis) (3, 8). Arrhythmias are rare and usually occur on the background of hypertensive left ventricular hypertrophy. Hyperaldosteronism (primary or secondary excess of mineralocorticoid) may induce

cardiovascular emergencies, both related to secondary hypertension or severe hypokalemia (may cause severe ventricular arrhythmias). Severe hypokalemia induces ECG abnormalities: QT prolongation, abnormal repolarization phase (slight depression of ST segment, flat T waves), U waves in precordial leads, prolonged P and PR interval duration (first degree atrioventricular block), wide QRS complex, ventricular arrhythmias (ventricular extrasystoles, ventricular tachycardia with evolution through ventricular fibrillation refractory to defibrillation). The severity of ECG abnormalities correlates with low potassium level (14, 15, 22, 24).

Primary adrenal insufficiency (Addison's disease) has humoral response causing hypoglycemia, hyponatremia, hypochloremia, hyperkalemia. Cardiovascular consequences are represented by hypotension and syncope and specific electrocardiographic changes related to electrolyte disturbances. Hyperkalemia causes various ECG abnormalities related to potassium levels: sharp, narrow base T waves, intraventricular conduction abnormalities (large QRS complexes exceeding 0.12 seconds), left axis deviation and then the decrease of P wave until disappears, ST-segment abnormalities (pseudo - lesion), arrhythmias and atrioventricular conduction disturbances. At high potassium concentrations (above 7.5 mEq / l) - cardiac arrest (14, 15, 22, 24).

Pathology of the adrenal medulla

Pheochromocytoma - chromatin tumor of the adrenal medulla is characterised by excessive secre-tion of catecolamines (adrenaline and noradrenaline). The clinical manifestations are represented by paroxystic seizures, paroxystic or permanent hypertension, sometimes orthostatic hypotension, arrhythmias, angina episodes. Electrocardiographic abnormalities in pheochromocytoma are nonspecific: negative T waves, left ventricular hypertrophy, sinus tachycardia, short PQ interval, supra ventricular arrhythmia (atrial premature beats, paroxystic or permanent atrial fibrillation) (13).

Adrenergic myocarditis and cardiomyopathy have clinical manifestation as: left ventricular failure or cardiac asthma, pulmonary oedema or severe arrhythmia (atrial fibrillation, premature ventricular beats or ventricular tachycardia). All of them represents emergencies and requires specific therapeutic measures. All these clinical and electrocardiographic signs of pheochromocytoma disappear together with the removal of the tumor (13).

CONCLUSIONS

Electrocardiographic changes in endocrine disorders are quite frequent and may be provoked by hormone excess (ex. hyperthyroidism) or by secondary electrolites disturbances (hypokalemia associated with primary or secondary hyperaldosteronism). Sometimes these electrocardiographic changes may be related to other cardiac abnormalities – such as arterial hypertension or left ventricular hypertrophy often associated with acromegaly or pheochromocitoma. Ignoring the cardiovascular events that may occur in the evolution of endocrine diseases, may induce increased mortality due to cardiovascular complications. In the majority of cases these electrocardiographic signs disappear together with the treatment of endocrine disease.

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PHARMACOTHERAPY VERSUS PSYCHOTHERAPY IN PANIC DISORDER. REVIEW

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PHARMACOTHERAPY VERSUS PSYCHOTHERAPY IN PANIC DISORDER.REVIEW (Abstract) Panic disorder is one of the most frequent psychiatric conditions and the incidence is increasing. More worrying than the number of patients is the major negative impact over personal, social and economic functioning. Due to the increasing risk of death, whether by suicide or by cardiovascular disease, in patients suffering of panic disorder a prompt, adequate intervention is essential. This condition has a chronic evolution, with low rates of remission and a significant negative impact over quality of life and over social and economic aspects. In this article we presented different aspects related to the therapeutic management of panic disorder, with special reference to pharmacotherapy and its specific classes and psychotherapy, particularly Cognitive-Behavioral Therapy. These two therapeutic categories proved to be the most efficient in both acute and long-term symptomatology, with improvement of anxiogenic rumination, avoidance behavior or agoraphobia. Pharmacotherapy, whether with antidepressants or benzodiazepines, is recommended to be followed by psychotherapy. SSRIs and SSNRs are recommended as first-line medication, while benzodiazepines are necessary only in acute phases or until the therapeutic effect of the above-mentioned medicines is obtained. In treatment-resistant patients at patients who do not respond sufficiently to any first-line treatments it is necessary to use other classes of medicines, such as atypical antipsychotics. **Keywords**: PANIC DISORDER, PSYCHOTHERAPY, COGNITIVE-BEHAVIOURAL THERAPY, PHARMACOTHERAPY.

Panic attack is one of the most important types of anxiety disorder. In Romania, due to the high level of risk, the acute episode of a panic disorder was included in the list of psychiatric emergencies, based on Order 488, point 159 of April 2016, from Mental Health Law. Since over 70% of the people who attempted suicide had an associated anxiety disorder, this Order is well justified. Panic attack is also independently associated with suicide risk, and comorbidity of panic attack with personality disorder is characterized by a higher risk of attempted suicide over either disorder alone (1, 2, 3, 4). Thus, anxiety associated with personality disorder was correlated with an increase in suicide attempt from 1.7 to 2.6 times (5).

Secondly, cardiorespiratory involvement in panic attack physiopathology can worsen its course or more, it can lead to death in population with the above-mentioned comorbidities (6,7,8). In the case of a cardiovascular disease, the anxiety symptoms in arrhythmic patients are associated with a higher severity of atrial fibrillation (9,10) and atrial fibrillation recurrence (10). Comparing the QT interval on electrocardiogram in a control group and patients suffering from panic disorder, Atmaca found that the QT in the latter group was significantly longer, prolonged QT being associated with arrhythmic events and sudden death (11).

In patients with panic disorders, the high risk of death, whether by suicide or cardiovascular disease, makes a prompt intervention mandatory.

Panic disorder has a chronic course and a low rate of remission with a significant negative impact not only on life quality but also on social and economic aspects (12,13).

Considering all these aspects, the main purpose of this article was to present data in the literature regarding different approaches of panic disorders, especially the adequate management of acute phase, which requires special emergency care. Therefore, the efficacy of pharmacological and psychological therapy, especially the cognitive behavioral therapy, used separately or combined will be analyzed.

To obtain the articles for this review, we searched PubMed using the following key words: “panic disorder”, “pharmacotherapy” and “psychotherapy”. The accepted studies were only those comparing pharmacological therapy with psychotherapy.

First line treatment

First line treatment of panic disorder is represented by selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs). According to meta-analyses and randomized clinical trials, Citalopram, Fluoxetine, Fluvoxamine, Paroxetine, Sertraline and Escitalopram are the first therapeutic option (5).

A study of Azhar (14) compared the efficacy of Fluvoxamine treatment alone and Fluvoxamine combined with Cognitive Behavioral Therapy (CBT) and CBT alone. The best results were obtained by combining the two therapeutic methods, with significant improvement of the following parameters tested before and after nine weeks of treatment: frequency of panic attacks reduced from 16.7 to 4.07 per week, catastrophic belief score from 100 to 31.79 and the scores from Hamilton Anxiety Rating Scale reduced from 37.14 to 22.86. The group that received only CBT obtained similar results, sometimes even better than the others for the above-mentioned variables. The Fluvoxamine alone group had the lowest scores for the mentioned parameters, therefore psychotherapy is essential for improving anxiety symptoms.

Venlafaxine, an SNRI, is also efficient in reducing the severity of panic disorder symptoms (5).

Treatment with Benzodiazepines

In the acute phase of a panic disorder, benzodiazepines are the most frequent recommended medicines. Their main advantage is represented by the short period of time necessary to achieve the therapeutic effects.

Watanabe et al. (15) presented the benefits of benzodiazepine therapy versus combined benzodiazepines and behavioral therapy. The conclusion is that the combination therapy might be superior to behavior therapy alone in terms of the global severity of symptoms, phobic avoidance behavior and social functioning.

In acute phase, combined therapy is ef-

ficient in reducing the frequency of panic attacks. On long term, six to twelve months after treatment completion it was noticed that psychotherapy alone is superior to the one that associates benzodiazepines (15). This situation can be explained by the fact that long-term administration of benzodiazepines causes cognitive dysfunction (16). Besides that, it is required to use them for short periods and in minimal doses (17) because this class of anxiolytics increases the risk of dependence and relapse (12).

Considering that panic disorder is characterized by catastrophic beliefs about the physical, mental or social consequences, the associations with drugs that diminish the cognitive capacity will only emphasize the anxiogenic ruminations and thus will have a negative impact on disorder course (17).

Management of treatment-resistant panic disorders

Panic disorder consists of recurrent paroxysmal panic attacks, along with the worry regarding future panic attacks and phobic avoidance behavior. Over 50% of patients on treatment continue to experience panic attacks symptoms and fail to obtain remission. Half of the cases that do remit will present relapses later in life (18).

Two types of criteria regarding remission of panic disorder have been proposed (19,20). The first criteria include: absence of panic attacks, absent or reduced agoraphobic avoidance behavior, Hamilton Anxiety Score (HAMAS)≤ 7-10, absence of functional impairment, absent or reduced depressive symptoms, Hamilton Depression Scale (HAMDS) ≤ 7. The second criterion includes the following elements: Panic Disorder Severity Scale (PDSS) ≤ 3 and HAMDS ≤ 7. Treatment resistance is defined as the failure to meet any of the above-mentioned remission criterion after at least six months of optimal treatment (19,20).

According to Mu-Hong and Shih-Jen (18) recommendations, CBT or pharmacotherapy should be the first option in panic disorder treatment, while combining the two of them should be indicated as second-line and administered to patients resistant to treatment. If favorable results are not obtained with any of these strategies, augmentation with atypical antipsychotics is an established drug treatment strategy. From this category Aripiprazole, Quetiapine and Olanzapine proved to increase the efficacy of baseline antidepressant treatment. The results with using other drugs, such as GABAergic antiepileptics, D-cycloserine and anti-inflammatories are inconsistent and need additional evidence to sustain their inclusion in the treatment of treatment-resistant panic disorders.

CBT or/and pharmacotherapy

Even though some studies support the superiority of CBT over pharmacotherapy (21), it is generally accepted that their combination is more effective than CBT alone. A global improvement of symptoms but without an improvement on quality of life had been recorded. However, when long-term outcomes were examined combining CBT with pharmacotherapy proved unsatisfactory. The negative effects can be explained by the fact that the learning process during CBT is influenced by drug therapy (12), especially with benzodiazepines, which on long term had been associated with impairment of cognitive function (15,16). In the study by Krysta et al. (16) the anxiolytic agent is represented by Escitalopram and Duloxetine, which improve attention and executive function, Tianeptine, which improves attention and short-term memory, and Sertraline and Mirtazapine.

Meuldijk et al. (22) suggest that concise care with CBT and/or pharmacotherapy (three months) has similar outcomes as

classic therapy, which requires a longer period of treatment of three or six months to one year or even more. This approach involves 45-minute weekly CBT sessions for seven weeks consecutively. Following this method, the clinical results are obtained earlier, and patients are more satisfied with this type of care.

Treating panic disorders with CBT has certain advantages to pharmacotherapy: fewer side effects, lower relapse rate, and a higher rate of treatment adherence. Pharmacological treatment has many side effects and the risk of relapse is higher, resulting in discontinuation of treatment. In practice CBT is less used than pharmacotherapy especially due to the lower availability of specialized personnel (23, 24). Having patients repeatedly confront safe, but anxiogenic situations, as it happens in exposure therapy, results not only in a reduction in frequency of panic attacks but also a reduction of the fear of having another panic attack, compared to the administration of drugs alone which only reduces the frequency of attacks (21).

At the basis of these results stands the neurobiological change, which appears secondary to the psychotherapy. In panic disorder there is an improper cortical inhibition, for example from prefrontal cortex to an overactive limbic system, from amygdala and insular cortex and a poor modulation to the hippocampus (18). In the case of patients who followed CBT, exposure to anxiogenic situations did not over activate the amygdala. This deactivation represents a more efficient control over limbic system to the prefrontal cortex, which manages more efficiently anxiogenic triggers, by more rational analyze of the exposure (21).

Initiation of whichever therapy requires careful analysis of individual characteristics of patients with panic disorder. Therefore, CBT should be taken in consideration as first option in patient who, for example, worry excessively about or cannot tolerate the side effects of prescribed medicines. On the other hand, pharmacotherapy can be considered as first-line strategy for a patient who presents more somatic symptoms (palpitation, paresthesia, sweating etc.) than cognitive ones (persisting fear for not experiencing another panic attack) (18, 25, 26).

From our review and summing up all the studies above presented, in the treatment of panic disorder we can used pharmacotherapy, whether antidepressants or benzodiazepines, but it is recommended to be associated with psychotherapy. As first line treatment it is recommended selective serotonin reuptake inhibitors or serotonin and norepinephrine reuptake inhibitors, while benzodiazepines are necessary only in acute phase or until the first two treatment lines determined a therapeutic effect. For the resistant cases, it is required to use other group of drugs such as atypical antipsychotics.

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IRON HYPOTHESIS OF CARDIOVASCULAR DISEASE: STILL CONTROVERSIAL

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IRON HYPOTHESIS OF CARDIOVASCULAR DISEASE: STILL CONTROVERSIAL (Abstract): Iron hypothesis has been a controversial subject for over 30 years as many studies support its role as a risk factor for cardiovascular disease, while other studies found no evidence to support it. The conflicting results are accounted for by the non-homogeneity of trial design in terms of population inclusion criteria and different endpoints, non-uniform use of parameters for assessing iron role, and incomplete understanding of the mechanisms of action. The nature of iron is dual, being of crucial importance for the human body, but also toxic as "free iron" induces oxidative stress. Under physiological conditions, there are efficient and complex mechanisms against iron-induced oxidative stress, which could be reproduced for creating new, intelligent antioxidants. Iron depletion improves the cardiovascular prognosis only if serum concentration is at the lowest limit of normal ranges. However, low iron levels and the type of dietary iron intake correlate with atherosclerotic cardiovascular disease, influence the ischemic endpoints in the elderly, and exert negative impact on heart failure prognosis. So far, the causal relation and involved mechanisms are not fully elucidated. Iron overload is a difficult and frequent condition, involving the cardiovascular system by specific pathogenic pathways, therefore determining a particular form of restrictive cardiomyopathy and vaso-occlusive arterial damage. **Keywords**: IRON HYPOTHESIS, OXIDATIVE STRESS, CARDIOVASCULAR DISEASE, IRON DEFICIENCY, BODY IRON STORES

IMPORTANCE OF IRON IN THE HUMAN BODY

Although the total amount of human body iron is very small (4-5 g), it is of vital importance. Iron is a metabolically active micronutrient with the unique property to donate and accept electrons, so it exists in a range of oxidation states, the most common being the bivalent or ferrous (Fe^{2+}) and trivalent or ferric (Fe^{3+}) forms. The human body uses this property in the main enzymatic and non-enzymatic cellular processes. Dietary iron is absorbed mainly by duodenal enterocytes using specific transmembrane transport mechanisms, depending on the heme or non-heme iron form. Systemic iron metabolism regulation is controlled by hepcidin through the specific receptor ferroportin which optimizes and synchronizes its prompt use, preserves iron stores, and prevents uptake by enterocytes. Over 80% of circulating iron is directed to hemoglobin synthesis, whereas the remaining 5-15% provides structure and function

to various enzymatic proteins (cytochromes, myoglobin, iron-sulfur complexes). Therefore, iron is involved in the proper oxygen transport and storage, regulation of cellular energy processes, cellular apoptosis and growth, inflammation, gene regulation, DNA and neurotransmitter synthesis, myogenesis, and xenobiotic metabolism. The action of iron is achieved by binding to transferrin, the specific "carrier" which delivers it to target cells using soluble receptors – sTfR. In the absence of a physiological pathway for excretion, iron excess is stored as ferritin (10-20%), mainly in the liver, spleen and bone marrow cells. Intracellular iron metabolism is regulated by a complex regulatory protein system (1, 2).

IRON HYPOTHESIS

The role of iron in cardiovascular disease was first mentioned in the '60s, when the relationship between iron deficiency anemia in inflammatory diseases and heart failure (HF) was postulated, but the issue was abandoned (3, 4). The American researcher and clinician Jerome L. Sullivan is the first to rediscover and theorize this relationship based on a series of clinical observations, namely that HF is usually a rule in iron overload diseases, and iron stores are higher in men and post-menopausal women. Sullivan gathered these observations in the "iron hypothesis" published in 1981. This hypothesis states that postmenopausal women are at higher risk for cardiovascular disease not only by changing their hormonal profile, but also due to the accumulation of stored iron with age (5).

Based on studies available at that time (6, 7), Sullivan proposes a new paradigm that iron may modulate the ischemic risk and atherogenesis through a unifying mechanism (8). The pathogenic mechanism of the paradigm is oxidative stress. Iron has the propensity of binding reactive oxygen species through Fenton reaction: $Fe^{2+} + H_2O_2 = Fe^{3+} + OH^- + HO^\cdot$ and Haber-Weiss reaction: $O_2^{\cdot -} + Fe^{3+} = O_2 + Fe^{2+}$. Hydroxyl radical ($HO^\cdot$) resulting from these reactions is most harmful to cells and tissues. However, the cytotoxic potential of iron is possible only in case of free iron, also known as "serum catalytic iron". Under physiological conditions, the human body neutralizes free iron by its complete binding to transferrin and ferritin. Any situation in which storage capacity is exceeded or there is an inadequate iron supply will result in overproduction of reactive oxygen species, with cytotoxic effect on cardiomyocyte and arterial wall. The relationship is bidirectional because vascular inflammation, common in cardiovascular disease, also induces oxidative stress. The consequence is the stimulation of iron release from ferritin and low affinity of transferrin for iron, namely generation of circulating catalytic iron. The mechanism of action of free iron on arterial vessel is the subject of numerous experimental studies. Some studies show that iron induces endothelial dysfunction, the promoter of atherosclerosis (ATS). In an original study, Kartikasari et al. noticed the increasing rate of monocyte adhesion to human umbilical endothelial cells treated with serum from patients with hemochromatosis, followed by the expression of adhesion molecules (VCAM, ICAM, E-selectin) in direct relation to the level of free iron (9). Recently, in a study investigating endothelial dysfunction and inflammation induced by iron oxide nanoparticles, Zhu et al. confirmed the toxic action of catalytic iron on human aortic endothelial cells. The authors suggested that the

interaction was mediated by monocytes that release free iron through phagocytosis, but the nanoparticles also have a direct structural and functional cytotoxic effect on vascular endothelium (10). An interesting observation reported by other studies on iron deficiency is the increase in nitric oxide production and iNOS synthase activity that paradoxically alter the intracellular metabolism and induce the release of catalytic iron (1, 10). The relationship with the initiation of atherogenesis is another process demonstrated in the 80's and is based on LDL oxidation and formation of fatty streaks, as the earliest stage in atheroma plaque formation. Recent studies also support iron intervention in ATS progression. Exposure of red blood cells to oxidized plaque material induces erythrocyte lysis and generation of ferrohemoglobin, which is converted by oxidation to feri- and ferilhemoglobin. This chain of chemical reactions destabilizes the heme group that is oxidatively cleaved and releases iron. Thus, plaque lipid oxidation is a feed-forward process which promotes atheroma development, while free iron exerts its cytotoxicity on endothelial cells. Interestingly, the lipids of atheroma plaque isolated from the arterial vessel are not harmful to endothelial cell, while the exposure to red blood cells induces cytotoxicity (11).

LESSONS FROM CLINICAL TRIALS

Iron hypothesis is even today a topic of debate and controversy as there are numerous studies that equally support or deny the initial view. The first remarkable study that supports the iron hypothesis is KIHD, a Finnish prospective trial that investigated over 5 years a cohort of 1,931 men aged between 42 and 60 who had no symptomatic ischemic heart disease (IHD) at entry (12). The conclusion is that serum ferritin level $\geq$ 200 μg/L (considered to be normal) in subjects with high dietary intake of red meat is associated with a 2.2-fold higher risk of acute myocardial infarction (AMI), and the effect is enhanced by LDL-cholesterol. The findings of this reference study are also supported by the Rotterdam trial (13). It supports the relationship with the risk of AMI in the elderly, but again emphasizes the synergistic role of serum ferritin and cardiovascular risk factors, like diabetes, smoking, high LDL-cholesterol. As there are many conflicting data, two recent meta-analyses synthesized the information regarding the relationship between IHD and ferritin level (11,337 patients) or total iron-binding capacity with transferrin (14, 15). The conclusion is that each 50 μg/l increase in ferritin above the level of 200 μg/l increases by 2.4% the IHD risk, and the effect is synergistic with other cardiovascular risk factors (14). As to transferrin, considered an antioxidant molecule due to its ability to bind free iron, an inverse relationship between its level and IHD risk was found (15). The role of ferritin in promoting atherothrombosis is demonstrated by studies focused on the risk of carotid disease. SHIP study suggests a relationship with the prevalence of carotid damage in 2,443 participants recruited from the general population (16). The results are in agreement with the Bruneck study, the first of this kind, in which ferritin is considered to be one of the strongest predictors of incidence and progression of preexisting carotid lesions assessed by ultrasonography for 5 years, its effect being also amplified by LDL-cholesterol (17).

However, there are just as many studies suggesting that iron has a neutral effect on

cardiovascular risk (18). Meta-analyses and studies offer several explanations for these conflicting results (19). The first would be non-homogeneous study design related to different inclusion criteria, cutoffs used to define iron deficiency/overload, various end-points such as all-cause mortality, IHD, AMI mortality, stroke. A second key issue arises from the method to assess iron status (in most studies by measurement of ferritin, but also serum iron, transferrin saturation, dietary iron intake etc) and the interpretation of results (related to the impact of other risk factors). A good example is the study conducted by Sung et al. showing that ferritin is independently correlated with early coronary ATS estimated by calcium score, when not only the influence of traditional risk factors but also transferrin, markers of inflammation, and other risk factors are eliminated (alcohol, diet, type of physical activity, components of the metabolic syndrome) (20). The limits of interpretation might be related to the markers used to define iron role. For example, currently there are only two epidemiological studies using the sTfR/ferritin ratio, considered optimal for iron status description in the human body. These studies noted the weak but consistently positive relationship with the risk of carotid ATS or the occurrence of a first AMI (21, 22). A promising perspective is the use of catalytic iron as a marker of cardiovascular risk, based on observations of two recent studies (23, 24). Rajapurkar et al. support both the relationship between catalytic iron levels and the prevalence of cardiovascular disease and its importance in acute coronary syndromes (25). The 10-month follow-up of a cohort of 1,701 patients with acute coronary syndromes concluded that the new marker is able to detect early a heart attack (within 3 hours of the onset of chest pain) and to identify the high-risk subgroup for all-cause mortality (3.97-fold) and adverse cardiac events apart from traditional risk factors. Finally, the results could be influenced by information related to the mechanisms of action of iron, insufficiently used in studies. For example, there are voices that argue that iron does not increase the cardiovascular risk directly but by amplifying the harmful vascular effects of homocysteine, assessed by flow-mediated vasodilatation (26). In an article published this year, Hsieh et al. report remarkable findings regarding hepcidin, which acts as an intrinsic cardiac hormone with a predominantly local effect. The authors note increased hepcidin levels in human cardiomyocytes pretreated with ferrous iron, which has a cytotoxic effect. The conclusion elegantly demonstrates that hepcidin can protect cardiomyocytes from iron-induced apoptosis through the extrinsic apoptotic pathway and GATA-4/Bcl-2 inactivation, a myocardial survival factor (27). Therefore, hepcidin contributes to the maintenance of myocardial structure. This study completes and offers a molecular explanation for previous data regarding the low level of hepcidin and increased 3-year mortality in HF or its correlation with increased left ventricular mass (28). On the other hand, there are observations on upregulation of hepcidin specific to ischemic myocardium after myocardial infarction (29). In the light of the study by Hsieh, it can be speculated that increased levels of hepcidin have the potential to diminish catalytic iron cytotoxicity derived from oxidative stress, thus limiting the infarct size (27). Finally, an interesting conclusion is that chemotherapeutic agents such as daunorubicin and doxorubicin induce cardiomyopathy by the same mechanism of iron-induced GATA-4 inactivation, namely feroptosis.

SERUM IRON LEVEL – PROTECTOR OR RISK FACTOR?

Based on the results of studies supporting the role of ferritin as a cardiovascular risk factor at levels close to the upper limit of the normal range, Sullivan launches a new assertion on the possible protective role of iron depletion against ischemic events, ATS lesions included (30). Salonen supports the idea reporting the reduction of the risk of ischemic events in all arterial areas, and of the number of revascularization procedures (12), and Meyers demonstrates the reduction of the risk of fatal AMI in men blood donors versus non-donors (31). Latest data from the multicenter controlled randomized trial conducted at the Department of Veterans Affairs Cooperative Studies Program on 1,277 patients with stable symptomatic peripheral arterial disease also suggest that reducing iron stores could improve the clinical evolution in certain subgroups of patients – young, smokers with low average concentrations of ferritin (32). Basically, iron depletion improves the cardiovascular prognosis only if serum concentration is at the lower limit of normal ranges.

Iron deficiency is well-documented to be associated with increased cardiovascular risk. Recent studies support the relationship with the high prevalence of IHD and carotid ATS, the influence on the ischemic endpoints in the elderly (33, 34), and its prognostic role in HF (35). The unsolved problem remains whether serum iron is causally related to cardiovascular disease, or is only a marker of chronic diseases associated with cardiovascular risk (19). Iron deficiency causes thrombocytosis associated with the risk of thrombotic complications (36), hypercoagulable state due to reduced deformability of microcytic erythrocytes, and increased viscosity (19). Finally, an important mechanism is iron deficiency anemia, a pejorative factor in the higher incidence and progression of chronic HF (2). Anemia in HF is the consequence of absolute iron deficiency resulting from decreased dietary intake, impaired intestinal absorption and transport mechanisms, blood loss after antiagregant and anticoagulant medication, abnormal hepcidin regulation etc. At the same time, HF is a state characterized by inflammatory status associated with increased levels of pro-inflammatory cytokines and oxidative stress. Inflammation leads to inadequate iron supply to meet the demand despite normal body stores, a condition called functional deficiency. Renal impairment and associated infections maintain inflammation and consequently iron deficiency. The fact is that anemia causes hypoxia, which induces increased nitric oxide concentrations causing vasodilatation and low blood pressure. It results in sympathetic stimulation that causes change in renal hemodynamics with decreased glomerular filtration rate, stimulation of renin-angiotensin-aldosterone system, and ADH secretion. The consequence is fluid retention and plasma volume expansion with a negative impact on the structure and function of the heart. Heart dilatation, increased ventricular mass, and apoptosis of cardiomyocytes are common events associated with cardiac dysfunction (35). Therefore, the association of anemia with HF and renal dysfunction (cardio-renal syndrome) is suggestively called the "triangle of death" (37). Despite this well-known pathogenic substrate, iron deficiency in HF was less studied. Opasich et al. noted that 20% of patients with systolic HF had absolute iron deficiency microcytic anemia, but in

57% of patients it was due to functional iron deficiency (38). Shortly after, Nanas published the only study to date in which iron deficiency was assessed by the standard method, bone marrow biopsy, and reported a high percentage (73%) in patients with advanced HF (39). Until now, only two observational studies reported the incidence of iron deficiency in the general population with HF, which seems to be more common in women, in advanced stages of disease with high NT-proBNP and C-reactive protein levels (40, 41). The fact remains that iron deficiency with or without anemia affects the myocardium and skeletal muscle, both organs having increased energy needs dependent on iron normal metabolism. Experimental data show that the myocardial molecular consequences are an increased expression of natriuretic peptides (ANP, BNP) and hepcidin, extracellular matrix remodeling, mitochondrial dysfunction, apoptosis, and reduced expression of TfR-1 (2,42). In the skeletal muscle, iron deficiency causes low oxygen transport and diffusion, decreased oxidative capacity, abnormal use of energy sources represented by glucose and fatty acids (2). The clinical consequence is decreased exercise ability. Also interesting are the clinical data that support the correlation with the risk of depression, predominantly in males (41). Also important is the negative impact on prognosis, iron deficiency being a strong predictor of the need for transplantation and 3-year risk of all-cause mortality (41, 43). On the other hand, the findings of seven studies on iron supplementation benefit in these patients are promising (2). Although the number of patients included is small, the follow-up period is short, and study design is heterogeneous, there is the assumption that iron deficiency should become a therapeutic target in HF. Its correction improves exercise ability, NYHA class, systolic dysfunction assessed by echocardiography, and mortality. FAIR-HF trial, the only multicenter placebo-controlled study that included both patients with and without anemia followed for 24 weeks, supports the beneficial effect of i.v. iron supplementation regardless of NYHA class, HF etiology, co-morbidities, hemoglobin level, with important impact on rehospitalization rate and good clinical tolerance (44). Similarly, a meta-analysis by Hunnicutt et al. published this year suggests that the incidence of IHD is related to low iron concentration and dietary heme iron intake. The causal relationship and the mechanisms involved remain to be elucidated (45). Lapice believes that the relationship with morbidity and mortality from IHD is not directly related to iron, but to dietary and lifestyle behaviors (smoking, alcohol consumption, level of physical activity) that can modulate the risk. For example, red meat containing heme iron has high levels of saturated fats and other oxidants (19).

Iron overload is a much more difficult to handle both clinically and therapeutically and the cardiovascular involvement is demonstrated by such iron storage diseases as hemochromatosis. In practice, there are also frequent conditions which require repeated blood transfusions, causing iron accumulation (hemosiderosis) in the absence of physiologic iron excretion mechanism. A unit of blood is equivalent to a dietary intake of 200 mg iron. A total body iron of over 7 g, corresponding to 30-50 units of red cells, can lead to signs of tissue toxicity. For this reason, it is necessary to monitor serum ferritin levels and at > 1000 μg/l, iron phlebotomy and chelating therapy become man-

datory (46). The cardiac response to iron overload is cardiomyopathy, typically developing in two stages - initially restrictive cardiomyopathy caused by fibrosis and myocardial ischemia invariably progressing to dilated cardiomyopathy with severe biventricular impairment (47). Diastolic dysfunction is considered an independent prognostic marker for the increased mortality rate in this category of patients. The pathophysiology of iron overload cardiomyopathy is mediated by oxidative stress that enables ischemia-reperfusion lesions even in the presence of normal coronary arteries. Specifically, iron regulates excitation-contraction coupling in cardiomyocytes via action on L-type Ca^{2+} channels, thereby causing diastolic and systolic dysfunction (48). Associated chronic tissue iron deposition and its electrophysiological effects determine interstitial fibrosis and cardiac specific conduction system damage, the cause of arrhythmias with prognostic impact. On the other hand, oxidative stress causes vascular impairment secondary to endothelial dysfunction and vascular stiffening (49). Iron overload also determines vaso-occlusive pulmonary disease with secondary pulmonary hypertension. Overall, vascular damage enhances heart dysfunction. Given high level of oxidative stress, antioxidant agents together with iron chelating agents is the rational therapy in these patients. Experimental studies suggest a cardiac protective role of taurine. A promising perspective is the use of Ca^{2+} channel blockers, especially of amlodipine, due to its coronary vasodilator and antioxidant action, but also the specific mechanism of iron action on L-type Ca^{2+} channels (50).

Even if the direct role of iron as a risk factor for cardiovascular disease is unclear, oxidative stress caused by catalytic iron is a certainty. Nature has effective weapons against this mechanism in physiological conditions, as is the formation of specific complexes (complex 1-Fe) with antioxidant role. The therapeutic use of this mechanism is considered by Gross`s team as innovative as these complexes could form the basis of the future design of "intelligent antioxidants". The authors demonstrate the mechanism of these complexes, which are bipolar, that is they can bind to both types of lipoproteins, LDL and HDL. The consequence is the catalytic decomposition of harmful species, i.e. permanent and targeted replacement of oxidized environment, in contrast to current antioxidants which have limited effect on reactive oxygen species and can damage the arterial wall. An interesting remark of the study is that directing the bivalent ferrous iron to these complexes by specific enzymatic methods may increase the antiatherogenic effect of HDL-cholesterol (51).

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UP DATES IN THE PATHOGENESIS AND DIAGNOSIS OF HEPATIC ENCEPHALOPATHY

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UPDATES IN THE PATHOGENESIS AND DIAGNOSIS OF HEPATIC ENCEPHALOPATHY (Abstract): In the pathophysiology of hepatic encephalopathy (HE), a serious complication of liver cirrhosis, different hypotheses, including increased blood ammonia levels, increased production and absorption of intestinal bacterial products are discussed. Recent studies demonstrated that *Helicobacter pylori* infection is associated with elevated blood ammonia levels. Inflammatory mediators and cytokines also play important roles along with hyperammonia in the pathogenesis of HE, while recent studies revealed correlations between CRP levels and length of hospital stay. Neuropsychological diagnostic tests, such as Psychometric Hepatic Encephalopathy Score, were especially designed for detecting minimal HE. Imaging investigations, like Computed Tomography or Magnetic Resonance Imaging, show characteristic aspects in advanced stages of liver disease. Recent studies revealed that Mini-Mental State Examination (MMSE) can show significant changes mostly in advanced stages of overt HE, but that it is not an appropriate tool in defining an encephalopathy-related status of patients with cirrhosis. **Keywords**: HEPATIC ENCEPHALOPATHY, LIVER CIRRHOSIS, PATHOGENIC

Cirrhosis represents the end-stage of chronic liver disease from any cause. In the natural history of liver cirrhosis there is a compensated phase (i.e. asymptomatic phase), followed by a decompensated phase, defined by the development of complications, such as portal hypertension and/or liver insufficiency, specifically ascitis, variceal hemorrhage, and/or encephalopathy (1). Hepatic encephalopathy (HE) is a serious complication of liver cirrhosis, negatively affecting both health-related quality of life and survival. Although it is now considered as a continuum of neurocognitive deterioration, HE is usually divided into minimal and overt HE according to mental status change. In minimal HE characteristic are the lack of a detectable brain dysfunction at clinical examination but subclinical psychomotor slowing and cognition deficits are present and clinically relevant. Overt HE is characterized by various symptoms encompassing neuromuscular abnormalities, disorders in intellectual functions, personality and consciousness, traditionally graded in four stages, after West-Haven criteria (2).

PATHOPHYSIOLOGY

HE is a multifactorial syndrome, involving metabolic and biochemical derangements, brain atrophy and edema, impaired cerebral perfusion and hyperpermeability of blood-brain barrier (3). HE occurs in the presence of insufficient hepatic clearance of toxins absorbed from the intestine, resulting in neurochemical abnormalities across the blood-brain barrier.

Specific hypothesis of HE

Ammonia

Cirrhosis represents the final common histological pathway for a wide variety of chronic liver diseases. The blood ammonia levels of cirrhotic patients are usually higher than that of normal people (4).

Ammonia is central in the disease process, contributing to alterations in neurotransmission, oxidative stress, cerebral edema and astrocyte swelling (5). This compound is mainly derived from nitrogenous products in the diet, bacterial metabolism of urea, and deamination of glutamine by the enzyme glutaminase. Ammonia enters the portal circulation from the gut and is converted to urea in the liver. In the presence of significant portosystemic shunting, with or without hepatocellular dysfunction, ammonia concentration rises in blood and crosses the blood-brain barrier. Exposure to increased brain ammonia results in structural alterations in astrocytes that cause swelling and brain edema. Over a long period of exposure to high ammonia levels, astrocytes develop structural changes, known as Alzheimer type II astrocytes (6).

New studies show roles of changes in active glutamine transport in brain edema development during HE. Excessive glutamine (Gln) synthesis in ammonia-overloaded astrocytes contributes to astrocytic swelling and brain edema. Much of the newly formed Gln is believed to enter mitochondria, where it is recycled to ammonia, which causes mitochondrial dysfunction (a “Trojan horse” mode of action). A portion of Gln may increase osmotic pressure in astrocytes and the interstitial space, directly and independently contributing to brain tissue swelling. Accumulation of excess Gln in mitochondria involves a carrier-mediated transport which is activated by ammonia. Excessive synthesis of Gln from ammonia and glutamate catalyzed by an astrocyte-specific enzyme, glutamine synthetase (GS) plays a major role in the pathogenesis of astrocytic swelling (7).

Bowel Bacterial Overgrowth

According to previous studies, the patients with liver cirrhosis have an increase in production and absorption of intestinal bacterial products, which can lead to a continuous flux of lipopolysaccharides and other toxic materials to the portal vein. In the liver, these substances are recognized by Kuppfer cells and thus trigger the release of tumor necrosis factor (TNF), which, together with other products of these cells, leads to hepatocyte lesion (8).

Compounds derived from bacterial activities in the gut can cause neurochemical changes in the brain. These gut-derived toxins (e.g., ammonia, benzodiazepine-like substances) are implicated in the pathophysiology of overt HE. In patients with liver disease or portosystemic shunting, these toxins are inefficiently detoxified, accumulate in the blood, cross the blood-brain barrier, and result in abnormalities, such as altered neurotransmission, astrocyte swelling, and impaired energy metabolism (9).

Helicobacter pylori

Not only bowel bacterial overgrowth must be discussed as an oligosymptomatic

cause of HE, but other bacterial infections in cirrhotic patients might be important, such as *Helicobacter pylori* infection. *H. pylori* infection can produce high blood ammonia concentration in these patients (8).

H. pylori, a bacterium which commonly infects human stomach, has urease activity, which is many times more potent than that of enterobacteria. The *H. pylori* urease in the gastric juice breaks down urea into ammonia and carbon dioxide, and the ammonia is then rapidly absorbed into the blood (4).

From thirteen prospective clinical trials which assessed the effects of H. pylori eradication in patients with liver cirrhosis and HE, seven of these showed a beneficial effect of eradication therapy on minimal HE. All of these studies were highly diverse in design and methodology (10). Eradication therapy in H. pylori-positive cirrhotic patients may have a beneficial influence on hyperammonemia and minimal HE, but evidence from well-designed clinical studies is weak (10).

Inflamation

It is now clearly evident that inflammatory mediators and cytokines play an important role along with hyperammonia in the pathogenesis of HE; possible mechanisms include cytokine-mediated changes in blood-brain barrier permeability, microglial activation, subsequent production of neurosteroids, and altered activity of peripheral benzodiazepine binding sites (6).

Inflammation in the presence of ammonia coactively worsens HE. Inflammation can result from hyperammonemic responses, endotoxemia, innate immune dysfunction or concurrent infection. Treatments currently focus on reducing inflammation and/or blood ammonia levels and provide varying degrees of success (5). It has been shown that the high correlation between CRP levels and the total in-hospital days corroborates the impact of the systemic inflammatory status, not only at the moment of hospital arrival, but also as an indicator of HE regression during the treatment with antibiotics (8). Other studies correlates the CRP levels with encephalopathy occurrence in cirrhotic patients, and many others have described the relevance of inflammation in HE neural physiopathology. These studies demonstrate for the first time that CRP levels are correlated with the hospitalization duration (11, 12, 13). The role of inflammatory cytokines in affecting the blood–brain barrier and increasing the ammonia diffusion in astrocytes was also well described by other authors. In conclusion, CRP reduction must be a clinical target during antibiotic treatment in HE when the values obtained are high (8).

Hyponatremia

In a recently observational study, it has been shown that hyponatremia was a common feature in patients with liver cirrhosis and its severity increased with the severity of liver disease. The existence of serum sodium concentration < 135 mmol/L was associated with greater frequency of HE compared with patients with serum sodium concentration > 135 mmol/L (14).

Other mechanisms for HE

✓ Increased benzodiazepine-like compounds in the brain.

✓ Accumulation of manganese in the basal ganglia: this is implicated in altered dopaminergic neurotransmission and extrapyramidal symptoms.

✓ Alterations in central nervous sys-

tem (CNS) tryptophan metabolites (e.g. serotonin): these changes may underlie some of the classic descriptions of altered sleep-wake cycles seen in early stages of encephalopathy (6).

DIAGNOSTIC OF HE

The diagnosis of HE first requires excluding other potential causes for encephalopathy. Metabolic disorders, infectious diseases, intracranial vascular events, and intracranial space-occupying lesions can present with similar neuropsychiatric symptoms and need to be excluded (15). Consideration of the possibility of HE arises when significant liver dysfunction is known or suspected to be present. Clinical or laboratory evidence of liver failure and/or portal hypertension is usually obvious (6).

The West-Haven grading system (table I), which classifies HE into categories, remains criticized because of its intense subjectivity and low intra- and inter-observer reliability. Therefore, several other more objective diagnostic tools (e.g. neuroimaging, neurophysiological methods or psychomotor tests), were implemented to diagnose both minimal and overt HE, but there is still an ongoing debate which of them should be a diagnostic standard (2).

TABLE I

West Haven criteria for classification of hepatic encephalopathy (6)

Grade	Description	Recommended Tests
0	No abnormality detected	
Covert HE	Normal mental status and neurologic examination Abnormal psychometric tests	>2 SD on 2 or more tests in PHES ICT: >5 lures CFF: cutoff frequency 39 Hz
I	Trivial lack of awareness Euphoria or anxiety Shortened attention span Impairment of addition or subtraction	Naming </= 7 animals in 120 sec Orientation in time and space
II	Lethargy or apathy Disorientation for time Obvious personality change Inappropriate behavior	Disorientation in time (>/=3 items incorrect): ✓ Day of the week ✓ Day of the month ✓ The month ✓ The year Orientation to place
III	Somnolence to semistupor Responsiveness to stimuli Confusion Gross disorientation Bizarre behavior	Disorientation to place (>/=2 items incorrect): ✓ State/country ✓ Region/country ✓ City ✓ Place ✓ Floor/ward Disorientation to time Reduction of Glasgow coma score (8-14)
IV	Coma, inability to test mental state	Unresponsiveness to pain stimuli (Glasgow coma score <8)

CFF, Critical Flicker/Fusion Frequency; HE, hepatic encephalopathy; ICT, Inhibitory Control Test; PHES, Psychometric Hepatic Encephalopathy score; SD, Standard deviation.

1. ***Blood ammonia***. No significant cor-

relation between serum ammonia level and stage of HE has been observed. Most authors agree that measurement of ammonia may be helpful in the initial evaluation of unexplained encephalopathy in patients with not known prior history of liver disease. Normal ammonia values in these patients would argue against a diagnosis of HE (15).

Measurement is not recommended routinely, because it does not change the approach to diagnosis or treatment of patients with suspected HE (6). The measurement of serum ammonia levels for the diagnosis of HE remains controversial: some authorities consider that the use of arterial blood is preferred (15), while others consider that venous ammonia level correlates with the severity of HE as arterial ammonia level does (3).

2. ***Psychometric testing***. Neuropsychological diagnostic tools show a number of practical advantages, making them potent candidates for the common use in HE assessment. Generally, they are simple to perform, not time-consuming and can be used in the outpatient clinic. For this purpose, both single paper-pencil tests (such as the block design test, the trail making test and the digit symbol test), as well as standardized batteries (i.e. the Repeatable Battery for the Assessment of Neuropsychological Status, the Wechsler Adult Intelligence Scale - Revised, the Auditory Verbal Learning, the Everyday Memory Questionnaire) have been implemented. The complex tools have a superior value in comparison to single modalities, because they cover a broad spectrum of neurocognitive deficits. However, among plenty of these tests, only the Psychometric Hepatic Encephalopathy Score (PHES) was especially constructed for detecting minimal HE. Others have a great importance in the diagnostic of cognitive alteration in elderly or neuropsychiatric disorders, in the estimation of the effects of psychoactive drugs or to measure intelligence, while their value in the field of HE remains to be established (2).

Critical Flicker/Fusion Frequency (CFF) test - is based on the hypothesis that retinal gliopathy (hepatic retinopathy) could serve as a marker of cerebral gliopathy in HE. It correlates well with paper and pencil psychometric tests used to diagnose covert HE. It enables discrimination of stage 0 HE from covert HE and overt HE at a cutoff frequency of 39 Hz, with a sensitivity of 55% and a specificity of 100%. It is useful for monitoring fluctuations in the severity of HE in response to precipitating factors or therapeutic interventions (6).

3. ***Electroencephalography (EEG***) - is rarely used clinically, it has some value in research settings (6). It is often performed in patients with their first presentation of HE or in any patient in whom the presentation is atypical in nature. The EEG may have some value in determining the advanced stages of HE, when characteristic triphasic waves are identified (15).

However, the EEG detects cortical neuronal activity and may not reflect a wide variety of neurophysiological events seen in HE . Moreover, it is not broadly implemented in the daily practice, because of its limited availability and the need of experienced personnel to appropriate interpretation of the obtained electroencephalograms (2).

4. ***Computed tomography (for porto-systemic shunts)***

Cirrhotic patients with persistent or recurrent HE commonly have large spontaneous portal-systemic shunts. Using multidetector computed tomography (CT) imag-

ing, it has been shown that large spontaneous porto-systemic shunts are significantly more common in patients with (71%) than without (14%) HE. This approach is ideal in patients with compensated liver disease, where signs and symptoms suggest HE, but the severity of liver disease is mild (15).

5. ***Magnetic resonance imaging (MRI).*** MRI has become a standard technique for the assessment of patients with neurologic manifestations. Patients with liver cirrhosis and no clinical manifestations of HE can have symmetrical high-signal abnormalities in the pallidum on T1-weighted images. Pallidal hyperintensity is not related to the grade of HE; rather, its absence in a patient with cirrhosis and neurologic manifestations suggests an alternative diagnosis. Decreased brain myo-inositol and elevated glutamine by magnetic resonance spectroscopy is characteristic of HE when detected (15).

6. ***Mini-Mental State Examination (MMSE***) is one of the most commonly used methods in the assessment of cognitive mental status. MMSE is one of above mentioned complex neuropsychological tools, that was occasionally used for identification patients with HE in clinical trials and could serve as a potential screening test for this purpose. MMSE assesses cognitive abilities: orientation (spatial and time), attention, concentration, calculation, immediate and short-term memory, visual-spatial orientation, praxis and language skills. Some of these functions are also altered in cirrhotic patients in the course of minimal HE and in stages 1 and 2 overt HE according to West-Haven criteria, long before neurocognitive decline is obvious and clinically easy to detect (2).

In a prospective, quantified electroencephalography study, the authors concluded (2) that MMSE is not an appropriate tool in defining an encephalopathy-related status of patients with cirrhosis and should not be used for this purpose in clinical trials. The authors showed that the score (summary scores and most of the items) is altered in patients with more advanced liver disease measured by Child-Pugh classification and there is a reduction of the most of MMSE items in patients with overt HE, but differences reached the statistical significance only in few items and mostly in more advanced stages of overt HE (i.e. in grade 2 and 3) and the numeric difference between MMSE scores was small and unlikely to be clinically useful.

CONCLUSIONS

Hepatic encephalopathy (HE) is a serious complication of liver cirrhosis, involving metabolic and biochemical derangements, brain atrophy and edema, impaired cerebral perfusion and hyperpermeability of blood-brain barrier. In the pathophysiology of this complication, different hypotheses, including increased blood ammonia levels, increased production and absorption of intestinal bacterial products, *Helicobacter pylori* infection which can produce high blood ammonia levels are discussed. Inflammatory mediators and cytokines also play important roles along with hyperammonia in the pathogenesis of HE, while recent studies revealed correlations between CRP levels and length of hospital stay. Of the diagnostic tools for HE, the measurement of serum ammonia levels remains controversial, because no significant correlation were found between serum ammonia level and HE stage. Of the neuropsychological diagnostic tests, only the Psychometric Hepatic Encephalopathy Score (PHES) was especially designed for

detecting minimal HE. Imaging investigation, such as CT or MRI, show characteristic aspects in advanced stages of liver disease. Also, recent studies revealed that MMSE is not an appropriate tool in defining an encephalopathy-related status, significant changes being found mostly in more advanced stages of overt HE.

In view of the complex aspects in the pathogenesis and diagnosis of HE, there is still a need for developing more specific tests for an objective evaluation of the entire spectrum of this complication, better management and treatment.

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PULMONARY REHABILITATION AND QUALITY OF LIFE IN LUNG CANCER PATIENTS

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PULMONARY REHABILITATION AND QUALITY OF LIFE IN LUNG CANCER PATIENTS (Abstract): Lung cancer is one of the most common types of cancer, often diagnosed in advanced stages. Chest surgery is the main component of curative treatment, the pre-operative functional status being predictive for post-operative morbidity. The potential benefits of pulmonary rehabilitation in lung cancer management appear in pre-operative period, improving the exercise capacity and increasing the number of patients sent to surgery, in post-operative period, decreasing the number of complications, and during the medical treatment of patients with advanced disease, improving the symptoms and increasing the quality of life. The quality of life (QOL) in patients with lung cancer is determined by factors related to patient (stage of disease, co morbidities) as well as treatment (surgery, chemotherapy, radiotherapy). The assessment of QOL is made through questionnaires, mainly related to therapeutic regimens and symptoms. Among the most used are Questionnaire of the European Organization for Research and Treatment of Cancer (EORTC QLQ - C30), Functional Assessment of Cancer Therapy - General (FACT - G), FACT - L (Lung) and the Lung Cancer Symptom Scale (LCSS). **Keywords:** LUNG CANCER, PULMONARY REHABILITATION, QUALITY OF LIFE

Lung cancer is one of the most common types of cancer, with a 5-year survival rate of approximately 15%. Given the long asymptomatic evolution of the lung cancer, in contrast to other tumor types, it is often diagnosed in advanced stages. The main symptoms include cough, hemoptysis, dyspnea, chest pain, weakness, loss of appetite. Therefore, the treatment goals for these patients are relief of symptoms and improving overall survival (1).

The increasing number of alternative lines of therapy and the decreased differences in the clinical effectiveness of drugs improve the survival rate but are often accompanied by severe side effects. In this setting, the importance of estimating the quality of life (QOL) arises although the inclusion of this parameter in lung cancer clinical trials was longtime neglected (1, 2).

Chest surgery is also a main component in the management of patients with lung cancer, the pre-operative functional status being predictive for post-operative morbidity (3). In order to improve it, in recent years appears increasingly more the need for integrate comprehensive pulmonary rehabilitation in clinical approach of the oncologic patient.

THE IMPACT OF PULMONARY REHABILITATION ON THE TREATMENT OF LUNG CANCER

Pulmonary rehabilitation is seen as a comprehensive, non-pharmacological and interdisciplinary patient-centered intervention (4). It targets the impairments derived from chronic pulmonary diseases and its role is well-established and evidence-based in Chronic Obstructive Pulmonary Disease but it began to be adapted to nontraditional areas, such as lung cancer, Chronic Obstructive Pulmonary Disease exacerbations, and critical illness (5).

The potential benefits of pulmonary rehabilitation in lung cancer management appear in three settings, according to Nici and ZuWallack: in pre-operative period, improving the exercise capacity and increasing the number of patients sent to surgery, in post-operative period, decreasing the number of complications and during the medical treatment in patients with advanced disease (tab. I).

TABLE I

The potential benefits of pulmonary rehabilitation in lung cancer

(after Nici and ZuWallack, 2010) (5)

PRE-OPERATIVE
Improves exercise capacity
POST-OPERATIVE
Optimizes airflow
Prevents complications such as hypoxaemia, hypercapnia, respiratory infections
Decreases the number of days with a chest tube
Decreases the length of hospital stay
Pain management
DURING MEDICAL TREATMENT
Improves the symptoms related to chemotherapy/radiotherapy
Increases the quality of life

The official statement of the American Thoracic Society and the European Respiratory Society on pulmonary rehabilitation, released in 2013, clearly states as "noteworthy advances" that pulmonary rehabilitation provided to individuals with lung cancer "has demonstrated improvements in symptoms, exercise tolerance, and quality of life" (6). Emerging data support the usefulness of the multiple interventions included in pulmonary rehabilitation for oncologic patients with lung carcinoma, especially for non-small cell lung cancer (NSCLC).

Surgical resection is an effective curative treatment for lung cancer but low exercise tolerance and multiple serious co morbidities, especially COPD and cardiovascular diseases, limit the access of those patients to surgery. Some small observational

studies concentrated on the impact of short-term pre-operative pulmonary rehabilitation: Bobbio et al. (7) found significantly increased maximal oxygen consumption after 4-week program while Jones et al. (8) obtained significant improvement of maximal oxygen consumption and of six-minute walk test (6-MWT) distance, baseline to pre-surgery, after 5 endurance cycle ergometry session / week. Pre-operative pulmonary rehabilitation seems to be useful even when lung cancer is associated with COPD, a limited number of training sessions leading to shorter length of post-operative hospital stay, fewer days with chest tube and improved lung expansion (9).

Post-operative pulmonary rehabilitation was more extensively studied, even in some randomized controlled trials. Cesario et al. (10) investigated the efficacy of a 4-weeks in-patient respiratory rehabilitation program, after lung resection in patients diagnosed with NSCLC. Several functional parameters – distance achieved at 6-minutes walk test, hemoglobin saturation, dyspnea on Borg scale – were improved after one month versus baseline although the comparison with a group of untreated patients as controls showed no significant difference. A recent 18-months study followed lung cancer patients (n=58) enrolled in a comprehensive rehabilitation program and patients with standard care (n=305): those who had pre-operative rehabilitation had a significant increase of FEV_1 and 6-MWT distance but no effect on post-operative morbidity (11).

Several other researchers also have shown that post-surgery pulmonary rehabilitation interventions improve the peak exercise capacity, walked distance and the main symptoms (dyspnea, fatigue) (12, 13). In terms of quality of life, the reported results are variable (14). A large randomized trial (LUNGEVITY) included 160 patients with histologically confirmed stage I-IIIA NSCLC, after curative-intent lung surgery and randomly assigned them to aerobic training alone, resistance training alone, combined aerobic and resistance training or progressive stretching sessions. This study is ongoing and aimed at identifying the optimal type of exercise training as well as discovering new insight into the physiological effects of such training (15). Glattki and colab. found favorable effects of 6-week multidisciplinary rehabilitation program on 6-MWT distance, FEV_1, FVC, dyspnea on Medical Research Council Scale (MRCS) in patients with lung cancer, both with or without COPD, with or without chest surgery, after completion of treatment (3).

The evidence concerning the role of pulmonary rehabilitation during the medical treatment for lung cancer relies mostly on case series of non-resectable stage III-IV cancer. These patients have a seriously affected quality of life due to severe symptoms such as fatigue, anorexia, dyspnea, pain, depression and anxiety, and also physical deconditioning all that resulting in disability (6). Many of them are unable to perform exercise training but chest physiotherapy and breathing exercise could help controlling the symptoms (16). A recent systematic review (17) evaluated the effect of physical exercise and nutritional interventions in patients with advanced stage NSCLC. The results suggested that exercise and nutrition "may have beneficial effects on unintentional weight loss, physical strength, and functional performance" but these findings were resulted from small studies at significant risk of bias.

There is need for further randomized controlled trials in order to clearly establish

the benefits of pre-operative and post-operative pulmonary rehabilitation, the type of physical training, and the optimal duration of the program in the setting of lung cancer.

NON-SURGICAL TREATMENT IN LUNG CANCER AND QUALITY OF LIFE

During 2001-2011, there were 43 studies conducted to measure health-related quality of life (HRQoL) of patients with lung cancer, 27 having as main objective HRQoL. Most articles illustrated the results of phase III clinical trials: 38 included patients with locally advanced non-small cell lung cancer (NSCLC), especially in stage III/IV, two studies included patients with small cell lung cancer (SCLC) in all stages and three studies included both patients with NSCLC and SCLC. Most studies have investigated the impact of platinum-based drug combinations, 6 studies have investigated the effect of gefitinib and 2 studies concerns of erlotinib(18).

Quality of life (QOL) in patients with lung cancer was assessed by questionnaires, mainly related to therapeutic regimens and symptoms. Among the most used are *Questionnaire of the European Organization for Research and Treatment of Cancer* (EORTC QLQ - C30), *Functional Assessment of Cancer Therapy-General* (FACT - G), FACT-L (*Lung*), *Lung Cancer Symptom Scale* (LCSS), *Hamilton Anxiety and Depression Scale* (HADS), *Brief Pain Index* (BPI).

The most commonly used is the EORTC QLQ-C30; it contains 30 criteria, available in 60 languages (18), while FACT-G questionnaire contains 27 physical, emotional, social, functional elements (19) and FACT-L - 37 items, the last one being suitable for lung cancer (20). Due to inhomogeneity of the patients and treatment regimens, it is difficult to compare the studies on the HRQoL.

However, most researches including comparisons between different chemotherapy regimens did not show significant differences in HRQoL between different treatment arms (21-23). Another group of studies report cautious assumptions of improving HRQoL (24, 25). Only Belani et al. (26) and Reck et al. (27) indicated HRQoL superiority of paclitaxel or docetaxel compared with vincristine or vinorelbine/cisplatin.

Regarding EGFR inhibitors, several authors showed that patients with highly advanced NSCLC treated with gefitinib had improved HRQoL (28) while others correlated that outcome with tumor response (29). Regarding erlotinib, Lilenbaum et al. (30) could not demonstrate significant improvement in progression-free survival, median survival and HRQoL compared to standard chemotherapy regimen but other researchers had positive results in HRQoL when erlotinib is administered in the second line of treatment (31).

LUX- Lung 3 study was conducted on a population of patients with advanced NSCLC and positive EGFR mutation and showed an unprecedented improvement in cancer-related symptoms and quality of life when treated with afatinib, an irreversible inhibitor of the ErbB receptor family as first-line therapy, compared to chemotherapy with pemetrexed and cisplatin, considered the standard of care in this population of patients (32).

Lung cancer or lung metastases often have symptoms for which palliative radiotherapy is effective and improves or maintains quality of life, for about one-third of

the affected patients (33, 34). Many studies assessed at least in one arm the use of palliative thoracic radiotherapy and evaluated QOL or symptoms relief. The researches included patients with NSCLC, with endobronchial brachytherapy alone or in combination with external radiotherapy or evaluated the use of palliative radiotherapy in patients with lung cancer other than NSCLC histological type.

Clinical trials that compared different regimens of palliative radiotherapy fractionation showed improved quality of life and survival in patients with good performance status who received high doses of radiation (TD = 30Gy/10fractions/3Gy/fraction) compared to lower doses (TD = 20Gy/5fractions/4Gy/fraction, 17Gy/2fractions, 10Gy/1fraction), which are mainly used in patients with reduced performance status. This type of radiotherapy could be interspersed between the series of chemotherapy, without causing delay in chemotherapy administration (35-37).

The impact of new radiotherapy techniques (IMRT - intensity modulated radiotherapy, IGRT - guided radiotherapy imaging) and PET - CT imaging integration in the palliative treatment of patients with pulmonary cancer is not clearly defined. Regarding the palliative role of endobronchial brachytherapy, recent analysis of 13 clinical trials concluded that external radiotherapy is superior to endo-bronchial brachytherapy and the addition of the last one has no advantages over external radiotherapy alone (37). So far, it was not demonstrated an advantage of concomitant radio - chemotherapy over sequential administration for symptoms palliation in patients with lung cancer (38).

Irrespective of the chosen therapeutic regimen, there is a great concern for ensuring the best quality of life as possible for the oncologic patients. An interesting study of Gore et al. (39) opposed patients with inoperable lung cancer to those with end-stage chronic obstructive pulmonary disease (COPD) in terms of medical and social care. Patients with COPD had significantly worse activities of daily living (ADL), depression and anxiety status, social functioning than the lung cancer patients and these findings were due to the fact that patients other than oncologic do not receive a holistic care that would include specialized palliative care.

CONCLUSIONS

Patients with lung cancer have a poor prognosis due to their oncologic condition and often to co morbidities. The appropriate selection of therapeutic regimen and inclusion of pulmonary rehabilitation in the management of these patients could lead to improving symptoms, increasing quality of life, less post-operative morbidity or, at least, fit with palliative care, thus helping lung cancer patients to cope with their ruthless disease.

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NEWS

OUTBREAK OF EBOLA IN WEST AFRICA

Ebola virus disease is associated with a case fatality rate of 30 to 90%, depending on the virus species. Specific conditions in hospitals and communities in Africa facilitate the spread of the disease from human to human. Three Ebola virus species have caused large outbreaks in sub-Saharan Africa: *ebolavirus*, *Sudan ebolavirus*, and the recently described *Bundibugyo ebolavirus*. Epidemics have occurred in the Democratic Republic of Congo, Sudan, Gabon, Republic of Congo, and Uganda. *Reston ebolavirus* circulates in the Philippines. It has caused disease in non-human primates but not in humans. The fifth species, *Tai Forest ebolavirus*, was documented in a single human infection caused by contact with an infected chimpanzee from the Tai Forest in Ivory Coast. Although this event indicated the presence of *Tai Forest ebolavirus* in West Africa, this subregion was not considered to be an area in which Ebola virus was endemic. New evidence implicates bats as the reservoir hosts for Ebola viruses, though the means of local enzootic maintenance and transmission of the virus within bat population remain unknown. Epizootics caused by Ebola viruses appear sporadically, producing high mortality among non-human primates and duikers and may precede human outbreaks. Epidemics produce acute disease among humans. As of June 10, 2014, the *Guinea* Ministry of Health announced a total of 376 suspect and confirmed cases of Ebola hemorrhagic fever (EHF), including 241 fatal cases, from which 233 cases across Guinea have been confirmed by laboratory testing to be positive for Ebola virus infection. The Ministry of Health and Sanitation of *Sierra Leone* reported 43 laboratory confirmed cases of EHF. An additional 117 suspect cases and 19 fatal cases were also reported in Sierra Leone on June 9. The Ministry of Health and Social Welfare of *Liberia* reported 1 new laboratory confirmed case and one death on June 7, 2014. Genetic analysis of the virus indicates that it is closely related (97% identical) to variants of Ebola virus (species *Zaire ebolavirus*) identified earlier in the Democratic Republic of the Congo and Gabon. (*CDC Report*, June 2014 & Baize et al. Emergence of Zaire Ebola Virus Disease in Guinea - Preliminary Report. *NEJM*, April, 2014)

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MULTIPLE SCLEROSIS AND PREGNANCY

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MULTIPLE SCLEROSIS AND PREGNANCY (Abstract): Multiple sclerosis is one of the main reasons for invalidation of young adults of both sexes. The disease is more common in women than in men. The illness begins most frequently in patients between the ages of 20 and 40 years, which is also the most fertile period for women. MS is an immune-mediated disease with chronic evolution marked by exacerbations and remissions that amplify the degree of disability. The most common clinical picture is the one with relapse and remission whose evolution is greatly improved after immunomodulatory treatment. We have revised the literature together with the data from the national multiple sclerosis society and the cases that are in the National Programme of Multiple Sclerosis, mainly the ones that are assigned to the regional center of Iaşi, at the Neurology Clinic inside the Clinical Rehabilitation Hospital Iaşi. Pregnancy is quite frequent in female patients with MS. Certain risks are present during pregnancy, birth and breastfeeding and certain protocols must be applied, such as interrupting the immunomodulatory treatment before the conception. Child delivery must be closely monitored and it must take into consideration the dysfunction that the patient has and be adapted to the existing deficits. There are some methods that may be used during delivery for female patients with multiple sclerosis in order to make this process smooth and reduce the risk of postpartum complications. Multiple sclerosis is an invalidating disease, with a high prevalence in women. Pregnancy in patients with MS is not such a natural phenomenon as in a healthy female and it requires a multidisciplinary team in order to ensure the safety of both the mother and the newborn. **Keywords:** MULTIPLE SCLEROSIS, PREGNANCY, BREASTFEEDING, BIRTH.

Multiple sclerosis is one of the main reasons for invalidation of young adults of both sexes. The disease is more common in women reports of 2:1 or even 3:1 being cited. The illness begins most frequently in patients between the ages of 20 and 40 years but can affect also children, adolescents and adults over 50 years (1). The prevalence is 30-150 cases per 100,000 inhabitants and depends on geographic location among others. Multiple sclerosis (MS) is a disease with increasing prevalence in temperate regions and also as we move away north or south of the 45th parallel.

MS is an immune-mediated disease with chronic evolution marked by exacerbations and remissions that amplify the degree of disability. The most common clinical picture is the one with relapse and remission whose evolution is greatly improved after immunomodulatory treatment (2). This form can go into secondary progressive form which requires changing the type of interferon therapy. There are primary progressive forms

which often do not respond to current immunomodulatory treatments. In the last years it was identified the so-called clinically and radiological isolated syndrome that can be treated effectively with some of the known immuno-modulatory therapies (3).

More than half of the female patients with MS are in their fertile period of life which means that while taking the treatment they resort to contraception methods. The efficacy of the disease -modifying therapy has made more and more female patients to want their first child in terms of a significant decrease in the annual rate of relapses and "relapse free" period. At several of the female patients with MS clinical and imaging stabilization was found and approaching or exceeding the age of 30 years has generated a desire for pregnancy which should develop in optimal conditions without adverse effects on the mother or newborn. There are risks related to the possibility of transmitting the disease, worsening health of the mother, the difficulties at birth, during breastfeeding and resumption of immunomodulatory therapy (4).

There were identified multiple genes that may negatively influence the risk of developing the disease in combination with certain environmental factors. Loci have been identified with the help of the major histocompatibility complex (MHC) and seem the most significant encoding IL2R and IL7R. If the mother or father has MS the risk for the children to develop the disease is 3-5 % compared with the general population where the probability is only 0.2 %. If both parents have MS risk increases to 29.5% (5). Among the environmental factors involved temperate climate, exposure to sunlight, disease prevalence in the geographical area of birth etc are involved. In the northern hemisphere the risk of disease is higher in offspring's born in spring compared to those born in winter. The same phenomenon takes place in the southern hemisphere and it is related to sunlight exposure of pregnant patient. Reduction of exposure to sunlight may cause a vitamin D deficiency which would not exert immunomodulatory effect under the influence of the MHC (6).

The health of the mother may suffer some aggravation especially after birth, but therapeutic strategies to prevent and counteract undesirable events exist. We can expect the apparent worsening of preexisting dysfunction or the appearance of new events.

Underlying dysfunction may be of motor type related to sphincters, digestive, sensory, fatigue and seem to be due to the state of pregnancy associated with weight gain. Excess weight can decrease motility already affected by demyelinization more or less extended to some fibers of the cortico -spinal tract. Existing spasticity and ataxic elements may contribute to gait problems.

The presence of demyelinating plaques localized in the spine and the impact of the pregnant uterus on thoraco-abdominal-pelvic cavity can generate or amplify urgency, constipation, mild ventilatory dysfunction. Some sensitive changes related to failure notification to the urinary passage through the urethra, the presence of paresthesia, constrictive or existing areas of hypoesthesia can be easily determined.

Depending on the effort and the temperature of the external environment, the increase in intensity and prolonged daily presence of the fatigue phenomenon can be present. Fatigue has among psychological components also motor ones that exacerbate when the body temperature or of the environment increase or by immersion in hot water. Fatigue can easily change bio-

rhythm prolonging the patients' rest and sleep (7).

The main event announcing the unfavorable evolution both during pregnancy and beyond is the relapse. Although the effect of pregnancy on the evolution of MS is of protective type exacerbations can occur both during pregnancy and after giving birth. PRIMS study says the annual rate of relapse during pregnancy decreases from 0.6 relapses / year before conception to 0.2 relapses /year in the last weeks of gestation. The danger of relapse seems to outline and amplify after birth when the annual rate reaches 1.2 relapses / year interfering with breastfeeding (8).

The occurrence of a relapse during pregnancy requires differentiated treatment depending on the stage of pregnancy. In the first trimester of pregnancy methylprednisolone may not be administered because there were identified cases of children born with malformations of the palate and cataract. Depending on the extent of dysfunction there can be taken into account relapse therapy with methylprednisolone 500-1000 mg/day for 3-5 days in the second and third quarters of the pregnancy. It is known that methylprednisolone can pass through the placenta and can induce preeclampsia, gestational diabetes, venous thrombosis, low birth weight of the child, postpartum adrenal insufficiency. Immunoglobulin (0.2-0.4 g/kg/day, 5 days) seems to be more secure and hassle-free. It is considered as belonging to the same relapse the dysfunction that occur which extend to 30 days after onset. Plasma exchange can be practiced, similarities in the evolution of pregnant patients who have myasthenia (9).

In the first quarter after birth the risk of a new relapse increases, in which case we have to choose between treatment of relapse or discontinuation of breastfeeding. There are therapeutic strategies using bolus methylprednisolone 3-5 days and evacuating the milk secreted after each injection.

The most important moment is the birth itself. The obstetrician and anesthetist must choose the safest solution for the mother and fetus taking into consideration the already existing neurological dysfunctions.

MS does not have any contraindications to vaginal birth or for cesarean. The decision for natural childbirth should take into account maternal age, existing neurological dysfunction at the time, the phenomenon of "fatigue", the fact that the mother is at her first pregnancy or not. The presence of a pyramidal syndrome with paraparesis requires special adaptation with a position in lateral decubitus with the lower limbs supported by a support. Labor and birth in water may be a solution with protective effect by the supporting force and push from the bottom up of the liquid and low body temperature of the pregnant patient in order to prevent fatigue.

Some studies of female patients with MS who gave birth vaginally say that forceps usage was more frequent in comparison with the healthy population. This maneuver would replace the motor and sensitive deficits of the mother with origin in the brain or spinal cord.

Caesarean operation is most commonly practiced type of birth in patients with MS being a deliberate choice that is practiced before the onset of labor in order to avoid possible complications (10).

There are some decisions to make about anesthesia. Most opinions converge towards the use of drugs such as Entonax, Pethidine or TENS machine. There is a preference for epidural anesthesia in comparison with the spinal one. The presemce of the anesthetic in high concentrations in CSF is associated with increased annual

rate of postpartum relapse being suspected a potentiation of low spinal demyelination (11). There were described several cases in which after the anesthesia it was found the so-called autonomous dysreflexia which consists of hypertension spikes, headache, cold sweats and anxiety (12).

Breastfeeding should be encouraged because there is no risk of disease transmission through breast milk. If the mother should receive a drug, it will reach the baby. Planning pregnancy is one of the important moments in the life of female patients with MS. There is some risk of disease worsening if the immuno-modulatory treatment is stopped for at least 15 months. In the first three months the birth control and immunomodulatory drugs should be stopped. For patients treated with glatiramer acetat only two months of interruption should be enough. At least three months after stopping natalizumab should the conception process begin. Fingolimod requires only two months distance to conceive from the moment of interuption. Special measures should be taken for patients who were treated with combined drugs or only with mitoxantrone. The aggressiveness of this product and its teratogenic impact requires initial contraceptive therapy 6 months after stopping administration (13).

Fertility in female patients with MS is under the influence of contraceptive medication and the modifying disease treatment. Interrupting them is the first step towards planning pregnancy. There are no reliable data on negative influence of immunomodulatory medication on fertility, the increased risk of miscarriage in patients undergoing treatment with interferon beta being cited. Animal studies have shown reduced fertility after treatment with Natalizumab, a phenomenon that is not met by the use of fingolimod. Mitoxantrone has negative effects on fertility which means stopping it long before conception. Reduced effect of contraceptives may be induced by concomitant administration of drugs such as Modafinil, Phenitoin and Carbamazepine. Mitoxantrone or cyclo-phosphamide medications may exert negative influences on spermatozoons and oocytes.

Spontaneous fertility appears to be influenced in MS patients in which the effect of the medication is combined with some couple problems. Sexual dysfunction is a consequence of erection problems, motor deficit, sensory deficit, sphincter deficit or psychological issues. Decreased libido is a phenomenon that applies to both sexes and reduced testosterone in male patients will affect fertility. To counter some of the aspects of the disease and the treatment some methods such as in vitro fertilization may be used. The risk is to increase the annual rate of relapses. Cryostorage or egg cryopreservation followed by selfgrafting may be another solution (14).

Fertility in men appears not to be negatively affected; the recommendations are between continuing the treatment and discontinuation for some time before conception.

Our experience covers a total of 450 patients of whom more than half are women. Most of the female population is at fertility age (18-40 years).

A significant number of patients had one or more children before being diagnosed with MS and treated. After starting treatment more patients expressed a desire to have children. Our recommendations supported starting immunomodulatory treatment and follow-up to stabilize the evolution of the disease. This stabilization occurred after a minimum of 3 years and maximum of 5 years. At this time some patients have given up the desire to have

off springs because of the disease. Until now they were born 28 healthy children of whom a pair of twins. Our patients had no relapse during pregnancy or breastfeeding. They restarted immunomodulatory treatment within four months after birth.

The EDSS (disability score) of the female patients who gave birth was 1.5. The largest EDSS was 4.5 in a person aged 33 years old with 9 years of disease with initial treatment with glatiramer acetate and after birth being switched to interferon beta. At this moment she continues with interferon beta. We have treated a person who has made a second child under treatment with interferon beta. The average age of those who gave birth was 30 years.

CONCLUSION

Multiple sclerosis is an invalidating disease, with a high prevalence in women. Pregnancy in patients with MS is not such a natural phenomenon as in a healthy female and it requires a multidisciplinary team in order to ensure the safety of both the mother and the newborn. More studies are needed in order to have a specific protocol in the future.

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THE IMPACT OF RESPIRATORY TRACT INFECTIONS ON THE NUTRITIONAL STATE OF CHILDREN WITH CYSTIC FIBROSIS

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THE IMPACT OF RESPIRATORY TRACT INFECTIONS ON THE NUTRITIONAL STATE OF CHILDREN WITH CYSTIC FIBROSIS (Abstract): Cystic fibrosis (CF) is a life-shortening, autosomal-recessive disorder characterized by intestinal malabsorption, impaired growth and lung disease. Recurrent pulmonary infections in children with CF are often associated with nutritional deficiencies. **Aim:** To emphasize the effects of recurrent pulmonary infections on nutritional status in children with CF. **Material and methods**: This retrospective study included 27 patients diagnosed with CF between 1994 and 2011 in the 3rd Pediatric Clinic of the Iasi "Saint Mary" Children's Hospital. The nutritional status was assessed according to ponderal index (PI), body mass index (BMI), Z score for weight and waist. Correlations between the age of onset of symptoms, age at diagnosis, and frequency of infectious episodes, identified bacterial agents and nutritional status were established. **Results:** Patients aged between 3 months old and 17 years old with an average of 49.48 months ± 9.83DS; sex ratio was 1.7:1. The patients were diagnosed late, one month to 112 months (average 41.11 months±9.4DS) from the first symptoms until the moment of diagnosis. The clinical forms of CF in the study group were: predominantly respiratory manifestations in 48.14% of cases, and the mixed type, with both respiratory and digestive symptoms, in 18.52% of cases. Delayed weight and/or height gains were identified in 85.19% of cases. The etiologic agents involved in pulmonary infections were *Staphylococus aureus* (48.14%), *Pseudomonas aeruginosa* (33.33%), *Stenotrophomonas maltophilia* (18.51%), *Haemophilus influenzae* (14.8%), *Klebsiella pneumoniae* (11.10%), *Moraxella catarrhalis* (7,40%), *Streptococcus pneumoniae* (7.40%), *Neisseria sica* (7.40%). Pulmonary infections caused by *Staphylococus aureus, Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* were more often associated with nutritional status abnormalities. **Conclusions:** In small children with CF pulmonary infections due to various causative agents cause a slow rate of growth (both weight and height). Good nutrition and adequate early treatment of pulmonary infections are beneficial for the general state of affected children and are very important in maintaining their health. **Keywords:** CYSTIC FIBROSIS, CHILDREN, MALNUTRITION, PULMONARY INFECTION.

Cystic fibrosis (CF) is a life-shortening, autosomal-recessive disorder that is characterized by intestinal malabsorption, impaired growth, and lung disease (1). Opti-

mal nutritional status is vital in children with CF to enhance their quality of life and prognosis (2, 3). Despite the hyper caloric diet, some children do not achieve the length and weight growth standards. Pulmonary recurrent infections in CF children are often associated with nutritional deficiencies.

MATERIAL AND METHODS

We conducted a retrospective study based on medical observation sheets that included 27 patients diagnosed with CF between 1994 and 2011 in the 3rd Pediatric Clinic of the Iasi "Saint Mary" Children's Hospital. Nutritional status was assessed according to ponderal index (PI), body mass index (BMI), Z score for weight and waist. In patients with pulmonary disease the micro bacteriological exam of hypopharyngeal aspiration was performed. In these patients, we established correlations between the ages of onset of symptoms, age at diagnosis, frequency of infectious episodes, identified bacterial agents, and nutritional status. Statistical data analysis was performed using *SPSS 19.0*. The error of statistical estimations was of 5%, corresponding to a 95% confidence interval.

RESULTS

Patients aged between 3 months old and 17 years old with an average of 49.48 months ±9.83 SD (mean age 4.1 years). Sex distribution showed that 17 patients were male (62.96%) and 10 females (37.04%), sex ratio: 1.7: 1.

Although the onset of disease was recorded in the first year of life in 77.77% of cases, the CF diagnosis was made in 70.37% of cases after the first year of life. So, without newborn screening for identification of CF, the patients were diagnosed late, after an average 41.11 months±9.4 SD (ranges one month to 112 months) from the time of the first symptoms until the moment of diagnosis.

The average age at the onset of the disease was 7.23 months±4.32 SD, ranging between 1 day and 13 months. The disease had an early onset, during the first 3 months of life, in 22.22% of cases.

The clinical forms of CF in the study group were: predominantly respiratory manifestations in 48.14% of cases, predominantly digestive symptoms in 33.33% of cases and the mixed type, with both respiratory and digestive symptoms, in 18.52% of cases.

The respiratory disease started in 25.9% of cases with bronchopneumonia and in 18.5% of cases with recurrent wheezing and chronic cough (fig. 1).

In patients with the mixed type of disease, we found that severe exocrine pancreatic failure associated with recurrent respiratory infections lead to severe forms of CF. Analyzing the relationship between the age of onset and the severity of pancreatic and respiratory failure, we found that an early onset is extremely often associated with severe forms of disease (r=0.696, p=0.418, 95% CI) (fig. 2).

Of the 27 patients, only 14.81% (4 patients) had normal weight. Delayed weight and/or height gains were found in 85.19% (23 patients) of cases, with various stages of low body weight according to Z score: 37.04% of cases (10 patients) – stage I dystrophy, 18.52% (5 patients) – stage II dystrophy, 7.41% (2 patients) - stage III dystrophy (fig. 3).

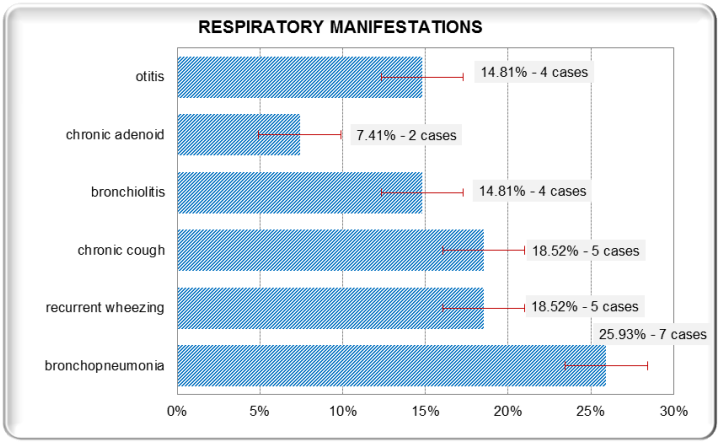

Fig. 1. Respiratory manifestation in children with CF

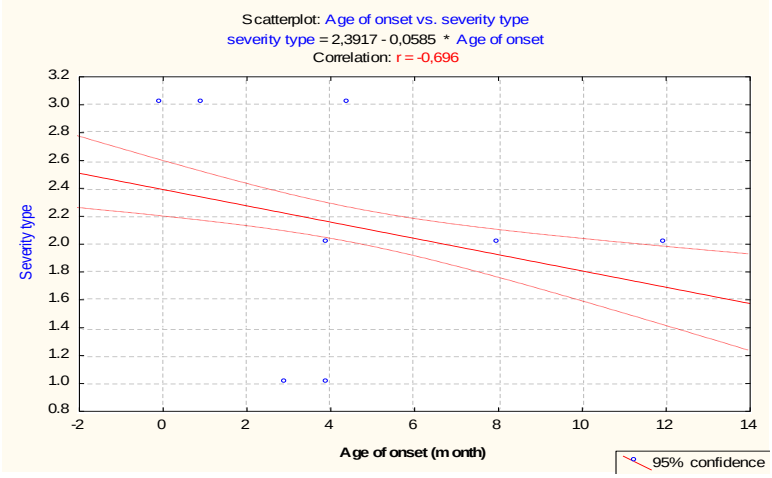

Fig. 2. Correlation between the age at onset and disease severity

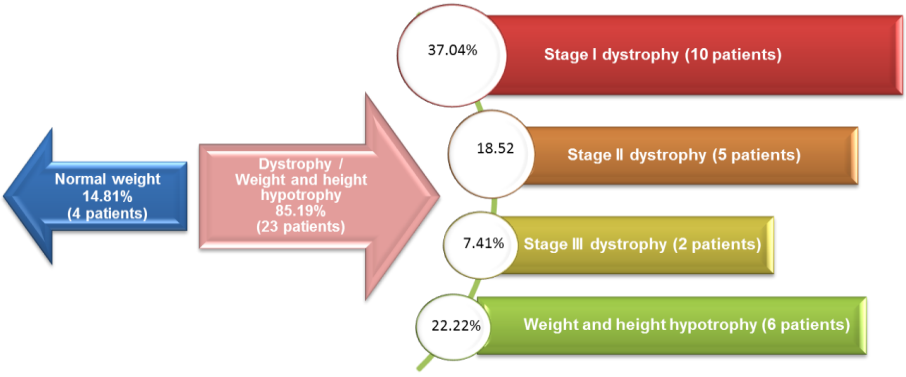

Fig. 3. Nutritional status in patients with CF

In the study series, we noticed that an early onset and recurrent pulmonary infections are highly related to an abnormal nutritional state, as demonstrated by multivariate analysis. Partial correlations from multivariate analysis between the age of onset, presence of recurrent infections (r=–0.434, p=0.0045), and nutritional state abnormalities (r=–0.517, p<<0.05) were statistically significant.

TABLE I
Multiple correlation of disease onset vs. impaired nutritional status and recurrent infections

Multiple correlation	**Estimated value**
Coefficient of multiple correlation	0.88694
Multiple R^2	0.78667
F	44.24963
p (95%CI)	0.00000
Std. Err. of Estimate	2.07712

Partial correlation disease onset *vs.*	**Correlation Coefficient (Beta)**	**Std.Err. (Beta)**	**B**	**Std.Err. B**	**t**	**p-*95% confidence interval***
Intercept			10.6428	0.55513	19.171	0.000000
Recurrent infections	-0.434356	0.13877	-3.9066	1.24819	-3.129	0.004549
Impaired nutritional status	-0.517684	0.13877	-4.3928	1.17761	-3.730	0.001039

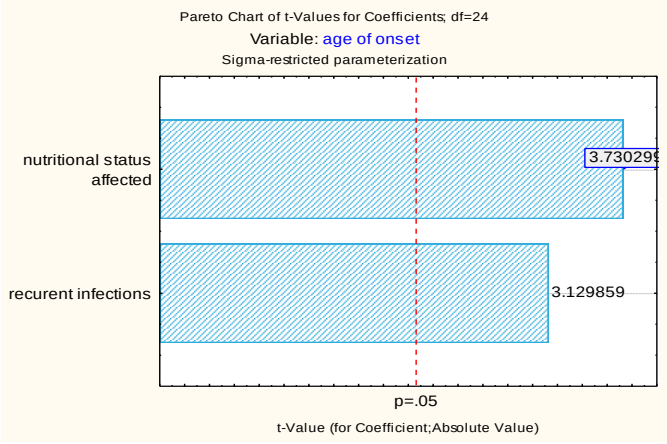

Fig. 4. Absolute value of statistics "t" in the evaluation of multivariate analysis (onset *vs.* impaired nutritional status, recurrent infections)

For the multivariate analysis of the implications of onset time in recurrent infections (nutritional state abnormalities) we applied logistic regression. Table I shows the predictive factors for recurrent infections incidence and nutritional state abnormalities.

The results prove that early onset determines both the occurrence of recurrent infections (b=-0.434-partial correlation indices) and nutritional state abnormalities (b=-0.517) (tab. II).

The causative agents of pulmonary infec-

tions were *Staphylococus aureus* (*S. aureus*) (48.14%), *Pseudomonas aeruginosa* (*Ps. aeruginosa*) (33.33%), *Stenotrophomonas maltophilia* (18.51%), *Haemophilus influenzae* (14.8%), *Klebsiella pneumoniae* (11.10%), *Moraxella catarrhalis* (7.40%), *Streptococcus pneumoniae* (7.40%), *Neisseria sica* (7.40%) (fig. 5).

TABLE II

Estimated parameters in the assessment report age of onset chance on the basis of risk factors under study

	onset – Param.	onset – Std.Err	-95% - Cnf.Lmt	+95% - Cnf.Lmt	onset – Beta (ß)	onset – St.Err.ß	-95% - Cnf.Lmt	+9% - Cnf.Lmt
Intercept	10.6428	0.55513	9.4971	11.7886				
Recurrent infections	**-3.90667**	1.24819	-6.4828	-1.3305	-0.43435	0.13877	-0.72078	-0.1479
Impaired nutritional Status	**-4.39286**	1.17761	-6.8233	-1.9623	-0.51768	0.13877	-0.80410	-0.2312

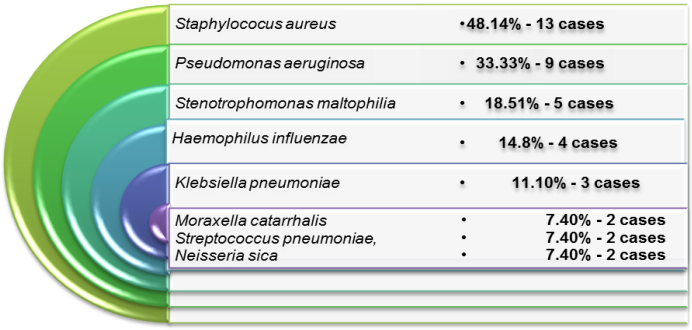

Fig. 5. Etiologic spectrum of respiratory infections

Pulmonary infections due to *S. aureus*, *Ps. aeruginosa* and *Stenotrophomonas maltophilia* were more often associated with nutritional status abnormalities.

DISCUSSION

In children, multiple diseases, CF included, are associated with weight and growth deficit. In the absence of neonatal screening, in 88.88% of cases the disease was diagnosed late, the average age at the time of diagnosis being 3.4 years old compared with a mean age of onset of symptoms of 7 months old. The clinical forms with pulmonary manifestations were the most common in children with CF, the clinical form with predominantly respiratory symptoms being found in 48.14% of cases, and the mixed forms, respiratory and digestive, in 18.52% of cases. In all 5 patients with respiratory and digestive forms, the association between exocrine pancreatic failure and recurrent respiratory infections caused severe disease progression.

Impaired growth (weight and/or height) was found in 23 patients (85.19% of cases). In this study group, the onset at a young age and repeated respiratory infections were correlated significantly (r = 0.94, CI = 95%) with impaired nutritional status. Staturo-ponderal hypotrophy (SPH) was identified in 6 patients with chronic lung infections: three patients with methicillin-resistant *S. aureus* (MRSA), one patient with *Ps. aeruginosa* and two patients MRSA and *Ps.aeruginosa* co-infection. Of the six pa-

tients with SPH, two children were diagnosed late, at the age of ten and eight, respectively. So, the earlier onset and the late diagnosis were associated with many respiratory infections and poor nutritional status. The main pathogen of CF lung infections is *S. aureus* (3, 4, 5, 6). The study by Beringer et al. found that about 50% of children with CF until the age of 10 had chronic pulmonary infections with *S. aureus* (7). Miall et al. compared 10 children with CF and MRSA versus 18 patients with CF negative for MRSA; they found deteriorations in height, weight, and BMI in the MRSA positive group, but only the change in height was statistically significant ($p = 0.039$) (8).

In patients with CF, *S. aureus* itself is not a common cause of morbidity and mortality, but because of the progressive destruction of lung tissue and worsening pulmonary obstruction, it favors co-infection with *Ps. aeruginosa* (8, 9, 10, 11). In our study, pulmonary infections with *S. aureus*, *Ps.aeruginosa* and *Stenotrophomonas maltophilia* were more often associated with poor nutritional status.

Despite adequate pancreatic enzyme replacement therapy and nutritional supplements, the weight and height gains were not satisfactory due to loss of appetite and frequency of catabolic stress during pulmonary exacerbations, requiring repeated hospitalizations. Recurrent respiratory infections, inappropriate nutritional intake and low compliance to substitution treatment caused altered nutritional status. The increased incidence of respiratory infections at a small age is associated with an abnormal nutritional status in CF children. On the other hand, nutritional status has a significant effect on pulmonary disease progression and survival in patients with CF (12, 13).

CONCLUSIONS

All patients presented delayed growth (both weight and height), CF onset at a young age and repeated lung infections. All patients with pulmonary infections caused by *S. aureus* (48.14% of cases) and *Ps. aeruginosa* (33.33% of cases) had a poor nutritional status. Newborn screening is essential for diagnosing CF and thus for preventing airway colonization by aggressive germs and so is the early initiation of an adequate nutritional management.

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NEWS

FACTORS RESPONSIBLE FOR RELATIVE POSITION OF INTERPROXIMAL PAPILLA IN HEALTHY SUBJECTS

A group of Korean researchers conducted a study that examined the factors that can be associated with the appearance of the interproximal papilla.Were examined one hundred and forty-seven healthy interproximal papillae between the maxillary central incisors (male, 74; female, 73; average age, 25.36±7.58 years). A digital photograph and periapical radiograph of the interdental embrasure were taken for each subject using a 1-mm grid metal piece. The recorded parameters were: the amount of recession of the interproximal papilla, contact point-bone crest distance, contact point-cemento-enamel junction distance, cemento-enamel junction-bone crest distance, inter-radicular distance, tooth shape, embrasure space size, interproximal contact area, gingival biotype, papilla height, and papilla tip form. The amount of recession of the interproximal papilla was associated with: 1) increase in contact point-bone crest, contact point- cemento-enamel junction, and cemento-enamel junction -bone crest distance; 2) increase in the inter-radicular distance; 3) triangular tooth shape; 4) decrease in the interproximal contact area length; 5) increase in the embrasure space size; 6) flat papilla tip form. The amount of gingival recession was not associated with the gingival biotype or papilla height. In the triangular tooth shape, the contact point-bone crest distance and inter-radicular distance were longer, the interproximal contact area length was shorter, and the embrasure space size was larger. The papilla tip form became flatter with increasing inter-radicular distance and cemento-enamel junction-bone crest distance. The relative position of the interproximal papilla in healthy subjects was associated with the multiple factors and each factor was related to the others. A triangular tooth shape carries a higher risk of recession of the interproximal papilla because the proximal contact point is positioned more incisally and the bone crest is positioned more apically. This results in an increase in recession of the interproximal papilla and flat papilla tip form. (Kim JH, Cho YJ, Lee JY, Kim SJ, Choi JI. An analysis on the factors responsible for relative position of interproximal papilla in healthy subjects. *J Periodontal Implant Sci*, 2013; 43 (4): 160-167)

Irina Grădinaru

CORRELATIONS BETWEEN STATE ANXIETY AND QUALITY OF LIFE IN METASTATIC BREAST CANCER PATIENTS

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CORRELATIONS BETWEEN STATE ANXIETY AND QUALITY OF LIFE IN METASTATIC BREAST CANCER PATIENTS (Abstract) **Aim:** to evaluate the correlations between perceived state anxiety during chemotherapy and quality of life in metastatic breast cancer patients. **Material and methods**: 62 metastatic breast cancer patients were evaluated during chemotherapy concerning age, living environment, marital status, social support and preexisting financial difficulties, the histological type of cancer, the site of the metastasis, the time from diagnosis, the type of surgical intervention, dexamethasone use, somatic comorbidities and the radiotherapy. The STAI-X1, BDI- IIA and the QLQ 30 (Quality of Life Questionnaire 30) plus BR 23 (Breast 23) questionnaires were applied. For the statistical analysis we used the *SPSS 13* package. **Results:** 24 subjects were experiencing low state anxiety (≤39), whilst 38 had significant state anxiety (>39). Statistically significant differences were encountered between the two compared subgroups concerning the living environment, the type of surgical intervention, the marital status, the social support and the mean BDI scores, adjusted means were calculated for the items considered to potentially influence quality of life. Social, emotional and role functioning had lower scores in the low state anxiety group. Fatigue, future perspective, chemotherapy induced side effects, breast symptoms, upset by hair loss and sexual functioning were more disturbing in the high state anxiety group. The general health/quality of life mean score was lower in the low state anxiety group. **Conclusions**: Higher state anxiety correlates with certain quality of life items, suggesting that psychological counselling and appropriate therapy induced side effects management should be a priority in the palliative care for metastatic breast cancer patients.
Keywords*:* CANCER, BREAST, ANXIETY, QUALITY OF LIFE, PAIN.

Breast cancer is the most frequently encountered form of malignancy in women and it represents around 28% of the reported cancers in Europe (1). The National Cancer Institute estimates that 12.7% of women born today will be diagnosed with breast cancer during their lifetime (2). In Romania over 6000 new cases, usually in an advanced stage, are diagnosed every year, with an incidence of 56.84/100000 and a reported mortality of 39.28/100000. A recent study of the International Agency for Cancer, published in 2010 in the British Medical Journal, describes an increase with 17% of the mortality generated by breast cancer in Romania in the last few years, most probable due to the dysfunctional strategies of early detection and treatment

(3). Cancer signifies a serious threat on someone's existence and there are studies that evaluate over 77% of the subjects having this diagnosis as experiencing anxiety symptoms generated by the disorder itself or by its therapy (4). In 2005, Burgess et al. described a decreasing annual prevalence of depression and anxiety of 50% in the first year, 25% in the second, third and fourth year and 15 % in the fifth year after being diagnosed with breast cancer (5).

Anxiety in cancer is a sensitive issue, generating relational problems with both medical staff and caregivers. Its prevalence at a psychopathological level is reported to be 10-30% (6). Moreover, its relation with quality of life is complex and bivariate. Although anxiety disorders are correlated with deficits in the quality of life, there are studies that postulate that their impact is not a significant one (7). Nevertheless, state anxiety was considered to be a risk factor for chemotherapy induced nausea and vomiting (8). In 2004, Schreier et al. found a negative correlation between state and trait anxiety scores and quality of life in a sample of 48 women receiving chemotherapy for breast cancer (9). It is of great interest to determine the causing factors and the specificity of anxiety in oncology, and its correlation with quality of life items, in order to have a better management of complex situations, leading to best palliative care.

MATERIAL AND METHODS

We conducted a clinical study at the Oncological Institute "Ioan Chiricuță" Cluj-Napoca, starting from January 2011 until November 2012, on a group of 62 women, aged over 18, suffering from metastatic breast cancer, evaluated in the day care hospital. All of the subjects were undergoing chemotherapy at the moment of the evaluation. The exclusion criteria were brain metastasis, psychiatric history, psychotropic medication administration (current or within the last 6 months), alcohol or illicit substances abuse/ addiction, confusion, unable or unwilling to sign the written consent, patients taking psychoactive medications, others than chemotherapy and premedication for chemotherapy. The STAI-X1, BDI-IIA and the EORTC QLQ 30 (Quality of Life Questionnaire 30) plus BR 23 (Breast 23) questionnaires were applied. The evaluated items were the age, the living environment, the marital status, the social support and pre-existing financial difficulties, the histological type of cancer, the site of the metastasis, the time from the announcement of the diagnosis, the type of surgical intervention, the use of dexamethasone as premedication, the somatic co morbidities and the radiotherapy. A cut off value of 39 was established for STAI-X1 scores, splitting the group into clinically significant state anxiety (>39) and low state anxiety (≤39). The two resulting groups were compared regarding the quality of life items. For the statistical analysis we used the *SPSS 13* package, including the T Student test. The distribution of the variables was tested with Skewnes and Kurtosis, and the general data were analyzed with the Chi Square test.

The study was approved by the medical ethics commission of the University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj-Napoca.

RESULTS

The age mean in the group was 54.37 years old, with a minimum of 30 and a maximum of 81. The majority lived in the urban area (79%), were married (77.4%) and bene-

fited from a strong social support (91.9%). Approximately half of the group reported important financial difficulties (51,6%). According to the TNM staging system, all patients were in the IVth stage evolution of the disease. Most of the subject had bone metastasis (51.61%). From the histological perspective, as expected, the majority (85.5%) were infiltrating ductal carcinomas, followed by the infiltrating lobular carcinomas (7.14%), the intraductal (3.2%) and the undifferentiated types (3.2%) and in a smaller percent we discovered lobular pleomorphic (1.6%) and medullar carcinomas (1.6%). A number of 34 patients had a history of unilateral breast mastectomy, 19 of them breast-conserving surgery, whilst 9 were considered to be inoperable. 48.4% underwent radiotherapy (tab. I).

Most of the participants were not suffering from other somatic illnesses (40 cases), part of them having however co morbidities (hypertension, diabetes mellitus, gastritis, goitre, anaemia, leyomioma, spondylitis, deep vein thrombosis).

According to the STAI X1 scores, 24 subjects (38.70%) were experiencing low state anxiety (≤39), whilst 38 of them (61.29%) had significant state anxiety (>39) (tab. II).

TABLE I

Previous therapies

	Yes(number)	No (number)	Yes (percent)	No (percent)
radiotherapy	30	32	48.4%	51.6%

TABLE II

BDI IIA scores

	STAI X1	N	Mean	Standard deviation
BDI	<= 39	24	11.0417	7.77666
BDI	>39	38	19.2895	8.47224

Statistically significant differences were encountered between the two compared subgroups (the low state anxiety and the high state anxiety one) concerning the living environment ($p<0.0001$), the type of surgical intervention ($p=0.010$), the marital status ($p=0.0001$), the social support ($p<0.0001$) and mean BDI scores.

Previously published research report showed that living environment (10), marital status (11) and depressive symptoms (12) have an influence on the quality of life. Moreover, the type of surgical treatment (breast-conserving versus mastectomy) (13), and the subject's perceived social support (14) also were proved to impact on the quality of life. In consequence, we used the general linear model in order to adjust the means of quality of life questionnaire for those items. After these corrections significant variations were observed for certain quality of life items between the two subgroups (tab. III).

The functional scales mean scores were significantly higher in subjects with low state anxiety.

Concerning the QLQ 30 symptom scales, the only item having a higher ad-

justed mean score in the higher state anxiety group was fatigue. The adjusted mean score for diarrhoea did not register a normal distribution.

Evaluating specific breast cancer items by QLQ 30 BR 23, significant variations were documented for future perspective; chemotherapy induced side effects, breast symptoms, upset by hair loss and sexual functioning, with higher mean scores for subjects with state anxiety over the cut off score of 39 points in STAI X1. The adjusted mean score for sexual functioning did not register a normal distribution (tab. IV).

TABLE III
Functional scales

	STAI X1	N	MEAN	Standard deviation	p
Prediction value for role functioning	≤ 39	24	48.3890	19.43992	0.0290
	≥40	38	35.6617	23.08044	
Prediction value for emotional functioning	≤ 39	24	74.9347	69.5731	0.0160
	≥40	38	56.4796	56.9373	
Prediction value for social functioning	≤ 39	24	69.4036	21.47722	0.0360
	≥40	38	59.0151	21.47474	

TABLE IV
Specific subscales

	STAI X1	N	Mean	p
Prediction value for future perspective	≤ 39	24	46.5890	0.013
	≥40	38	28.0632	
Prediction value for side effects	≤39	23	32.2360	0.016
	≥40	38	42.1011	
Prediction value for breast symptoms	≤39	24	16.2071	0.016
	≥40	38	25.5781	
Prediction value for upset by hair loss	≤39	16	32.9202	0.015
	≥40	26	54.0972	
Prediction value for sexual functioning	≤39		11.2968	0.004
	≥40		4.3518	

QLQ/ General health mean score was higher in the low state anxiety subgroup (p=0.011).

DISCUSSION

The STAI Y inventory is one of the most frequently administered anxiety self-report scales, especially in somatic co morbidities (16). The decision to only use the STAI X1 questionnaire was driven by the aim of the study, specifically the evaluation of state anxiety experienced in the context of both cancer diagnosis and outpatient chemotherapy. The statistical analysis of our data resulted in interesting results concerning the quality of life subscales, evaluated by the EORTC QLQ 30 and BR 23 questionnaire (17).

Regarding the functional scales, those refer to the last week in subject's life. Pa-

tients with dysfunctional role and social status were encountered in the higher state anxiety group, which is suggestive for a lower frustration tolerance and certain dysfunctional copying mechanisms involved in their adjustment to the somatic disorder. The differences in the emotional functioning mean scores are not surprising, considering the fact that they go along with STAI X1 evaluating the same issue.

Cancer treatment–related fatigue is categorized as chronic fatigue because it is present over a long period of time, interferes with functioning, and is not completely relieved by sleep and rest (15) and it is an important symptom to target in the treatment of cancer survivors (16). It is well recognized to generate depression and anxiety, and in our study it correlated with higher levels of state anxiety. Higher scores for chemotherapy side effects, breast symptoms and upset by hair loss were found in the high state anxiety group, suggesting that cancer therapies are perceived as aggressive and stressful.

The last months of sexual life was evaluated by BR 23 questionnaire. The results of our study suggest that dysfunctional sex life generated higher state anxiety during chemotherapy. Still, the relationship could be bivariate, as anxiety is known to be detrimental to sexual life (17). A pessimistic future perspective also generates a higher state anxiety during chemotherapy.

The limits of the study consist in the relatively small sample of evaluated subjects and in disregarding the trait anxiety of the subjects.

CONCLUSIONS

Higher state anxiety correlates with certain quality of life items, suggesting that psychological counselling and management of side effects induced by appropriate therapy should be a priority in the palliative care for metastatic breast cancer patients.

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No conflict of interests.

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NEWS

PARKIN PROTEIN - THE LINK BETWEEN TUBERCULOSIS AND PARKINSON'S DISEASE

Tuberculosis (TB) is a major public health problem worldwide through incidence and mortality. Parkinson's disease is a common neurodegenerative disorder that affects million people. The pathogenesis of TB is the result of interaction between foreign bacteria and the immune response of the host organism. In macrophages, mycobacteria are surrounded by a chain of ubiquitin protein that promotes bacterial destruction (xenophagy). The mitochondrial dysfunction has a causal role in Parkinson's disease pathogenesis. The malfunction mitochondria are disposed by autophagy. According to Cox and colleagues, the Parkin protein is a ubiquitin ligase that has an important role in ubiquitin-mediated xenophagy of mycobacteria and also in autophagy. They showed that Parkin gene polymorphisms that induce a Parkin protein deficit, increase susceptibility to bacterial infection with intracellular pathogens (*Mycobacterium tuberculosis*, *M. leprae* and *Salmonella enterica* serovar Typhi). Also, mutations in the Parkin gene increased susceptibility to Parkinson's disease. The malfunction of this protein is associated with a loss of nerve cells. The finding suggests that Parkin protein could be a target for new drugs from fight with tuberculosis infection. (Manzanillo PS, Ayres JS, Watson RO, Collins AC, Souza G, Rae CS et al. The ubiquitin ligase parkin mediates resistance to intracellular pathogens. *Nature*, 2013; DOI: 10.1038/nature12566).

Catalina Lunca

INFLUENCE OF METABOLIC SYNDROME PROFILE ON CARDIOVASCULAR RISK

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INFLUENCE OF METABOLIC SYNDROME PROFILE ON CARDIOVASCULAR RISK (Abstract): **Aim:** The trial was conducted on patients with metabolic syndrome hospitalized between 01.01.2012-31.12.2012 at Cardiovascular Rehabilitation Clinic of the Rehabilitation Hospital Iasi. **Material and methods:** Patients included in the study were analyzed according to age and sex, criteria for the clinical definition of the metabolic syndrome, known cardiovascular diseases, diabetes mellitus type 2 or type 1. **Results:** Metabolic syndrome components analysis showed statistical differences between the sexes, in case of certain cardiovascular risk factors. The frequency of cardiovascular risk factors (obesity, hypertriglyceridemia, hypertension, glycemic profile modification) was higher among women, the only risk factor most common in males was the decrease of HDL cholesterol. The statistically significant difference occurs in hypertriglyceridemia ($p = 0.001$) and HDL-Cholesterol ($p = 0.000$). Patients included in the study were studied depending on the frequency of the main cardiovascular diseases, secondary to the metabolic syndrome: ischemic heart disease, stroke, peripheral arterial disease of the lower extremities. **Conclusions:** Association of impaired glucose tolerance/diabetes mellitus, arterial hypertension and hypertriglyceridemia recorded an additional risk of developing CIC or ischemic stroke, independent of sex. **Keywords**: OBESITY, HYPERTRIGLYCERIDEMIA, HYPERTENSION, CARDIOVASCULAR RISK.

The metabolic syndrome (MS) is a more and more debated research objective, as its clinical and biological components: obesity, insulin resistance, impaired glucose tolerance or diabetes, hypertension and dyslipidemia, gives individuals an increased cardiovascular risk (1). Current studies have shown that metabolic syndrome increases the risk of adverse cardiovascular events (2) accelerates the age-related changes in blood pressure, both in the carotid arteries and in large arteries, similar in both sexes (4).

MATERIAL AND METHODS

The trial was conducted on patients with metabolic syndrome hospitalized between 01.01.2012-31.12.2012 at Cardiovascular Rehabilitation Clinic of the Rehabilitation Hospital Iasi.

The inclusion criterion was the presence of increased cardiovascular risk, defined according to the European Society of Car-

diology guidelines (5). Patients included in the study were analyzed according to:

- epidemiological data - age and sex;
- criteria for the clinical definition of metabolic syndrome (MS);
- known cardiovascular disease, diabetes mellitus type 2 or type 1.

Centralization of data was done in a nominal and numerical form. Statistical comparison of groups was performed according to the type of data, size of groups, the distribution of variables using Fisher's exact test, Shapiro-Wilk test, Mann-Whitney U test and ANOVA. Statistical analysis was performed with *SPSS Statistics 17.0* and *Microsoft Office Excel* 2007.

RESULTS AND DISCUSSION

1. Epidemiological data

We included 336 patients with a mean age of 62.7 years and age limits between 34 and 89 years. The analysis by sex targeted a total of 188 women (56%) and 148 men (44%) (fig. 1).

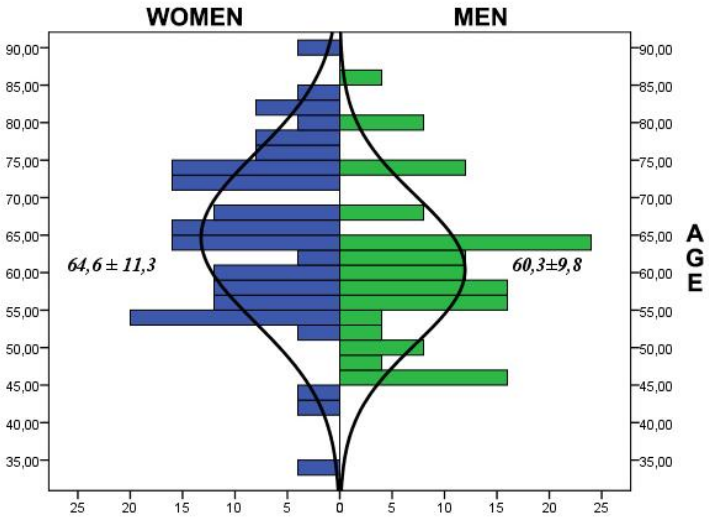

Fig. 1. Distribution of patients according to age, sex

2. Metabolic syndrome

Abdominal obesity

Abdominal obesity was present in 152 patients (45.2%), of whom 64 were men (42.1%) and 88 women (57.9%). By using Fisher's exact test (Exact Sig. (2-sided)) we obtained a p=0.294 (at a significance level of 0.05), concluding that there is no statistical difference between the sexes in terms of the presence of abdominal obesity.

Hypertriglyceridemia

Hypertriglyceridemia was present in 284 patients (84.5%), of which 120 were men (42.2%) and 164 women (37.8%). In the working group the median triglyceride value was 129.5 mg / dl higher among women (141 mg / dl) than among men (122 mg / dl) (fig. 2).

The analysis of triglycerides values corresponding to the two sexes showed that the data distribution is not comparable (ANOVA p = 0.162). *So*, there was no significant difference between triglycerides value according to sex (fig. 3).

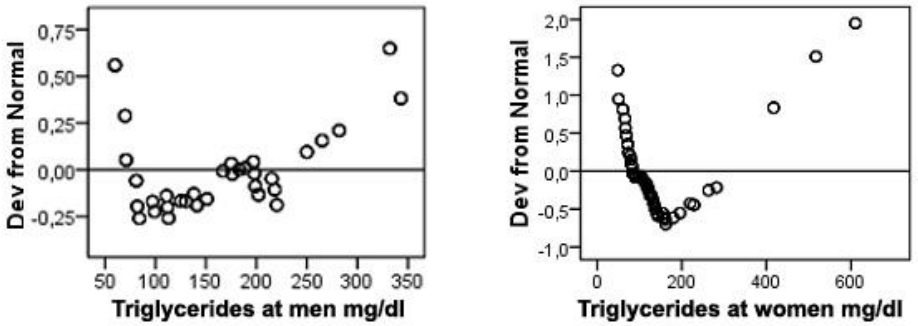

Fig. 2. Distribution of triglycerides values according to sex

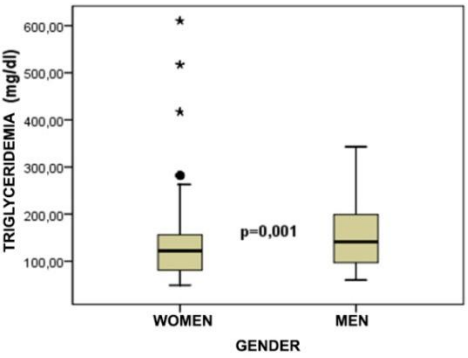

Fig. 3. TG variation by sex

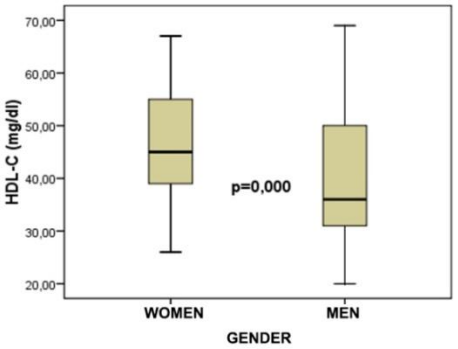

Fig. 4. Variation of HDL-C by sex

HDL-cholesterol

The presence of low HDL-C was found in 193 patients, of whom 97 were men (50.3%) and 96 women (49.7%). The statistical analysis showed a median of 40 mg / dl, with differences according to sex. Analysis of HDL-C values corresponding to the two sexes shows that the distribution of the data is comparable (ANOVA p = 0.032). *Finally*, there is a significant difference between triglycerides values according to sex (fig. 4, 5).

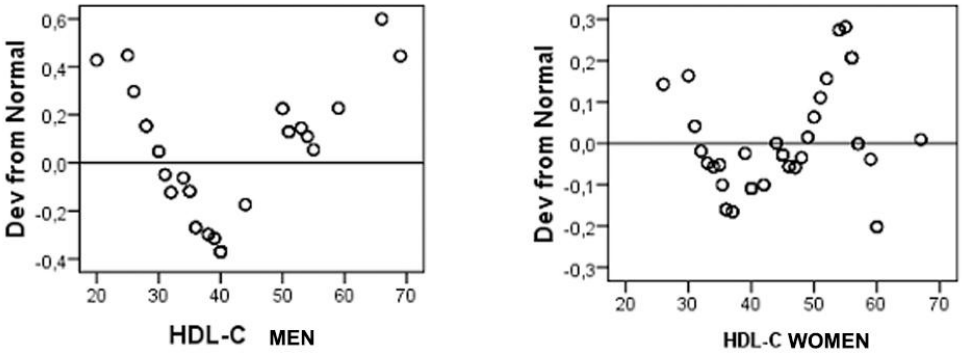

Fig. 5. Distribution of HDL-C by sex

Hypertension

Hypertension was present in 292 patients (86%), of whom 148 were women (56%) and 144 men (44%). The blood pressure median in the study group was 140 mmHg, with a range between 100 mmHg and 200 mmHg, and differences between sexes (fig. 6).

Analysis of BP corresponding to the two sexes showed that the data distribution is not comparable (p = 0.865). *Conclusion*: There is no significant difference between BP values according to sex (fig. 7).

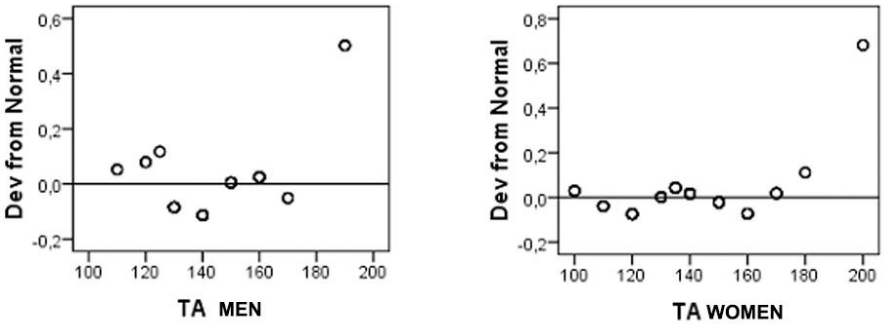

Fig. 6. Distribution of blood pressure (BP) by sex

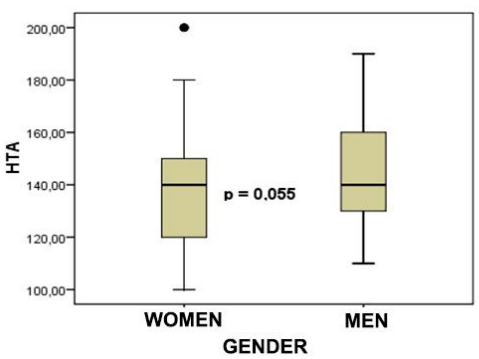

Fig. 7. Variation of BP by sex

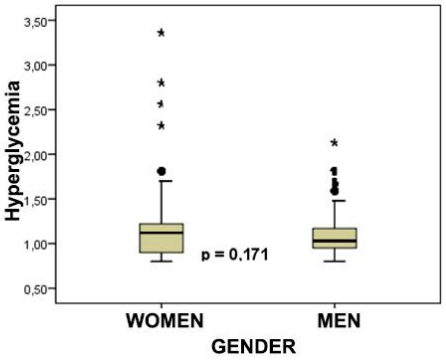

Fig. 8. Variation of glucose values by sex

Glycemic profile

In the study group there were 140 patients with decreased glucose tolerance and 84 patients with DM. The glycemia median in case of the study group was 109 mg / dl, varying between 80-336 mg/dl. The analysis of blood glucose values corresponding to the two sexes shows that the distribution of the data is comparable (ANOVA p = 0.028). The *conclusion* is that there is no significant difference between blood glucose levels by sex (fig. 8, 9).

3. Metabolic syndrome profile

The systematized analysis of the metabolic syndrome profile showed differences according to the sex of patients who were part of the study.

The risk factors studied had the following features:

• AHT was present in 292 patients (86.9%), with no statistical difference between sexes (p = 0.055), 148 women (56%) and 144 men (44%);

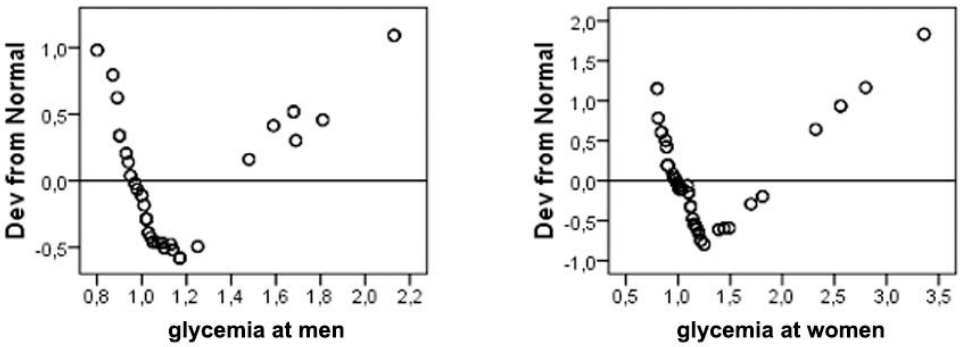

Fig. 9. Distribution of glycemia levels by sex

• hypertriglyceridemia was present in 284 patients (84.5%), with statistical difference between sexes (p = 0.001), 164 women (37.8%) and 120 men (37.8%);

• decrease in HDL-C was found in 193 patients (57.4%), with statistical difference between sexes (p = 0.001), 97 women (50.3%) and 96 men (49.7%);

• abdominal obesity was present in 152 patients (45.2%), with no statistical difference between sexes (p = 0.581), 88 women (57.9%) and 64 men (42.1%);

• change of glycemic profile was recorded in 224 patients (66.6%), without statistical difference between sexes (p = 0.206), 124 women (55.4%) and 100 men (44.6%).

Statistical analysis of Impaired glucose tolerance (IGT) / DM, AHT and increase of TG was present in 172 patients (51%), with no statistical difference between sexes (p = 0.206) in 92 women (53.5%) and 80 men (46.6%) (fig. 10).

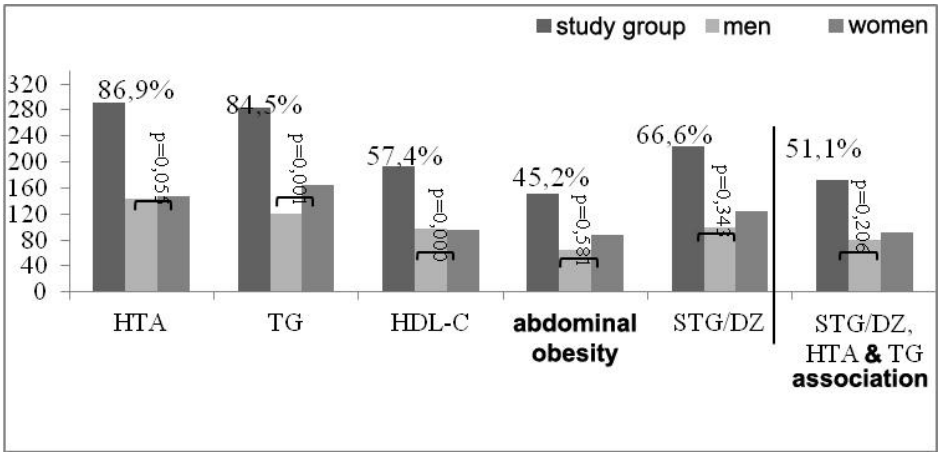

Fig. 10 – Metabolic syndrome profile

4. Cardiovascular risk associated with metabolic syndrome

Patients included in the study were studied depending on the frequency of the main cardiovascular diseases, secondary to metabolic syndrome and of unfavorable

combination of cardiovascular risk factors included in the definition of metabolic syndrome.

Ischemic heart disease

Ischemic heart disease was present in 168 patients (50%), 68 women (40.5%) and 100 men (59.5%), with statistically significant differences between sexes (p = 0.000).

The statistical analysis of the presence of IHD in patients who have in association IGT / DM, AHT and increased TG showed a statistically significant difference compared to those without this association (p = 0.000, Exact Sig. 2-sided).

Ischemic cerebral vascular accident (stroke)

The statistical analysis of the presence of stroke has not showed a statistically significant difference between sexes (p = 0.575, Exact Sig. 2-sided), it was present in 32 patients (9.5%), of whom 16 women (50%) and 16 males (50%). The statistic analysis of this association IGT / DM, AHT and increased TG in patients with ischemic stroke revealed a statistically significant difference compared to those without this association.

Peripheral artery occlusive disease (PAOD)

Peripheral artery occlusive disease was present in 13 patients (3.9%), with statistical difference between sexes (p = 0.019, Exact Sig. 2-sided), in 2 females (15.4%) and 11 men (84.6%). The statistical analysis of this association IGT / DM, AHT and increased TG did not objectify a statistically significant difference (p = 0.251, Exact Sig. 2-sided).

The results of this study were consistent with the literature data in case of hypertriglyceridemia, the other components of MS recording larger differences (9). The effects of IGT / DM, AHT and hypertriglyceridemia association were consistent with literature data, statistically significant in case of IHD's and ischemic stroke (4).

CONCLUSIONS

In the study group the metabolic syndrome profile showed statistical differences according to sex, in situations of HDL-C's decrease, being lower in male patients, and in case of hypertriglyceridemia, the triglycerides level was significantly higher in women.

In case IGT / DM, AHT and hypertriglyceridemia association we recorded an additional risk of development of IHD or ischemic stroke, independent of sex, requiring a multidisciplinary approach including lifestyle changes, pharmacological and surgical treatment (10).

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NEWS

CARDIOVASCULAR BENEFITS OF EXERCISE

It is well established that regular exercise results in significant cardiovascular health benefits, including improvements in blood pressure, lipid profile, body mass and composition, as well as objective measures of functional capacity. Moreover, it has been extensively demonstrated that individuals engaging in regular, moderate exercise are at lower risk for mortality, enjoying an average life expectancy 7 years greater than their sedentary counterparts. Such health benefits are evident at relatively modest 'doses' of exercise, amounting to around 6–10 metabolic equivalents per week. Indeed, exercise prescription is among the most cost-effective therapies available for cardiovascular disease prevention, and is devoid of the side effects common to most pharmacological interventions. Incremental benefits in coronary heart disease risk reduction are manifest with increasing activity, such that those achieving around 42 metabolic equivalents per week appear to gain the greatest benefit. By way of example, this would equate to brisk jogging for around 5 h per week. That the beneficial effects of exercise continue to increment above and beyond this point has never been demonstrated.(Abbas Zaidi, Sanjay Sharma. Exercise and Heart Disease. Future Cardiol. 2013;9(1):119-136).

Doina Butcovan

BONE MINERAL DENSITY IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE FROM NORTH-EASTERN ROMANIA

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BONE MINERAL DENSITY IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE FROM NORTH-EASTERN ROMANIA (Abstract) Inflammatory bowel disease (IBD) is associated with increased prevalence of bone demineralization. One of the risk factors for low bone density is the inadequate level of 25-OH vitamin D. **Aim:** To determine the degree of bone demineralization in patients with IBD and the main causes leading to this condition. **Material and methods:** A prospective study was carried out between April, 2011 and October, 2012 in 143 patients diagnosed with IBD at the Gastroenterology and Hepatology Centre of Iaşi.The IBD diagnosis was made on clinical, biological, and endoscopic criteria and confirmed histologically. The diagnosis of osteopenia/osteoporosis was based on World Health Organization criteria. **Results:** Osteopenia was found in 48.07% of the patients with ulcerative colits (UC) and in 56.41% of the patients with Crohn's disease (CD); osteoporosis was present in 18.26% of the patients with UC and 15.38% of those with CD. The main causes identified were inadequate vitamin D level, extended high-dose corticotherapy in patients with CD, BMI<18.5 kg/m2, and smoking, especially in the patients with UC. **Conclusions:** Bone demineralization and hypovitaminosis D are frequently associated with IBD and require specific treatment. **Keywords**: BONE DEMINERALIZATION, OSTEOPOROSIS, 25-OH VITAMIN D, INFLAMMATORY BOWEL DISEASES.

Low bone density is a condition frequently associated with inflammatory bowel diseases (IBD). The pathogenesis of bone demineralization is multifactorial. Even though the induction mechanisms have not been completely elucidated, the main risk factors are the duration of inflammatory bowel disease, protein-caloric malnutrition, prolonged corticotherapy, calcium and vitamin D deficiency, sex hormones deficit and tobacco consumption (1, 2, 3).

The mineral bone components (calcium and phosphates) are absorbed with the help of 1.25-di-hydroxyvitamin D, the active metabolite of vitamin D. Gastrointestinal tract anomalies, altering their absorption, are factors involved in bone homoeostasis.

According to the World Health Organization (WHO), *osteopenia* is a condition of decreased bone density, while *osteoporosis*is a condition of bone rarefaction, involving the alteration of bone micro architecture

anda secondary increase in fracture risk.

The aim of our study was to determine the degree of bone demineralization in patients with IBD and to identify the main causes leading to this condition.

MATERIAL AND METHODS

A prospective descriptive study was carried out on a series of 143 patients with IBD, hospitalized at the Institute of Gastroenterology and Hepatology in Iaşi between April 1, 2011 and October 15, 2012. The diagnosis of IBD was based on clinical, biological, and endoscopic criteria and confirmed histologically.

The demographic parameters (age, gender, place of residence, type of disease, duration, extension of lesions and degree of activity) as well as the treatment (corticotherapy – months of treatment and cumulative prednisone-equivalent doses) were analyzed. To establish the degree of disease activity, the Crohn's Disease Activity Index (CDAI) and the Ulcerative Colitis Disease Activity Index (Mayo-UCDAI) scores were used. Additionally, the body mass index (BMI) (kg/m²), smoking status and intensity (estimated in packs per year), history of fractures, and, in women, obstetrical antecedents were assessed.

*Exclusion criteria***:** Patients presenting other possible causes of bone demineralization and vitamin D deficiency (bowel resections, thyroid or parathyroid pathology, hypogonadism, diabetes mellitus, neoplasia, renal or hepatic failure, patients with menopause for more than 5 years).

Osteoporosis was assessed by osteodensitometry (biphotonic X-rays absorptiometry = DEXA) at the lumbar spine (L1-L4) and the upper end of the femur. The results were expressed as a T-score (standard deviations below or above the mean value for a younger subject in the general population). WHO classification of bone mineral density was used: normal (T-score > 1DS), osteopenia (T-score between -1 DS and -2.5 DS) and osteoporosis (T-score < -2.5).

The following biochemical values were determined: serum calcium (mg/dl), ionic Ca (mmol/l), alkaline phosphatase (UI/l), 25 hydroxy-vitamin D (ng/ml).

Data analysis was carried out using the *SPSS 16.0* software. The statistical significance threshold was p = 0.05.

In order to test the statistical hypotheses and for continuous and categorical variable data analysis, Student's t and chi-squared distribution were used. The analysis of variance (ANOVA) was used to obtain the statistical indices for the dependent variable (on the whole and by groups), and the Levene test for the analysis of homogeneity of dispersions.

RESULTS

The study included 143 IBD patients divided into two groups: *A:* 104 patients with Ulcerative Colitis (UC) and *B:* 39 patients with Crohn's Disease (CD).

Group A: Most patients (53.84%) were male, mean age 44,65 years. The average duration of the disease was 49.58 months. The extension of lesions was determined based on the Montreal classification; thus, of the 104 patients with UC, 19.23 % had proctitis (E1), 50% left colitis (E2), and 30.76% pancolitis (E3).

As to disease activity, 23.07% of the patients presented severe activity, 25.96% - moderate, 34.61% - slight activity, while 16.34% were in clinical and biological remission.

The body mass index (BMI) was <18.5 kg/m2 in 12 patients (11.5%), normal (BMI=18.5-24 kg/m^2) in 32 patients (30.8%) and high (BMI>24 kg/m^2) in 60 patients (57.69%). Sixty-two patients were

smokers or ex-smokers, with an average tobacco consumption estimated at 15.93 PY (packs-year).

As to UC treatment options, 94,23% of the patients were administered derivatives of 5 aminosalycilates, 42.3% were treated with corticosteroids administered orally or intravenously, with an average prednisone-equivalent dose of 2.82±2.4 g/patient; only 13.5% of the patients benefited from biologic therapy at enrolment.

Osteodensity at the lumbar spine and proximal end of the femur revealed: 34 patients (32.69%) had normal bone mineral density, 50 patients (48.07%) presented osteopenia, and 20 patients (19.23%) osteoporosis (fig. 1).

Group B: Predominantly female patients (53.84%), meanage 39.28 years. The average disease duration was 37.64 months.

Topographically, the Montreal endoscopic classification revealed: 20 colon (L2)-51.28%, ileum and colon-43.58%, while only 5.12% of the patients presented upper digestive tract disorders (L4/ L1+L4).

Disease activity at enrollment: 10 patients (25.64%) were in clinical and biological remission, 12 patients (30.76%) presented slightly-moderate activity, 15 patients (38.46%) – moderate-severe activity, while 2 patients (5.12%) presented highly severe activity. Twenty-two patients (56.41%) were smokers or ex-smokers with an average tobacco consumption of 11.12 PY.

More than half of the patients with CD (61.53%) underwent treatment with corticosteroids administered orally or intravenously, with an average prednisone-equivalent dose of 8.37±21.56 g/ patient. Approximately 30.76% of the patients underwent biological therapy (4 patients with Adalimumab, and 8 patients with Infliximab) at enrollment.

The BMI calculation revealed: 4 patients (10.25%) with CD were underweight (BMI<18.5 kg/m^2), 22 (56.41%) were normal weight, while 13 patients (33.33%) were overweight.

Osteodensiometry at the hip and femoral neck levels revealed 11 patients with normal mineral density, 22 patients (56.41%) with osteopenia, and 6 patients (15.38%) with osteoporosis (fig. 1).

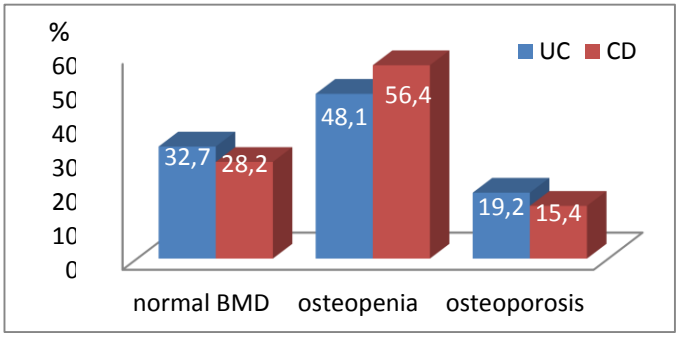

Fig. 1 Bone mineral density in patients with IBD determined by DEXA

Comparing the two 2 groups, it was noticed that bone mineral density was lower in the patients with CD compared to those with UC. T-score for both lumbar spine and hip was lower in CD than UC patients (lumbar spine: CD=-1.60±1.00; UC = -1.00±1.13, proximal end of the femur: CD=-0.93±0.84; UC=-0.31±0.90) (fig.2).

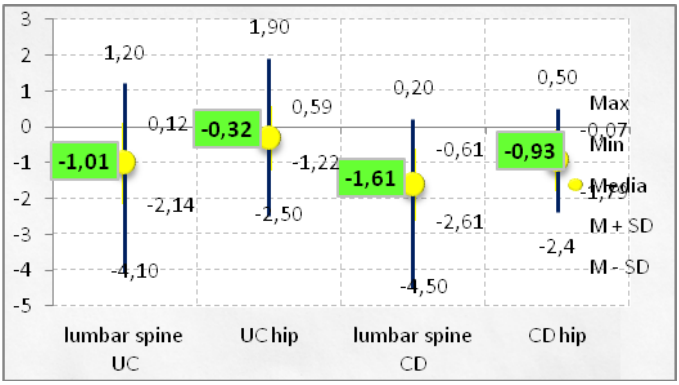

Fig.2. Distribution of the T-score in patients with IBD

TABLE I

Analysis of the risk factors for bone demineralization

Parameters	UC	Crohn's disease	p
BMI < 18.5 kg/m^2	12/104	4/39	> 0.05
Smoker	62/104	22/39	< 0.05
Average tobacco consumption (PY)	15.93	11.12	< 0.05
Average disease duration (months)	49.58	37,64	> 0.05
CS Treatment	44/104	24/39	< 0.05
Average CS dose (prednisone equivalent dose - g/patient)	2.82±2.4	8,37±21,65	< 0.05

As seen, the analysis of the risk factors did not reveal statistically significant differences between the two groups as far as BMI is concerned. Nevertheless, it can be noticed that tobacco consumption was an important risk factor in the patients with UC; moreover, CD patients showed a significantly higher need of corticosteroids, a dose 3 higher/patient compared to UC patients.

25-OH vitamin D determination revealed that CD patients had a significantly lower level compared to UC patients ($p<0.05$) (fig. 3).

There were no statistically significant differences between vitamin D level and the decrease in bone density.

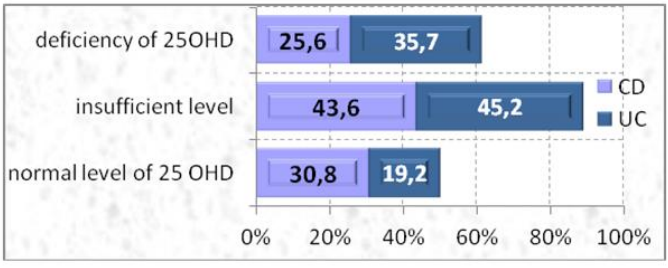

Fig. 3: The level of 25-OH vitamin D in the patients with IBD.

TABLE II

Hematologic and biochemical results in patients with IBD

Marker	UC	CD	p
Average lumbar T-score	-1.00±1.13	-1.60 ± 1.00	**<0.05**
Average hip T-score	-0.31±0.90	-0.93 ± 0.84	**< 0.05**
25-OH vit. D	22 ± 10,71	22.13±9.13	> 0.05
sericCa (ng/dl)	9.42 ± 0.43	9.51 ± 0.37	> 0.05
ionic Ca (mg/dl)	1.22± 0.53	1.19 ± 0.73	> 0.05
Alkaline phosphatase (mg/dl)	84.40 ± 20.82	88.86 ±17.50	> 0.05

DISCUSSION

Over the last years, extended epidemiological studies based on WHO criteria have proven the high prevalence of osteopenia (40-50%) and osteoporosis (10-25%) in the patients with IBD (4, 5). Our study revealed low bone density in 67.3% of the patients with UC and in 71.8% of the patients with CD. Nineteen percent of the UC patients and 15.4% of the CD patients had osteoporosis. These data are in agreement with the data published in the literature. We noticed a smaller percentage of osteoporosis in the patients with Crohn's disease, which can probably be explained by the small number of CD patients compared to UC patients. Osteopenia was found in 48.1% of the UC cases and 56.4% of the CD cases. The obtained data are in agreement with data from similar studies carried out in Italy, Greece and Switzerland (6, 7, 8).

Vitamin D deficiency was significantly higher in the CD patients (35.7%) compared to UC patients (25.6%), despite the fact that the course of UC was relatively longer - 49.58 months compared to 37.64 months in CD. As far as the disease activity is concerned, there were no statistically significant differences. Data in the literature show that 25-OH vitamin D levels vary by season (9). Thus, in all patients, 25-OH vitamin D was determined in the interval May - August (in order to avoid seasonal differences).

The cumulative dose of corticosteroids was inversely correlated with bone mineral density (5, 10, 11). The significant decrease in BMD occurs when the prednisone dose exceeds 7.5 mg/day (5, 12). In our study, it was noticed that approximately 61.53% of the CD patients and 42.3% of the UC patients benefited from treatment with corticosteroids at enrollment or during the disease course, with an average cumulative dose of 8.37 g in the CD group, compared to 2.82 g in the UC group. As to the smoker/non-smoker status, significantly more smokers were found among the UC patients.

Osteoporosis is a condition that can be prevented; thus, a limited use of corticosteroids is recommended, but only in severe activity outbreaks and for a short period. Equally recommended in patients with IBD is osteodensitometry, as early as possible after the diagnosis, since bone demineralization starts early within in the first 2-3 months of disease (5). Some of these patients are young and did not present other risk factors; a bone disease was present at diagnosis in patients with IBD, probably due to the hypovitamnosis D, phenomenon also confirmed by our study.

Osteodensitometry should be repeated every 12-18 months, even though the first

osteodensitometry indicated a normal bone density, since demineralization occurs in time, due to the inflammatory activity of the disease, vitamin D malabsorption and deficient bone calcium deposits.

CONCLUSIONS

Bone mineral density was significantly lower in the CD patients as compared to UC patients. Corticosteroids used in over half of the cases at a dose 3 times higher in CD patients resulted in bone demineralization especially at hip level. 25 OH vitamin D level was lower in the newly diagnosed patients and partially and directly proportionally influenced BMD at the hip level.

Although clinically this complication is noticeable especially with the occurrence of osteoarticular pains and fractures, it requires adequate knowledge and treatment, as well as special attention from gastroenterologists and endocrinologists in order to increase the quality of life of the patients with IBD.

ACKNOWLEDGEMENTS This work was supported by the Managing Authority of the Sectorial Operational Programme for Human Resources Development through the project "Inter-university partnership for increasing the medical doctoral research quality and interdisciplinarity by granting doctoral scholarships – DocMed.net", POSDRU/107/1.5/S/78702).

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METABOLIC SYNDROME IN OBESE CHILDREN AND ADOLESCENTS

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METABOLIC SYNDROME IN OBESE CHILDREN AND ADOLESCENTS (Abstract). **Aim:** To investigate the risk factors for metabolic syndrome (MS) in children and adolescents. **Material and methods:** Investigation conducted in the interval October 2010 - June 2011 in 3103 school children aged 7-18 years. After body weight, height, waist circumference, and blood pressure were measured, 262 school children were found to be obese, of which 120 agreed to testing for defining the metabolic syndrome: triglycerides (TG), cholesterol, HDL and LDL cholesterol, blood glucose, insulinemia, OGTT (oral glucose tolerance test), SGOT, SGPT, urea, creatinine, and ESR. **Results and discussion**: Using IDF 2009 modified criteria, the prevalence of MS in the study series was 55.8%. The most common criteria for defining MS were: TG ≥ 95th percentile, BP ≥ 95th percentile, and blood glucose ≥ 100mg/dl. **Conclusions:** Applying IDF 2009 criteria, 55.8% of the obese subjects presented 3-5 criteria, thus meeting the diagnostic criteria for MS in children. **Keywords:** OBESITY, METABOLIC SYNDROME, CHILD

Child obesity tends to become a global epidemic as both overweight and obesity are increasingly common in developed and developing countries. In recent years, the high incidence was accompanied by an increasing frequency of obesity complications occurring at younger and younger ages (1, 2).

Metabolic syndrome (syndrome X or insulin resistance syndrome) associates obesity (predominantly abdominal), dyslipidemia (hypertriglyceridemia, high LDL, low HDL), arterial hypertension, hyperglycemia, and future risk of developing diabetes, cardiovascular complications and stroke.

There are many controversies about the definition of MS in children. The problem is complicated by the fact that the components of MS syndrome occur progressively and thus not all young patients present a full picture. Furthermore, pathophysiological changes and syndrome components are influenced by growth, and puberty, and not only by gender and ethnicity as in adults (3). Due to these features, adult criteria, IDF (International Diabetes Federation), WHO (World Health Organization), NCEP-ATP III (National Cholesterol Education Program - Adult Treatment Panel III), should be used with great caution in children (4, 5).

For defining the diagnostic criteria for pediatric MS, IDF 2009 modified criteria are divided according to age-groups starting with age 6 years (6).

MATERIAL AND METHODS

The study group included 120 obese

children, selected by overweight screening from 3103 school children aged 7 to 18 years from 8 schools in Galati, Romania. Height, body weight, waist circumference (WC) and blood pressure (BP) were measured, and body mass index (BMI) was calculated; for BMI interpretation the CDC age and gender specific charts were used (7).

In the interval October 2010 – June 2011, after obtaining the informed consent for participation in the study, the 120 obese children (61 girls and 59 boys) were investigated at the Galati "Sf. Ioan" Emergency Children Hospital: triglyceride (TG), cholesterol, HDL and LDL cholesterol, fasting blood glucose, insulinemia, OGTT (oral glucose tolerance test), SGOT, SGPT, urea, creatinine, ESR.

To define pediatric MS we used IDF criteria adapted to children: WC ≥ 90 percentile for age and sex, blood glucose ≥ 100 mg/dl, HDL colesterol ≤ 50th percentile for girls, and ≤ 40 mg/dl for boys, TG ≥ 95th percentile, and BP ≥ 95th percentile for age and sex (8). According to 2009 consensus, the presence of at least three criteria is needed for defining MS, abdominal obesity not being a mandatory criterion.

There are no age-, gender-, race-, and pubertal status-specific insulinemia reference values. In this study we used the following reference values: normal <15 µU/ml, borderline 15-20 µU/ ml, high >20 µU/ml. A mathematical model of glucose-insulin interrelationship, based on a set of feedback loops (Homeostasis Model Assessment = HOMA) was used to draw a graph of the predicted blood glucose and insulinemia values at different degrees of insulin resistance and β –cell deficiency. HOMA-IR index was calculated according to HOMA-IR formula = basal insulinemia µU/ml x fasting glucose (mmol/l)/ 22.5.

Data were loaded and processed using statistical functions of *Excel*, *EpiInfo* and *SPSS*. The used statistical methods were t-Student test, χ^2 test, relative risk, Kaplan-Meier survival curve.

RESULTS

Of the 3103 school children, in 589 (18.9%) weight was over the healthy range for age and height. Of these, 262 (8.4% of all school children) were classified as "obese" having a BMI ≥ 95%. Informed consent to participate in the study was obtained from 120 children and their parents.

Of the 120 investigated obese children, 35 (29.2%) had severe obesity (BMI ≥ 97 percentile for age and gender). Waist circumference (WC), as a marker of visceral obesity and insulin resistance, showed values above the 90th percentile in 69.2% (83 of the 120 obese children), with a slightly higher proportion of males (54, 2%) ($\chi 2 = 2.13$, df = 1, p = 0.144) (fig. 1). There was a correlation between the degree of obesity and waist circumference, the percentage of children with WC above the 90th percentile ranging between 79-83%, in children with moderate and severe obesity this being prevalently visceral (fig. 2).

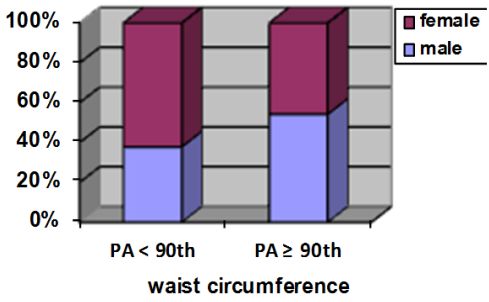

Fig.1. WC and gender correlation

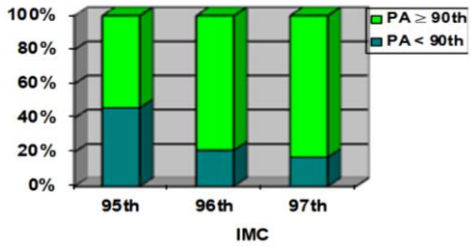

Fig. 2. BMI and WC correlation

The distribution of subjects according to systolic/diastolic blood pressure (SBP/DBP) showed that SBP was above the 95th percentile in 13-14% of the subjects and DBP over the 95th percentile in 9.2% (proportionately, more common in male versus female subjects, 32.2% and 22.9%, respectively, difference statistically insignificant) (fig. 3).

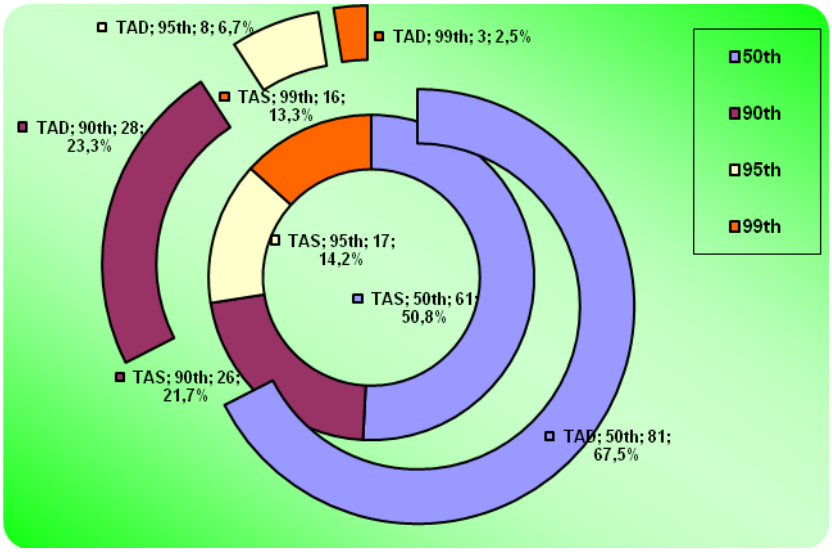

Fig. 3. Distribution of subjects according to blood pressure values

In the study obese children, blood triglyceride levels were: TG ≥ 95th percentile (150 mg/dl): 32 cases (26.6%), TG < 95th percentile (150 mg/dl): 88 cases.

HDL cholesterol levels in the study children were: females - 61 children: HDLc ≤ 50 mg/dl: 46 cases (65.5%), HDLc > 50 mg/dl: 5 cases (34.4%); males - 59 children: HDLc ≤ 40 mg/dl: 39 cases (42.3%), HDLc > 40 mg/dl: 19 cases (57.6%)

Total cholesterol (mg/dl): acceptable level under 170 mg/dl: 62 cases (51.6%), borderline levels between 170-199 mg/dl: 40 cases (28%), elevated ≥ 200 mg/dl: 18 cases (15%).

Fasting glucose levels above the reference values (≥ 100 mg/dl) were found in only 12 of the study children (10%), with no significant gender, BMI and WC differences, all of them meeting the criteria for metabolic syndrome.

OGTT was performed in 108 children, regardless of fasting glucose level or family history, as a measure of obesity. In sample 4, blood glucose levels above 199 mg/dl were found in only a male subject with BMI 95th percentile and WC above the 90th percentile. Glucose tolerance was found decreased in only 2 children, one male and one female, both with WC above the 90th percentile.

Of the 120 investigated children, insulinemia, as a marker of insulin resistance, was determined in only 17. In all these children insulinemia was above 15 µUI/ml. All calculated HOMA-IR values were greater than 3, which can be interpreted as insulin resistance.

MS prevalence in the 120 obese children (BMI ≥ 95th percentile) using IDF 2005 consensus criteria revised in 2009 was 55.8% (67 of 120 children had at least three diagnostic criteria for MS) (tab. I).

TABLE I

IDF criteria for the diagnosis of metabolic syndrome adapted to children by using age and gender specific percentile charts

Diagnostic criteria	Number of cases	Number of cases diagnosed with metabolic syndrome
WC ≥ 90th percentile	83	67 (80,7%)
Fasting glucose ≥ 100 mg/dl	12	12 (100%)
HDLc ≤ 50th percentile	93	67 (72,04%)
TG ≥ 95th percentile	32	32 (100%)
BP ≥ 95th percentile	33	33 (100%)

DISCUSSION

Although components of IDF criteria were present in 72% to 100% of the investigated obese children, only 67 of them (55.8%) met the criteria for MS diagnosis by the presence of at least three of the five criteria.

In our study, of the five IDF 2009 criteria, the most reliable were: triglycerides ≥ 95th percentile, BP ≥ 95th percentile, and fasting glucose ≥ 100 mg/dl (in 100% of the cases that met the criteria for MS diagnosis).

Second in importance was WC ≥ 90th percentile (although 83 of the cases had values suggestive of MS, only 67 met the diagnostic criteria).

Less reliable proved to be HDLc ≤ 50th percentile; although present in 93 of cases, only 67 (72.04%) met at least three IDF criteria for the diagnosis of MS.

This study along with numerous other studies, confirms that MS is present not only in obese adults but also in obese children, even though the clinical and biological picture is not complete at this age, it gradually progressing with advancing age.

Longitudinal studies are needed to assess the evolution of these parameters in relation with advancing age and maintenance or not of obese status.

Data reported by various researchers on the incidence of MS in obese people range between 11.5% and 60-80% in relation to patient age, degree of obesity, ethnicity, presence or absence of diabetes mellitus.

The incidence of MS in child obesity is still insufficiently studied, and the reports differ because they do not use the same evaluation criteria, which have changed over time. For example, when the same group of obese people was assessed by different criteria, MS was present in different percentages (10): IDF 2009 criteria = 58%, NCEP-ATP III criteria = 42 %, and WHO criteria = 10%. Another important issue is the gradual occurrence and worsening with time of its manifestations, so that the percentage will vary with study series age homogeneity. In the present study, the percentage of obese children in which MS by the presence of at least three IDF criteria began to develop is 55.8%. These results, surprising at first sight, are however in agreement with those published by Preda

that found 58% (9). However, regardless of used definition, the prevalence of MS was significantly higher in obese (42.9%) compared to normal weight people (2.8%) (10).

The above data clearly show that the phenomenon occurs and needs to be stopped even during childhood, when manifestations are not yet severe or irreversible as in the elderly. From this perspective, children and youth are an important target group because early healthy eating habits along with a healthy lifestyle make the most effective method of living in health. School and education are a means and an important environment for overweight prevention and WHO identified school as the main starting point in the fight against obesity (11, 12).

CONCLUSIONS

In our study, of the 3103 school children (7-18 years), 589 (18.9%) were overweight for age and height, and 262 (8.4% of children) were obese having a BMI $\geq$ 95.

MS (IDF 2009 criteria) was recorded in 55.8% of the 120 obese school children from which the informed consent for participation in the study was obtained.

The need for longitudinal studies to evaluate the evolution of these parameters in relation to patient age and maintaining or not obese status is emphasized.

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STUDY ON THE EFFECTIVENESS OF THE KINETIC METHOD IN PATIENTS WITH RHEUMATIC DISEASES AND TEMPOROMANDIBULAR JOINT DYSFUNCTION

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STUDY ON THE EFFECTIVENESS OF THE KINETIC METHOD IN PATIENTS WITH RHEUMATIC DISEASES AND TEMPOROMANDIBULAR JOINT DYSFUNCTION (Abstract): selecting the appropriate treatment decision is essential for achieving optimal results in the management of algo-dysfunctional syndrome of the temporo-mandibular joint (TMJD). The study aims to decide on the most effective (symptomatic control, preserved motility) kinetic program in patients with TMJ involvement. **Material and Methods**: prospective observational study on 83 consecutive patients with rheumatic diseases and TMJ dysfunction. Clinical assessment (pain, noises, muscle spasm, range of motion, ROM) was performed at baseline and after 3 months of specific kinetic rehabilitation program. Change in clinical parameters and TMJ index was reported, $p<0.05$. **Results**: over 45% TMJ involvement at baseline as defined by TMJ index (mean value of 13.56) and only 36.66% at 3 months ($p<0.05$). Significant improvement in pain (presence, severity) was demonstrated at 3 moths ($p<0.05$): 18.05% spontaneous pain, 75.9% provoked pain, with 12.11% respectively 2.41% decreased in nocturnal respectively diurnal pain. Significant decrease ($p<0.05$) in joint noises at movements: 27.71% when opening and 12.04% when closing the mouth, 8.43 at protrusion and 3.61% at retraction, while 18% at the side movements. **Conclusions**: Complex accurate kinetic reeducation is mandatory for achieving correct posture (head, neck and trunk), normal mastication, swallowing and respiration, as well as correction of neuromuscular imbalances in patients with TMJD secondary to rheumatic disorders. **Key words:** TEMPORO-MANDIBULAR JOINT DYSFUNCTION, THE FUNCTIONAL ASSESSMENT, TREATMENT, REHABILITATION.

About 40% to 70% of adults have experienced at least one symptom related to the temporo-mandibular joint dysfunction (TMJD), but in the majority of cases it was treated superficially leading to worsening of the symptoms. Due to the complexity and variability of the manifestations, the painful TMJD can initiate significant structural and functional changes, finally leading to impaired work capacity, reduce opportunities to carry out daily activities, and subsequent impaired quality of life (1).

Both inflammatory and degenerative rheumatic conditions may secondary involve TMJ being responsible for a range of pain and mandible dysfunction. (2). Pain, joint cracklings and affected restricted range of motion (ROM) are the mainstay of the clinical background of TMJ participation (2). Therefore, it is widely recognized that the kinetic therapy, through its effects and the applied noninvasive methods, can improve the existing symptoms and may change the patient's opinion regarding the condition of suffering.

Establishing a treatment plan customizes all general data regarding the clinical, functional and laboratory aspects, as well as the synopsis of all clinical and biological, functional, psycho-socio-economic and behavioral indices. Subsequent kinetic approach requires an accurate functional diagnosis of the TMJ involvement and a choice of the optimal therapeutic solution, based on the theoretical and practical achieved experience, but also on general and local treatment.

Moreover, the kinetic treatment is essential in the setting of persistent TMJD aiming to maintain or restore the functionality at this level leading to structural, functional and aesthetic changes. Choosing the appropriate treatment plan coupled with appropriate therapeutic management is the ideal option for achieving superior results in the treatment of the painful-dysfunctional syndrome of the TMJ.

Given the complexity and variability of the algo-dysfunctional TMJ syndrome, the current study aims to decide on the most effective kinetic program in patients with TMJ involvement.

MATERIAL AND METHODS

We performed a prospective observational study during October 2009 and September 2011 on eighty-three consecutive patients with different inflammatory and degenerative rheumatic disorders and secondary TMJ involvement in order to elaborate a complete and correct protocol for TMJD evaluation and treatment.

The study was approved by the Ethics Committee and all patients signed the informed consent before enrollment.

Clinical signs and symptoms of the TMJ involvement were systematically registered at baseline and at three months, including: pain, muscle spasm, noises and bruxism, as well as range of motion (ROM).

Specific medication, physical and kinetic management were promoted in all patients according to the disease and the stage (acute, sub acute, chronic) of the TMJD, aiming to control muscle spasm, referred pain and to restore mandible motility rebalancing the agonist and antagonist muscles groups of the neck and face and proprioceptive recovery. Orthodontic care was required to restore normal occlusion.

All patients were included in a complex kinetic program: 3 sessions per week for the first two weeks, 2 sessions per week for the next two weeks and 1 session per week up to 3 months. The duration of a session was settled between 60 to 90 minutes, intending to obtain the local and general relaxation of the patient.

Several methods and rehabilitation techniques were currently used including general massage of the head and neck; relaxing massage for muscles spasm control (temporalis, masseter, medial and lateral pterigoids, suprahyoids, infrahyoids, digastrics); techniques for detecting and treating trigger points (face, head, neck); passive and active stretching exercises lowering of the mandible, side movements, protrusion and retraction; passive stretching exercises performed through specific movements of the trunk and neck; techniques of intra- and extra-oral mandible

manipulation; resistance exercises for opening and closing the mouth; corrective exercises for posture (head, neck, trunk); proprioceptive exercises of TMJ; diaphragmatic breathing exercises and swallowing rehabilitation exercises.

Statistical analysis. Both descriptive (mean, standard deviation, percentage) and analytic statistics (t-test) were used to analyze difference between visits; statistical significant "p" was settled below 0.05.

RESULTS

Pain

Baseline clinical exam has revealed TMJ pain at palpation (external auditory canal, pretragus, jaw muscles) and mobilization of the mandible, but also during chewing in all patients. After three months, we demonstrated the decrease in pain intensity, as well as the increase in the number of patients presenting moderate and mild pain compared to the initial assessment as shown in figure 1; besides, two patients had no pain. Only 18.05% of cases presented with spontaneous pain and up to 75.9% provoked pain at visit 2; in addition we reported a decrease of 12.11% in the nocturnal pain and 2.41% in the diurnal TMJ pain.

Noises

At the initial assessment 50% of the analyzed cases complained noises at the level of the TMJ: more advanced stage of the disease, more frequent noises in the joint. After three months of treatment we revealed the decrease, even the absence of the joint noises during different movements as follows: 27.71% noises when opening the mouth, 12.04% noises when closing the mouth, 8.43% at protrusion, 3.61% at retraction, while 18.07% of cases presented with noises at the side movements (fig. 2).

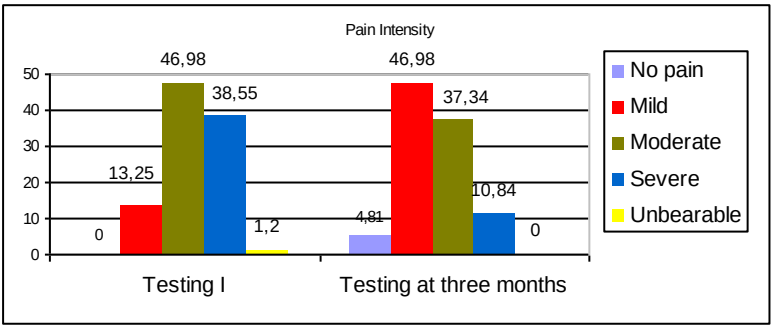

Fig. 1. Pain evidence in tested patients

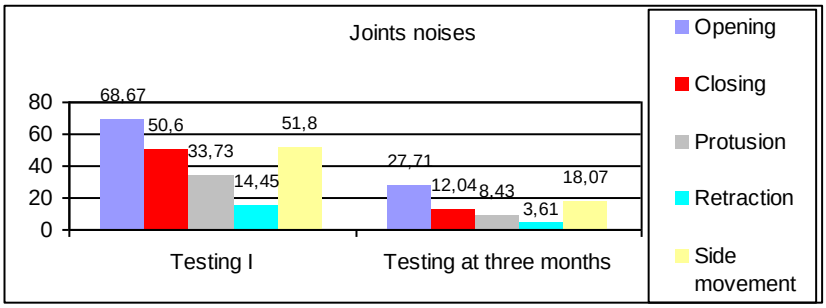

Fig. 2. Joint noises

Muscle testing

The manual muscle testing was performed in all patients on masseter, temporalis, medial and lateral pterigoid, digastric, sternocleidomastoidian and longus colli muscles, looking for the presence of pain, spasm and atrophy. The initial testing showed pain in the majority of cases (84.61%) in the masseter muscle, as well as in 65.38% of cases in the temporal muscle, the main masticators muscles. After three months of specific kinetic treatment, we demonstrated a decrease of pain in the tested muscles, about 25 to 30%. Keeping the same algorithm of muscle pain, the main masticators muscles (temporal, masseter) remained the most affected.

Muscle spasm and muscle atrophy

Muscle spasm was accompanied or caused by pain and was reported at baseline in about half of patients (51.91%) in the masseter, 26.92% in the temporal, 13.46% in the median and lateral pterigoids, and in 17.30% in the sternocleidomastoidian muscle. Muscle spasm significantly decreased ($p<0.05$) after three months of treatment and allowed proprioceptive mandible movements in various positions with increasing amplitude and severity.

On the other hand, muscle atrophy was present in a small proportion of patients as compared with other parameters both in the initial and final testing.

Range of motion

Baseline assessment showed a decrease in the ROM at the TMJ as follows: 15.38% for opening of the mouth, 29.9% for the side movement, 21.15% for protrusion, 1.92% for the retraction movement. Limitation of the mandible mobility resulted in diet restriction, disruption of daily activities and impaired quality of life. After three months of kinetic treatment, ROM at the TMJ significantly ($p<0.05$) improved, the majority of patients showing even normal parameters, with significant progress of quality of life.

TMJ index

Furthermore, a TMJ index was calculated for each patient; the TMJ index is centralizing data on pain (severity and duration), joint noises, vicious habits, joint ROM and muscle status. We obtained an average value 13.56 at the initial testing, meaning a TMJ involvement of over 45%. After three months, TMJ involvement was reported in only 36.66%, indicating a decrease in joint dysfunction along with an increase in the physical and psychological comfort of the patients (fig. 3, tab. I).

TABLE I

Analysis of the TMJ index

TMJ INDEX	No	MED	Standard deviation
Initial Testing	83	15.01	3391
Testing after 3 months	83	11	2798

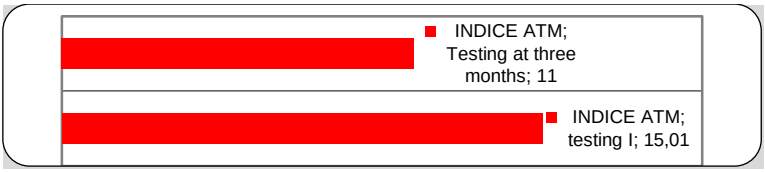

Fig. 3. Evolution of the TMJ index

Moreover, in our study we obtained a correct posture of the head, neck and trunk, a normal mastication, deglutition and respiration, as well as the increase of the range of motion in the TMJ, overcoming the neuromuscular imbalances.

DISCUSSION

It is actually widely recognized the benefit of kinetic therapy in the rehabilitation of the algo-dysfunctional syndrome of the TMJ, with subsequent improvement of quality of life (3). Besides, key goals of the rehabilitation process in patients with different rheumatic conditions affecting TMJ involve: local and general relaxation; the control of the painful symptoms, muscle spasm and residual tensions; the increase of the range of motion; the restoration of the morphology and aesthetic aspect of the face. Rebalancing the agonist and antagonist muscle groups of the neck and face, muscular coordination, proprioceptive rehabilitation, as well as restoration of the normal body movement through an accurate mental representation of different mandible functions and maintaining a correct posture of the head, neck, trunk and scapular-humeral joint are also promoted in the complex TMJ rehabilitation process (1, 6). All these objectives can only be achieved by an early, aggressive, sustained and continuous treatment.

Three main stages are actually recognized in active rehabilitation of TMJD including rehabilitation through visual control of the patient, rehabilitation of the patient without visual control, only by touch and the performance of the movements without visual or tactile control, proprioceptive rehabilitation(4).

In the current context of modern medicine emphasizing the weight of patient's comfort and quality of life, we have quoted the relationships that can be established between different therapeutic backgrounds and the clinical-biological, socio-economical and psycho-behavioral parameters, as well as the individual post-therapeutic feedback.

The TMJ index shows the degree (%) of the global dysfunction of the TMJ; in our study the assessment of this index was systematically done in all patients indicating a positive course of TMJD and demonstrating the benefit of complex kinetic approach. Furthermore, we have demonstrated a significant improvement in all parameters defining the TMJ index including pain, muscle and joint involvement.

After three months of kinetic treatment, only 37% of patients presented with dysfunction of TMJ secondary to different rheumatic disorders; data was comparable with the literature (6).

It can be assumed that complex multidisciplinary (rheumatologist, dentist, kinetic therapist, physiotherapist and psychologist) work-team is essential for achieving positive results in the rehabilitation of patients with algo-dysfunctional syndrome of the TMJ. In addition, the active role of patient should also be mentioned. The main goal of the rehabilitation team is to improve as much as possible the TMJ status, to restore the patient's capacity to live in an integrated manner, physically and psychosocially and, finally, to improve quality of life. The complex issues generated by the algo-dysfunctional syndrome of the TMJ capture and attract the interest of practitioners and researchers in the field. Therefore, our research has tried to highlight new emerging concepts on TMJ rehabilitation, particularly in the settings of rheumatic field.

CONCLUSIONS

Correct and complex analysis of patient's medical records (medical history, clinical and functional laboratory tests) is essential in order to provide the optimal therapeutic approach.

The kinetic program depends on the quality of therapist-patient communication, motivation, perseverance, as well as patient collaboration, being the most important issues in TMJ rehabilitation. Clear, well established objectives should be encouraged advancing long-term improvements. A short-term care is classically associated with increased risk of the recurrence and, even, complications.

Furthermore, accurate kinetic recovery is a prerequisite condition for a proper mastication in patients with algo-dysfunctional TMJ syndrome.

A significant improvement in the TMJD syndrome was reported after the kinetic management in patients with various (inflammatory, degenerative) rheumatic disorders and secondary TMJ involvement as suggested by the modification of the TMJ index, leading to better quality of life.

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REZISTENȚA LA CLOPIDOGREL – FACTOR DE RISC PENTRU PACIENȚII CU SINDROAME CORONARIENE ACUTE

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CLOPIDOGREL RESISTANCE – RISK FACTOR IN PATIENTS WITH ACUTE CORONARY SYNDROMES (Abstract): Clopidogrel is an ADP receptor antagonist, an inhibitor of platelet activation and aggregation. The effect is due to its selective and irreversible blockade of the P2Y12 receptor. The concept of clopidogrel resistance emerged since several years ago. This biological finding has a very important impact especially in patients with acute coronary syndromes, explaining by this their worse evolution. Mechanisms of this resistance are very complex including: differences in intestinal absorption, hepatic conversion to the active metabolite through cytochrome 3A4 (CYP3A4) activity, drug interactions, individual variations in the activity of hepatic cytochrome P450 izoenzymes, platelet receptor polymorphisms and also clinical factors. According to this resistance patients are divided in two groups : "low or non-responders" (with a high risk of thrombosis) and "high - responders" (with a high risk of bleeding). Carriers of CYP2C19*2 or *3 alleles have been shown to respond poorly to standard doses of clopidogrel due to reduced metabolic activation of the drug. So, they are likely to develop subsequent thrombotic events. Starting from these, a lot of studies have been performed in order to elucidate the mechanisms of this resistance offering new perspectives regarding a proper administration of antiplatelet therapy in patients with acute coronary syndromes. **Key words:** CLOPIDOGREL, ACUTE CORONARY SYNDROME, GENETIC POLYMORPHISMS

Sindroamele coronariene acute (SCA) definesc un spectru larg de afecțiuni clinice: angina instabilă, infarctul miocardic non-Q şi infarctul miocardic cu supradenivelare de segment ST. Evoluția şi, implicit, prognosticul acestor bolnavi rămâne o problemă importantă, care impune o spitalizare selectivă şi adesea repetată, în special a celor cu risc crescut. Riscul cumulat pentru un eveniment ischemic în timpul fazei acute şi al următoarelor trei luni postevenIment este de aproximativ 50%, datorită timpului lung necesar stabilizării plăcii aterosclerotice (1). Pacienții cu SCA au un risc major de apariție a unor complicații severe ajungînd uneori la deces. Medicația antiplachetară constituie baza tratamentului antitrombotic în SCA, eficiența ei având dovezi incontestabile, datorită numeroaselor trialuri randomizate (2). În ultimii 10 ani s-au realizat progrese remarcabile în ceea ce priveşte strategia terapeutică a

SCA, inclusiv în terapia antiplachetară, incluzând dozele utilizate, durata tratamentului şi folosirea terapiei duale sau triple cu aceşti agenţi (3). SCA au un substrat anatomic comun, iar observaţiile anatomopatologice, angioscopice şi biologice au demonstrat că angina instabilă şi infarctul miocardic reprezintă în realitate aspecte clinice diferite ale unui mecanism fiziopatologic comun, respectiv ruptura sau eroziunea plăcii aterosclerotice, cu grade diferite de tromboză şi embolizare distală supraadăugate (4). Terapia antiplachetară este în mod curent folosită la pacienţii cu risc înalt de aterotromboză şi a devenit „*piatra de temelie"* în tratamentul bolii coronariene (5). În pofida succeselor obţinute în domeniul terapiei antiplachetare, pacienţii ce urmează un asemenea tratament au un risc crescut pentru producerea unui eveniment trombotic nou (6). Tienopiridinele (ticlopidina şi clopidogrelul) sunt inhibitori ai adenosin difosfatului (ADP), ducând la inhibarea agregării plachetare. Ticlopidina a fost investigată într-un singur studiu (7), intoleranţa la acest medicament fiind relativ frecventă datorită tulburărilor gastrointestinale sau reacţiilor alergice. În plus, ca efecte secundare mai pot apare neutropenia sau trombocitopenia. Din acest motiv, ticlopidina a fost înlocuită de clopidogrel care este unul dintre cei mai recomandaţi inhibitori plachetari la nivel mondial, cu toate că farmacodinamica variază substanţial la nivelul individual (8), astfel încât pacienţii cu răspuns suboptimal manifestă un risc major pentru un eveniment cardiovascular nou (9). La pacienţii cu sindroame coronariene acute, clopidogrelul se recomandă atât pentru tratamentul acut cât şi pentru cel de durată, de minim 9-12 luni. Acest preparat poate fi recomandat şi pentru terapia imediată sau de lungă durată la pacienţii care nu tolerează acidul acetilsalicilic (10), precum şi celor la care s-a implantat un stent (11). De-a lungul timpului au fost realizate teste pentru evaluarea gradului de inhibiţie plachetară la pacienţii trataţi cu clopidogrel: LTA – *light transmision aggregometry* ("standard de aur'' pentru predicţia evenimentelor cardiovasculare), VASP – *vasodilator stimulated phosphoprotein* şi PRI – *platelet reactivity index* (12). VASP este considerat ca fiind cel mai specific test pentru identificarea gradului de inhibiţie a receptorului plachetar P2Y12 (13). Unele studii au evidenţiat efectul inhibitor al clopidogrelului în combinaţie cu acidul acetilsalicilic (14). Cu toate că în cazul clopidogrelului beneficiul clinic a fost demonstrat, variaţiile genetice individuale, ar putea explica inhibiţia plachetară insuficientă, care reprezintă factorul de risc major pentru un nou eveniment trombotic. Conceptul de „*rezistenţă la clopidogrel"* datează de câţiva ani şi are două implicaţii: una clinică - legată de evoluţia şi implicit prognosticul nefavorabil al unora dintre pacienţii cu SCA şi, cea de a doua – practică, care implică necesitatea introducerii în uz a unor noi agenţi antiagreganţi plachetari, care să nu dezvolte fenomenul de rezistenţă (15). Studii recente (16) au arătat că aproximativ 31% dintre pacienţii cu SCA prezintă rezistenţă la Clopidogrel, aceasta fiind definită ca răspuns slab în ceea ce priveşte antiagregarea plachetară, această categorie de pacienţi având implicit o evoluţie clinică şi un prognostic nefavorabile. Printre enzimele hepatice care sunt implicate în acest proces, un rol cheie îl are citocromul CYP2C19, care la rândul său prezintă numeroase izoforme răspunzătoare de variaţiile activităţii catalitice, corelate cu variaţiile procesului de conversie al clopidogrelului în

metaboliții săi activi. Din punctul de vedere al vitezei de conversie a clopidogrelului pacienții pot fi metabolizanți lenți sau rapizi. Astfel, unii pacienți (”low – responders” sau ”non-responders”) pot prezenta un răspuns slab sau lipsă de răspuns în ceea ce privește procesul de antiagregare plachetară (urmarea fiind riscul crescut de tromboze). În această categorie se încadrează şi pacienții cu tromboză precoce intrastent (17). Altă categorie de pacienți (aşa- numiții ”high – responders”) prezintă un răspuns exagerat manifestat clinic prin risc crescut de sângerare.

În privința mecanismelor acestei rezistențe au fost emise mai multe teorii. Studiile de farmacocinetică au demonstrat faptul că clopidogrelul este un medicament care necesită o serie de transformări pentru a genera metabolitul activ, acesta din urmă fiind inhibitorul real al receptorului ADP, P2Y12 (18). După absorbția la nivel digestiv, 85% din doza de Clopidogrel este hidrolizată de către esteraze într-un metabolit lipsit de activitate antiagregantă. Restul de 15% urmează două trepte de oxidare care implică citocromul P450s şi astfel este generat metabolitul activ, care se leagă ireversibil de receptorul P2Y12, inhibând astfel agregarea plachetară. Transformarea „*in vivo*”, a prodrogului – clopidogrel, în metabolitul activ, reprezintă un pas critic în atingerea efectului antiplachetar. Clopidogrelul transformat în metabolitul activ, care blochează receptorul ADP - P2Y12, inhibă astfel activarea plachetară mediată de ADP. Această metabolizare este dependentă de activitatea izoenzimelor hepatice aparținând familiei citocromului P450 (CYP2C19, CYP1A2, CYP2B6, CYP2C9 şi CYP3A4). Răspunsul la clopidogrel este în consecință influențat de o cascadă de reacții răspunzătoare de formarea metabolitului activ (19). Metabolizarea clopidogrelului la nivel hepatic are ca prim pas transformarea acestuia în 2-oxo-clopidogrel, urmată de conversia în metabolitul activ**.** Citocromii CYP1A2, CYP2B6, CYP2C9 şi CYP3A4/5 sunt recunoscuți ca fiind implicați în metabolismul clopidogrelului, dar importanța fiecăruia, cât şi etapa exactă în care aceştia sunt implicați este controversată (20). Anumite publicații (21) indică CYP3A4 ca factor major implicat în metabolismul clopidogrelului, în timp ce altele conferă citocromilor CYP1A2, CYP2B6 şi CYP2C19 acest rol (22). Kazui *et al.* (23) au arătat că CYP2C19 contribuie substanțial la metabolizarea clopidogrelului, în timp ce CYP3A4 are rol doar în conversia în 2-oxo - clopidogrel. Conform altor studii (24, 25, 26, 27, 29), nu ar exista o legătură directă între CYP3A5 şi efectul antiplachetar al clopidogrelului. În prezent, cel puțin 25 de polimorfisme ale unui singur nucleotid (*single nucleotide polymorphisms* - SNP), au fost descrise în gena care codifică CYP2C19, din care alela CYP2C19*2 (exonul 5), este cea mai cunoscută şi studiată. Ea se pare că induce o scădere semnificativă a concentrației de metabolit activ seric şi reduce astfel inhibiția agregării plachetare (24). Prezența alelei ce determină pierderea funcției enzimatice (CYP2C19*2) este cel mai studiat polimorfism, fără a exista, însă, un consens. Lee *et al.* (25) au arătat că există diferențe etnice în ceea ce privește frecvența polimorfismelor. Se pare că alela CYP2C19*3A este mai frecventă în populația asiatică decât în cea americană (25), considerându-se că 20-30% din populația SUA este heterozigotă pentru polimorfismele CYP2C19*2, în timp ce 2-3% sunt homozigoți, cu activitate enzimatică nulă, date similare celor din populația europeană (26). Deşi celelalte SNP sunt rare, sunt

necesare studii suplimentare, pentru a elucida rolul mai puțin cunoscut al acestor polimorfisme CYP2C19. Recent a fost raportat că heterozigoții compuși CYP2C19*3A - CYP2C19*2A, au un risc crescut de tromboză acută intrastent (27). Pacienții tratați cu clopidogrel și care au alela CYP2C19*2A, ar avea un risc crescut pentru evenimentele ischemice recurente (28), prin comparație cu alela CYP2C19*17 care a fost asociată cu o exacerbare a activității enzimatice, fiind considerate o mutație cu „câștig de funcție", care poate crește riscul de sângerare în timpul tratamentului cu clopidogrel (29). Studii recente au evidențiat influența variantei alelice C3435T (rs1045642) a genei *ABCB1* în absorbția la nivel intestinal a clopidogrelului la pacienții cu boli cardiovasculare (30).

Aceste evidențe susțin pe de o parte ideea necesității unor teste sau determinări care să identifice pacienții ce ar putea prezenta acest fenomen de rezistență, înainte de începerea tratamentului, iar pe de altă parte se impune realizarea unor preparate noi care să inducă fenomenul de rezistență și, eventual, să nu necesite metabolizare hepatică.

În prezent, cele mai cunoscute, specifice și sensibile metode de determinare a SNP-urilor (care pot varia în funcție de polimorfismul vizat), sunt:

- **TaqMan assays** (Applied Biosystems, Life Technologies, Pleasanton, CA, USA)**,** care se bazează pe discriminarea alelică prin Real-Time PCR în sistem TaqMan. Pentru fiecare alelă este utilizat același set de primeri și câte o sondă cu fluorescență specifică. Prin degradarea sondelor de către polimerază (cu proprietați exonucleazice 5'-3'), în timpul amplificării se va emite (doar de către sonda hibridizată), o anumită fluorescență. Aceasta va permite discriminarea alelică sub forma unui cod de culori ;

- **Reverse Hybridization** se bazează pe o amplificare PCR, urmată de hibridizarea ampliconului format, pe un „*strip"*, marcat în prealabil cu sonde specifice fiecărei alele, pentru ca apoi, printr-o reacție cu un anticorp biotinilat și o enzimă, să se obțină o reacție de culoare caracteristică;

- **Secvențierea** ampliconului de interes este singura metodă capabilă să identifice toate mutațiile și polimorfismele din regiunea de interes. De asemenea, secvențierea este și o metodă de validare a rezultatelor obținute prin metodele alternative, dar rămâne o metodă complexă, consumatoare de timp și fonduri.

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NOUTĂȚI

ROLUL TOR ÎN REGLAREA CELULELOR STEM

Factorul țintă al rapamicinei (TOR - Target of Rapamycin) controlează un sistem de semnalizare care modulează creşterea celulară şi diviziunea prin detectarea nivelurilor de nutrienți, energie şi stress. Semnalizarea mediată de TOR este o componentă crucială a țesuturilor şi organelor, care traduce semnalele în comportamente celulare. TOR are rol în apariția neoplaziilor, a bolilor degenerative şi în procesul de îmbătrânire, dar modul de acțiune nu a fost foarte clar elucidat. Natura ubicuitară a semnalizării TOR şi dificultatea analizării țesuturilor în cursul turnover-ului au limitat înțelegerea modului în care această kinază funcționeză în organism. Viermii Planaria au reprezentat un model experimental pentru studiul reglării TOR la nivelul organismului. Aceşti viermi sunt cunoscuți pentru capacitatea lor de regenerare celulară, iar studiul lor ar putea oferi noi indicii privind elaborarea unor terapii anticanceroase. TOR are un rol esențial în regenerarea celulară la Planaria, inhibiția sa împiedicând creşterea acestor viermi. Aceste observații sugerează că blocarea TOR în celulele care prezintă anomalii ar putea preveni proliferarea neoplazică. Smed-TOR, un omolog al TOR de la planarii este exprimat ubicuitar, inclusiv de către celulele stem şi țesuturi diferențiate. Inhibiția TOR prin interferența ARN inhibă proliferarea celulară, permițând studiul neoblaştilor cu o capacitate proliferativă redusă în cursul regenerării şi turnover-ului celular. Semnalizarea TOR este necesară pentru răspunsul neoblaştilor la amputație şi proliferarea la nivel de blastema. În absența semnalizării TOR, regenerarea are loc doar în țesuturile diferențiate. În plus, TOR este esențial în menținerea echilibrului între diviziunea celulară şi moartea celulară, iar disfuncționalitățile acestui sistem duc la degenerare tisulară şi lipsa creşterii în prezența nutrienților. (Harshani Peiris T., Weckerle F, Ozamoto E, Ramirez D et al. TOR Signaling Regulates Planarian Stem Cells and Controls Localized and Organismal Growth. *J CellScience*, 2012 DOI: 10.1242/%u200Bjcs.104711, Advance Online Publication: http://jcs.biologists.org/content/early/2012/03/16/jcs.104711.abstract)

Teodora Vremeră

TOXOPLASMOZA PULMONARĂ LA PACIENTUL IMUNODEPRIMAT

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PULMONARY TOXOPLASMOSIS IN IMMUNOSUPPRESSED PATIENT– (Abstract): Pulmonary toxoplasmosis occurs mainly in immunosuppressed patients and its diagnosis mainly relies upon biological confirmation of the parasite. We present the case of a 47 years patient in medullar aplasia after induction chemo-therapy for acute lymphoblastic leukemia that developed pulmonary infiltrates of parasitic origin. The diagnosis of pulmonary toxoplasmosis was established after identification of the parasite in brochioloalveolar lavage fluid (BAL) and peripheral blood. Serological tests are of limited utility in immunosuppressed patients. We used classical methods for the diagnosis of parasitosis but they are being replaced by molecular methods. Polymerase Chain Reaction (PCR) allows a highly specific and sensitive diagnosis on any sample but it cannot be performed in any center. **Key words:** PULMONARY TOXOPLASMOSIS, IMMUNOSUPPRESED PATIENT, BRONCHIOLOALVEOLAR LAVAGE FLUID, CEREBRAL LESIONS.

Toxoplasmoza este o infecţie parazitară insidioasă, frecvent oligo-simptomatică şi insuficient diagnosticată, al cărei agent etiologic este *Toxoplasma gondii***.** Ciclul parazitului implică gazdă definitivă pisica sau unele feline sălbatice, iar cea intermediară, poate fi reprezentatăde orice mamifer vertebrat, inclusiv omul (1).

Cele două forme parazitare sunt reprezentate de tahizoid, care se multiplică intratisular şi bradizoid, o formă rezistentă intrachistică. Gradul afectării organelor şi sistemelor, severitatea manifestarilor clinice, evoluţia bolii şi prognosticul depind de integritatea sistemului imun (2, 3).

MATERIAL ŞI METODE

S-a inclus în studiu pacientul M.T. în vârstă de 47 de ani, diagnosticat cu leucemie limfoblastică acută, supus chimioterapiei de inducţie cu prednison, vincristină, adriamicină şi imatinib (cromozom Philadelphia pozitiv). La 2 săptămâni de la terminarea tratamentului pacientul a prezentat tuse seacă, uscată şi sindrom febril. Pacientul a fost internat in Clinica de Hematologie cu sindrom febril, rinoree şi insuficienţă respiratorie. Examenul hematologic a relevat pancitopenie şi creşterea gradientului de oxigen alveolo-arterial. Radiografia toracică a evidenţiat infiltrate pulmonare bilaterale, difu-

ze. Se instituie tratament cu antibiotice cu spectru larg suspicionându-se o pneumopatie . Starea generală a pacientului se alterează progresiv cu apariția obnubilării şi a unei stări confuzionale. Examenul prin tomografie computerizată a permis identificarea unei leziuni frontale drepte cu edem perilezional şi aspecte similare în regiunea talamică stângă. Puncția lombară a evidențiat o presiune de 180 cmH_2O, leucocite $1/mm^3$, proteine 30 mg/dl, glucoză 40 mg/dl. Testul ELISA a fost pozitiv (fig. 1).

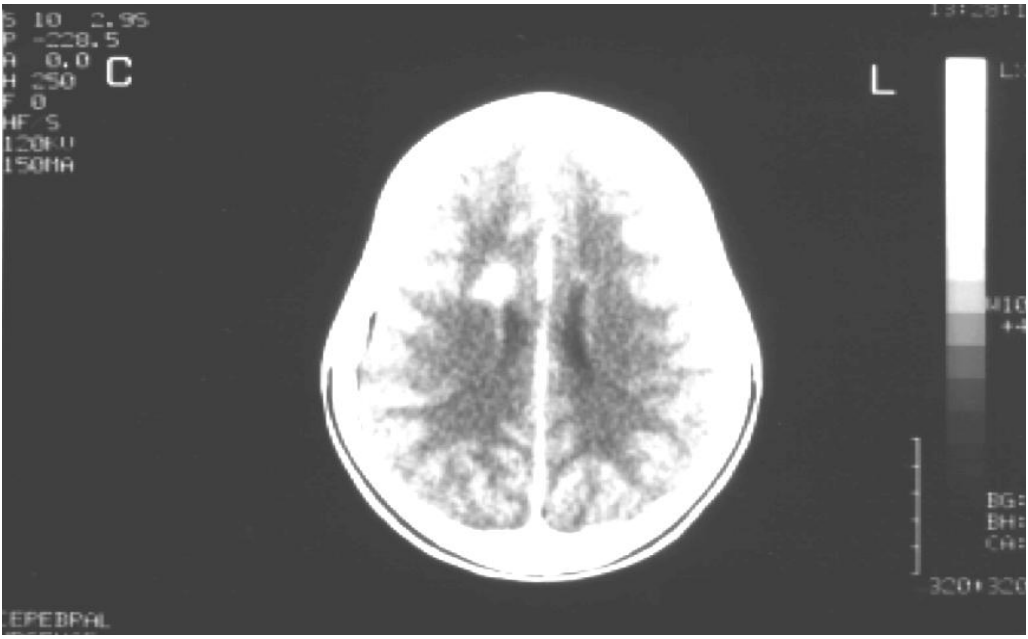

Fig. 1. Tomografia computerizată cerebrală

REZULTATE ŞI DISCUȚII

Examenul serologic pentru *T. gondii* a fost pozitiv pentru IgG şi negativ pentru IgM. Lavajul bronhioloalveolar (LBA) a identificat prezența de *T. gondii*.

Deşi manevrele terapeutice au fost intensive, insuficiența respiratorie a progresat rapid şi s-a impus suport ventilator. Pacientul a decedat în următoarele 72 de ore prin insuficiență de organ multiplă.

Ca şi in cazul nostru, toxoplasmoza pulmonară se traduce frecvent prin pneumopatie interstițială febrilă care asociază tuse seacă şi dispnee şi poate evolua spre insuficiență respiratorie acută. Datorită frecvenței scăzute a acestui sindrom clinic, diagnosticul de toxoplasmoză pulmonară poate fi uşor omis mai ales în contextul unor expresii clinice şi radiologice nespecifice (4, 5).

Vizualizarea directă a tahizoizilor pe preparatele Giemsa se caracterizează printr-o sensibilitate redusă şi depinde de medicul anatomopatolog care realizează interpretarea. În acest context se impun efectuarea unor tehnici de diagnostic moderne, rapide şi efective de tipul PCR, dar limitate de posibilitățile tehnice ale spitalelor din sistemul de stat.

Diagnosticul toxoplasmozei pulmonare

este astfel dificil şi se caracterizează prin absența unui aspect patognomonic clinico-radiologic deoarece rareori poate fi considerată cauză majoră a pneumoniei. În cazul pacienților imunosupresați infecția cu *T. gondii* poate fi mascată de alte semiologii infecțioase ceea ce face ca diagnosticul corect biologic şi serologic să fie delicat, cu o rată de supraviețuire de până la 15% (6).

Serologia constituie baza diagnosticului la pacienții imunocompetenți, dar interpretarea testelor serologice specifice devine dificilă în condițiile unui status imunodeprimat. Pacienții sunt frecvent IgG specifici, iar IgM apare doar în 6-12% din cazuri. Avantajul observării directe a prezenței parazitului în lichidul obținut prin lavaj bronhoalveolar(LBA) are o valoare predictiv pozitivă de 90% şi poate fi argumentată până la 100% prin biopsie pulmonară (7).

Introducerea metodelor moleculare (PCR) poate ameliora considerabil diagnosticul toxoplasmozei mai ales în formele pulmonare. Aşa cum arată studiile de specialitate, sensibilitatea PCR este de 100% în cazul toxoplasmozei indiferent de laboratorul în care se efectuează explorarea. În consecință, rata de supraviețuire poate creşte până la 60% la pacienții imunodeprimați infestați cu toxoplasma (8).

În cazul prezentat, toxoplasmoza a fost diagnosticată prin examinarea LBA, plămânul fiind al 2-lea organ afectat în cazul toxoplasmozei extracerebrale. Infecțiile cu oportuniști la pacienții imunosupresați nu sunt rare şi explică deterioarea stării clinice cu sindrom de detresă respiratorie acută şi MOFS diagnosticate în cazul inclus in studiu.

T. gondii modulează expresia genică a celulelor endoteliale cerebrale favorizând migrarea prin bariera hemato-encefalică. Mortalitatea este mai mare la pacienții imunodeprimați. Seroprevalența creşte direct proporțional cu înaintarea în vârsta, iar afectarea toxoplasmotică devine multiorganică. Dacă la pacienții imunocompetenți infecția este, în general, asimptomatică, la cei imunodeprimați tabloul clinic este sever şi reprezintă de obicei reactivarea unei afecțiuni subclinice prezentate anterior. Simptomele localizării cerebrale asociază febră, cefalee şi stare confuzională care pot progresa până la comă şi deficite neurologice focale. Limfadenopatia nu este frecvent întâlnită, iar parazitul poate fi responsabil de inflamarea neurogenică ce apare în diferitele tipuri de cefalee. Simptomele evoluează în zile şi săptămâni iar toxoplasma se localizează, predominant, în ganglionii bazali determinând hemicoree, hemibalism, parkinsonism sau tremor. Encefalita difuză apare în stadiile incipiente chiar în condițiile unui examen imagistic normal. Analiza LCR relevă pleiocitoză mononucleară cu creşterea proteinorahiei şi niveluri normale sau scăzute ale glicorahiei (4, 6).

CONCLUZII

Afectarea pulmonară în toxoplasmoza la pacienții imunodeprimați este frecvent, sublinică şi asociază determinări cerebrale. Investigarea LBA este o metodă eficientă de diagnostic. Mortalitatea este crescută, cauza principală fiind apariția insuficientei multiple de organ (MOFS)determinată de afectarea multiorganică.

Si in cazul nostru tusea, sindromul febril şi insuficiența respiratorie s-au dovedit a fi simptomele de alarmă, situație comună pentru pacienții imunodeprimați infestați cu *T. gondii*.

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NOUTĂȚI

QUORUM QUENCHING: O NOUĂ STRATEGIE DE LUPTĂ ANTI-INFECȚIOASĂ PRIN INTERFERENȚA SISTEMELOR DE COMUNICARE INTER-BACTERIANĂ

Numeroase funcţii bacteriene, precum virulenţa şi producerea de biofilme, sunt controlate de un mecanism de comunicare depedent de densitatea celulară cunoscut sub numele de Quorum Sensing (QS). Acest mecanism se bazează pe eliberarea unor molecule mici, difuzibile, care permit bacteriilor să-şi coordoneze comportamentul în funcţie de densitate. Interferenţa cu aceste sisteme de semnalizare, numită Quorum Quenching (QQ), reprezintă o perspectivă atrăgătoare pentru combaterea infecţiilor bacteriene. Controlarea producerii biofilmului de către sistemele QS extinde, în perspectivă, aplicabilitatea QQ, făcând posibilă rezolvarea problemelor legate de formarea biofilmelor implicate în boala periodontală. În plus, dat fiind faptul că biofilmul creşte rezistenţa bacteriilor la acţiunea substanţelor antimicrobiene, QQ ar putea reprezenta o nouă metodă de luptă contra tulpinilor nosocomiale multirezistente. (Romero M, Acuna L, Otero A. Patents on quorum quenching: interfering with bacterial communication as a strategy to fight infections. *Pat Biotechnol* 2012; 6(1): 2-12).

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NEW DATA OF BLOOD TRANSFUSION IMPORTANCE IN PATHOLOGY

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NEW DATA OF BLOOD TRANSFUSION IMPORTANCE IN PATHOLOGY (Abstract): Blood transfusion is a very important aspect of clinical hematology. An essential particularity is the correctly use of blood products because it can cause immediate or delayed complications. Transfusion security and efficiency requires integrated strategies and the purpose of the transfusion is to provide the patient with blood components to improve their hematologic status. From a single total blood donation can be obtained multiple type of blood components. Transfusion centers are capable of separating plasma erythrocytes, or of other products such as platelet concentrates, or cryoprecipitate named 'labile blood products'. On the other side plasma derivates, are made by fractionation of plasma. All the blood components to bring benefit must be viable when transfused. The blood or the resulting compounds must be preserved, transported and stored under conditions that meet quality criteria and follow the standardized procedures. **Keywords**: BLOOD PRODUCTS, BLOOD TRANSFUSION

Transfusion of blood components and the differential diagnosis or treatment of acute transfusion reactions represent conditions of great importance for clinicians, nurses, clinical laboratory and the transfusion centers. This is a reason to discuss some base directions concerning some of the blood transfusion preanalytical tests, use of blood products for transfusion in pathology and management of transfusion reactions. In a healthy person who does not have a bleed, a hemoglobin concentration of 70-80 g/L is normally sufficient to maintain oxygenation of the tissues. This is the partial effect of an improved microcirculation caused by a low blood viscosity. Therefore, in this context, erythrocyte transfusion is normally not necessary unless hemoglobin falls below this level. However, if the compensation mechanisms are not sufficient to adequately increase the cardiac output, for example due to heart failure and/or pulmonary function or sepsis, a higher level (90-100 g/L) may be appropriate.

General principles in blood transfusion

There must be documented procedure, standards and a good laboratory practice in

quality management system for the safety of the patient. There are some basic blood transfusion rules such as (1):

1 - assessment of the need for transfusion

2 - assessment of the benefits and risks of transfusion

3 - patient will receive that blood component he needs

4 - administration of blood or blood derivatives will be made taking at least the blood group into the OAB, Rh, Kell systems of the donor and the recipient

5 - correct and complete performance of the pre-transfusion compatibility test

6 - precise patient identification

7 - last check at the patient's bed

8 - using alternatives to blood transfusion whenever is possible and reducing the number of transfusions that are not necessary.

The pre-transfusion protocol

Pre-transfusion testing include laboratory testing necessary for compatibility between the blood of the transfusion recipient and the blood product intended for transfusion.

This process includes the following (2):

a) determination of ABO and Rh (D) blood group in the patient; in the case of infants and infants up to 6 months, the Coombs direct test will also be performed to confirm the ABO blood group; b) in special situations (newborns, infants, children, multiple transfusion, fertile age women, transplants, immunosuppressed, immunized), it is necessary to carry out blood group determinations in other erythrocyte antigenic systems;

c) detection of irregular anti-erythrocyte antibodies in immunized and multiple transfusion patients;

d) verifying the macroscopic aspect, integrity, ABO and Rh (D) blood group in the blood unit or blood component selected for compatibility testing

e) compatibility test between patient and total blood unit or blood component. All three complementary techniques - saline test, papain enzyme assay at 37°C, indirect Coombs test will be performed.

The last pre-transfusion control means:

a) checking the identity of the patient;

b) checking the macroscopic appearance and integrity of the transfusion unit;

c) checking the compatibility between the selected blood unit and the patient:

- determination of ABO and Rh (D) blood group in the patient;

- ABO and Rh (D) blood group determination at the transfusion unit;

- documentary verification;

- recording in the score sheet.

Traceability of blood components

Refers to the principle that all blood/blood components must be traceable from the donor to the patient and in reverse from the patient to the donor. It is an integral part of providing a safe blood service to patients. Traceability, which is a preliminary condition for hemovigilance, can be defined as the ability to follow each unit of blood or blood components from the time of collection from the donor to the destination, whether it is a patient, a manufacturer of medicinal products or a disposal center, and vice versa. This include the amount of recorded information and of measures that allow the tracking and identification of each stage of the activity, from the person's admission to donation to the therapeutic use of blood and human blood components; traceability allows establishing a link between the donor and one or more recipients

and from the recipient to the donor, being established using a unique national system for identifying blood units and individuals (3).

Traceability is essential for identify:

• retrospectively a potentially infected donor in case of transmission of an agent to a beneficiary;

• retrospectively a potentially infected recipient in the case of an infected donor;

• beneficiaries in case of systemic problems that expose beneficiaries to the risk or episodes of serious side effects.

Also, traceability can provide information about the total number of transfused patients, blood units or components issued or used and blood donors who have provided transfused blood units or components.

Blood products used in specific clinical situations

Acute blood loss

Initial measures taken to manage acute blood loss are aimed to stop bleeding by treating any traumatic, surgical or obstetric source and keeping the blood flow within the organs in critical condition. If organ and tissue oxygenation is severe affected, red blood cell transfusion may be needed to improve tissue oxygenation. The need for a erythrocyte transfusion should be evaluated individually and based on clinical signs and critical evaluation of laboratory test results. Hemoglobin and hematocrit values should be measured frequently but considering that they may be a poor indicator of blood loss in the acute situation.

Massive transfusion can be defined as replacing the patient's total blood volume with donated blood in less than 24 hours, replacing 50% of the blood volume within 3 hours, or transfusion to compensate blood loss that exceeds 150 mL per minute (4). Early recognition of lost blood amount and effective action are needed to prevent the shock and its consequences. Effective communication between clinicians, diagnostic laboratories and blood banks is of major importance and a massive transfusion procedure/protocol (MTP) is recommended.

Correction of coagulopathy in this context requires the judicious use of blood component therapy (erythrocytes, platelets, fresh frozen plasma, and, in some cases, cryoprecipitate or fibrinogen concentrate and pharmacological agents).

Chronic anemia

The treatment of chronic anemia due to deficiency of iron, folic acid/vitamin B_{12} should be primarily directed to correct the cause of anemia. Transfusion of erythrocytes is rarely necessary. When measures to correct the cause of anemia are ineffective, erythrocyte transfusion may be considered (5). The decision to perform a transfusion should be based on the clinical condition of the patient, considering the level of hemoglobin, the duration of anemia and the presence of conditions affecting the transport of oxygen, such as pulmonary insufficiency, cardiac disease or cardiovascular disease. In chronic diseases such as uremia and malignancies, increasing anemia is significantly associated with increased fatigue, which has a significant negative impact on functional abilities and patient quality of life. Transfusion therapy in chronic anemia, such as that associated with malignancy, usually has the goal of maintaining hemoglobin levels between 80 and 100g/l, while a level of >100g/L may be adequate for some patients, to avoid angina or heart failure.

The need for transfusion should be as-

sessed individually and should consider the fact that different patients need different transfusion support to maintain an acceptable social situation and maintain their physical performance but also to experience a satisfactory quality for the life. Regular transfusion therapy, although effective, has several disadvantages. The effect is short-lived and there is a risk of overloading with iron, which can harm the body. Treatment with erythropoiesis stimulating agents (ESA) to maintain hemoglobin up to 120 g/L is usually effective in the treatment of anemia in patients with renal impairment but not all patients respond to this treatment. ESAs should be used with caution in cases of malignant tumors and its use is contraindicated in some cancers, while in other cases it may be ineffective. Larger use of ESA in the treatment of chronic anemia will depend on their cost, effectiveness and safety. In patients with thalassemia and those with severe, chronic, life-threatening hemolytic anemia, transfusion therapy involves regular lifetime blood transfusions, usually given every 2-5 weeks, to maintain hemoglobin pre-transfusion greater than 90-105 g/L.

It has been shown that this moderate transfusion regimen promotes normal growth, allows normal physical activity, appropriately suppresses bone marrow hyperactivity in most patients, and minimizes iron accumulation. Higher hemoglobin pre-transfusion levels (110-120 g/L) may be more appropriate in patients with heart disease or other medical conditions, and in those patients where adequate bone marrow suppression is not achieved at lower hemoglobin levels.

In siclemia, the indications for transfusion therapy with erythrocytes are anemia and vascular occlusion. Transfusion therapy is not normally indicated in patients with hemoglobin values greater than 70 g/L. In the presence of vascular occlusion, the goal of transfusion therapy is to prevent or stop intravascular formation of sickle-shaped cells by diluting or replacing circulating pathological erythrocytes with the normal one. Patients with sickle cell disease should be transfused with erythrocytes that has no hemoglobin S. Patients with these conditions are unlikely to develop vaso-occlusive episodes when the percentage of hemoglobin S is less than 30%. Exchange of erythrocytes may be necessary in major elective surgery, ocular surgeries, and to prevent or treat acute vaso-occlusive episodes.

Acute autoimmune hemolytic anemia (AAHA)

Transfusion should normally be avoided in this context but may be necessary in life-threatening situations despite serological incompatibility (6). This anemia is caused by the acute destruction of the red blood cells by autoantibodies directed against its own antigens from the erythrocyte membranes or against modified structures of the patient's red cell membrane. The target antigens of these autoantibodies are often antigens present in most blood donors, and transfusion is therefore less effective. In an autoimmune hemolytic anemia, it is very important to correctly diagnose the type of AAHA

1. **Warm antibody autoimmune hemolytic anemia** type Ig G (with IgG type circulant autoantibodies that bind to erythrocyte antigens at a temperature of 37°C. It is mainly found in lymphoproliferative syndromes (type B, LLC) lupus erythematosus disseminated and occasionally with different cancers/may be idiopathic. Rare-

ly, autoantibodies may be IgM / IgA type and hemolysis are predominantly extravascular.

2. **AAHA with cold-IgM antibodies** (Cold agglutinin disease)-manifested as a chronic disease, often associated with macroglobulinemia, LLC, or in transient forms during the complications of infectious diseases encountered in children.

The autoantibodies are IgM type and intravascular hemolysis is possible

3. **AAHAI mixed type** were the autoantibodies are IgG and IgM.

Thrombocytopenia

The purpose of platelet transfusion is to prevent and treat hemorrhage in patients with thrombocytopenia or platelets dysfunction. The decision to transfuse the platelet components should be based on the platelet count, the clinical condition of the patient and the clinical context. Normally, before deciding on the use of platelet transfusion, the cause of thrombocytopenia should be determined (7). Platelet transfusions are not indicated in all causes of thrombocytopenia and may be contraindicated in certain conditions.

In thrombocytopenia due to primary or secondary bone marrow failure, thrombocyte therapeutic transfusions are indicated for patients with active hemorrhage associated with thrombocytopenia. Thrombocyte therapeutic transfusions are used when the platelet count is less than $5\text{-}10x10^9$/L and in the absence of hemorrhage or another platelets-consuming factor. The presence of fever, local lesions, coagulation disorders and/or other unwanted symptoms may require prophylactic transfusions to maintain a platelet count of $>15\text{-}20x10^9$/L.

In chronic failure of platelet production caused by aplastic anemia or myelodysplasia, long-term prophylactic transfusions should be avoided due to the risk of HLA/HPA alloimmunization and platelet refractoriness. Many patients do not suffer from severe hemorrhage, despite the $5\text{-}10x10^9$/L platelets. If platelet transfusions are needed, the interval between transfusions should be evaluated individually.

In thrombocytopenia and surgical procedures or invasive procedures platelet transfusions should be considered to maintain a platelet count greater than $50x10^9$/L in patients to be undergoing a major invasive procedure or in situations where direct hemostasis is not possible (liver or transbronchial biopsies). Higher platelet levels ($>100x10^9$/L) may be required in clinical contexts involving major bleeding risks (intraocular or neurosurgical procedures) (8).

In case of patients with platelet function disorders rarely require platelet transfusions. The cause of impairment of the function (drugs affecting the platelet function) should be identified and corrected, if possible, prior to surgery or invasive procedures. Platelet transfusion should be considered in patients with platelet function disorders when the patient requires an invasive procedure and there is an increased risk of bleeding. In this context, the platelet count is not a reliable indicator of the risk of bleeding and the platelet transfusion should be continued until the risk of bleeding has ceased. Transfusion could also be indicated in spontaneous hemorrhages that do not respond to simple local treatment or other measures.

Clinical indications
for fresh frozen plasma (FFP)

The purpose of FFP transfusion is to correct clotting irregularities in a patient

with hemorrhage with a deficiency of multiple coagulation factors. FFP has very few indications. It can be used in situations where there are several deficiencies in coagulation factor or in case the deficiency of a single coagulation factor if a concentrate for the coagulation factor with reduced pathogens is not available. The clinical effects of FFP transfusion are often difficult to evaluate. The clinical response may indicate the efficacy of fresh frozen plasma transfusion, especially in hemorrhagic patients (9). When FFP is transfused to correct abnormal levels of hemostatic variables, the effect should be monitored both to support decision-making on continued treatment. Monitoring clinical signs and hemostatic variables will help assess the suitability of the assistance and help in selecting components but should not delay the initial release of FFP.

Surgical bleeding and massive transfusion

There is considerable debate about the proper mixing of blood components used in early management of patients with massive blood loss. Observational studies have suggested that an initial percentage of FFP: Erythrocytes of 1:1.5 is associated with decreasing mortality. These transfusion protocols should be supplemented with clinical signs and appropriate coagulation tests. FFP should be used to maintain prothrombin time and partial thromboplastin time at approximately 1.5 times normal or less and to maintain fibrinogen above 1.0 g/L.

Disseminated intravascular coagulation (DIC)

Activation of fibrinolytic and coagulation systems consumes proteins and coagulation inhibitors, fibrinogen and platelets. Clinical presentation may range from major hemorrhage (with or without thrombotic complications) to a compensated condition diagnosed only with laboratory tests (10). Acute DIC is most likely to occur when the coagulation (and fibrinolytic) system is massively activated by the underlying disease, and natural inhibitors do not balance this massive activation. The primary cause of DICC should be treated. FFP, plasma support, platelets and fibrinogen or cryoprecipitate concentrate may be necessary if the patient is hemorrhagic. An initial dose of 15 ml/kg of FFP is recommended, although there is evidence that a dose of 30 mL/kg causes a higher recovery of coagulation factor levels.

Deficiencies of a single coagulation factor

FFP can only be used when a coagulation factor concentrates required to treat single coagulation factor deficiency is not available. Currently, it is mainly applied only to the rare inherited deficiency of the coagulation factor V.

Hepatic diseases

The bleeding tendency is caused by the reduction in coagulation factor synthesis, dysfibrinogenemia, thrombocytopenia and increased fibrinolysis. If there is active bleeding, support treatment with FFP may be necessary. The response to the FFP cannot be predicted when it comes to correcting abnormal test results before invasive procedures in patients with liver disease. The routine use of FFP has been challenged in this case. If FFP is given, coagulation tests will be repeated, and future therapy will be determined based on the results of these tests.

Thrombotic Thrombocytopenic Purpura (TTP)

The accumulation of high molecular weight molecular weights of Willebrand factor (HMW-vWF) due to a deficiency of the metalloproteinase enzyme (ADAMT13) leads to the activation and consumption of platelets. Plasma exchange with FFP should be performed as soon as possible, preferably within 24 hours of symptom manifestation. Daily plasma exchange should continue for at least 2 days after remission

Clinical use of granulocytes

The purpose of granulocyte transfusion is to improve the clinical status of patients with severe and infected neutropenia who do not respond to appropriate antibiotic therapy. These are rarely necessary. Granulocyte transfusion should only be used in patients with neutropenia that meet all the following criteria:

- the number of neutrophils is less than 0.5 x 10^9/L due to congenital or acquired medullary insufficiency;
- more than 24-48 hours, proven or highly probable fungal or bacterial infection, lack of response to appropriate antifungal therapy or antibiotic therapy;
- the hope of recovering the number and function of neutrophils or if bone marrow transplantation is planned;
- patients with severe or congenital granulocytic dysfunction, regardless of granulocyte count, or with a fungal infection or bacteria that is proven or suspected to be life-threatening, and who do not respond to appropriate antibacterial or antifungal therapy for at least 48 hours. Prophylactic granulocyte transfusions are not recommended (11).

Transfusion in obstetrical practice

Major obstetric hemorrhage remains an important cause of maternal death. Inappropriate transfusions during pregnancy and the postpartum period expose the mother to the risk of hemolytic disease of the fetus and newborn (HDFN) in subsequent pregnancies. Prevention and treatment of anemia in pregnancy (usually iron deficiency) avoids unnecessary blood transfusion (12). A blood count should be checked at the antenatal booking visit and at 28 weeks (allowing sufficient time to treat iron deficiency before delivery). Oral iron replacement is appropriate for most patients, but intravenous iron (after the first trimester) may produce a more rapid response and should be used in women intolerant of oral iron. Transfusion is rarely required in hemodynamically stable pregnant women with Hb >70 or 80 g/L unless there is active (or a high risk of) bleeding. Cytomegalovirus (CMV) seronegative red cells should be provided for elective transfusions in pregnancy but standard, leuco depleted units may be used in an emergency to avoid delay (13). Successful management depends on well-rehearsed multidisciplinary protocols, rapid access to red cells (including emergency group O negative units) and excellent communication with the transfusion laboratory. Access to cell salvage reduces use of donor blood and early administration of tranexamic acid may reduce mortality. Pregnancies potentially affected by HDFN should be managed by specialist teams with facilities for early diagnosis, intrauterine transfusion and support of high dependency neonates. Clinical audits show that many transfusions in pregnancy, especially in the postpartum period, are inappropriate and could be prevented by better antenatal monitoring and

the targeted use of iron supplements (14). The decision to transfuse should be made after careful clinical assessment rather than based on a specific Hb concentration.

CONCLUSIONS

The decision to apply for blood transfusion therapy is taken by the clinician only in cases where all alternative methods have been used to solve the problems related to the treatment of the patient.

The doctor bears responsibility for the benefit or risks of blood transfusion and all risks should be considered, especially immunological and infectious. Nevertheless, the transfusion benefit must be greater than the risks.

Plasma management allows most blood transmissible infections to be transmitted and the use of blood and blood derivatives proper, means the use of blood products exclusively for the treatment of conditions that cause morbidity or mortality that cannot be prevented or treated by other means.

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ROLUL IgE SPECIFICE LA COPIL ÎN EVOLUȚIA ŞI PROGNOSTICUL ALERGIEI LA PROTEINELE LAPTELUI DE VACĂ

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3. Clinica a V-a Pediatrie

THE ROLE OF SPECIFIC IgE TO EVOLUTION AND PROGNOSIS OF COW'S MILK PROTEIN ALLERGIES IN CHILD (Abstract): Cow's milk allergy affects 8% of infants less than 1 year of age. The allergy is usually transient, with most children tolerating ingested cow'milk by age 3 years. This prospective study analyzes the clinical course, development of tolerance and risk for other atopy (asthma, rhinoconjunctivitis, atopic dermatitis) in children with cow's milk allergy. **Material and methods:** We followed 71 infants hospitalized between January 2006 – January 2010 in two clinic of Pediatrics from Iaşi and Galați with gastro-intestinal, respiratory and skin signs and symptoms of cow's milk allergy. In this study were identified atopic symptoms and diseases, family history of atopy, measured serum total IgE levels and was evaluated development of tolerance to cow's milk. IgE levels was measured at diagnosis, 12 months after diagnosis and recovery tolerance to cow's milk. Patients were followed to acquire tolerance to cow milk. **Results**: The median age of patients was 7.57 months ±2.73DS. IgE-mediated cow's milk allergy was detected in 40.85% (29 cases) of children at diagnosis. After 12 months of follow 7 (24.14%) of 29 cases initially IgE positive became negative. The first rechallenge was carried out 12 months after diagnosis at mean age 1.6 years (95%CI, 1.5-1.6 years) and the result was positive in 12 cases of IgE negative group. All children (100% of cases) with IgE-negative cow'milk allergy were tolerant by 3.0 years old ($P < 0.0001$) compared to 70.73% in children with positive IgE. In the end 17.24% of patiens with IgE-mediated cow milk allergy presented respiratory and skin atopic sings. Are there significant differences about the persistent cow'milk allergy between the group of children with positive IgE compared to negative IgE. ($p = 0.1918$, 95% CI). **Conclusions**: Most children recover their tolerance to cow milk during childhood and those with negative IgE even at young ages. Patients with positive IgE have an increased risk for allergic diseases, food and inhaled allergens sensitization and development of persistent cow's milk allergy. **Key words**: COW'S MILK ALLERGY, IgE, SENSITIZATION, TOLERANCE, ATOPY

Alergiile alimentare, definite ca reacții alimentare netoxice cu mediere imună (1), reprezintă un domeniu relativ nou de cercetare, studiile statistice ale ultimelor decenii indicând faptul că acest tip de patologie afectează 1-2% din populația adultă şi se ridică până la un procent de 8% în cazul copiilor (2). La ora actuală, cele mai stu-

diate reacții alergice alimentare sunt cele mediate de IgE, dar, fiind vorba de tulburări deosebit de complexe, este foarte probabil să fie implicate mai multe mecanisme.

Cercetarea reprezintă un studiu prospectiv în care s-a urmărit evoluția clinică a alergiei la proteina laptelui de vacă (APLV) şi redobândirea toleranței la laptele de vacă, precum şi factorii de risc ce determină dezvoltarea altor alergii la copiii cu APLV. S-au analizat factorii care favorizează instalarea toleranței la laptele de vacă, precum riscul apariției astmului bronşic, rino-conjunctivitei, dermatitei atopice şi sensibilizarea la lapte de vacă persistența la copiii de vârsta mică.

APLV afectează 2% - 3% din copii in primul an de viață (1, 2, 3). De cele mai multe ori alergia este tranzitorie, majoritatea copiilor redobândindu-si toleranța la laptele de vacă (LV) în jurul vârstei de 3-4 ani. Copiii care au prezentat intoleranța la proteinele laptelui de vacă non-IgE şi-au redobândit mai repede toleranța la LV decât cei cu reacție mediată prin mecanism IgE, dar cu siguranța sunt şi alți factori necunoscuți până în prezent implicați în redobândirea toleranței la LV.

De asemenea, antecedentele de alergie alimentară reprezintă un factor de risc crescut în dezvoltarea altor boli alergice.

Studiile de specialitate arată că alergia alimentară creşte riscul pentru dezvoltarea astmului, rinitei, iar reacția la LV prin mecanism IgE creşte riscul apariției bolilor alergice. Deşi există date în literatura de specialitate cu privire la evoluția clinică şi dezvoltarea toleranței la proteinele laptelui de vacă, puține studii prospective au urmărit evoluția bolii de la naştere.

MATERIAL ŞI METODĂ

S-a efectuat un studiu prospectiv derulat pe o perioadă de 5 ani (ianuarie 2006- ianuarie 2010) pe un lot de sugari selectat timp de un an (2006) din două spitale din regiunea Moldovei, Spitalul Clinic de Urgență pentru Copii "Sf. Ioan" Galați, Compartimentul de gastroenterologie şi Spitalul Clinic de Urgență pentru Copii "Sf. Maria" Iaşi ce prezentau alergie la proteina laptelui de vacă. Alergia la proteina laptelui de vacă a fost confirmată prin testul de probă şi contraprobă ce reprezintă gold standardul în stabilirea acestui diagnostic.

Analiza a cuprins un număr de 71 copii cu vârste cuprinse între 0-12 luni ce prezentau un tablou clinic sugestiv pentru alergie la proteina laptelui de vacă, simptome digestive, asociate sau nu şi cu unele semne cutanate şi/sau respiratorii apărute după ingerarea laptelui de vacă, internați în două clinici din regiunea Moldovei. La copiii prezenți în studiu s-au identificat antecedente personale şi/sau familiare de atopie sau alergie. S-au urmărit datele anamnestico-clinice, evaluarea IgE specific, dieta de excludere şi proba de provocare.

Pentru analiza statistică a datelor am folosit o *SPSS 11.0*. Testele utilizate au fost specifice tipului de date analizate (cantitative, calitative) dintre care amintim testul Pearson χ^2, testul Fisher, testul t Student şi ANOVA ce au fost aplicate în comparații statistice. Curbele de supraviețuire au fost trasate utilizând metoda Kaplan-Meier, iar diferențele dintre grupuri au fost analizate cu ajutorul unui test log-rank.

În corelația multivariată s-a utilizat analiza logistică regresivă în care s-a studiat efectul mai multor factori cu privire la dezvoltarea toleranței la laptele de vacă. Datorită schimbărilor în IgE ca răspuns la proteina laptelui de vacă, au fost efectuate două analize: una folosind datele disponibi-

le la diagnostic şi alta folosind date disponibile după 12 luni de la diagnosticare. Factorii care sunt incluşi în studiu au fost antecedente familiale de atopie, fumatul parental, numărul de fraţi (0-1 sau 2) cu simptome alergice, prezenţa de animale cu blană.

Pozitivitatea IgE la proteina laptelui de vacă s-a măsurat la 12 luni de la diagnosticare. La copiii cu APLV am analizat efectele independente ale expunerii la lapte de vacă, istoria de APLV, antecedente familiale de atopie, prezenţa de animale cu blană, fumatul părinţilor, precum şi numărul de fraţi mai mari cu astm, rinoconjunctivita alergică, eczema atopică şi cu sensibilizare la alergeni.

Scopul studiului de a stabili evoluţia clinică a APLV precum şi a factorilor care favorizează apariţia toleranţei la LV. Copiii au fost urmăriţi până la dobândirea toleranţei la LV.

REZULTATE

În perioada analizată din cei 26.938 copiii internaţi în clinicile luate în studiu 4.485 au prezentat afecţiuni gastrointestinale dintre care 817 (18,22%) au fost alergii la proteina laptelui de vacă. Raportat la numărul total de internări incidenţa alergiei la proteina laptelui de vacă a fost de 3,03%. Prevalenţa sugarilor cu vârste mai mici de 12 luni ce prezintă alergie la proteina laptelui de vacă este de 8,69% din numărul total de copii diagnosticaţi cu APLV. Aceste rezultate sunt confirmate şi datele din literatură care menţionează faptul că simptomele alergiei alimentare se manifestă cu atât mai frecvent cu cât vârsta este mai mică (4).

Vârsta medie a fost de 7,57 luni ±2,73DS. La copiii incluşi in studiu, precum şi la lotul martor s-a urmărit frecventa apariţiei eczemei atopice, rinoconjunctivitei alergice, astmului, precum si a sensibilizării la alţi alergeni alimentari sau inhalatori. De asemenea, au fost urmăriţi factorii de risc implicaţi in apariţia manifestarilor alergice (formă digestivă, cutanată, respiratorie sau mixtă). Din lotul iniţial s-a analizat subgrupul copiilor care au dezvoltat toleranţă la proteina laptelui de vacă.

Rezultatele dozării IgE specific la proteina laptelui de vacă a evidenţiat faptul că 42 sugari (59,15%) au prezentat IgE negativ în timp ce 29 (40,85%) dintre aceştia prezentau IgE pozitiv. Pe parcursul urmăririi valorile IgE au prezentat modificări semnificative.

Distribuţia celor 71 copii cu APLV la momentul diagnosticului în funcţie de statutul de IgE specifice la LV este prezentată în figura următoare (fig. 1).

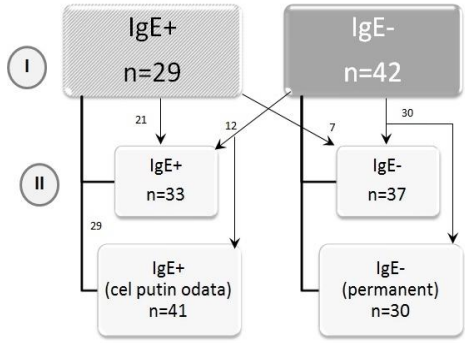

Fig. 1. Distribuţia cazurilor cu APLV la începutul tratamentului şi la 12 luni în funcţie de IgE specific

Rezultatele studiului evidenţiază faptul că numărul cazurilor cu valori pozitive ale IgE specific a scăzut, din cele 42 cazuri pozitive iniţial după 12 luni de urmărire 7 cazuri (24,14%) au devenit negative, iar la 12 (28,57%) din cele 29 cazuri cu IgE specific negativ statusul s-a schimbat. Se remarcă faptul că statusul IgE specific s-a schimbat la 19 cazuri (26,76%) în timp ce

41 cazuri (57,75%) au fost cel puțin odată IgE pozitiv, iar 30 cazuri au fost permanent negativ (42,25%).

Readministrarea laptelui de vacă s-a făcut la 12 luni după diagnostic la o vârsta medie de 1,6 ani (95%CI, 1,5 - 1,6 ani). Rezultatul testului a fost negativ (IgE s-a menținut negativ) la 37 cazuri (88,1%) iar la 12 (28,57%) copii IgE s-a pozitivat manifestându-se prin urticarie, exantem sau ambele, acestea fiind mai frecvente pentru copii cu IgE negativ – 35 cazuri comparativ cu frecvența la copiii la care IgE s-a pozitivat (94,59% *vs.* 16,67%, p=0,00271, 95% CI), în acest grup de copii mai des s-a întâlnit eczema atopică (41,67% - 5 cazuri vs. 10,81% - 4 cazuri, p=0,003) şi diaree (58,33% - 7 cazuri *vs.* 16,22% - 6 cazuri, p=0,037).

La prima probă de încărcare manifestările clinice au apărut la cei cu IgE prezent la aceştia predominând manifestările de tip: urticarie, exantem, wheezing recurent, în timp ce la cei care inițial IgE a fost negativ au fost mai des întâlnite: diaree, dermatita atopică, vărsături, falimentul creşterii.

Dezvoltarea toleranței

În sublotul cu IgE negativ toleranța la laptele de vacă a apărut semnificativ mai repede comparativ cu lotul cu IgE prezent. La vârsta de 5 ani toleranța s-a întâlnit la toți copii cu IgE (30 cazuri) negativ comparativ cu 70,73% în grupul copiilor cu IgE pozitiv (29 cazuri). La finalul studiului (vârsta medie 3,64 ani±1,35 SD) 5 cazuri (17,24%) cu IgE prezent inițial au rămas în continuare alergici la laptele de vacă aceştia prezentau semne clinice respiratorii şi cutanate. La vârsta medie de 1,6 ani 36 cazuri (48,65%) şi-au redobândit toleranța la proteina laptelui de vacă (PLV) (fig. 2). Sunt prezente diferențe semnificative în persistența APLV între grupul copiilor cu IgE + comparativ cu IgE- (Log-Rank test – p=0,1918, 95% CI).

Studiul factorilor de risc privind persistența manifestărilor alergice la proteina laptelui de vacă a fost realizată prin analiză multivariată ce a avut la bază regresia logistică, rezultatele fiind prezentat în tabelul următor. Au fost analizate antecedentele familiare de atopie, prezența de animale cu blană, fumatul părinților, precum şi numărul de frați cu manifestări alergice, eczema atopică şi cu sensibilizare la alergeni (tab. I).

TABELUL I

Estimarea parametrilor de şansă şi risc ai persistenței manifestărilor alergice

	Valoare Estimată - OR	*95% Interval de confidență*	
		Minim	**Maxim**
IgE pozitiv	4,54	2,08	6,87
Antecedente familiare de atopie	2,26	1,26	4,06
Fumat parenteral	1,19	1,29	2,71
Numărul fraților alergici	1,05	0,85	1,97
Prezența animalelor cu blană	1,24	0,73	2,06

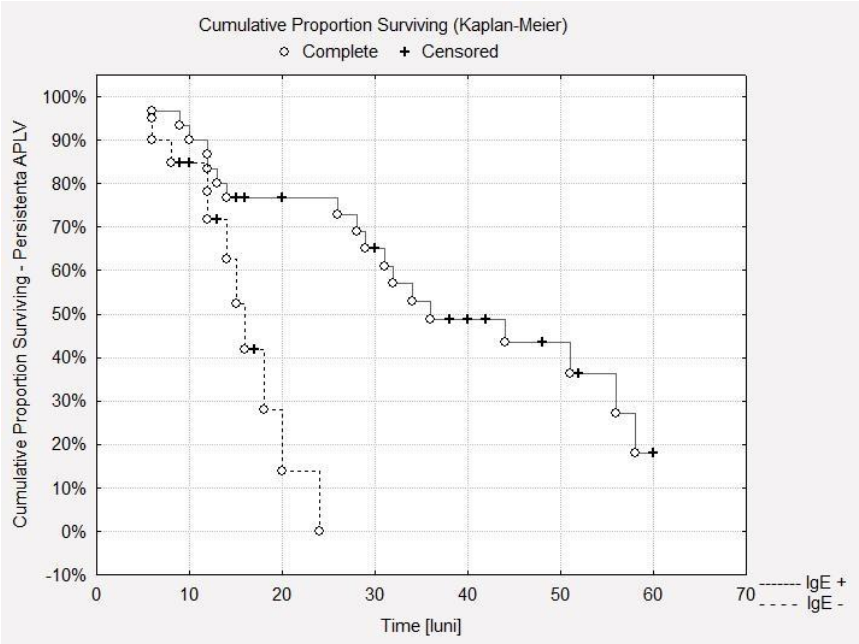

Fig. 2. Curba Kaplan-Meier privind persistența APLV în funcție de statusul IgE

DISCUȚII

Reacția de tip IgE la laptele de vacă crește riscul pentru a dezvolta persistență în APLV, iar copiii din acest grup au avut o vârsta semnificativ mai mare în momentul redobândirii toleranței la proteina laptelui de vacă. În același timp copiii cu IgE negativ și-au redobândit toleranța la vârsta de 5 ani. Am observat că dezvoltarea tardivă a sensibilizării tip IgE (la 12 luni după proba de încărcare) ar putea prezice persistența APLV după vârsta de 2 ani. Apariția anticorpilor IgE specifici pentru LV poate fi tardiva comparativ cu momentul apariției manifestarilor clinice. Creșterea tranzitorie a IgE alimentare specifice este relativ frecventă, dar numai sensibilizarea persistentă la alergenii alimentari crește riscul apariției manifestărilor respiratorii, sugerând ideea ca sensibilizarea tranzitorie este diferită de sensibilizarea persistentă. În momentul redobândirii toleranței un număr semnificativ (17.24%) din cei cu manifestări clinice de toleranță la LV vor rămâne totuși sensibilizați la proteina laptelui de vacă.

Urticaria ca simptom la proba de încărcare cu LV este un factor de risc pentru persistenta APLV până la vârsta de 2 ani. Alte studii nu au raportat o relație între simptomele clinice și vârsta de redobândire a toleranței la LV (5). Sensibilizarea la ou este un factor predictiv important al dezvoltării tardive a alergiei (6,7). De asemenea, expunerea precoce la LV determină dezvoltarea tardivă a toleranței la LV. Astfel, expunerea scurtă sau inconstantă la cantități mici de LV este un factor de risc pentru dezvoltarea sensibilizării IgE la LV(8) Studiile la animale au arătat că vârsta expunerii și cantitatea de antigen influențează apariția toleranței (9). Copiii cu APLV mediata IgE prezintă un risc crescut pentru alte boli alergice decât cei IgE negativ. Durata sensibilizării la alergenii alimentari precum și la

alergenii inhalatori se corelează cu riscul dezvoltării astmului şi a rinitei alergice. În studiul nostru am observat că intensitatea sensibilizării la LV la 12 luni de urmărire este un factor predictiv pentru rata de redobândire a toleranței şi dezvoltarea tardivă a altor boli alergice. Studiul de față demonstrează faptul că nivelul crescut al IgE specific laptelui de vacă este un factor predictiv semnificativ pentru persistența APLV, aspecte menționate şi de alți autori (5,6).

CONCLUZII

Cei mai mulți copiii îşi redobândesc toleranța în timpul copilăriei, iar cei cu IgE negativ chiar la vârste mici. Majoritatea copiilor cu APLV au prezentat reacții mediate IgE la LV, iar aceştia prezintă un risc crescut pentru bolile alergice, sensibilizarea la alergenii alimentari şi inhalatori, precum şi APLV persistentă. Valoarea prognostică a sensibilizării IgE la laptele de vacă depinde de vârstă; determinarea la 12 luni de la diagnostic este un factor predictiv pentru persistența APLV.

APLV mediată IgE persistă adesea la copiii de vârsta şcolara şi este un factor de risc şi pentru alte atopii; APLV non–IgE, din contra, este o afecțiune tranzitorie a sugarului şi copilului mic.

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INFECŢIA CU *CLOSTRIDIUM DIFFICILE*: NOI PROVOCĂRI

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CLOSTRIDIUM DIFFICILE INFECTIONS: WHAT IS NEW? (Abstract): *C. difficile* is the most common infectious cause of healthcare-associated diarrhea but now is increasingly recognized as a cause of diarrhea in outpatients and persons without apparent health care contacts. Emergence and spread of new epidemic clones of *C. difficile* 027 (PCR-ribotype) and 078/126 (toxinotype) with increase toxin production, an aditional binary toxin and high level resistance to fluoroquinolones and increasing incidence of more rapidly progressive severe disease, require prompt clinical recognition and new tools to predict severity and to prevent recurrences. Although antibiotics are effective at inhibiting *C. difficile* and treating symptoms, these drugs could not reestablish normal bowel flora and the rate of recurrences is 25%. During the past years we assisted to an impressive search for new and more effective therapy that shoud be save, with low potential for the development of resistance, with low levels of systemic absorbtion and high levels of active drug in the colon and should be associated with a low rate of recurrence after treatment. By consequence, different approaches to the management of recurrent infections have been studied such as new antibiotics (fidaxomicin), human monoclonal antibodies against *C. difficile* toxins A and B, intravenous human immunoglobulin, active immunization, and probiotic therapy. **Key words**: CLOSTRIDIUM DIFFICILE, INFECTIONS, DIAGNOSIS, TREATMENT.

Clostridium difficile este principalul agent al diareei asociate îngrijirilor medicale, dar, odată cu creşterea numărului de genotipuri implicate în izbucnirile epidemice din spitale se pune problema unor surse externe care sunt importate din mediul comunitar în cel spitalicesc, situaţie similară cu cea a infecţiei cu *Stapphylococcus aureus*.

Creşterea incidenţei infecţiei cu *C. difficile* (ICD) în lume (este implicat în 15-25% din totalitatea cazurilor de diaree asociată antibioterapiei) atrage după sine o rată semnificativă a mortalităţii, aceasta fiind datorată exacerbării virulenţei tulpinilor de *C.difficile* şi creşterii vulnerabilităţii gazdei.

Căile de transmitere ale bacteriei patogene pot fi foarte complexe şi pot fi luate în discuţie: transmiterea directă de la om a om, contactul cu mediul înconjurător, contactul cu animale, transmiterea aerogenă, prin vectori şi ingestia de alimente şi apă. Deşi *C. difficile* a fost izolat din diferite tipuri de carne (6-11% în carnea de vită din magazine în Canada) nu au fost studii care să documenteze ca factor de risc pentru infecţia cu *C. difficile* consumul unui anumit produs alimentar (1, 2).

Epidemiologie. Infecţia cu *C. difficile* s-a schimbat în ultimii ani, astfel: incidenţa

bolii a crescut (de 8 ori în Quebec şi de 4 ori în Germania), afecţiunea este mai severă (rate mai mari de şoc septic, peforaţie şi colectomie - până la 18%), ICD este mai refractară la terapia standard (eşec clinic cu metronidazol de 25% în 2004 faţă de 9% în 2002) şi rate de recurenţă care s-au dublat la persoanele peste 65 ani, emergenţa şi răspândirea unor noi clone (ribotipul 027 şi 078 producătoare de toxină binară). Ribotipul PCR 078 este un exemplu de tip emergent de *C. difficile* cu transmitere de la animale la oameni şi cu o virulenţă crescută la om, dar, a cărui prevalenţă variază de la ţară la ţară (1, 2, 3).

Etiopatogenie. Dezvoltarea infecţiei cu *C.difficile* are la bază prezenţa a trei factori: vîrsta avansată sau prezenţa unor comorbidităţi, perturbarea microbiotei intestinale ca urmare a terapiei cu antibiotice sau cu agenţi antitumorali şi expunerea la forme vegetative sau spori de *C.difficile* (4).

Terapia cu antibiotice joacă un rol principal în patogeneza infecţiei cu *Clostridium difficile* prin dezechilibrul creat la nivelul microbiotei colonului. Antibioticele se caracterizează prin capacităţi diferite de a inhiba *C.difficile* şi de a stimula producerea toxinei. Cele care nu au o activitate semnificativă asupra *C. difficile* dar care au o capacitate mare de a perturba microflora intestinală (ex. ceftriaxona) favorizează apariţia ICD atît în perioada de terapie cît şi în perioada de recuperare a microflorei. De asemenea, rezistenţa la antibiotice a *C. difficile* are un rol important în epidemiologia ICD. Tulpinile rezistente la clindamicină, la fel ca şi în cazul celor rezistente la fluoroquinolone sau cefalosporine rezistă într-un mediu în care celelate microorganisme au fost suprimate pe parcursul terapiei, ducînd la apariţia infecţiei digestive. Capacitatea de a induce producerea de toxină este mai mică în prezenţa unor antibiotice ca piperacilină-tazobactam şi tigeciclină faţă de cefotaximă (5).

Cel mai adesea sunt implicate în producerea diareei cu *C. difficile*: clindamicina, penicilinele, cefalosporinele şi fluoroquinolonele. Clindamicina afectează pe termen lung flora intestinală, ceea ce creşte fereastra de susceptibilitate pentru ICD. În ultima perioadă cefalosporinele se poziţionează pe primul loc între antibioticele capabile să favorizeze ICD. (5).

Manifestări clinice. Infecţia cu *C. difficile* poate îmbrăca următoarele forme de manifestare: (1) diaree, (2) ileus (3) megacolon toxic. La pacienţii cu vârsta ≥ 65 ani, având comorbidităţi semnificative şi la cei din terapie intensivă se consideră ICD ca fiind severă chiar în absenţa semnelor care definesc colita severă (6). Deşi afectează cu predilecţie colonul, în ultima perioadă au fost comunicate un număr crescut de cazuri cu afectare ileală, multe dintre ele cu evoluţie fatală (7).

Pentru stratificarea gradului de severitate se iau în calcul următoarele caracteristici ale pacientului: vârsta > 60 ani, temperatura>38,3°C, albuminemia < 2,5 mg/dl, leucocite> 15.000/mmc, colită pseudomembranoasă, internarea în secţii de TI şi se acordă cîte 1 punct pentru primele 4 caracteristici şi cîte 2 puncte ultimelor două. Se consideră formă severă de boală dacă punctajul acumulat este ≥ 2 (8).

Diagnostic de laborator. Testarea ICD trebuie realizată la toate persoanele cu suspiciunea de diaree infecţioasă la care testele pentru enteropatogenii obişnuiţi sunt negative şi la toţi pacienţii cu diaree care au avut o spitalizare prealabilă de cel puţin 72 ore (9).

Testele de diagnostic pentru infecţia cu *C. difficile* se clasifică în teste pentru (1)

produşi ai *C. difficile* (glutamat – dehidrogenaza (GDH), acizi graşi aromaţi, toxina A şi/sauB), (2) metode de cultură pentru detecţia *C. difficile* producătoare de toxină (cultura toxigenică), şi (3) teste pentru determinarea genelor *C. difficile*. Testele de citotoxicitate în cultură celulară (CCA) şi cultura toxigenică sunt considerate ca metode standard de referinţă pentru detecţia *C. difficile.* Recent, aceste metode consumatoare de timp, care necesită o utilare specifică a laboratorului au fost înlocuite prin teste imunoenzimatice (EIA), rapide şi uşor de realizat: EIA de detectare a GDH, EIA de detectare doar a toxinei A, şi EIA de detectare a ambelor toxine A şi B (9).

Tratament. Măsurile generale în cazul ICD sunt: întreruperea antibioticelor, măsuri suportive, evitarea agenţilor antiperistaltici, măsuri de izolare pentru prevenirea transmiterii enterale.

Metronidazolul şi vancomicina au fost principalele opţiuni terapeutice încă de la sfîrşitul anilor 70, cînd a fost recunoscut rolul *C. difficile* în patogenia infecţiei digestive. Metronidazolul este medicaţia de primă-intenţie pentru *terapia unui episod iniţial* de ICD formă uşoară-moderată (500mg x3/zi x10-14 zile) (9, 10).

În scopul limitării emergenţei enterococilor şi stafilococilor rezistenţi la vancomicină şi al diminuării costurilor, utilizarea vancomicinei în spitale este limitată doar la cazurile care nu răspund la metronidazol sau la pacienţii cu forme severe de ICD sau cu multiple recurenţe (125 mg/zi po x 10-14 zile).

În cazul *formelor severe*, complicate de ICD se poate administra vancomicina oral (intrarectal dacă există ileus)(doză de 500 mg po şi 500 mg în 100 ml ser fiziologic intrarectal/zi) asociată sau nu cu metronidazolul (500mg x3/zi).

În terapia unui *episod iniţial sau a unei prime recurenţe* a ICD, în situaţia în care nu se poate administra terapia orală se recomandă metronidazol 500 mg i.v. x3/zi timp de 10 zile atît în formele severe cît şi în celelalte non-severe, asociat cu vancomicină intracolonic 500 mg în 100 ml ser fiziologic la fiecare 4-12 ore şi/sau vancomicină 500 mg - o priză/zi pe sondă nasogastrică (6).

Recurenţele ICD survin cel mai adesea la 7-14 zile după întreruperea terapiei sugerînd mai curînd o recădere decît o reinfecţie. Recăderea se înregistrează la 15-35% din pacienţii care au avut doar 1 episod anterior şi la 33-65% din cei care au prezentat ≥ 2 episoade anterioare acestea datorîndu-se unei continue expuneri la patogen (fie prin reinfectare din mediul înconjurător, fie recădere de la o sursă endogenă) (11)

Nu se indică metronidazol pentru un episod ulterior primei recurenţe a ICD sau pentru terapia pe termen lung datorită potenţialului de neurotoxicitate cumulativă (10).

Procentul de recăderi după tratamentul cu oricare din cele două antibiotice de elecţie este similar (14-15%) (8).

Tratamentul celei de-a doua recurenţe şi ale celor ulterioare se realizează cu vancomicină 125 mg po timp de cel puţin 10 zile. Poate fi luată în considerare strategia de tip o doză de 125 mg la fiecare 3 zile timp de 3 săptămîni.

Dacă terapia orală nu poate fi administrată pentru tratamentul ICD la > a doua recurenţă se recomandă metronidazol 500 mg x3 i.v./zi timp de 10 -14 zile plus cu vancomicină intracolonic (retenţionată intrarectal) 500 mg în 100 ml ser fiziologic la fiecare 4-12 ore şi/sau vancomicină 500 mg-o priză/zi pe sondă nasogastrică. Vancomicina ar putea fi înlocuită cu teicoplanin 100 mg x 2/zi în toate cazurile

mai sus menţionate (6).

În prezent rezistenţa la metronidazol a *C. difficile* ajunge la 6%, fiind o rezistenţă de tip heterogen. Testarea sensibilităţii la antibiotice s-ar impune deoarece asistăm la o creştere a circulaţiei tulpinilor cu CMI a metronidazol de peste 2mg/l, şi ar permite detectarea tulpinilor cu heterorezistenţă şi cu rezistenţă la rifaximin, în condiţiile în care ne confruntăm cu o rată mare de eşec la terapia cu metronidazol. Efectuarea antibiogramei se recomandă în următoarele situaţii: dacă este o formă severă de boală şi se are în vedere o altă terapie decât vancomicina, dacă evoluţia clinică nu este favorabilă în cursul terapiei, în caz de izbucnire epidemică, dacă este un episod de recurenţă şi pentru monitorizarea anuală a trendului de rezistenţă la antibiotice într-un spital sau o anumită regiune (12).

Dacă simptomele se agravează sau nu se ameliorează după 3-4 zile de tratament se consideră eşec terapeutic, situaţie în care se recomandă switch-ul cu vancomicină 125-250 mg p.o la fiecare 6 ore (dacă pacientul primea metronidazol) şi se solicită consult chirurgical în vederea unei eventuale colectomii.

Noi strategii terapeutice. Dat fiind eficienţa limitată a terapiilor actuale pentru ICD sunt în curs de derulare un număr impresionant de studii care evaluează eficienţa unor noi agenţi terapeutici.

Fidaxomicina este primul reprezentant al antibioticelor macrociclice orale, cu acţiune bactericidă importantă împotriva *C. difficile*, lipsită de rezistenţă încrucişată cu alte antibiotice. Spectrul său de activitate asupra florei intestinale normale este limitat (fiind inactiv pe *Candida*, bacterii Gram-negative, numeroşi Gram-pozitiv şi anaerobi); atacă doar bacteriile patogenice şi protejează flora gastro-intestinală normală, spre deosebire de vancomicină şi metronidazol care suprimă creşterea florei endogene normale (13)

În studiul comparativ fidaxomicină versus vancomicină, Louie şi col înregistrează rezultate similare privind rata de vindecare clinică în cele două loturi, beneficiul maxim al fidaxomicinei fiind la cei cu recurenţe (15% vs 25%) (14).

Tigeciclina prezintă activitate inhbitorie împotriva *C. difficile* în colonul şoarecilor de laborator şi nu permite producerea de toxină (15).

Într-un studiu multicentric, randomizat, dublu-orb, placebo-controlat, recent publicat de Lowy şi colaboratorii a fost evaluată eficienţa a doi anticorpi monoclonali neutralizanţi cu acţiune împotriva toxinei A şi B administraţi concomitent cu antibioterapia standard. Rezultatele obţinute relevă o reducere semnificativă a ratei recurenţelor la pacienţii care au primit anticorpi faţă de placebo (8% *vs* 32%) (16).

Imunoglobulinele cu administrare intravenoasă s-au dovedit a fi urmate de un răspuns clinic favorabil la un subgrup de pacienţi cu numeroase recurenţe ale ICD. Doza poate fi de 300 mg/kg sau aceeaşi doză în 5 perfuzii zilnice (17).

Administarea de materii fecale colectate de la persoane sănătoase, prin colonoscopie, la nivelul tuturor segmentelor colonului, sau infuzia pe tub nasogastric au dat cele mai bune rezultate în tratamentul recurenţelor (11).

Eficienţa probioticelor (*Lactobacilus GG, Saccharomyces boulardii*) în profilaxia şi tratamentul infecţiei cu *C. difficile* nu este încă dovedită. Aceste produse nu se recomandă la pacienţii cu risc pentru translocarea bacteriană şi nici la pacienţii cu catetere venoase centrale (18).

Preparatele care conţin *Lactobacillus*

casei imunitass par să prevină ICD, fapt susţinut de un studiu prospectiv în care nu a apărut nici un episod de ICD la cei care au primit antibiotice şi *Lactobacillus* faţă de 17% episoade la lotul fără *Lactobacillus* (19).

Este posibil ca o strategie viitoare să se baze pe asocierea dintre un antibiotic şi o terapie imunologică.

CONCLUZII

Noile abordări de întrerupere a ciclului infecţiei cu *C. difficile*, concomitent cu acordarea unei atenţii speciale administrării unei antibioterapii adecvate şi cu punerea în practică a măsurilor de prevenţie şi control ar putea duce la limitarea substanţială a aceastei infecţii cu o prevalenţă în creştere şi greu de manageriat.

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NOUTĂȚI

PROGRESIA TULBURĂRILOR DE SOMN ŞI INCIDENŢA BOLILOR CARDOIVASCULARE

Tulburările respiratorii prezente în cursul apneei de somn sunt generatoare de complicaţii cardiovasculare. Sleep Heart Health Study a înrolat 2721 pacienţi (vârsta medie 62 de ani, 57% femei), fără boală cardiovasculară la intrarea în studiu, care au fost supuşi la 2 polisomnografii la interval de 5 ani. S-a consemnat incidenţa bolilor cardiovasculare, incluzând infarctul de miocard, insuficienţa cardiacă sau stroke. Pacienţii la care s-a evidenţiat o rată crescută a evenimentelor cardiovasculare au avut cei mai crescuţi indici de apnee obstructivă şi centrală. Diferenţa dintre indexul apnee-hipopnee la pacienţii cu şi fără complicaţii cardiovasculare a fost de 2,75 evenimente/oră (interval de încredere 95%, 0,1 versus 1,75, p=0,04). Studiul evidenţiază relaţia reciprocă care există între tulburările de respiraţie din timpul somnului şi complicaţiile cardiovasculare, persistenţa unor afecţiuni cardiovasculare pe o perioadă de peste 5 ani, determinând apariţia unor tulburări respiratorii nocturne. (Chami HA, Resnick HE et al. Association of incident cardiovascular disease with progression of sleep-disordered breathing, *Circulation 2011*, 123: 1280-1286)

Corina Dima-Cozma

PREVALENȚA INFECȚIEI CRONICE CU VIRUS HEPATITIC B (VHB) ÎN COLECTIVITĂŢI ÎNCHISE ŞI COMPORTAMENTUL LA RISC

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PREVALENCE OF CHRONIC HEPATITIS B VIRUS (HBV) INFECTION IN CLOSED COMMUNITIES AND RISK BEHAVIOUR (Abstract): **Aim:** To determine the prevalence of chronic hepatitis B virus infection in closed communities and the relations between the risk behavior and VHB infection. **Material and methods:** In this study we included persons deprived of personal freedom at Bacau Correctional Facility. The subjects filled out a questionnaire, which contained identification and epidemiologic data (detention history, evaluation of the risk factors), as well as an informed consent. All subjects were tested for HBsAg. **Results:** According to the performed tests, the general prevalence of hepatitis B and C in this correctional facility was 15,34%, with a prevalence of hepatitis B of 10,74%. Although these figures show a higher prevalence in this population than within the general population, they are lower than the figures reported in correctional facilities from other countries. **Conclusions**: The results confirm the fact that the population in correctional facilities is at high risk for hepatitis B infection, the ways of disease transmission being already known (infected blood, injected drugs, tattooing, homosexual relationships). **Key words:** HEPATITIS B VIRUS, PREVALENCE OF HBV INFECTION, RISK BEHAVIOR, CORRECTIONAL FACILITIES

În ciuda existenței unor metode eficiente de prevenire a infecției cu VHB, aceasta continuă să reprezinte o importantă problemă de sănătate publică pe plan mondial. Peste 65% din populația lumii trăieşte în zone cu prevalență crescută a infecției cu VHB (1), aceasta reprezentând a IX-a cauză de deces pe glob şi prima cauză de mortalitate prin carcinom hepatocelular (CHC) în țări din Asia de Sud-Est şi Africa Subsahariană (2). Conform datelor OMS (3), dar şi a unor studii (4), estimarea prevalenței infecției VHB în populația generală indică existența a 350-400 milioane de persoane cu infecție persistentă. Decesele cauzate de afectarea acută sau cronică determinate de VHB se ridică la 600.000 – 1 milion pe an (3,4). În fiecare an, la nivel mondial, sunt afectate între 10 şi 30 milioane de persoane, multe dintre ele fiind copii şi tineri (5).

Literatura de specialitate atestă existența unor grupuri populaționale cu risc crescut de infecție (4,6). În rândul lor se pot deosebi mai multe subgrupuri: utilizatorii de droguri intravenoase, partenerii sexuali

ai purtătorilor cronici de VHB, persoane cu parteneri sexuali multipli, inclusiv homosexuali, heterosexuali cu istoric de BTS - boli cu transmitere sexuală, deținuții - în funcție de numărul de condamnări, de anii petrecuți în instituții corecționale). Acestora li se adaugă şi personalul care deserveşte penitenciarele (mai ales cei care au un contact prelungit şi strâns cu deținuții - personal sanitar, supraveghetori).

Legat de persoanele private de libertate, OMS şi-a exprimat temerea că sistemul penitenciar (care la nivel mondial deserveşte aproximativ 9 milioane deținuți pe an) reprezintă un rezervor de menținere şi amplificare a transmiterii infecției în comunitate după liberarea deținuților (3). Trebuie menționat că 85% din persoanele intrate în penitenciar sunt asociate cu un comportament la risc preîncarcerar, comportament pe care îl continuă în penitenciar şi îl transmite şi celor neiniției (7). De aceea, multe țări propun ca penitenciarele să fie ținta majoră a programelor de prevenție primară, secundară şi terțiară (7,8).

Prevalența acestor boli în penitenciar este mare, comportamentul la risc este un fapt comun, programele de prevenție sunt rare (8-11) sau inexistente, iar dinamica (turnoverul) efectivelor în/şi din astfel de instituții este crescută. La aceasta se adaugă agregarea importantă a unor grupuri provenite din comunități având crescut comportamentul la risc, dar care au un nivel socioeconomic şi educațional scăzut (9).

Scopul acestui studiu a fost stabilirea prevalenței infecției virale B în colectivități închise la persoanele cu comportament la risc, iar scopul secundar a fost stabilirea de corelatii dintre diferiți parametri caracteristici (perioada de detenție, numărul de condamnări, consumul de droguri cu administrare intravenoasă, numărul partenerilor sexuali, orientarea sexuală) şi rata infecției virale hepatice B.

MATERIAL ŞI METODĂ

În studiu au fost incluşi rezidenții Penitenciarului Bacău pe o perioadă de 3 ani (2007-2010). După semnarea consimțământului informat, subiecții au completat un chestionar epidemiologic şi au fost supuşi unui examen clinic general. Chestionarul epidemiologic a cuprins date de identificare şi date epidemiologice (istoricul detenției/detențiilor, evaluarea factorilor de risc: administrare de droguri injectabile, tatuare, hetero/autoagresiuni, partajul obiectelor de toaletă, intervenții chirurgicale sau stomatologice, călătoria în străinătate şi eventual în zone cu endemicitate crescută). Toți subiecții au fost testați pentru prezența Ag HBs prin metoda ELISA.

Datele statistice au fost prelucrate cu ajutorul programului *SPSS 16.0*. Am utilizat pentru compararea valorilor testul Chi pătrat, iar valorile neparametrice au fost interpretate cu ajutorul testului Mann-Whitney de comparare a rangurilor.

REZULTATE

Din totalul de 500 deținuți din Penitenciarul Bacău, 326 au semnat consimțământul informat şi doar 141 au completat chestionarul epidemiologic.

Vârsta medie a lotului a fost 30,94 ± 9,67 ani (femei: 32,84 ± 8,92 ani, bărbați: 30,65 ± 9,77 ani). Pentru a vedea dacă s-au înregistrat diferențe semnificative statistic între valorile vârstei pe diferitele categorii de subiecți incluşi în lotul de studiu, am folosit testele de semnificație statistică. Testul de normalitate Kolmogorov - Smirnov a aratat că valorile vârstei nu respectă legea de distribuție normală ($Z = 1.805$, $p = 0.003$, SS), deci pentru a compara aceste

valori am folosit doar teste neparametrice. Am folosit testul Mann-Whitney de comparare a rangurilor, care a aratat că nu există diferențe semnificative statistic între valorile vârstei pe sexe (U = 5200.500, p = 0.084, NS), şi nici între subiecții pozitivi/negativi pentru Ag HBs (U = 4068.500, p = 0.052, NS), respectiv pentru anticorpi anti VHC (U = 2172.000, p = 0.229, NS). Ponderile pe sexe ale persoanelor identificate pozitiv nu au diferit semnificativ statistic în nici unul dintre cazuri (p=0,367).

A fost completat un număr de 141 chestionare, adică 43,25% dintre subiecții incluşi în studiu au acceptat să răspundă la această formă de interviu. La 197 (60,43%) dintre deținuții incluşi în studiu s-au efectuat analize medicale complete. Au existat 31 de persoane (22,0%) pentru care au fost realizate analizele medicale complete şi care au acceptat să completeze şi chestionarul, neexistând situații de p.p.l incluse în studiu şi care să nu fi răspuns măcar la una dintre cele două forme de investigare folosite. Întregul lot a fost însă testat pentru prezența Ag HBs.

În urma analizelor efectuate s-a obținut o prevalență a Ag HBs în lot de 10,74%. Majoritatea celor incluşi în studiu au făcut parte din grupa de vârstă 20-45 ani, prezența Ag HBs fiind preponderentă la aceeaşi grupă de vârstă.

Pentru ambele sexe mediul de proveniență a fost preponderent cel urban. Proporția pacienților cu infecție virală B din mediul urban a fost de 68% față de mediul rural unde s-a înregistrat o proporție de 32% (OR 1,61, p=0,344).

Cei mai mulți subiecți cu infecție virală B au fost: de etnie română (92%), aveau terminate 12 clase (liceul) ca nivel de studii 70,2%, nivelul socio-economic era mediu spre scăzut (60,9%, p=0,32), aveau statusul marital incert (divorțat, văduv sau necăsătorit) (30,5%), fiind recidiviști sau având condamnări cu o durată de cel puțin 2 ani.

În acest studiu s-au luat în calcul mai mulți factori de risc pentru transmiterea infecției virale B (tab. I).

TABELUL I

Factori de risc corelați cu VHB la pacienții din comunități închise

Factori de risc	OR	p
Istoric familial de VHB	14,157	<0,001
Locuit în străinătate >1 lună	0,974	0,038
Tatuaje	2,850	0,023
Boli cu transmitere sexuală	2,061	0,173
Homosexualitate	16,132	<0,001
Leziuni autoprovocate	1,211	0,714
Utilizare de droguri intravenoase	2,705	0,026

Tatuajele au fost realizate în mare proporție (82,3%) în penitenciar (de cele mai multe ori de colegii de cameră) şi doar 8,9% au apelat la saloane specializate (înainte de intrarea în penitenciar). Cu toate acestea, Ag HBs a fost detectat la 36,9% din cei care şi-au realizat tatuajul în penitenciar. Dintre pacienții cu VHB 16,6% au recunoscut că utilizau obiecte de toaletă comune (OR 2,04, p=0,132).

DISCUȚII

Infecția cu VHB recunoaşte drept căi majore de transmitere calea sanguină şi cea sexuală; calea de transmitere verticală (materno-fetală) nu a făcut obiectul studiului nostru.

Prevalența infecției cronice virale B în Penitenciarul Bacău a fost de 10,74% la grupa de vârstă 20-45 ani. Această prevalență este crescută față de prevalența de 5,05% identificată în studiile anterioare

(12) la aceiaşi grupă de vârstă, însă în populaţia generală a României, ceea ce relevă riscul crescut pe care îl au subiecţii aflaţi în colectivităţi închise.

Structura populaţiei penitenciare a influenţat unele rezultate. Faptul că în perioada desfăşurării studiului populaţia de sex feminin a Penitenciarului Bacău era de aproximativ 5% din total a determinat o prevalenţă mai mare a infecţiei VHB în rândul persoanelor de sex masculin. Aceste rezultate sunt în concordanţă cu cele obţinute într-un studiu (12) efectuat la nivel naţional, în care probabilitatea infecţiei cronice cu VHB era de 1,55 ori mai mare la bărbaţi ca la femei. Spre deosebire de acest studiu însă, mediul de proveniență (urban sau rural) are un rol opus. Dacă în studiul citat mai sus probabilitatea de a fi infectat cronic cu VHB era de 1,32 ori mai mare în mediul rural (fapt explicat prin standardul scăzut socio-economic, sanitar şi educaţional, cultura specifică şi practicile tradiţionale), în studiul nostru datele arată că există de 0,693 ori mai puţine şanse ca o persoană din mediul rural să fie pozitivă pentru VHB decât o persoană din mediul urban. Explicaţia este simplă: la nivel penitenciar, grupurile cu risc provin în special din mediul urban, aici aceste grupuri fiind în număr mai mare, având mai mulţi membri, care se angajează mai frecvent în activităţi comportamentale cu risc crescut pentru infecţie. Un alt factor protector este reprezentat de nivelul de studii – subiecţii cu studii universitare au şanse (risc) mai mici de a dezvolta infecţia cu VHB decât cei al căror nivel de pregătire se opreşte la şcoala primară, gimnaziu sau liceu. Deoarece etnia majoritară a fost cea română, a rezultat un număr mai mare de persoane de etnie română cu VHB prezent, decât de etnie rromă (23 versus 2), ducându-ne la concluzia că la populaţia română avem de 1,191 ori mai multe şanse de identificare a Ag HBs decât la rromi – un risc slab-moderat. Trebuie luată în considerare însă şi acurateţea cu care cei care au completat chestionarul şi-au declarat etnia. Statusul marital este un aspect important – grupul celor necăsătoriţi şi divorţaţi era de aproape două ori mai numeros decât al celor căsătoriţi în ceea ce priveşte prezenţa Ag HBs.

Un comportament la risc, frecvent întâlnit printre deţinuţi, este tatuarea. Relaţia dintre comportamentul la risc al deţinuţilor şi transmiterea virusurilor hepatice în rândul acestora este bine stabilită în literatura de specialitate (13-15). Tatuajele nu reprezintă un factor de risc pentru transmiterea VHB. Un alt comportament la risc este reprezentat de relaţiile sexuale cu parteneri de acelaşi sex (homosexualitatea). Acestea determină un risc pronunţat (de 3,897 ori mai multe şanse) pentru infecţia cu VHC. Numărul de condamnări şi anii petrecuţi în detenţie duc la un risc, deşi slab, de a achiziţiona infecţia cu virusurile hepatice. Aceste argumente sunt în favoarea aserţiunii potrivit căreia detenţia, în general, prin modul de viaţă şi comportamentul pe perioada executării pedepsei are un rol important în transmiterea infecţiei cu virusuri hepatice şi în perpetuarea unui important rezervor de virus, care ulterior (la eliberare) se va întoarce în comunitate. Rezultatele sunt contrare studiului citat mai înainte (12) potrivit căruia detenţia nu se corelează din punct de vedere epidemiologic cu infecţia VHB.

Alt comportament la risc este reprezentat de leziunile autoprovocate în perioada detenţiei. Ele au un risc slab pentru infecţia VHB şi se corelează cu numărul de condamnări şi anii de detenţie.

În studiul efectuat nu am găsit ca fiind

factor de risc pentru infecția cu VHB administrarea de tratamente injectabile la domiciliu, împărțirea obiectelor personale de igienă, accidentele de circulație, acupunctura, frecventarea saloanelor de cosmetică/manichiură/pedichiură, intervențiile chirurgicale sau spitalizările.

Un fapt demn de semnalat, recunoscut de întreaga literatură de specialitate (16-19), este faptul că vaccinarea pentru VHB elimină complet riscul de a mai contracta această boală.

Este posibil ca realizarea unui astfel de studiu în facilități corecționale cu o altă structură a populației să conducă la date mai apropiate de cele din alte țări. Totuşi datele obținute susțin necesitatea imperioasă a elaborării unor programe de screening, prevenție şi tratament în rândul persoanelor instituționalizate, dar şi întreprinderea unor inițiative legislative care să sprijine astfel de programe.

CONCLUZII

Datele obținute demonstrează că infecția virală B este mai frecventă în colectivitățile închise decât în populația generală, în concordanță cu creşterea expunerii la factorii de risc. Factorii de risc se suprapun cu cei ai populației generale, cu o frecvență crescută a utilizării de droguri intravenoase, a tatuajelor şi a comportamentului bi/homosexual.

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NOUTĂȚI

VALOAREA PREDICTIVĂ A INDICILLOR ANTROPOMETRICI PENTRU MORTALITATEA ŞI MORBIDITATEA PACIENȚILOR DIALIZAȚI

Evaluarea *status*-ului nutriţional al pacienţilor dializaţi este importantă deoarece malnutriţia protein calorică este un factor care influenţează morbiditatea şi mortalitatea acestora. Scopul acestui studiu a fost să argumenteze valoarea predictivă a parametrilor antrpopometrici şi modalitatea în care aceste două caracteristici se corelează. Studiul a inclus 242 pacienţi. Măsurătorile antropometrice au fost făcute în timpul iernii şi s-au referit la date privind pliul cutanat, circumferinţa braţului, înălţime şi greutate. Procentul de grăsime corporală a fost calculat în funcţie de pliul cutanat prezent în regiunea tricepsului, bicepsului, suprascapulară şi suprailiacă. Indicele de masă corporală (BMI) rata catabolizării proteinelor şi factorii de risc cardio-vasculari au fost de asemenea valori luate în discuţie. În urma analizei datelor au fost găsite corelaţii semnificative între parametrii antopometrici (analiza statistică folosită fiind regresia Cox extinsă). De asemenea, fiecare parametru investigat s-a corelat independent cu mortalitatea şi morbiditatea pacienţilor investigaţi. Parametrul cu valoarea predictivă cea mai mare a fost circumferinţa braţului, indicele de masă corporală fiind considerat doar un factor de risc. (Stosovic M, Stanojevic M, Ogrizovic S, Jovanovic, The predictive value of anthropometric parameters on mortality in haemodialysis patients. *NephrolDial Transplant.* 2011; 26: 1367-1374).

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MALADIA PEPTICĂ ÎN CIROZA HEPATICĂ: ROLUL INFECŢIEI CU *HELICOBACTER PYLORI* ŞI CONDUITA TERAPEUTICĂ

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PEPTIC ULCER DISEASE IN LIVER CIRRHOSIS: ROLE OF *HELICOBACTER PYLORI* INFECTION AND THERAPEUTICAL APPROACH (Abstract): Peptic ulcer has frequently been associated with liver cirrhosis. The death rate for peptic ulcer in cirrhotics has been reported to be five times higher than in general population. The underlying mechanisms are poorly understood. Different factors have been claimed to be involved, such as alterations in serum gastrin level, gastric acid secretions, mucosal blood flow and decreased prostaglandin production in gastric mucosa. Moreover, *Helicobacter pylori* infection, when accurately assessed, is detectable in most peptic ulcer cirrhotics. Since the *H. pylori* infection strongly correlates with peptic ulcer in general population, it is necessary to clarify the role of *H. pylori* in the pathogenesis of peptic ulcer in cirrhosis before eradication can be proposed as a preventive measure. **Key words**: PEPTIC ULCER DISEASE, LIVER CIRRHOSIS, HELICOBACTER PYLORI

Se estimează că infecţia cu *Helicobacter pylori* (*H. pylori*) reprezintă cea mai frecventă infecţie întâlnită la om, peste 50% din populaţia globului şi peste 60% din populaţia României fiind infectată cu *H. pylori*. Este unanim recunoscută implicarea sa în producerea gastritei cronice, ulcerului gastric şi duodenal, neoplasmului gastric şi limfomului gastric MALT, dar locul său în tabloul clinico-evolutiv al cirozei hepatice rămâne un subiect controversat (1).

Astfel, relaţia dintre infecţia cu *H. pylori*, generatoare de patologie ulceroasă gastroduodenală şi ciroza hepatică – boală cronică invalidantă – devine o problematică medicală importantă şi o provocare pentru sistemul sanitar, incomplet elucidată la ora actuală.

INFECŢIA CU *H. PYLORI* ÎN CIROZA HEPATICĂ

Literatura de specialitate a fost invadată de semnale frecvente privind rolul bacteriei descoperite în 1983 de către Warren şi Marshall în patogeneza bolilor extragastrice, în prezent *H. pylori* fiind obiect de cercetare pentru aproape toate specialităţile medicale. Întrebarea care încă îşi aşteaptă răspunsul este în ce măsură un microorganism cu localizare exclusiv gastrică intervine în mecanismele patogenice ale unor maladii extragastrice (2).

H. pylori interferă atât cu mijloacele de apărare ale stomacului (cantitatea şi calitatea mucusului, secreţia de bicarbonat), cât şi cu cele de agresiune (secreţia de gastrină, HCl, pepsină). *H. pylori* este implicat în etiologia ambelor localizări ale bolii ulceroase (duodenal şi gastric), proporţia celor infectaţi fiind mai ridicată în primul caz. S-a demonstrat că *H. pylori* determină tulburări în secreţia hormonilor polipeptidici gastrointestinali la nivelul antrului gastric, cu rol determinant în ulcerogeneză. Se admite în prezent că *H. pylori* are un efect hipergastrinemiant, corelat pozitiv cu creşterea secreţiei acide şi peptice, atât la subiecţii cu ulcer, cât şi la cei fără boală ulceroasă (3).

Ulcerul *H. pylori* pozitiv este frecvent întâlnit într-o serie de afecţiuni : boli hepato-biliare (ciroza hepatică, litiaza biliară, hepatocarcinom), boli cardio-vasculare (cardiopatia ischemică, ateroscleroza cerebrală), boli autoimune (tiroidita autoimună), boli cutanate alergice (urticaria cronică idiopatică), boli hematologice (anemia sideropenică), boli neurologice (boala Parkinson), boli metabolice (diabetul zaharat), boli respiratorii (bronşita cronică, astmul bronşic). În majoritatea bolilor studiate, prevalenţa infecţiei cu *H. pylori* este mai ridicată decât la populaţia de control. Rămâne de stabilit rolul patogen al bacteriei şi dacă localizarea sa în alte organe este primară sau secundară suferinţelor respective (2).

Deşi infecţia cu *H. pylori* este frecvent asociată cirozei hepatice, rolul său patogenetic prin mecanisme în mare parte de tip imun este încă un subiect controversat şi incomplet elucidat (3, 4).

Prevalenţa infecţiei cu *H. pylori* la pacienţii cirotici este raportată în limite foarte largi, diferenţele datorându-se atât mijloacelor diferite de diagnostic utilizate, precum şi posibil criteriilor de selecţie a bolnavilor.

Astfel, 14 studii recente înrolând, un număr de 1499 de bolnavi diagnosticaţi cu ciroză hepatică, au observat o seroprevalenţă medie de 70,2%, situându-se între 43,5% şi 93,9% (3, 5, 6).

Utilizând metode invazive de diagnostic (examen histologic, testul rapid la urează, tehnici de cultură), prevalenţa infecţiei cu *H. pylori* în ciroza hepatică a înregistrat o reducere notabilă, fiind în medie de 46,1%, după cum reiese din 21 de studii care au inclus 1331 de bolnavi (3).

Un studiu recent efectuat pe 186 de cirotici demonstrează că prevalenţa infecţiei cu *H. pylori* evaluată prin teste serologice a fost semnificativ mai ridicată faţă de examenul histologic (66% vs. 44%). Sensibilitatea testului serologic la bolnavul cu ciroză hepatică este de 85,4%, iar specificitatea de 38,4%, net redusă faţă de lotul de control (7). Acest fenomen se poate explica prin reducerea imunocompetenţei, precum şi prin reacţia încrucişată dintre anticorpii anti-*H. pylori* şi antigenele organismului gazdă. Alţi autori au observat o prevalenţă similară a infecţiei cu *H. pylori* la bolnavii cirotici cu cea a lotului de subiecţi sănătoşi (8).

Cercetările din ultimii ani au adus în discuţie posibile corelaţii între prevalenţa infecţiei cu *H. pylori* şi profilul etiologic al suferinţelor hepatice. Astfel, există studii care arată o proporţie ridicată de bolnavi cu ciroză hepatică alcoolică infectaţi cu *H. pylori* comparativ cu etiologia post virală (9), sunt autori care notează preponderent etiologia virală în asociere cu infecţia cu *H. pylori* (8, 10) şi, în sfârşit sunt date care nu afirmă nici o asociere semnificativă în acest sens (11).

Referitor la corelaţia dintre severitatea cirozei hepatice evaluată prin scorul Child-Pugh şi infecţia cu *H. pylori*, se nasc de asemenea controverse. Majoritatea autorilor concluzionează că insuficienţa hepatică nu influenţează prevalenţa infecţiei cu *H. pylori* (5, 6). Totuşi, un studiu recent remarcă o corelaţie invers proporţională între severitatea suferinţei hepatice şi prevalenţa infecţiei cu *H. pylori* (8). Se pare că *H. pylori*, bacterie cu un echipament enzimatic foarte bogat care îi conferă o rezistenţă deosebită, nu reuşeşte să supravieţuiască în mediul gastric ostil al bolnavului cirotic.

ULCERUL PEPTIC

Ulcerul gastro-duodenal, una dintre cele mai comune suferinţe ale tubului digestiv recunoaşte o patogeneză multifactorială. În mod convenţional, factorii patogenetici se clasifica în două categorii : factori de agresiune (acidul clorhidric, pepsina, antiinflamatoarele non-steroidiene, fumatul, refluxul duodeno-gastric) şi factori de apărare (secreţia de mucus şi bicarbonat, prostaglandinele, celulele epiteliului de suprafaţă, microcir-

culaţia sanguină). *H. pylori* este o verigă patogenetică ce interferă atât cu mijloacele de agresiune, cât şi cu cele de apărare.

Majoritatea datelor publicate în literatură afirmă o prevalenţă crescută a bolii ulceroase la pacienţii diagnosticaţi cu ciroză hepatică, cu o incidenţă raportată anual de 10 ori mai mare faţă de populaţia generală (5, 12).

Ulcerul peptic este responsabil de 7,4--16% din totalul hemoragiilor digestive superioare la bolnavul cirotic. Rata mortalităţii prin ulcer peptic este de circa 5 ori mai ridicată la ciroze decât în populaţia generală, la aceasta contribuind tulburările de coagulare şi trombocitopenia specifice insuficienţei hepatice (3).

Relaţia ciroză hepatică-ulcer peptic este complexă şi controversată. Mecanismele ulcerogenezei din ciroza hepatică prezintă încă numeroase lacune care urmează a fi acoperite de cercetări viitoare. Au fost incriminaţi o serie de factori precum : infecţia cu *H. pylori*, secreţia clorhidro-peptică, perturbările mucoasei gastrice din gastropatia portal-hipertensivă, consumul de antiinflamatorii non-steroidiene, etilismul cronic. În ciroza hepatică se descriu o multitudine de evenimente care se derulează la nivelul mucoasei gastro-duodenale : reducerea nivelului de prostaglandine E2, secreţie inadecvată de mucus şi bicarbonat, creşterea retrodifuziunii ionilor de hidrogen, alterarea fluxului sangvin mucosal (13).

Calvet si col. au condus un amplu studiu, concluzionând că factorii de risc majori asociaţi bolii ulceroase din ciroza hepatică sunt reprezentaţi de infecţia cu *H. pylori* şi sexul masculin (14).

Prevalenţa ulcerului *H. pylori* pozitiv în ciroza hepatică, stabilită prin metoda serologică variază între 44-95% în cazul ulcerului duodenal şi între 50-87% în localizările gastrice (5, 6). Seropozitivitatea *H. pylori* este considerată de cei mai mulţi autori ca fiind un factor de risc independent în producerea ulcerului la bolnavul cu ciroză hepatică (14).

În conformitate cu două studii recente în care s-au prelevat biopsii de la nivelului *antrum*-ului gastric, infecţia cu *H. pylori* a fost detectată la 40% din pacienţii cirotici cu ulcer peptic, respectiv la 51,2% dintre bolnavii diagnosticaţi concomitent cu ulcer duodenal şi ciroză hepatică (15, 16).

În condiţiile în care pentru diagnostic se apelează simultan la examenul histologic, cultură şi test respirator cu uree marcată, infecţia cu *H. pylori* se decelează la o proporţie mare de cazuri (94% din 19 bolnavi cirotici şi ulceroşi fiind infectaţi cu *H. pylori*) (17).

Un studiu care a evaluat 60 de bolnavi cu ciroză hepatică şi ulcer, utilizând atât examinarea histologică cât şi testul rapid la urează, cu prelevare de biopsii antrale şi corporeale a evidenţiat infecţia cu *H. pylori* la 88% din cazurile cu ulcer duodenal şi la 83% din cazurile cu ulcer gastric (18).

S-a semnalat, de asemenea că există o prevalenţă semnificativ mai ridicată a ulcerului gastric la bolnavii cirotici infectaţi cu *H. pylori* faţă de lotul de control (22% vs. 3%, $p < 0,0001$), în timp ce prevalenţa ulcerului duodenal este similară în cele două grupuri (16% vs. 19% $p = 0,5$). În acest studiu s-a observat că ulcerul peptic *H. pylori* pozitiv a fost responsabil de 12% din hemoragiile digestive superioare (18).

TRATAMENTUL INFECŢIEI CU *HELICOBACTER PYLORI* ÎN CIROZA HEPATICĂ

Având în vedere prevalenţa înaltă a ulcerului peptic în ciroza hepatică, precum şi rolul ulcerogen de necontestat pe care îl deţine infecţia cu *H. pylori*, tratamentul de eradicare al infecţiei este pe deplin justificat, fiind un factor decisiv în creşterea ratei de vindecare a ulcerului, reducerea recurenţelor şi complicaţiilor, scăderea duratei şi costurilor de spitalizare şi, în final reducerea ratei mortalităţii la aceşti bolnavi.

Există, însă şi dificultăţi în alegerea schemei de eradicare. Oricum pacienţii cirotici primesc o multitudine de medicamente (diuretice, dizaharide, β-blocante), astfel incât un tratament antibiotic adiţional este greu de acceptat de către bolnav şi privit cu rezervă de către clinician.

Pe de altă parte, insuficienţa hepatică influenţează în mare măsură farmaco-cinetica medicamentelor. În ciroza hepatică se constată o prelungire a timpului de înjumătăţire al Metronidazolului, ceea ce se asociază cu o creştere a frecvenţei reacţiilor secundare, în timp ce are loc şi o reducere a concentraţiei serice a 14-OH-claritromicinei (metabolitul activ al Claritromicinei), conducând la scăderea eficienţei terapiei. În plus, studii recente semnalează că infecţia cu *H. pylori* alterează activitatea citocromului P-450, ceea ce determină perturbarea metabolizării Omeprazolului la nivel hepatic (3).

Un studiu randomizat incluzând 83 de pacienţi cu ciroză hepatică a comparat rata eradicării infecţiei cu *H. pylori* după dublă terapie administrată timp de 2 săptămâni (Omeprazol 20 mg x 2/zi şi Amoxicilină 1000 mg x 2/zi), cu triplă terapie administrată timp de 1 săptămână (Omeprazol 20 mg x 2/zi, Claritromicină 250 mg x 2/zi şi Tetraciclină 500 mg x 2/zi). S-a obţinut o rată de eradicare similară între cele două regimuri terapeutice (87,8% vs. 85,7%) (19).

Eficienţa dublei asocieri în eradicarea infecţiei cu *H. pylori* a fost constatată şi în alte studii, obţinându-se eradicarea în 21 de cazuri din 26 trataţi (81%), respectiv în 91% din 11 pacienţi cirotici trataţi (20).

Alţi autori au verificat regimurile terapeutice sub forma triplei terapii: rata eradicării a fost de 86% din 22 de pacienţi trataţi cu Omeprazol 20 mg x 2/zi, Claritromicină 500 mg x 2/zi şi Amoxicilină 1000 mg x 2/zi timp de 1 săptămână (21), respectiv de 88% din 27 de pacienţi trataţi utilizând aceeaşi schemă timp de 10 zile (22).

Un studiu recent a evaluat eficienţa triplei terapii (inhibitor de pompă de protoni, Amoxicilină 1000 mg x 2/zi şi Claritromicină 500 mg x 2/zi) la două loturi de bolnavi cu *H. pylori* pozitivi: 59 diagnosticaţi cu hepatită cronică şi 104 cu ciroză hepatică. Rata eradicării a fost de 84,7%, fiind similară în cele două loturi (hepatită cronică 88,1% vs. ciroză hepatică 82,6%). Nu s-au constatat diferenţe semnificative între grupul cu ciroză hepatică Child A (85,7%) faţă de grupul cu ciroză hepatică Child B/C (76,5%). Singurul parametru corelat cu rata eradicării a fost nivelul de ALT: la pacienţii cu nivelul ALT mai mic decât dublul valorii normale, rata eradicării a fost mai înaltă, sugerând astfel că eradicarea infecţiei cu *H. pylori* este condiţionată de gradul de inflamaţie necrotică de la nivel hepatic. Autorii au concluzionat că tratamentul de eradicare a infecţiei cu *H. pylori* la pacienţii cu afecţiuni hepatice este eficient şi sigur (23).

Un alt studiu prospectiv realizat într-un centru terţiar din Atena şi desfăşurat pe o perioadă de 7 ani, a evaluat 365 de pacienţi consecutivi diagnosticaţi cu ciroză hepatică. Ulcerul peptic a fost decelat endoscopic la 67 de bolnavi, în studiu fiind înrolaţi 30 dintre aceştia, care au fost ulterior testaţi pentru *H. pylori*: 63,3% *H. pylori* pozitivi, 36,7% *H. pylori* negativi. Pacienţii *H. pylori* pozitivi au primit tratament de eradicare (Omeprazol 20 mg x 2/zi, Amoxicilină 1000 mg x 2/zi şi Claritromicină 500 mg x 2/zi) timp de 1 săptămână, apoi Omeprazol 20 mg/zi 3 săptămâni. Pacienţilor *H. pylori* negativi li s-a administrat Omeprazol 20 mg/zi timp de 4 săptămâni. Evaluarea endoscopică s-a efectuat după 4 săptămâni de la terminarea tratamentului, la 12, respectiv 24 de luni. Pacienţii la care nu s-a obţinut eradicarea *H. pylori* au primit cvadruplă terapie 2 săptămâni, apoi Omeprazol 20 mg/zi încă 2 săptămâni. Rata eradicării după prima linie de tratament a fost de 79%, iar după a doua linie de tratament de 95%. Recurenţa ulcerului a fost observată în 17 cazuri (majoritatea – 13 în primul an, 4 în al doilea an). Interesant de semnalat a fost faptul că recurenţa

ulcerului s-a produs în 44% din cazuri în lotul pacienţilor *H. pylori* pozitivi trataţi şi 90% din cazuri în lotul bolnavilor cu status *H. pylori* negativ. Autorii subliniază ca eradicarea infecţiei cu *H. pylori* nu previne recurenţele ulcerului peptic la toţi bolnavii cirotici şi propun un tratament de lungă durată cu Omeprazol în toate cazurile, indiferent de statusul *H. pylori* (24).

Pe de altă parte, Villalan şi col. au arătat că eradicarea infecţiei cu *H. pylori* la pacienţii cu ciroză hepatică nu reduce rata recidivelor ulceroase, ceea ce sugerează că terapia de eradicare nu trebuie aplicată la ciroticii cu ulcer peptic (25).

Recent s-a dovedit că rata de eradicare a infecţiei cu *H. pylori* la bolnavii cirotici ulceroşi este strict dependentă de genotipul CYP2C19, deschizându-se astfel noi perspective asupra selecţiei pentru tratament a acestor pacienţi (26).

CONCLUZII

Deşi ulcerul peptic este mai frecvent la pacienţii cu ciroză hepatică, relaţia dintre *H. pylori* şi ulcerul peptic dezvoltat la pacienţii cirotici este incomplet elucidată, rezultatele studiilor publicate fiind heterogene şi contradictorii. Disfuncţia hepato-celulară constituie un factor suplimentar de agresiune asupra mucoasei gastro-duodenale la care se sumează factorii ulcerogeni clasici, inclusiv infecţia cu *H. pylori*.

Majoritatea datelor din literatură afirmă o rată înaltă de eradicare a infecţiei cu *H. pylori* la pacienţii cu ciroză hepatică şi ulcer peptic, tratamentul de eradicare fiind eficient şi sigur. Cu toate acestea, eradicarea infecţiei cu *H. pylori* nu previne recidivele ulceroase la toţi pacienţii cirotici, fiind necesară terapia cu inhibitori de pompă de protoni de lungă durată în toate cazurile, indiferent de statusul *H. pylori*.

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PHYSICIAN-PATIENT COMMUNICATION IN CHRONIC DISEASES

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PHYSICIAN-PATIENT COMMUNICATION IN CHRONIC DISEASES (Abstract): Chronic diseases represent an important public health issue, given that for many years they are the main cause of death worldwide and the specialists foresee they will soon become the main cause of disability as well. In this paper, the authors analyze the particularities of communication between physicians and patients with chronic diseases, the bidirectional nature of the physician-patient relationship, as well the barriers in establishing an effective relationship, together with the solutions in each case. Patients with chronic disease are particularly special, so that a very important element in the management of their disease is the communication they have with their physicians. Effective physician-patient communication is known to increase the adherence of the patient to the proposed treatment, to help the physician in establishing faster the correct diagnosis and in obtaining better therapeutic result, this leading to professional satisfaction. The effective relationship will have a positive impact over the medical system as well, as the admission in the hospital will be shorter, and, consequently, the costs will be lower. Anxiety, fear or unrealistic expectations of the patient, together with the large amount of work of the physician, his fear of litigation, of physical or verbal abuse from the patient are some of the most important barriers that can be managed to improve the quality of the medical act. The skills needed to communicate effectively with the patient must be learnt, as people do not born with them, and they can be acquired through postuniversitary training. Education of the patients is another issue that must be accomplished to obtain an effective physician-patient relationship, as they will become real partners in their own medical care. **Keywords**: PHYSICIAN-PATIENT COMMUNICATION, CHRONIC DISEASE, BARRIERS, THERAPEUTIC ADHERENCE.

Chronic diseases are characterized by a slow evolution over a long period of time (1), with the symptoms occurring gradually. These diseases are frequently associated with functional disorders or disability, existing, as well, an increased risk for complications to occur (2). World Health Organization divides chronic diseases in four categories: cardiovascular diseases (such as heart attacks and stroke), cancer, respiratory chronic disease (such as chronic obstructed pulmonary disease and asthma) and diabetes (1).

Currently, chronic diseases are the main cause of death in the world, with ischemic coronary disease and stoke holding constantly the first places in the last 15 years (3). More, it is suggested that the incidence

of these diseases will significantly increase in the next couple of years, to become the leading cause of disability as well (4). As a reaction to these alarming projections, collaborative care gains more and more attention (4), the patient being considered a partner in the decision-making process regarding his/her healthcare (5).

Particularities of the patient with chronic disease

Prolonged evolution of the chronic diseases and delayed symptom occurrence explains why these diseases are diagnosed usually in elderly, life stage associated with an increased number of comorbidities- the most frequent being the cardiovascular group, as well as with polytherapy (5). More, psychological disturbances- such as depression, often accompany the chronic diseases, increasing in this way the morbidity and mortality, for which diagnosis and treatment of depression becomes critically important (6).

The moment when they find out the diagnosis of chronic disease is very difficult to bear for most of the patients as they feel vulnerable, confused, without control, being in risk to alter the self-image (7). Likewise, patients with chronic diseases are constrained to endure the difficulty of secondary psychological problems, and if we count the social disturbances, the burden becomes even more difficult to endure, the patients being constrained to make changes both regarding the image of their bodies and their future expectances (8).

Most of the chronic diseases require long life treatment, this being an additional stressor both at physical and psychical level, with the risk to precipitate the symptomatology, being therefore necessary a particular attention to these issues, in order to establish the proper treatment.

Patient centered medicine

Although the patient-centered medicine precepts have their roots in Ancient Greek School of Cos, they were not applied since the beginning, being rather preferred the paternalistic type physician-patient relationship, when the physician alone establishes the treatment, in the virtue of his professional knowledge, while the patient is considered the beneficiary of the medical services, without any possibility to be an active part in the decision-making process (9-11). However, in the last years, the emphasis lays on the collaboration between physician and patient and the involvement of the patient in the decision-making process regarding the therapeutic plan (9, 11, 12). This approach has a series of important advantages, like: facilitates the right diagnosis in a short time interval as the patient will cooperate better, and implicitly adequate recommendations for his treatment (6), as well as the patient adherence to therapy with the subsequent improvement of the results (13), increases the patient complacency and decreases the expenses of medical care as the hospital admission time period will last shorter (6). On the other hand, the risks associated to failure in the effective communication process between the physician and the patient often materialize in losing the trust of the patient, which will lead to lack of his adherence to treatment (6).

Physician-patient with chronic disease relationship – a bidirectional relationship

Therapeutic relationship between the

physician and the patient is considered "the heart and art of medicine" (9), and an essential role in building this relation is acknowledged to effective communication between the two protagonists. Starting with obtaining of appropriate medical history and continuing with the choice of the optimum therapy, the physician-patient relationship rests on communication, and the physician's role in improving the evolution of chronic disease is crucial.

In collaborative care, therapeutic relationship is mutual and dynamic, always existing a bidirectional exchange of information (10, 12, 14, 15). Thus, on the one hand is the fundamental information coming from the patient side, regarding his endurance reflected in symptoms (16) and his inner state, anxiety and emotions (15), as well as those regarding the expectations (9), preferences and concerns (16) or the accepted risk level (13). On the other hand, lays the information coming from the physician side, regarding the disease and the treatment (13). This information must be transmitted using an appropriate language, according to the patient capacity to understand the notions, this being an essential aspect to be an active part in the therapeutic process-making (9, 15).

The successful physician- patient communication materialize in an accurate and quick diagnosis, in psychological counseling and appropriate therapy recommendation, with the final aim being the improvement of the patient's health and healthcare. In this way, the patient will gain self-confidence, will be motivated and optimistic regarding his health- essential items, given that the optimum psychological state may lead to improvement of the results (9).

Plato stated almost two and a half millennia ago that "the greatest mistake in the treatment of diseases is that there are physicians for the body and physicians for the soul, although the two cannot be separated". Combining the concerns regarding the physical suffering and the inner distress is vital to build an effective doctor-patient relationship.

More, efficient communication between the physician and the patient helps the former to understand expectations and experience of the patient, as well as the social context in which the latter lives (9).

An important role in collaborative care have also the emotional problems of the patient with chronic disease, assessed during the same effective information exchange, as well as using the cognitive-behavioral principles and combining the communication skills with evidence-based knowledge (4).

To accomplish this challenge, the physician must gain the proper skills, both interpersonal and communication-related, which will later lead to accumulation of the proper information (9).

Benefits of the effective physician-patient communication

There are numerous benefits of the effective physician-patient communication, about to both.

Thus, the patient can regulate his emotions and can understand the medical information, which will allow him to identify his own needs, perceptions and expectations. In this way, the patient will feel he is involved in the therapeutic process and will adhere easier to the proposed treatment, the quality and results of the care will improve, and his satisfaction will increase.

The physician, by combining his profes-

sional training with the interpersonal communication skills will succeed in establishing the right diagnosis in a shorter period and obtain better therapeutic results, which will lead to professional satisfaction, decrease in the stress level and decreased exhaustion risk.

Not least, effective physician-patient communication, quick diagnosis and patient's adherence to therapy has a positive impact over the medical system, as the admission in the hospital will be shorter, and, consequently, the costs will be lower (9).

Patient's adherence to treatment assumes more than his simple compliance to therapy, being the result of his direct involvement in establishing the therapeutic plan. When the patient is asked about different ways to manage his disease, he will feel more responsible and will follow easier the recommendations regarding the medication, diet or life style (5, 17).

Therefore, the characteristics of the effective physician-patient relationship include a relaxed environment for the medical interview, discussing various therapeutic options and the regimen which is to be put into practice, as well as scheduling the check-up visits- to assess the efficiency of the administered therapy according to the way the disease will evolve (17).

The physician must pay attention when he seeks words to be used during the discussion with the patient. Communication assumes also non-verbal language, so that the attitude, gestures, and mimic, will have at least the same influence in the transmission of the information (15). When the physician communicates as the patient expects, the latter will be more willing to provide the information required to establish the right diagnosis (incomplete information from the patient may delay and negatively influence establishment of the diagnosis as well as the evolution of the disease), to follow the medical recommendation, to adhere the prescribed treatment (9). All of these will finally result in: recovery (18), increased pain tolerance, decrease in tumor growth, improvement in the mental health and a shorter hospitalization (9).

In a therapeutic relationship based on effective communication the patient could understand the prognosis of his disease, the aim of the care, the expectations regarding the evolution of the disease and the necessity of his own involvement in the treatment (19, 20). These aspects are likewise important for the situation when it is a need to establish the end-of-life treatment, an issue that may have a significant influence over the disease (10, 12, 21), especially because in some chronic disorders dementia sets earlier, and if hypoxia is added, will result a cognitive decline, which becomes more and more severe with aging (5). Therefore, in order to facilitate the establishment of treatment in the event of severe degradation of the decisional competence of the patient along with evolution of the chronic disease, a pro-active attitude it is needed by approaching this subject before the installation of the physical and cognitive decline: identification of medical issues that might occur during the evolution of the disease, the attitude that must be approached in different moments in time and building a therapeutic individualized plan (10, 22).

The communication skills of the physician will lead to an increased self-confidence regarding the patient's participation in discussions related to his disease

and the healing process (18).

Communication skills regard alike the content of the information and the way it is transmitted and received, including: skills for careful and active listening, empathy, using open answer questions (9), clarifying the psychological issues, positive body language (15), supporting the patient to verbalize the emotions, exploring the patient's perspectives (6).

Frequent meetings between the patients and their physicians provide the latter multiple opportunities to optimize the communication relationship, to monitor the treatment or to detect new medical issues, related or not with the initial disease. Information exchange should focus on building up a partnership and counseling the patient.

In order to gain the battle with the disease, the patient needs to be acquainted with it, needs to know how it acts, who is helping it and its weak points, a precious ally in this endeavor being the doctor himself.

Barriers in establishing a therapeutic relationship

A series of barriers may hinder the establishing of a therapeutic relationship between the physician and the patient with chronic disease. These may come from the side of the patient, such as anxiety, fear or unrealistic expectations, or from the side of the physician, generated by the large amount of work, fear of litigation, fear of physical or verbal abuse from the patient (9), discouraging patients to express their fears, concerns, expectations or asking for additional information (13).

It is thus created a vicious chain: if the patient does not have all the information in his terms, if he does not express his concerns, the therapeutic plan is at risk to fail (13). More, there is the risk that patient who is not pleased regarding the communication he had with his physician, to accuse the latter more often for malpractice when the result of the therapy is not the expected one, and more, not for lacking medical knowledge, but for gaps in communication (9, 23)

The moment when the patients find out the diagnosis of their chronic disease, wears the burden of deep feelings: emotion, concerns regarding the disease, shock etc., so that there is a risk that these feelings will monopolize the thoughts of the patient, making him to forget most of the information received, the more so as his cognitive state is damaged (8). These issues are additional barriers in communication and have a negative impact towards understanding the disease, being often associated with non-adherence to therapy (5, 8).

Solutions to establish a therapeutic relationship

There are some solutions the physician can bring forward to support the patient with chronic disease. They regard provision of printed information, for example flyers, provided that the language is appropriate, and the patient can read them. Likewise, if the patient agrees, instruction can be forwarded to family members that wish to be part of the therapeutic plan (8). Before ending the medical visit, the physician should make sure that the patient understood the information received by revising the notions, which will be resumed and strengthened in every subsequent check-up (5, 8, 20). Provision of the medical information both verbally and written increases the chances that the patient will follow the

recommendation (5, 20). Depending on the education level of the patient and his desire to find out additional information, the physician may recommend various other genuine resources, such as books, websites or support groups, where the information is explained using appropriate language. Some studies show that the possibility to contact the physician off work through email or telephone improved the physician-patient communication and the latter's adherence to therapy (7). Likewise, another encouraging element in facing the disease might be the case presentations of various famous people who survived with the same disease or the results of reliable studies in this field (24).

Even though in an effective therapeutic relationship discussion between the patient and his physician should emphasize the understanding of notions related to the course of the disease, its impact on the patient's life, the risks and benefits of each therapeutic option, many of the medical interviews are encumbered by a technical language, difficult to be understood by the patient (20). To be truly helpful for the patient, medical information should be complete, including the aim, the manner and schedule for administration, and possible side effects of the therapy. In this way are eliminated misunderstandings related to the medication administration, and chances for adherence to the therapy will increase accordingly (5). Moreover, information should be provided correctly and promptly, adjusted to the medical condition, patients' needs and preferences. The patient and his family will thus face better the diagnosis, will become aware of the available supportive measures, while the doubt and suffering will proportionally decrease (25).

As the disease worsens, the patient will adapt more and more difficult, will be overwhelmed by negative feelings, such as: physical and psychical helplessness, decreased self-esteem, independence and hope, fear facing the disease, concerns regarding the decreased capacity to cope with the disease, which will lead to decreased ability to decide and seek for the needed support (8, 26). In supporting the patients, the physician may offer explanations regarding how the disease progress, explanations regarding the interpretation of the laboratory investigation results and therapeutic plan, all these leading in the end to a diminished impact of the disease towards the patient (5).

In building the therapeutic relationship between the physician and the patient with chronic disease, the focus should also be on the education of the patient, as an integral part of a therapeutic whole: increasing the level of knowledge about his disease and effective implication of the patient in the management of his own disease. This will result in improving the quality of life, the independence and the degree of (re)integration in society, elaboration of a plan to modify the life style and helping the patient to accomplish these changes (24).

Communication skills can be learned

Communication skills are not innate, nor accumulated along with the clinical experience. They can, however, be learned and will remain embedded by continuous practice (6, 9, 11). Ideally, the process of learning these skills starts in the first years after entering the faculty (4, 9), and they will be supplemented along the professional life by participation in postgraduate courses, for example. The educational pro-

cess dedicated to learning physician-patient communication skills must focus on the practical aspect. For this purpose, role play can be very useful, with or without actors mimicking patients. Some special techniques can be useful in the educational process, such as: WEMS (Waiting, Echoing, Mirroring, Summarizing) and NURSE (Naming, Understanding, Respecting, Supporting, Exploring) (6).

Physician's and patient's opinion towards successful communication do not always overlap, the physician having the tendency to overestimate his own communication skills. Thus, a study performed by Tongue *et al.* shows that 75% of the surveyed orthopedic surgeons were satisfied by the manner they communicated with their patients, while only 21% of the patients were content of the communication they had with their physicians. More, the patients tend to assess the professional capacity of their physician also in the view of the latter's ability to transmit information regarding the disease their patients suffer of and the proposed therapeutic plan (9).

CONCLUSIONS

Nowadays, the chronic diseases represent the main cause of mortality and morbidity across the world, with a prediction that their incidence will significantly increase. To effectively manage his disease, the patient suffering of a chronic disease should learn to live with it and adjust his life style in a manner that will ensure a better evolution of the disease. Physician-patient communication represents a key element in accomplishing this essential desideratum.

The benefits of effective communication between the physician and the patient are well known and studied, these having a serious impact both over the medical aspects which reside mainly in a favorable evolution, as well as over the psycho-emotional aspects, which have many times an important impact on the patient with chronic disease.

Effective communication between the physician and the patient assumes, on the one hand, building up communication skills during the academic and postuniversitary training of the physicians, so that they will be able to effectively combine the medical knowledge and the empathy and emotional support offered to their patients, and on the other hand education of the patients so that they will become real partners in their own medical care.

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DISLIPIDEMIA – FACTOR DETERMINANT AL CARDIOPATIEI ISCHEMICE

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DISLYPIDEMIA AS A RISK FACTOR FOR CARDIOVASCULAR DISEASE (Abstract): The aim of the study was to evaluate dislypidemias as risk factor in the countryside. **Material and method**: There have been evaluated 413 patients from the countryside (Jorăşti, Galaţi County), in the period 2008-2010. There was performed history taking, physical examination, an ECG and biological findings (glycemia, colesterolemia, HDL-colesterolemia, trygliceridemia, creatinenemia). **Results**: In these patients there was noticed a higher prevalence of the dislypidemias in women (65.7%) than in men (50.29%). Both dislypidemias and hypertension were more frequent in patients with cardiovascular disease or in those with high risk of cardiovascular disease than in those with a small or absent risk both in men and in women. In 208 cases there have been present all the elements of the metabolic syndrome. **Conclusions**: The modifiable risk factors have a very important part in the development of cardiovascular diseases in the countryside. Using proper programmes of prophylaxis there can be hope for a decrease of the morbidity and mortality of cardiovascular disease. Key words: CARDIOVASCULAR RISK FACTORS, DYSLIPIDEMIAS, CARDIOVASCULAR DISEASE

Dislipidemiile cuprind totalitatea modificărilor calitative şi cantitative ale lipidelor din sânge (creşteri sau scăderi). În practica medicală termenul este restrâns la hiperlipidemiile care sunt însoţite şi de scăderea Col-HDL. Hiperlipidemiile reprezintă creşterea în sânge a colesterolului şi/sau trigliceridelor peste valorile normale. Valorile normale reprezintă o medie statistică populaţională şi corespund la cea mai scăzută mortalitate şi morbiditate cardiovasculară. Colesterolul circulant are rol central în patogeneza aterosclerozei (1,2).

Dislipidemia este un important factor de risc cardiovascular, iar în cele mai multe situaţii, ea se însoţeşte de diabet zaharat, obezitate şi hipertensiune arterială (entităţi reunite în sindromul metabolic). Studiile recente au subliniat implicarea dislipidemiilor în afecţiuni nu doar din sfera cardiologiei, diabetologiei, nutriţiei ci şi în patologii din sfera gastroenterologiei, hepatologiei, nefrologiei, reumatologiei, neurologiei şi bolilor infecţioase.

Studiile observaţionale au demonstrat că reducerea incidenţei a principalilor trei factori de risc majori, fumat, dislipidemie şi hipertensiune arterială este responsabilă de peste 50% din reducerea mortalităţii cardiovasculare.

EVALUAREA RISCULUI CARDIOVASCULAR

Bolile cardiovasculare reprezintă în continuare prima cauză de morbiditate şi mortalitate în ţările civilizate. Riscul cardiovascular este reprezentat de totalitatea acţiunii factorilor care produc boli cardiovasculare aterosclerotice. Prin controlul acestor factori pot fi influenţate aterogeneza şi consecinţele

ei clinice : cardiopatia ischemică, boala cerebrovasculară şi arteriopatia periferică (3,4).

Factorii de risc cardiovascular :

1. Stilul de viaţă : dietă aterogenă (bogată în grăsimi saturate, colesterol şi calorii), fumatul (> 10 ţigări/zi), excesul de alcool, sedentarismul, stresul psiho-social
2. Vârsta : bărbaţi > 45 ani, femei > 55 ani sau postmenopauză
3. Istoric familial de cardiopatie ischemică sau alte boli cardiovasculare cu instalare precoce (< 55 ani pentru tată sau frate şi < 65 ani pentru mamă sau soră)
4. Caracteristici biochimice şi fiziologice : hipercolesterolemie (creşterea Col-LDL > peste 130 mg/dL), Col-HDL < 40 mg/dL (< 1 mmol/L) pentru barbaţi şi < 50 mg/dL pentru femei ; hipertrigliceridemie > 150 mg/dL ; hipertensiune arterială (chiar controlată medicamentos) ; diabet zaharat ; obezitate.

La evaluarea riscului cardiovascular poate fi eliminat un factor de risc în cazul în care Col-HDL > 60 mg/dL (1,6 mmol/L).

MATERIAL ŞI METODĂ

Lotul de studiu a cuprins un număr de 413 pacienţi din mediul rural care s-au prezentat la CMI Dr. Anghel Lucreţia- Joraşti , judeţul Galaţi în perioada 2008-2010.

Evaluarea pacienţilor s-a făcut prin :

I Anamneză – a cuprins întrebări privind :

1. vârsta, sexul, starea civilă, nivelul de instruire, ocupaţia
2. antecedente heredo-colaterale : deces prin boală cardiovasculară sub 55 de ani, cancer diagnosticat sub 65 de ani, diabet zaharat, hipercolesterolemie familială
3. antecedente personale patologice : existenţa de boli cronice, boli cu transmitere sexuală, intervenţii chirurgicale, medicaţie relevantă
4. stilul de viaţă : alimentaţia, consumul de alcool, activitatea fizică, fumatul

II. Examenul clinic obiectiv – inclusiv măsurarea înălţimii şi a greutăţii, a taliei, a perimetrului abdominal, (pentru obezitatea abdominală, normal sub 94 cm la bărbaţi şi 80 cm femei) calcularea indicelui de masă corporală, măsurarea tensiunii arteriale (3 valori la interval de 5 minute) şi a pulsului

III. Investigaţiile au cuprins : EKG, glicemie, colesterolemie, HDL-colesterolemie, trigliceridemie, creatininemie, sideremie.

REZULTATE ŞI DISCUŢII

Boala cardiovasculară este responsabilă de 17 milioane de decese în fiecare an. Specialiştii estimează că în zece ani numărul deceselor va ajunge la 25 de milioane pe an. Potrivit studiului EuroAspire III, 2, 50% din totalul deceselor din Europa sunt provocate de bolile cardiovasculare. România se afla în primele 4 locuri în lume în ceea ce priveşte mortalitatea de cauză cardiovasculară. Riscul de apariţie a bolii cardiovasculare la populaţia adultă din România în următorii 10 ani este de 3,5%.

Pe linia aceloraşi eforturi de prevenţie , studiul nostru a urmărit evidenţierea rolului important pe care dislipidemiile îl joacă în apariţia bolilor cardiovasculare.

Repartiţia pe sexe a pacienţilor peste 60 de ani incluşi în studiu

Din studiile efectuate pe lotul de pacienţi se obsevă că valorile colesterolului total sunt în medie mai mari la femei (65,70%) decât la bărbaţi (50,29%).

Atât femeile câ şi bărbaţii cu boală cardiovasculară prezentă şi cu risc crescut de boală cardiovasculară prezintă valori ale colesterolului mult mai mari faţă de cei cu risc scăzut sau fără risc de boală cardiovasculară. Din totalul de 413 pacienţi bărbaţi şi femei, 110 sunt cu boală cardiovasculară prezentă iar dintre aceştia 74 (62,27%) sunt cu valori crescute ale colesterolului. Din totalul de 413 pacienţi 65 sunt fară risc de boala cardiovasculară, iar dintre aceştia 9 pacienţi sunt cu valori crescute ale colesterolului.

Se observă în lotul de femei o prevalenţă

PACIENȚI FEMEI	NR. PACIEN?I	TA		COLESTEROL TOTAL		HDL COLESTEROL			TRIGLICERIDE		
		HTA	TA normală	Val. normale	Val. crescute	Val. normale	Val. crescute	Val. scăzute	Val. normale	Val. crescute	Val. scăzute
CU BOALĂ CARDIOVASCULARĂ PREZENTĂ	67	40	27	16	51	62	3	2	58	7	2
CU RISC CRESCUT DE BOALĂ CARDIOVASCULARĂ	72	60	72	12	60	64	5	3	63	9	0
CU RISC SCĂZUT DE BOALĂ CARDIOVASCULARĂ	67	20	47	22	45	60	3	4	59	6	2
FĂRĂ RISC DE BOALĂ CARDIOVASCULARĂ	36	4	32	33	3	32	0	4	36	0	0
TOTAL PACIENȚI	242	124	118	83	159	218	11	13	216	22	4
		54,24%	48,76 %	34,30 %	65,70 %	90,08 %	4,55 %	5,37 %	89,26 %	9,09 %	1,65 %

PACIENȚI BĂRBAȚI	NR. PACIEN?I	TA		COLESTEROL TOTAL		HDL COLESTEROL			TRIGLICERIDE		
		HTA	TA normală	Val. normale	Val.crescute	Val. normale	Val.crescute	Val. scă zute	Val. normale	Val. crescute	Val. scăzute
CU BOALĂ CARDIOVASCULARĂ PREZENTĂ	43	16	27	20	23	41	0	2	34	8	1
CU RISC CRESCUT DE BOALĂ CARDIOVASCULARĂ	86	50	36	33	53	74	5	7	76	8	2
CU RISC SCĂZUT DE BOALĂ CARDIOVASCULARĂ	13	1	12	9	4	10	1	2	12	0	1
FĂRĂ RISC DE BOALĂ CARDIOVASCULARĂ	29	7	22	23	6	27	2	0	28	1	0
TOTAL PACIENȚI	171	84	97	85	86	152	8	11	150	17	4
		49,12 %	56,73 %	49,71 %	50,29 %	88,89 %	4,68 %	6,43 %	87,72 %	9,94 %	2,34 %

ridicată a hipercolesterolemiei atât în cazul pacientelor cu afecţiuni cardiovasculare prezente (76,1%), cât şi în cazul celor cu risc crescut de boală cardiovasculară (83,3%).

Asocierea cu hipertensiunea arterială este de asemenea semnificativ mai importantă la pacientele cu boală cardiovasculară (59,5%) şi la cele cu risc crescut de boală cardiovasculară (83,3%), faţă de cele cu risc scăzut (asociere de 29,8%) sau absent (asociere de 11,1%).

În cazul bărbaţilor se observă de asemenea o prevalenţă ridicată a hipercolesterolemiei la pacienţii cu boală cardiovasculară prezentă (53,5%), precum şi la cei cu risc crescut de boli cardiovasculare (61,6%), dar corelaţia este mai mică decât în cazul femeilor.

De asemenea în cazul lotului de bărbaţi asocierea cu hipertensiunea arterială este semnificativ mai importantă la pacienţii cu boală cardiovasculară (37,2%) şi la cei cu risc crescut de boală cardiovasculară (58,1%), faţă de cei cu risc scăzut (asociere de 7,5%) sau absent (asociere de 24,1%).

Frecvenţa mai mică a dislipidemiei şi a hipertensiunii la pacienţii de sex masculin cu boli cardiovasculare sau cu risc de boli cardiovasculare faţă de pacienţii de sex feminin este legată probabil de existenţa asocierii mult mai importante la lotul de bărbaţi cu ceilalţi factori de risc (fumat, consum de alcool)

Riscul de a dezvolta disfuncţie endotelială creşte cu numărul factorilor de risc prezenţi.

În funcţie de antecedentele heredocolaterale :

- Deces prin boală cardiovasculară la rude de gradul I-II : 2 cazuri la bărbaţi şi 17 cazuri la femei
- DZ tip 2 la rudele de gradul I – II : 4 cazuri la bărbaţi şi 11 cazuri la femei
- Hipercolesterolemie familială la rudele de gradul I – II : 1 caz la femei

Într-un număr de 208 cazuri pacienţii au prezentat sindrom metabolic, cu o frecvenţă mai mare la femei 124 (59,62%) şi 84 bărbaţi (40,38%).

În 89 de cazuri pacienţii au prezentat asocierea de multipli factori de risc : fumat, hipertensiune arterială, HDL < 40 mg/dL, istoric familial de boală cardiacă ischemică (BCI) precoce (BCI < 55 ani la rude de sex masculin şi BCI < 65 ani la rude de sex feminin), vârsta (bărbaţi > 45 ani, femei > 55 ani)

Studiul demonstrează importanţa factorilor de risc modificabili în apariţia bolii cardiovasculare. El se aliniază eforturilor concertate ale specialiştilor români ce urmăresc scăderea mortalităţii cardiovasculare cu 10% în următorii cinci ani. Este necesară elaborarea unei strategii care să îmbine atitudinile individuale (evaluare, stratificarea riscului cardiovascular, aplicarea intervenţiei, reevaluarea rezultatelor) cu cele populaţionale (accesibilitate crescută la măsurile de prevenţie cardiovasculară), având drept obiectiv fundamental reducerea morbidităţii şi mortalităţii cardiovasculare (5).

Unele măsuri pe care ţări vest-europene le-au adoptat şi la care medicii de familie din ţara noastră s-ar putea ralia cu succes sunt :

- realizarea de cercetări naţionale şi internaţionale privind nutriţia şi bolile cardiovasculare
- integrarea în serviciile de sănătate, în special cele primare, a recomandărilor privind o alimentaţie corectă şi echilibrată
- implementarea de programe de promovare a unei alimentaţii corecte
- programe de informare în şcoli şi instituţii educaţionale
- realizarea unui sistem de monitorizare a comportamentelor privind sănătatea, factorii de risc, nutriţia, bolile şi mortalitatea la nivelul populaţiei (6,7,8).

CONCLUZII

Studiul demonstează rolul foarte important jucat de factorii de risc modificabili

pentru apariţia bolilor cardiovasculare la populaţia rurală din România.

Atât femeile cât şi bărbaţii cu boală cardiovasculară prezentă, cât şi cei cu risc crescut de boală cardiovasculară prezintă valori ale colesterolului mult mai mari faţă de cei cu risc scăzut sau fără risc de boală cardiovasculară.

Hipertensiunea arterială este mai frecventă la pacienţii cu boală cardiovasculară cât şi cei cu risc crescut de boală cardiovasculară faţă de cei cu risc scăzut sau absent. Aceasta oferă speranţa că prin măsuri adecvate de prevenţie se poate obţine o scădere a incidenţei bolilor cardiovasculare şi deci a morbimortalităţii asociate.

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NOUTĂŢI

STUDIUL TOXICITĂŢII EXTRACTULUI APOS DE *TRIGONELLA FOENUM-GRAECUM*

Utilizarea plantelor medicinale în terapie este o practică frecventă în ţările în curs de dezvoltare. Pentru multe dintre aceste plante lipsesc informaţiile privind posibilele efecte adverse. Prezentul studiu evaluează toxicitatea seminţelor de schinduf (*Trigonella foenum-graecum* (L.)) la animale de experienţă gestante. Extractul apos liofilizat obţinut din seminţele speciei a fost administrat în doze de 500 şi 1000 mg/kg/zi unor femele gestante de şoarece pe parcursul întregii perioade de gestaţie. S-au observat o scădere în greutate a fetuşilor, o creştere a ratei de mortalitate a fetuşilor, precum şi o creştere a incidenţei malformaţiilor fetale. Seminţele de *Trigonella foenum-graecum* (L.) influenţează negativ funcţia de reproducere şi au potenţial teratogen (Khalki L, Ba M'hamed S, Bennis M, Chait A, Sokar Z. Evaluation of the developmental toxicity of the aqueous extract from *Trigonella foenum-graecum* (L.) in mice. *J Ethnopharmacol* 2010 ; 131 : 321-325).

Anca Miron

EDITORIAL

FIGHTING FOR A SUCCESSFUL AGEING

"Who wants to live forever?" and Freddie Mercury were in our minds and our hearts more than thirty years ago. Of course, it meant something completely different than now. Well, I do not know about "forever" but most of us would want to live longer and, at the same time, to enjoy life as much as possible without any burden associated with old age. We want to live longer but with the same physical and mental strength as in our younger years, we want to enjoy ourselves with the same intensity as we used to, and we want to stay as far away as possible from diseases, disabilities, and dependence.

This trend of thought is increasingly popular as the number of elderly increases, being of great interest not only for the beneficiaries but also for the medical and social services. The response to this increasing demand has been developing over the last twenty years as a general concept with several denominations: healthy ageing, successful ageing, positive ageing, ageing well, ageing productively (1).

The broad concept of active and healthy ageing was proposed by the World Health Organization (WHO) as the process of developing and maintaining functional ability that enables wellbeing in older age (2). On a broader scale, healthy ageing means optimizing opportunities for physical, social and mental health to enable older people to take an active part in society without discrimination and to enjoy an independent and good quality of life. It means considering the concepts of independence, autonomy, purposeful and meaningful existence, and the opportunity to participate and contribute. To fulfill these aspirations, people need to maintain adequate physical and mental health status (3, 4).

But it is not that simple as it sounds. It is well documented that ageing is influenced by genetic factors and modifiable factors (that we can safely rename as our own lifestyle-dependent factors). Rethinking our lifestyle from the earliest period of our life is really the best start for a healthy ageing. But what can be done for those who "missed the starting gun" and are facing, in older age, this terrible challenge-changing life-time habits (most of them being the very source of self-enjoyment-good food, good wine, a cigarette or two, lazy Sunday afternoons) with new, healthier habits, most of them totally hateful-untasty food, water instead of wine, no cigarettes whatsoever and a lot of exercise, usually in terrible weather or embarrassing attempts to do aerobics.

When to start? Where to start? The good news is that it is never too late - the challenge is how to do it and actually enjoying it at the same time.

Normal ageing is intimately related to two very important phenomena that we must face and should fight, sarcopenia and obesity, and it is widely believed that first important steps in healthy ageing mean a good understanding of these two elements.

Sarcopenia is an age-related process of skeletal muscle loss associated with declining physical performance. It was first described by Rosenberg in 1989, who gave it

the name from the Greek "sarx" = meat and "penia" = loss (5). It is highly prevalent among older subjects, reaching 15–24% in 60–70-year-olds, over 50% in subjects aged 80+, and close to 70% in nursing home residents (6). Sarcopenia is associated with a risk of adverse outcomes such as physical disability, poor quality of life and death, which promote it as a major public health problem in the world.

As the studies on sarcopenia multiplied in the last 20 years, they brought to attention that new parameters were needed for a more accurate diagnosis, such as determination of muscle mass, muscle strength and physical performance. New techniques were recommended as golden standard in the diagnosis of sarcopenia instead of the well-known and somewhat warned anthropometric measurements: computed tomography, magnetic resonance imaging, dual energy X-ray absorptiometry, and bioimpedance analysis for the determination of muscle mass, handgrip strength, knee flexion/extension and peak expiratory flow for muscle strength, and short physical performance battery for physical performance. All these tests proved that there is a progressive mismatch between mass and strength that occurs because of a progressive deterioration of muscle “quality”, including decrease in fiber size and number, intrinsic reduction of contractility in the intact fibers, fat micro and macroinfiltration, increase in collagen, modification of the motor unit, and impaired neurological modulation of contraction.

Evidence has also emerged that muscle strength is more important than muscle mass as a determinant of functional limitation and poor health in older age. The loss of muscle strength rather than muscle mass is called dynapenia, and some have argued that the term sarcopenia should be replaced by dynapenia, which is better suited to describe age-associated loss of muscle strength and function.

Determining the most appropriate reference value of muscle mass for the purpose of making a diagnosis of sarcopenia is still an important issue in different populations in the world. To establish low muscle mass, some studies have assessed the total or appendicular skeletal muscle in young adults as the reference value. For a clinically applicable definition, correction factors such as height, body mass index, and body fat have been used, and that brought us back to obesity and its leading part in ageing.

Obesity is defined as abnormal or extensive fat accumulation that negatively affects health. Until recently, its diagnosis was based on the values of Body Mass Index (BMI). However, recent studies pointed out that new parameters, which include measures of body composition, fat and fat-free mass are of greater importance than BMI, and therefore should be determined when diagnosing obesity, especially in elderly population.

It is common knowledge that the association between sarcopenia and obesity, most frequently encountered in the ageing population is an entirely different entity, called sarcopenic obesity, which is associated with higher risk for mortality than sarcopenia and obesity by themselves (7).

These new concepts influenced the physicians’ approach to changing the lifestyle in elderly persons. It is now agreed that keeping a constant, vigorous physical activity will guarantee an improved longevity by decreasing the risk of sarcopenia and sarcopenic obesity, frailty, and chronic diseases. It will also guarantee a better

quality of life as it improves functional health, cognitive functions and reduces the risk of falls and fractures. However, people tend to become less active as they get older and even less active if they have chronic dyspnea or chest pain or osteoarticular problems. Evaluating the degree and type of physical activity that the patient qualifies for and fitting it into patient's type of life is vital and it requires a good relationship with the patient who should be convinced of the benefits of these alterations in his/her life (8).

It is also a well-known fact that good eating habits and healthy foods are keys for staying healthier for longer. Many of the diseases that torment elderly people are the results of dietary factors, some of them acting since childhood. Education and a healthy diet since infancy tend to promote and maintain healthy habits throughout the rest of our life (9).

How easy is to change old (and pleasant) eating habits in later life? With old age there will be different nutritional requirements and fighting cachexia and sarcopenic obesity should become one of the main goals. Combining specific nutritional needs with restrictive diets for the inevitable metabolic and cardiovascular diseases and making them look good is almost a "bridge too far".

Even considering the inevitable cognitive and psychological changes among old people, everybody wishes to remain useful, alert and socially interconnected. But the barriers are innumerable: retirement, age discrimination, neglect, abuse in all its forms, etc. Sustained education should promote better ways of using the huge amount of energy and lifelong experience the elderly people have. Health and social programs should promote and actively increase the employment of elderly people either in their own field, by training and transferring knowledge and experience to younger generations, or in new jobs that would require small investments in retraining (10).

Involving older people in volunteering activities is another important option, as it helps keeping them useful and avoids social exclusion. Explaining these opportunities to our ageing population would be the main tool we have in promoting healthy mental and functional ageing. We should never forget that the environment helps determine how active older people can be in society-the more accessible and age-friendly the environment, the more active old people can be. Participation in all types of activities builds and consolidates social connections and improves self-esteem and wellbeing.

It is not an easy task to promote and ensure healthy ageing, but the good news is it is not a one-man task, but a multidisciplinary team problem. We age differently, so the goal of the team will be to find personalized recipes that will increase our chances of positive ageing. It is and should be based on a strong and confident relationship between all team members and the patient. If the patient is not fully committed the results will be disappointing no matter how skilled the team is. So, maybe all those of us who are not elderly yet should change a thing or two in our lifestyle and start fighting for a happy and long healthy ageing!

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NEWS

OBESITY AND INFLUENZA

Influenza is an infectious disease with self-limiting evolution, with the exception of age extremes and patients with chronic diseases where life-threatening complications may occur. Recent studies have shown the role of obesity in increasing the risk of severe complications of flu infection but also the influence of obesity on flu transmission. Thus, according to an epidemiologic study published in *The Journal of Infectious Diseases* in September 2018, the obesity can prolong the duration of viral shedding which influence influenza transmission. The study was conducted in the period 2015-2017 and included 1783 participants aged between 0 and 92 years. In the study group, the prevalence of obesity was between 2% - 42%, about 6 times higher in adults than in children. The results showed that the mean predicted influenza virus shedding duration was higher in children (7.2 days) than in adults (5.5 days). Also, regardless of the age group, influenza B virus recorded a shedding duration longer than influenza A virus. The same study showed that the influenza A virus shedding duration increased by 40% for symptomatic obese adults (5.23 days) compared to non-obese adults (3.68 days) but has not established any association between obesity and the influenza B virus shedding duration. The researchers motivated the increase in the virus shedding duration by chronic inflammation in patients with obesity due to immune depression. This study confirms the need for measures to prevent and control obesity as well as flu vaccination amongst the population at risk of developing severe complications or death (Maier HE, Lopez R, Sanchez N, Sophia Ng, Gresh L, Ojeda S, Burger-Calderon R, Kuan G, Eva Harris, Balmaseda A, Gordon A. Obesity Increases the Duration of Influenza A Virus Shedding in Adults. *J Infect Dis* 2018; 218(9): 1378-1382).

RESILIENCE - INTERDISCIPLINARY APPROACH AND PERSPECTIVES

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RESILIENCE-INTERDISCIPLINARY APPROACH AND PERSPECTIVES (Abstract): Resilience is defined as the ability to maintain or successfully regain cognitive health after facing significant stress. Therefore, this concept helps us understand why some individuals cope with stressful situations much better than others. To explore the concept of resilience, reference should be made to the allied concept of adaptation strategies. In the geriatric population, chronic illness is a common problem that could reduce the impact of coping strategies and resilience, especially when the illness is associated with pain and disability. Depression and functional independence are also correlated with resilience and are important in modulating the subsequent rehabilitation. In the geriatric population, functional recovery after major stress is influenced by many factors. Adaptive ability and frailty play an important role, interacting in determining the final functional status. Acute or chronic stress reduced physical activity, poor nutritional status, and depression can trigger or accelerate frailty. **Keywords:** RESILIENCE, FRAILTY, CHRONIC ILLNESSES.

Definition of resilience in relation to successful ageing

In the literature there is a multitude of different types and definitions of resilience, this term being used in different contexts. In a general sense, resilience has been defined as the psychological ability or capacity of a person to respond to stressful situations (1).

According to the American Psychological Association (APA), resilience is defined as "the process of adapting well in the face of adversity, trauma, tragedy, threats or significant sources of stress" as well as bouncing back from difficult experiences (2). Thus, resilience has been recognized as an important personal attribute in life that contributes to the ability to cope with stress and cultivate cognitive health and wellbeing.

Resilience is a concept that has also gained ground in gerontological research because it has been linked to successful aging and quality of life (3, 4).

As the ability to resist adversity or circumstances that cause a significant decrease in quality of life (3), resilience can be improved and supported by certain protective factors (PFs) that facilitate this process by modifying the effects of risks associated with stress-related conditions (5, 6). So far, several studies have examined the relationship between the various protective factors and resilience. In the studied literature, three such factors have been identified frequently: social support, physi-

cal health and psychological self-efficacy.

With physical activity and lack of disease as components, health, in its turn, can promote and improve resilience, generating a general wellbeing (7). Several studies have found that physical health is reflected in resilience scores (8, 9, 10).

The physical characteristics associated with high resilience include independence in activities of daily living (determined by ADL); daily physical activity/physically active; better physical health; fewer chronic diseases (7).

The study of the relationship between physical health and resilience in the elderly is relevant because functional capacity and health status can decline with age (10).

Rowe and Kahn (11) developed the first theoretical model of successful aging in the late 1990s, a model that included three interactive components: avoiding disease and disability, high cognitive and physical function, and engagement with life. High performance in each of these three areas results in the absence of disease and disability, which leads to the definition of successful aging as "high cognitive and physical functional capacity and active engagement with life". In addition to successful aging, resilience is also associated with health-related quality of life (HRQoL).

Protective factors contribute to support resilience in the process of overcoming adversity, allowing elderly people to age successfully. Including high functional capacity, active engagement and absence of disease and disability, successful aging promotes independence among the elderly (7).

The psychological, physical and social aspects of resilience help overcome age-related stressful situations (12). Thus, psychological resilience is a process of using positive adaptive behaviors when dealing with adversity, such as loss of functional independence due to dementia or depression (13, 14).

From another perspective, at physical level, resilience is the ability to maintain good health and restore the optimal body function when faced with a stressful event, such as after hip fracture (15).

Social resilience is the ability to cultivate, engage in and maintain positive relationships, proving an increased ability to adapt and recover from adverse events and social isolation (8). Recent research suggests that resilience can help override the challenges associated with aging by overcoming hardship despite the adversity (3, 12, 16). The resilience process is outlined by the following model (fig.1).

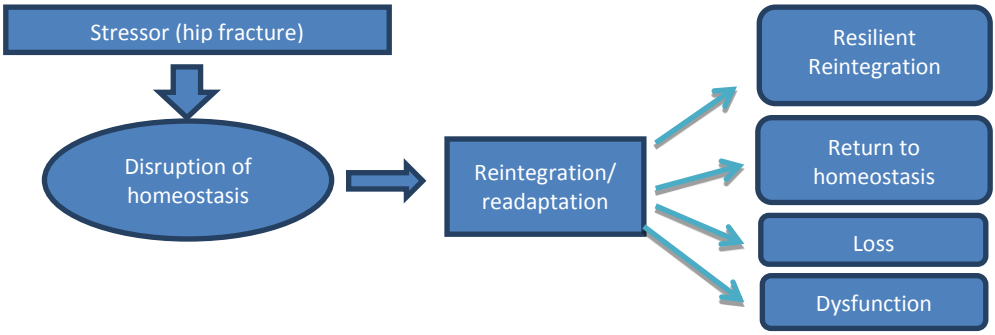

Fig. 1. Resilience model adapted from Resnick B (13). The model outlines the cause and effect pathway of facing adversity, leading to four possible reintegration outcomes. Faced with adversity, homeostasis is disrupted resulting in reintegration. Ideally resilient reintegration is reached, but homeostasis, loss, or dysfunction are possible.

Resilience and frailty

Frailty has been widely used as a term to describe the very old people with disabilities and multiple chronic conditions. It has also been attributed to people living in nursing homes. More recent studies tend to place greater emphasis on the relationship between the concept of frailty and its opposite, resilience (17).

Lekan *et al*. (18) found in their study that participants characterized frailty by such objective physical features as disability, weakness, and balance disorder. In addition, subjective psychosocial aspects, including positive attitudes, social support, and spirituality, motivate in their turn healthy self-care behaviors, thus suggesting that resilience helps prevent frailty by mitigating or reversing its negative effects.

Conditions requiring surgery are particularly stressful for the patient and can interfere with homeostasis. For this reason, the presence of frailty should be evaluated preoperatively and subsequently treated postoperatively. It was found that the prevalence of frailty was much higher among patients undergoing surgery compared to the general population.

Acute and chronic stress, low levels of activity, depression and low nutritional status can initiate or accelerate the frailty status (19).

Techniques for the determination of resilience

There is a large number of scales or tools that have been developed to measure the concept of resilience, a high level of reliability and validity being demonstrated for the Resilience Scale (20).

A comprehensive assessment of resilience scales suggested that Brief Resilience Scale (BRS), Connor-Davidson Resilience Scale (CD-RISC) and the Resilience Scale for Adults (RSA) could provide the best psychometric assessments and the most significant results of the measurements. However, Windle *et al*. (21) determined no true "gold standard" of the measures they evaluated.

Resilience levels among the elderly vary according to the population surveyed and the measurement scale used; a high resilience was reported in the range of 14% -35%.

In a study in adults aged 50 years and over, 14.5% of the sample proved to be very resilient, based on General Health Questionnaires (GHQ-12) scores. The results have shown that high levels of resilience are associated with older age, suggesting that younger old are not always as resilient as those who are older. Increased resilience rate in other studies, using the CASP-19 questionnaire, the 10-item Connor-Davidson Resilience Scale (CD-RISC 10) and GHQ-12 ranges from 31% to 46% (22, 23, 24).

Key characteristics of increased resilience in adults aged 65 years and over, outlined in the literature, include mental, social and physical factors (tab. I).

TABLE I

Key characteristics of resilience

Mental	Social	Physical
Coping and adaptive models **Gratitude** **Happiness** **Lack of cognitive failure cognitive** **Mental health** **Optimism** **Hopefulness** **Positive emotions**	Communion Contact cu family and friends Self-rated successful aging Social support Social support seeking Strong, positive relationships	ADL independence High mobility Physical health Self-rated successful aging

Resilience in geriatric pathology

With aging, there may be a decline in physical, cognitive and psychosocial activity, and thus an increasing number of seniors suffer from chronic diseases. These patients additionally associate low physical performance, decreased pain tolerance, potential decrease of life expectancy, isolation tendency, and loss of self-confidence (25).

Among the geriatric population chronic illnesses are common and can reduce resilience, especially when these diseases are associated with pain and disability (26). However, in the context of such specific conditions as spinal cord injuries, amputation and neoplasms, some authors believe that the elderly seem more resilient than young people when faced with chronic diseases. This is due to their lived experiences that made them more mentally strong when faced with physical problems (19).

In patients with cardiovascular disease, psychological resilience has been shown to independently predict the physical and mental health-related quality of life, but its relationship with depression has not been examined. Low psychological resilience is associated with depression, especially with its affective features (27, 28, 29).

Older patients (over 65 years) with heart disease have higher resilience than younger patients (< 65 years).

Chronic pain, the most common symptom encountered in medical history, is present in 25% -50% of patients. The most common pain complaints in the elderly are osteoarthritis, especially in the lumbar or cervical region (approximately 65%); musculoskeletal pain (approximately 40%); peripheral neuropathy (35%); and chronic joint pain (15% -25%). Psychological resilience can protect against the adverse effects of chronic pain. However, due to some life circumstances and old age-related challenges (loss of life partner, social exclusion), resilience could work differently in the elderly compared to adults (30).

In their study, Morete *et al.* (31) concluded that the elderly with chronic pain have lower resilience, more severe depression and lower quality of life than those without chronic pain.

Hip fracture, for example, is common in elderly patients and is generally associated with other comorbidities and physiological changes with impact on quality of life. Thus, the concept of resilience can explain why this impact varies among these patients. In their study Rebagliati *et al.* (19) have assessed the relationship between resilience, frailty, and quality of life, and to what extent these factors might affect the postoperative rehabilitation outcome. These factors were analyzed by a multidisciplinary assessment at the beginning and at the end of the rehabilitation program. It has thus been demonstrated that there is a negative correlation between disability and resilience, interacting with the frailty level. Resilience and quality of life are positive predictors of functional status at discharge.

Adaptive capacity interacts with the disease in orthopedic surgery: a low plasticity level predicting a lower degree of rehabilitation in elderly patients with hip fracture.

Depression, satisfaction, and functional independence are also correlated with resilience, being important in modulating rehabilitation outcome, as these personal characteristics can be modifiable during rehabilitation. Thus, resilience plays a significant role in achieving optimal functional rehabilitation in elderly people with hip fracture. The results suggest the introduc-

tion of early routine assessment of resilience in future outcome studies in rehabilitation (32).

CONCLUSIONS

In our observation, multidimensional assessment of the elderly before commencing rehabilitation becomes a necessity. Screening for low adaptive capacity and frailty at the initial assessment allows patients with a low recovery potential to be identified so that each patient to receive a personalized treatment program.

From this perspective, it is very important to evaluate all variables that could affect the performance: cognitive impairment, depression, family support and environment.

Resilience can be an important aspect in the process of fighting chronic pain; its assessment could contribute to a thorough/holistic approach to elderly patients.

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COMORBIDITĂŢI ALERGICE ÎN ASTMUL BRONŞIC LA COPIL

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ALLERGIC COMORBIDITIES IN CHILDHOOD BRONCHIAL ASTHMA (Abstract): Bronchial asthma is the most frequent chronic disease of childhood. The association of this disease with allergic rhinitis increases the prevalence and severity of bronchial asthma to 60% compared with 2% in subjects without allergic rhinitis. Food allergy can be expressed in a variety of respiratory symptoms, especially when IgE mediated immune responses are involved. In children, the prevalence of bronchial asthma associated with food induced atopic eczema is 2-8% and respiratory clinical picture can be a component of the multisystem anaphylactic reaction or of chronic respiratory disease like bronchial asthma, serous otitis. The association of other allergic comorbidities, like sinusitis, ocular allergies, lymphoid hypertrophy, sleep obstructive apnoea, leads to the conclusion that allergic bronchial asthma (extrinsic) is a systemic disease, with onset at any age, and the dominant clinical feature depends on the child's genetic pattern. **Key words**: BRONCHIAL ASTHMA, FOOD ALLERGY, ATOPIC ECZEMA, ALLERGIC RHINITIS, OCULAR ALLERGY

Bolile alergice sunt cele mai frecvente boli cronice la populaţia pediatrică, afectând aproximativ 25-30% dintre copii: 15-20% - dermatita atopică, 7-10%- astmul bronşic, 15-20%- rinita şi rino-conjunctivita alergică (1).

Noţiunea de „marş alergic" se referă la istoria naturală a afecţiunlor alergice, care se caracterizează printr-o anumită secvenţialitate în perioada copilăriei sau mai târziu (2). Sensibilizarea alimentară în absenţa manifestărilor clinice este un indicator al predispoziţiei genetice.

Comorbidităţile alergice ale astmului bronşic pediatric sunt: sindromul eczemă/ dermatită atopică (SEDA), rinita alergică şi alergiile oculare.

Alergia alimentară se poate prezenta printr-o varietate de simptome respiratorii, mai ales prin implicarea mecanismelor imune Ig E mediate. Aceste simptome respiratorii, produse prin ingestia sau inhalarea particulelor alimentare din aer, pot fi componente ale reacţiilor multisistemice, anafilactice sau boli respiratorii cronice ca astmul bronşic, otita medie seroasă. Prevalenţa astmului bronşic în alergia alimentară este dificil de apreciat (2-8% la copil).

Epidemiologia relaţiei alergie alimentară--alergie respiratorie poate fi de 3 tipuri:

1. simptome respiratorii în timpul alergiei alimentare cu manifestări clinice cutanate sau gastrointestinale, sugerând manifestări clinice sistemice, până la şoc anafilactic.
2. simptome specifice rinitei alergice induse de alergia alimentară.
3. simptome specifice astmului bronşic in-

TABELUL I
Prevalenţa estimată a alergiei alimentare în reacţiile astmatic-induse

Populaţia studiată	Prevalenţa estimată
Copii cu Astm bronşic	57%
Copii cu alergie la lapte	29%
Aliment ca trigger pentru wheezing în timpul reacţiilor acute	<5%
Copii cu SEDA	17-27%

duse de alergia alimentară (3).

Alimentele responsabile de simptome respiratorii în cadrul anafilaxiei sunt: oul, laptele, arahidele, soia, peştele etc. Detectarea IgE specifice pentru ou la vârsta de sugar se corelează cu specificitate de 97% şi sensibilitate de 50% cu apariţia alergiei respiratorii la vârsta de 7 ani şi reprezintă un marker predictiv pentru atopie, mai bun decât nivelul IgE totale în sângele cordonului ombilical. Creşterea concentraţiilor serice de IgE specifice pentru cel puţin unul din patru alergeni alimentari majori (ou, lapte, soia sau grâu) la vârsta de 1 an se corelează cu sensibilizarea la aeroalergeni la vârsta de 5 ani, iar menţinerea la valori crescute a anticorpilor specifici la vârsta de 2 ani a dovedit capacitate predictivă semnificativă pentru apariţia rinitei alergice şi a astmului alergic după vârsta de 5 ani (4).

O investigaţie recentă pe 5149 de indivizi, majoritatea copii alergici la arahide, a demonstrat prezenţa sindromului funcţional respirator manifestat prin dispnee, wheezing, tiraj intercostal, respiraţie zgomotoasă şi congestie nazală în proporţie de 42-56% din cazuri. Jumătate din aceste reacţii au afectat mai mult de un organ sau sistem, dacă pacienţii erau astmatici, faţă de cei fără astm bronşic (21-33%; $p < 0.001$) (5).

Rinita alergică indusă de alergia alimentară reprezintă 70% din simptomele respiratorii observate la testele de provocare alimentară (6). Rinita alergică tipică, în asociere cu alte manifestări clinice cutanate (SEDA) şi/sau gastrointestinale în timpul reacţiei acute alimentare este rară. Pinnock et al. (citat de 3), au investigat relaţia între ingestia de lapte şi producerea de mucus nazal şi nu s-a putut stabili o corelaţie pozitivă.

Astmul bronşic izolat indus de alergia alimentară este rar întâlnit în practica alergologică, dar relaţia aliment ca factor trigger - astm bronşic a fost frecvent semnalată în cadrul reacţiei sistemice la alimente. O analiză publicată pe 300 de bolnavi cu astm bronşic, cu vârsta cuprinsă între 7 luni şi 80 de ani, a evidenţiat, la 12% dintre pacienţi, istoric de alergie alimentară, iar alimentul, ca trigger inductor de wheezing, a fost prezent la 6 cazuri (2%), toţi fiind copii cu vârsta între 4-17 ani (3). Businco et al. (citat de 7) studiază 42 de copii cu vârsta cuprinsă între 10-76 de luni cu eczemă atopică prin alergie la proteina laptelui de vacă şi demonstrează asocierea astmului bronşic în timpul testului de provocare alimentară la 27% din cazuri. Rezultă că prevalenţa relaţiei aliment - wheezing este mai mare la pacienţii tineri cu boli atopice (tab. I).

Creşterea riscului alergiei respiratorii la pacienţii cu alergie alimentară este demonstrat pentru alergia la ou. Sensibilizarea alergică la ou în copilărie a fost identificată ca factor de predicţie pentru boala alergică a tractului respirator în adolescenţă şi viaţa adultă în 55% din cazuri; dacă este asociat SEDA, valoarea predicţiei creşte la 80%. Răspunsul imun mediat prin anticorpi IgE specifici la aliment este responsabil de simptomele respiratorii. Aceşti anticorpi se leagă de receptorii IgE de mare afinitate ai bazofilelor şi mastocitelor din tractul respirator, cu activare celulară şi degranulare, cu eliberare de citokine pro-inflamatorii, ca histamina, triptaza, leucotriene şi prostaglandine. Aceşti mediatori sunt responsabili de reacţia alergică imediată, caracterizată prin

vasodilataţie, bronhospasm şi hipersecreţie de mucus, care determină simptomatologia clinică. Aceşti mediatori specifici pot contribui şi la reacţia alergică tardivă (întârziată), care apare la 4-8 ore după răspunsul alergic imediat. Mastocitele eliberează mediatori către celulele endoteliale modificând expresia moleculelor de adeziune pentru eozinofile, bazofile şi leucocite. În plus, triptaza poate activa celulele endoteliale, crescând permeabilitatea vasculară. Leucocitele, prin proteaze, distrug ţesuturile, determinând faza tardivă a răspunsului imun. Inflamaţia cronică produce hiperreactivitate bronşică. Celulele T specifice cu memorie contribuie la exacerbările astmatice ca răspuns la acelaşi stimul antigenic alimentar.

Alte simptome respiratorii induse de alimente sunt rare, dar grave. Astfel, inflamaţia alergică a mucoasei nazale poate cauza disfuncţia trompei lui Eustachio şi otită medie seroasă cu exsudat recurent, mai ales în alergia la proteina laptelui de vacă.

Sindromul Heiner (hemosideroza pulmonară indusă de alergia la proteina laptelui de vacă) se caracterizează prin episoade recurente de pneumonie asociate cu infiltrate pulmonare, sângerări gastrointestinale, anemie prin deficit de fier şi insuficienţa creşterii. Acest sindrom rar este asociat cu hipersensibilitate non-IgE mediată la proteinele laptelui de vacă.

Simptomele respiratorii induse de inhalarea alergenelor alimentare sunt rar semnalate la copil, dar se pot produce în timpul gătitului sau al manipulării alimentelor.

În ceea ce priveşte aditivii alimentari, s-a semnalat o prevalenţă de 5% a rinitei în relaţie cu ingestia de glutamat monosodic.

În concluzie, alergia alimentară la un bolnav cu astm bronşic trebuie suspectată în următoarele condiţii:

a) astmul acut este neexplicat şi rezistent la tratament;
b) astmul apare după ingestia unor alimente particulare;
c) copiii cu astm bronşic care asociază şi alte manifestări ale alergiei alimentare (ex. anafilaxie, SEDA).

Punctele cheie în afirmarea relaţiei alergie alimentară – simptome respiratorii sunt:

a) simptomele respiratorii acute induse de aliment sunt tipic asociate cu simptome cutanate şi gastro-intestinale;
b) alergia la ou, lapte, arahide, soia, peşte asociază simptome respiratorii;
c) alergia la ou în copilărie (teste pozitive) are rol predictiv pentru dezvoltarea tardivă de alergie respiratorie şi astm;
d) astmul bronşic indus de alimente este mai frecvent la vârsta pediatrică decât la adult;
e) copiii cu SEDA confirmat prin test de provocare alimentară au un risc crescut de a dezvolta astm bronşic indus alimentar;
f) reacţiile alergice induse de alimente pot creşte hiperreactivitatea bronşică la bolnavii cu astm moderat sau sever, dar fără simptome de astm acut;
g) rolul alergiei alimentare în otita medie seroasă este rar;
h) sunt descrise reacţii astmatice la aditivi alimentari, dar sunt neobişnuite;
i) reacţiile astmatice induse de alergia alimentară, pot fi factori de risc pentru reacţii fatale sau nonfatale sistemice.

Rinita alergică, ca inflamaţie a mucoasei nazale, exprimată clinic prin rinoree anterioară şi posterioară, strănut, obstrucţie şi/sau prurit nazal, reprezintă principala comorbiditate alergică a astmului bronşic pediatric.

Mucoasa nazală şi mucoasa bronşică au asemănări structurale şi funcţionale (8). Deoarece majoritatea bolnavilor cu astm bronşic au rinită alergică, s-a sugerat, pentru asocierea astm bronşic – rinită alergică, conceptul „o cale aeriană unică, o singură boală” (9). Totuşi, nu toţi bolnavii cu rinită alergică au astm şi există unele diferenţe între cele două boli (10). Deseori, asocierea dintre cele două forme de manifestare a alergiei respi-

ratorii nu este diagnosticată, deoarece simptomele sunt mai evidente la un organ.

Procesul inflamator alergic se instalează şi se autoîntreţine prin aceleaşi celule inflamatorii, mediatori şi citokine, având aceleaşi faze ale răspunsului alergic (precoce şi tardiv). Diferenţele majore structurale constau în existenţa unui pat vascular bogat în mucoasa nazală şi a musculaturii netede de la nivelul bronhiilor care au proprietăţi contractile şi impact patogenic în astmul bronşic (proliferare, secreţie de mediatori proinflamatorii şi citokine) (11).

La sugar şi copilul mic, rinita alergică este diagnosticată cu dificultate, deseori fiind confundată cu rinita infecţioasă. Persistenţa simptomelor mai mult de două săptămâni impune diagnostic diferenţial cu o cauză ne-infecţioasă de rinită.

Copilul cu rinită alergică dezvoltă mai frecvent unele complicaţii, ca otita medie seroasă şi rino-sinuzita cronică.

Epidemiologia rinitei alergice este mai puţin studiată. Studiul multinaţional ISSAC (12) apreciază prevalenţa rino-conjunctivitei alergice diferit de la o ţară la alta, cu valori de 0,8- 14,9% la categoria de vârstă 6-7 ani şi de 1,4-39,7% la 13-14 ani. În România, pe teritoriul municipiului Cluj–Napoca, utilizând metodologia ISSAC, au evidenţiat o prevalenţă a rinitei alergice la vârsta de 7 ani de 16,6% şi la 13-14 ani o prevalenţă de 13,6%, prevalenţă subestimată la copil. Rinita alergică s-a asociat cu astmul bronşic la 34%, iar astmul bronşic s-a asociat cu rinita alergică la 61,1% (1). Rinita alergică este un factor de risc pentru astmul bronşic. La sugarul la care diagnosticul de rinită alergică a fost formulat de medic, riscul pentru astmul bronşic la 11 ani este dublu (13).

Alte comorbidităţi alergice sunt otita medie seroasă, sinuzita, eczema, faringita, hipertrofia limfoidă, apneea obstructivă în somn, alterarea vorbirii, conjunctivita.

În anul 1999, Organizaţia Mondială a Sănătăţii recomandă (11):

a. bolnavii cu rinită alergică persistentă vor fi evaluaţi pentru astm bronşic anamnestico-clinic, prin explorări respiratorii şi prin răspunsul la bronhodilatatoare;
b. bolnavii cu astm bronşic vor fi examinaţi pentru depistarea rinitei alergice;
c. strategia terapeutică va fi combinată;
d. tratamentul corect al rinitei alergice scade numărul de exacerbări ale astmului bronşic, scade necesarul de medicaţie antiastmatică şi scade riscul de evoluţie spre remodelare bronşică.

Tratamentul cu glucocorticoizi intranazal poate ameliora simptomele astmatice. Administrarea concomitentă de corticosteroizi intranazali şi inhalaţi scade complianţa la tratament a bolnavilor, dar ameliorează formele severe de boală. Asocierea antihistaminicelor orale anti H1 de generaţia a II-a, ameliorează astmul bronşic la bolnavii care asociază rinita alergică.

Monoterapia cu antileucotriene reprezintă o opţiune terapeutică în formele uşoare de astm bronşic asociat cu rinita alergică. Imunoterapia specifică sublinguală a rino-conjunctivitelor alergice poate preveni apariţia astmului bronşic iar, în astmul bronşic persistent uşor asociat cu rinita alergică persistentă moderată/severă, are efecte favorabile.

Alergiile oculare reprezintă un grup heterogen de situaţii patologice la nivelul ochiului, dintre care doar pentru rino-conjunctivita alergică sezonieră (intermitentă) sau persistentă s-a demonstrat relaţia cu astmul bronşic. Conjunctivita vernală, keratoconjunctivita atopică şi conjunctivita gigantopapilară pot asocia sindromul SEDA sau astmul bronşic.

În concluzie, alergia este o boală sistemică, care debutează la orice vârstă, iar manifestarea clinică dominantă este determinată de particularităţile genetice ale copilului.

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NOUTĂŢI

ANTIENDOTELINELE DE TIP A - UN EŞEC ÎN TRATAMENTUL HTA SEVERE

Endotelina 1 (ET-1) este un vasoconstrictor de patru ori mai puternic decât angiotensina II care acţionează prin intermediul a 2 receptori relativ ubicuitari (ET-A şi ET-B). Blocajul acestora a părut a fi o cale promiţătoare în tratamentul HTA. Un blocant specific al receptorilor ET-A, darusentan, a fost încercat, fără rezultate încurajatoare în tratamentul insuficienţei cardiace. În tratamentul HTA mai multe studii au comparat darusentanul cu un placebo. Astfel Studiul HEAT-2 a demonstrat un efect semnificativ, la 6 săptămâni la hipertensivi moderaţi. Studiul DAR-201 a confirmat aceste rezultate pentru 115 pacienţi cu HTA rezistentă. Două noi studii de fază III au fost realizate la pacienţi cu HTA severă : primul, pe 718 pacienţi, a demonstrat un oarecare efect al darusentanului adăugat la triterapia iniţială a subiecţilor, celălalt a eşuat în încercarea de a demonstra, pentru 849 de pacienţi, o scădere semnificativă a valorilor TA la 14 săptămâni. Efectele secundare semnalate au fost de cele tip vasodilatator, asociate cu o hepatotoxicitate dependentă de doza administrată. Deşi s-a dovedit a fi eficace în studii mici de fază II darusentanul nu a demonstrat a avea constant un efect aditiv la hipertensivul sever de fază III, determinând stoparea utilizării sale cu referire la această indicaţie (Enseleit F et al. Darusentan, a selective endothelin A receptor antagonist, for the oral treatment of resistant hypertension. *Ther Adv Cardiovasc Dis* 2010 ; 4 : 231-240).

Al.D. Ciochină

FOOD PATTERNS IN PEOPLE WITH OBESITY

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FOOD PATTERNS IN OBESE PEOPLE (Abstract): **Aim**: The objective of the study was to examine dietary patterns in patients with obesity. **Material and methods**: We evaluated a group of 71 subjects with excess weight, who completed a validated food frequency questionnaire. Analyzing the results of the questionnaire, we were able to determine the average daily food intake. We applied the factor analysis and the cluster analysis on the results obtained from the questionnaire in order to identify the food patterns. We calculated correlation coefficients between pattern scores and clinical and biological parameters. **Results**: Three patterns derived from the analysis of the questionnaire: a traditional pattern characterized by increased intake of soups, sauces, and medium intake of food groups from prudent pattern; a prudent pattern, characterized by high intake of cereals, fats/oils, fish, meat, potatoes, vegetables, medium intake of nonalcoholic beverages, eggs, with low intake of sugar, snacks, alcohol; and a western-like pattern, which included an increased intake of sweets/snacks/alcohol and low intake of food groups from traditional pattern. Men had the tendency to adopt more frequently the traditional pattern, than women. **Conclusions**: In the study group, we identified three food patterns which could help in analyzing food intake in patients with obesity and in promoting a healthy lifestyle. **Keywords**: OBESITY, DIETARY PATTERN, FOOD GROUPS.

Obesity is a complex disease with a high risk of developing insulin resistance and type 2 diabetes. Obesity is a major public health problem that already has epidemic proportions. According to the World Health Organization (WHO), 1.9 billion adults are overweight, of whom 650 million are obese, and the prevalence of obesity is expected to increase in the coming years (1). In Romania, obesity is a worrying problem, with 52.4% of the adult population being overweight among which 21.3% are obese (2); in the region of Moldavia we have previously reported that more than half population in rural areas have excess weight (3). People with obesity are at high risk of developing insulin resistance and type 2 diabetes. Diabetes mellitus, the main non-transmissible chronic illness, has also an alarming increase, and can be a co-morbidity, but often a consequence of obesity (4).

Obesity is a complex disease with social and psychological dimensions that affects all ages, all social backgrounds and all coun-

tries, regardless of their economic development. Obesity is more than a consequence of excess eating and sedentary lifestyle. Failure of weight loss diets requires a more in-depth understanding of energy needs and a more sophisticated analysis of behavioral, hormonal and autonomic responses to weight loss (5). Sleep and microbiota disorders are just two of the many pathogenic causes of obesity (6, 7). The medical consequences of overweight, mainly cardio-metabolic disorders (8), are often outweighed by social aspects (discrimination, stigma, accountability) (9) or those generated by the nutritional approach of the disease itself (10, 11).

Nutritional evaluation consists of anamnestic, clinical, dietetic and biological data (12), but food research is an essential element of this evaluation. The study of food intake is based mainly on validated questionnaires (13), especially those on food frequency, as we used in previous studies in the general population (14) or in individuals with diabetes (15). Food frequency questionnaires (FFQ), as well as other methods used in nutritional epidemiology, have many biases. The study of foods or food groups, detailing component nutrients, cannot give a causal relationship in the etiology of chronic diseases. That is why the studies of food patterns providing a comprehensive view of the interactions and synergies of foods and beverages have become increasingly important and can correlate with health and disease (16). The results from studying these interactions can be used to prevent diseases, by including nutritional recommendations related to food patterns in the general food guidelines (17). The relationship of food patterns with obesity has been much studied all over the world (18), and the method has also been previously introduced in Romania (19).

The purpose of the study was to define food patterns determined by factorial analysis in a group of subjects with obesity.

MATERIAL AND METHODS

We performed an observational study on 71 patients with obesity at the Clinical Center of Diabetes, Nutrition and Metabolic Diseases of "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, conducted between January and November 2016. The inclusion criteria were: adults >18 years, presence of obesity (BMI >30 kg/m^2), type 2 diabetes controlled with diet or oral antidiabetics. The exclusion criteria were: age < 18 years, type 1 diabetes, BMI <30 kg/m^2, type 2 diabetes insulin treated. Dietary data were obtained using a version of the EPIC-FFQ, translated, adapted and validated previously for the Romanian population (13). Our version of FFQ has 130 food items. The subjects completed the FFQ by choosing the frequency for the consumption of the foods included in the list, over the past 12 months (never or less than once a month, 1-2 times per month, once a week, 2-4 times per week, 5-6 times per week, once a day, 2-3 times per day, 4-5 times per day, 6 times per day). Then, the completed FFQs were analyzed by the FETA-Software, from which the average daily intake of nutrients (carbohydrates, proteins, lipids, MUFA, PUFA, SFA, vitamins, minerals) and energy was obtained. We used in our analysis 13 food groups, measured in grams.

We also collected socio-demographic data, anthropometrical indices: weight, body mass index (BMI), waist circumference (WC), blood pressure (BP), biological parameters: total cholesterol, low-density lipoprotein-cholesterol (LDL), high-density lipoprotein-cholesterol (HDL), triglycerides, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), gamma-glutamyl transferase (γGT), C-reactive protein (CRP), fibrinogen. Subjects were weighed using a calibrated scale and measured with a stadi-

ometer. BMI was calculated by the formula: weight over height (in meters) squared. Eligible subjects were those with BMI over 30 kg/m^2, according to WHO criteria. In addition, BP and WC were measured (normal values for WC were <80 cm in women and <94 cm in men).

For statistical analysis we used descriptive tests, t-independent test, Pearson correlation coefficients. A *p* value of <0.05 was considered statistically significant. Factorial analysis (principal component analysis - PCA) was used to identify food groups that inter-correlated and formed certain patterns. To verify the applicability of PCA, Kaiser-Mayer-Olkin (KMO) and Bartlett Sphericity Test (BTS) were applied. The number of patterns was determined by considering load factor values greater than 0.3. Cluster analysis was performed to group the subjects into exclusive dietary patterns, based on their adherence to the dietetic components identified by PCA. The database was developed and analyzed in SPSS 20.

Prior to entering the study, all patients signed an informed consent, after reading and understanding its content. The Ethical Committee of the University approval was obtained before the study began.

RESULTS

We evaluated a group of 71 subjects with obesity and type 2 diabetes with an average age of 60.14 years.

Of the whole study group, 29 (40.8%) were men and 42 (59.2%) were women.

The clinical and anthropometric characteristics are presented in first table.

Statistically significant differences were observed in the total caloric and cholesterol intake between men and women (the mean values being higher in men) (tab. II).

Regarding the average daily intake of each food group, statistically significant differences were found for alcohol, eggs, meat and derivatives, soups and sauces intake between sexes (higher mean values in men) (tab. III).

Only 60 subjects answered the questions about the frequency of meals per day and other habits, and we noticed that little more than half consumed three main meals a day, one third skipped breakfast and about a quarter had the habit of eating in front of the TV. Those who chose some unhealthy habits had a higher daily energy intake (tab. IV).

TABLE I

Clinical characteristics of participants by sex

	sex	N	Mean	Std. Deviation	Std. Error Mean	P sig.
Weight	men	29	106.690	16.8313	3.1255	**0.001**
	women	42	92.869	13.7624	2.1236	
BMI	men	29	35.403	5.1971	.9651	0.843
	women	42	35.626	4.1937	.6471	
Wc	men	29	114.38	12.718	2.362	0.284
	women	42	111.38	10.574	1.632	
Bp	men	29	142.86	16.225	3.013	0.505
	women	42	139.98	18.849	2.908	

TABLE II

Average daily intake of energy and macronutrients by sex

	Mean	Std. Deviation	Mean		Std. Deviation		
	Total (N=71)		Men	Women	Men	Women	p sig
Energy (kcal/day)	1699.5262	742.37505	1912.4276	1552.5228	939.23970	533.18080	**0.044**
Carbohydrates (g/day)	205.6651	100.19772	229.4128	189.2678	129.85793	70.35750	0.097
Fibers (g)	18.8012	9.91014	20.4426	17.6678	12.81822	7.22812	0.249
Proteins (g/day)	83.0740	43.32740	95.0810	74.7834	57.83740	27.35322	0.052
Cholesterol (mg)	338.3182	138.92943	385.1487	305.9829	135.64883	133.28553	**0.017**
Lipids (g/day)	59.7739	31.36680	65.8800	55.5578	41.05183	22.00689	0.175
MUFA(g/day)	21.0316	12.04735	23.4493	19.3623	15.82403	8.35487	0.162
PUFA(g/day)	12.1471	6.78136	13.3086	11.3452	9.03563	4.59841	0.233
SFA(g/day)	20.7639	10.56615	22.6144	19.4861	13.01545	8.41588	0.223
Carbohydrates(%)	48.24	6.330	47.31	48.88	5.581	6.790	0.308
Lipids (%)	31.61	5.708	30.59	32.31	5.193	5.998	0.214
Proteins (%)	19.44	3.413	19.59	19.33	3.905	3.074	0.761
SFA (%)	11.01	2.675	10.62	11.29	2.111	2.998	0.307
MUFA (%)	11.06	2.672	10.79	11.24	2.320	2.903	0.494
PUFA (%)	6.39	1.535	6.10	6.60	1.345	1.639	0.187

MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids, SFA=saturated fatty acids

TABLE III

Average intake of food groups by sex

Daily average (g)	Mean	Std. Deviation	Mean		Std. Deviation		
	Total (N=71)		Men	Women	Men	Women	P sig
Alcohol	119.03002	77.2013	129.4614	41.1169	151.95110	71.80335	**0.006**
Cereals and derivatives	231.9699	149.02062	244.0310	223.6419	187.47817	117.20555	0.575
Eggs	23.063	15.2156	27.569	19.952	15.9361	14.0556	**0.037**
Fats/oil	12.5904	8.30017	11.9690	13.0195	9.10881	7.77801	0.064
Fish and derivatives	32.2038	28.42182	37.0866	28.8324	38.18815	18.83420	0.232
Fruits	283.4585	171.07224	306.9345	267.2488	203.91498	144.60246	0.340
Meat and derivatives	131.3068	93.57995	161.0912	110.7414	115.78394	68.81387	**0.025**
Milk and derivatives	241.0452	154.58116	231.0200	247.9674	151.44089	158.15898	0.653
Nonalcoholic beverages/juice	429.3423	330.70357	467.3090	403.1271	371.49805	301.24015	0.425
Nuts, seeds	5.151	10.4526	4.086	5.886	6.8478	12.3757	0.480
Potatoes	63.4210	40.25166	62.5997	63.9881	43.73367	38.19938	0.888
Soups and sauces	231.5401	168.95362	300.7845	183.7286	216.96741	104.18304	**0.010**
Sugar/snacks (confectionary)	10.8887	11.86935	10.9490	10.8471	12.21343	11.77516	0.972
Vegetables	304.37	184.310	350.65	272.41	239.292	127.793	0.116

TABLE IV

The main eating behaviors that resulted from the questionnaire

		Frequency(N)	Percent (%)	Energy (kcal)
3 daily meals	daily	33	55.0	1598
	often	16	26.7	1856
	rarely	11	18.3	1890
Skipping breakfast	yes	18	30%	1843
	no	42	70%	1667
High-energy meal after 9 p.m.	often	7	11.7	1934
	rarely	53	88.3	1692
Eating during night	yes	9	15.0	1969
	no	51	85.0	1676
Eating in front of the TV	yes	16	26.7	1861
	no	44	73.3	1669

As for the frequency of food consumption, we noticed that men consumed significantly more pork, bacon, sausages, liver pates, fries, eggs, vegetable soups, meat soups, alcohol, garlic, tomatoes ($p<0.005$), while women consumed significantly more margarine ($p <0.005$). Whole bread, cereals, pods, corn and dried fruit were consumed equally. Otherwise, the differences were not statistically significant (data not shown).

Most of the subjects consumed sunflower oil, but for cooking they preferred to use butter. Also, almost half of the subjects who usually consumed milk chose to consume whole milk (tab. V).

By correlating the frequency of food consumption with anthropometric indices, we found that the weight correlated positively with the consumption of hamburger ($r = 0.264$, $p = 0.026$), lamb ($r = 0.396$, $p = 0.001$), meat pie ($r = 0.260$, $p = 0.038$), biscuits ($r = 0.260$, $p = 0.029$), melons ($r = 0.333$, $p = 0.005$) and negatively with consumption of tofu, dairy desserts ($r = -0.241$, $p = 0.043$). WC was positively correlated with lamb consumption ($r = 0.375$, $p = 0.001$), melon ($r = 0.312$, $p = 0.008$) and negatively with consumption of dairy desserts ($r = -0.269$, $p = 0.023$). BP correlated negatively with avocado consumption ($r = -0.267$, $p = 0.024$).

TABLE V

Fats and milk intake by preference

Food		Percent	Food		Percent
Oil	sunflower	88.1	Cooking fat	sunflower oil	14.0
	olive oil	11.9		margarine	7.0
Visible meat fat	most	15.5		butter	55.8
	a part	19.7		solid margarine	4.7
	little	63.4		poly-unsaturated margarine	16.3
	none	1.4		lard	2.3
Milk daily	none	26.8	Milk type	fatty	25.8
	150ml	42.3		powdered	18.2
	300ml	22.5		whole	47.0
	450ml	5.6		semi skimmed	9.1

By correlating the frequency of food consumption with some biological parameters, cholesterol was found to correlate positively with consumption of fatty fish (r = 0.343, p = 0.004) and peanut butter (r = 0.239, p = 0.046). LDL-cholesterol correlated positively with the consumption of fatty fish (r = 0.278, p = 0.023) and negatively with the consumption of lentils (r = -243, p = 0.048). HDL-cholesterol correlated positively with consumption of black bread (r = 0.298, p = 0.016), peanut butter (r = 0.313, p = 0.011) and negatively with sausage consumption (r = -0.257, p = 0.039), liver pate (r = -0.282, p = 0.023). γGT correlated negatively with coffee consumption (r = -0.254, p = 0.034) and positively with consumption of fatty fish (r = 0.282, p = 0.018). Transaminases correlated positively with the consumption of wine (r = 0.252, p = 0.036), and negatively with coffee consumption (r = -0.290, p = 0.015).

To determine food patterns we used factorial analysis, and subsequently cluster analysis. The KMO value was 0.782, which allowed further analysis. An Eigen values >1.0 criterion was considered, which allowed the identification of 4 factors (food categories), representing 67.3% of the total variance of food intake. Factor loadings >0.3 was considered to have an increased contribution to each category (tab. VI). The first category explained 38.4% of the variance and was named *mixed foods*, characterized by increased intake of cereals and derivatives, fats/oils, fish and derivatives, fruits, meat and derivatives, milk and derivatives, potatoes, vegetables. The second category explained 11.4% of the variance and was named *traditional foods*, characterized by an increased intake of soups and sauces and an average intake of meat and derivatives, fruits and vegetables. The third category explained 9.3% of the variance and was named *protein foods*, characterized by increased intake of non-alcoholic beverages, eggs and moderate intake of meat and derivatives. The fourth category explained 8% of the variance and was named *sugars*, characterized by high intake of sugar, snacks and alcoholic beverages.

TABLE VI

Food categories derived from factor analysis based on factor loadings

Food groups + alcohol	Food categories			
	mixt foods	*traditional foods*	*protein foods*	*sugars*
Alcohol	.102	.194	.163	.817
Cereals and derivatives	.818	.038	.261	.074
Eggs	.241	.081	.619	.127
Fats/oil	.873	-.113	.176	-.105
Fish and derivatives	.774	.255	.201	.098
Fruits	.709	.409	.143	-.030
Meat and derivatives	.524	.505	.386	.288
Milk and derivatives	.460	.286	-.600	-.016
Nonalcoholic beverages/juice	.260	.124	.747	-.074
Nuts, seeds	.278	.046	.132	-.482
Potatoes	.756	.042	.002	-.076
Soups and sauces	.232	.825	.069	.092
Sugar/snacks (confectionary)	.339	-.568	.146	.563
Vegetables	.753	.431	.033	.065

Regarding the correlations between these food categories and some clinical and biological parameters, we found a positive correlation between fibrinogen and the mixed foods category (r = 0.289, p = 0.019). (tab. VII).

TABLE VII

Correlations between food categories and other parameters

Parameters	*Mixt foods*		*Traditional foods*		*Protein foods*		*Sugars*	
	r	p	r	p	r	p	r	p
Age	.049	.684	-.021	.860	-.136	.258	-.030	.804
BMI	-.038	.755	-.141	.241	.000	1.000	-.150	.213
WC	-.047	.697	-.080	.509	-.050	.679	-.006	.959
BP	.084	.484	.003	.979	-.033	.785	-.011	.927
Glycemia	-.109	.367	-.118	.328	.082	.497	-.180	.132
GGT	-.053	.665	.200	.097	.125	.303	.191	.114
Cholesterol	-.038	.753	-.053	.661	.130	.283	.057	.642
LDLc	.083	.507	-.038	.758	.137	.269	.107	.387
HDLc	-.123	.328	-.041	.745	-.065	.607	-.002	.988
Triglycerides	-.027	.823	.057	.639	.113	.353	-.069	.571
CRP	-.085	.482	-.173	.153	.108	.376	.001	.991
Fibrinogen	.289*	.019	-.128	.307	.088	.482	.117	.350

Based on the four food categories, a cluster analysis was subsequently performed, resulting three exclusive clusters representing three food patterns (traditional pattern – with 67.62% of the participants, prudent pattern – with 4.22% of the participants and Western-like pattern – with 28.16% of the participants) (tab. VIII, fig. 1).

The *traditional pattern* had a high correlation with traditional foods and moderated with the other food categories. The *prudent pattern* had high scores for mixed foods and low scores for sweets/snacks/alcohol. The *Western-like pattern* had a high positive association with sweets/snacks/alcohol and a negative association with traditional foods (tab. IX).

TABLE VIII

Cluster analysis based on food categories scores

Food categories derived from factor analysis	Traditional pattern	Prudent pattern	Western-like pattern
	Food categories score		
Mixt foods	-.26564	3.01666	.18505
Traditional foods	.36202	.96494	-1.01358
Protein foods	-.10526	.69427	.14849
Sugars	-.15964	-.51663	.46063

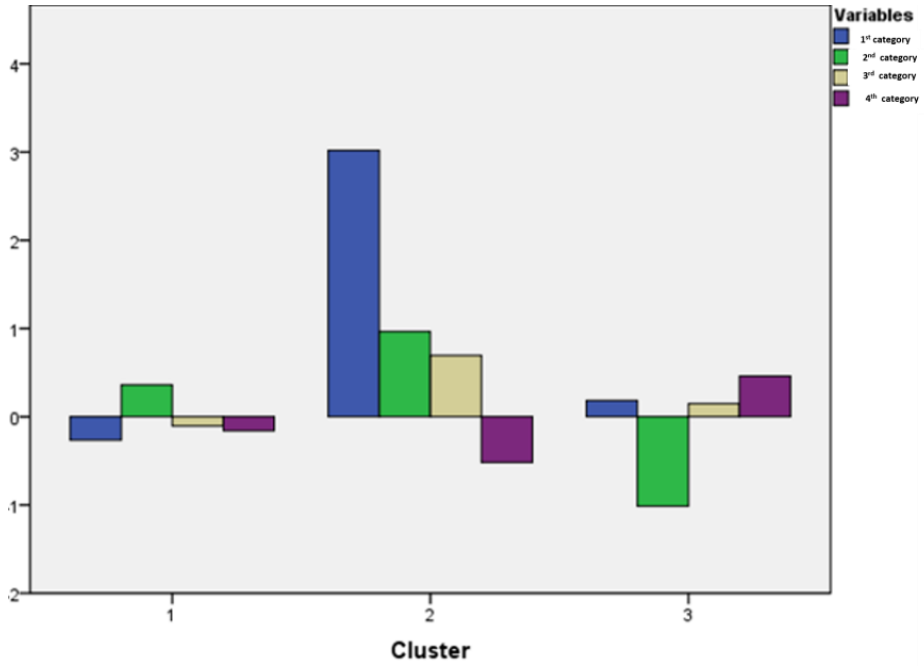

Fig. 1. Food patterns

TABLE IX

Description of food patterns identified in patients with obesity

PATTERN	Traditional	Prudent	Western-like	p
FOODS	High intake of Soups and sauces Medium intake of food groups from prudent pattern	High intake of cereal, fats/oil, fish, meat, potatoes, vegetables Medium intake of nonalcoholic drinks, Eggs, Low intake of sugar, snacks, alcohol	High intake of alcohol, sugar, snacks Low intake of soups, sauces	
Energy (kcal/day)	1539.30	1823.46	1617.84	0.238
Carbohydrates (g/day)	184	224	190	0.166
Proteins (g/day)	73	86	81	0.348
Lipids (g/day)	50	68	54	0.014
HbA1c (%)	6.8%	6.7%	7.4%	0.134
Waist-to-hip ratio	0.99	0.97	1	0.187

DISCUSSION

Increasing the burden of chronic disease requires an alternative and complementary approach to the relationship between diet and the risk of these diseases.

We presented in this study the dietary

patterns identified in people with obesity, highlighting the significant differences in the frequency of food consumption by gender, certain dietary preferences and analyzing the possible association between food patterns and biological parameters. The interpretation of the questionnaires revealed that the daily intake of macronutrients was consistent with the intervals recommended by the guidelines (20).

For those who had a high-energy meal after 9 p.m., those who had the habit of eating in front of TV or during night, those who skipped breakfast and those who did not have 3 main meals per day, the average energy intake was higher. Only half of the participants had regularly 3 main meals per day. In a study that used three 24-hour questionnaires applied over a year to 499 people, following the frequency and timing of the meals, breakfast and meals in the city, it was found that the habit of eating several times a day was associated with the lowest risk of obesity (21).

Four categories of foods were identified in our study participants. The first (mixed foods) includes cereals and derivatives, fats/oils, fish and derivatives, fruits, meat and derivatives, milk and derivatives, potatoes, vegetables. The second (traditional foods) includes an increased intake of soups and sauces and the average intake of meat and derivatives, fruits and vegetables. The third category (protein foods), characterized by high intake of eggs, non-alcoholic beverages and moderate intake of meat and derivatives. The fourth category (sugars) includes sugar, snacks and alcoholic beverages. Subsequently, we continued the analysis of these categories using cluster analysis, which allowed the subjects to be divided into three distinct food patterns based on the consumption of foods from the four categories derived originally. *The traditional pattern* was adopted by the largest group, more than half of the participants (67.62%), and was characterized by the high intake of soups and sauces, and medium intake of foods from the prudent pattern. The *prudent pattern* was adopted by a low percentage of subjects (4.22%) and was characterized by a high intake of cereals, fats/oils, fish, meat, potatoes, vegetables, a medium intake of eggs and nonalcoholic drinks and a low intake of sugar, snacks, alcohol. The *Western-like pattern* was adopted by 28.16%, including an increased intake of sweets/ snacks/alcohol and low intake of soups, sauces. Interestingly, our study showed that even if the patients following a prudent pattern had the highest energy intake, they also had the best cardio-metabolic parameters, whereas the patients following a Western-like pattern had the worst metabolic parameters (WC, HbA1c).

A study conducted in Taiwan on middle-aged and elderly adults found that the food pattern rich in animal proteins, saturated fats, sweets, sodium and food additives was positively associated with C reactive protein (22). However, we did not find the same results. A cross-sectional study of 138 overweight and obese Egyptian women showed that the most common unhealthy eating habits associated with the risk of obesity were skipping breakfast, eating in front of the TV, frequent intake of "snacks" and sweets and low portions of fruit and vegetables (23). Regarding gender differences, although not statistically significant, it was noted that most men adopted the traditional diet more than women (75.9% vs. 61.9%), for the other patterns the percentages are similar.

We found that the frequency of red meat consumption (lamb in our study) was positively associated with anthropometric indices (hence associated with obesity and central obesity) and negatively with HDL-cholesterol. The evidence from The National Health and Nutrition Examination Survey

(NHANES) highlighted the importance of investigating the effects of red meat consumption on the health status (including obesity and central obesity) (24, 25).

Fatty fish consumption was positively associated with serum levels of cholesterol, LDL-cholesterol and γGT. It would be interesting to further investigate cooking methods of fish in Romania (especially frying), which could possibly explain this unusual finding. Black bread and peanut butter were positively associated with HDL-cholesterol, as expected.

We found an inverse association between coffee intake and serum transaminases and γGT levels. Coffee intake could have potential benefits (inhibit inflammation, reduce cardiovascular disease, reduce induction of the cytochrome P450 system) (26, 27) that could be related to some antioxidants found in coffee. These compounds may inhibit the increase in serum transaminases and γGT levels. Other studies also found this inverse association (28).

The study has several limitations: the small number of subjects, the lack of comparison with normal weight individuals and people without diabetes, the observational nature of the study. Thus, we should consider a further study on a larger sample.

CONCLUSIONS

In this study we identified three major food patterns in subjects with obesity, that could help us implement behavior and lifestyle changes in these individuals.

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NEWS

INCREASED INTEGRATION OF EXTRACELLULAR DNA IN THE ENVIRONMENTAL METAGENOME IN THE PRESENCE OF MUTAGENS LIKE BROMOACETIC ACID

A study by Mantilla-Calderon D *et al.*demonstrated that bromoacetic acid can increase transformation rates in microorganisms. Bromoacetic acid is a drinking water disinfection byproduct. Natural transformation allows for the uptake and integration of foreign DNA, which is a form of horizontal transfer of genes. This can be responsible for the acquisition of virulence and antibiotic resistance. The model microorganism used in the study was *Acinetobacter baylyi* ADP1. The transformation rate in this study was dependent on concentration of bromoacetic acid and its ability to cause DNA damage. Thus, transcription of recA gene was enhanced by 20 to 40%. This, in turn, facilitates the integration of extracellular DNA within the acceptor bacterial cell during DNA repair. (Mantilla-Calderon D, Plewa MJ, Michoud G, Fodelianakis S, Daffonchio D, Hong P. Water disinfection byproducts increase natural transformation rates of environmental DNA in *Acinetobacter baylyi* ADP1. *Environ Sci Technol.* 2019 May 3. doi: 10.1021/acs.est.9b00692.)

ASSOCIATED RISK FACTORS FOR PANCREATIC CANCER: POTENTIAL TARGETS IN EARLY DETECTION STRATEGIES

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ASSOCIATED RISK FACTORS FOR PANCREATIC CANCER: POTENTIAL TARGETS IN EARLY DETECTION STRATEGIES (Abstract) : The common risk factors for pancreatic cancer (PC) are smoking, high alcohol consumption, obesity, family history of PC and dietary factors. Chronic pancreatitis facilitates the progression to cancer, resulting in the occurrence of precancerous lesions. Genetic factors variation or mutation contribute to increase risk of PC**.** The biomarker combination CA19-9 (Carbohydrate antigen 19-9 , also known as sialyl-Lewis) along with MUC5AC (Mucin 5AC - this gene has been linked to mucus hypersecretion**)** is also suggestive for PC diagnosis. Transabdominal ultrasound, contrast enhanced computed tomography or magnetic resonance imaging are used to detect PC, but positron emission tomography with fluoro-D-glucose is clearly superior in early detection of small tumors. **Keywords**: PANCREATIC CANCER, RISK FACTORS, EARLY DETECTION.

Pancreatic cancer (PC) is an aggressive and invariably deadly disease without any improvements in patient outcome over the last 2 decades. The estimated number of deaths in 2013 was around 39,000, making PC the fourth leading cause of cancer deaths in the USA, with over 45,220 new cases diagnosed every year (1).

EPIDEMIOLOGY

The worldwide incidence of pancreatic ductal adenocarcinoma (PDAC) is 6-12/100,000/year (2) and it is seldom diagnosed before 55 years old. PDAC can be defined as a disease of elderly populations with the highest incidence in people older than 70 (3).The incidence of PDAC varies across regions and populations, possibly because of the environment and/or the exposure to certain risk factors.

RISK FACTORS FOR PC

The etiology of PC represents the subject of numerous meta-analyses and pooled analyses in order to establish several risk factors. The most common risk factor is

tobacco smoking, followed by obesity, high alcohol consumption, history of pancreatitis and diabetes, family history of PC and possibly selected dietary factors (4).

Acute pancreatitis (AP), a sterile inflammation initiates from acinar cell injury following a series of impairment of pancreatic enzymes activation processes, increased inflammatory responses and ultimately cell death with the complication of multiple organ failure (5, 6). The most common causes of pancreatitis include alcohol, gallstones, toxins, hyperlipidemia, and trauma, with a small number of idiopathic cases. Traditionally AP is self-limited with complete resolution of function after the acute event, but sometimes there may be stricture formation, leading to pancreatic flow obstruction and recurrent AP (RAP). Recurrent episode of AP would progress to chronic pancreatitis (CP), which is a well-defined risk factor for PC (1). There are three stages of CP development: stage one (the pre-pancreatitis phase), associated with risk factors for this transition, like alcohol consumption and tobacco smoking (6). Then, stage two (AP form) with release of inflammatory cytokines which, in the severe forms, it could activate macrophage dependent stellate cells, which finally could lead to fibrosis. The third stage is represented by the progression to CP driven by the factors that modulate immune responses (7).

In one recent study of 731 prospectively enrolled patients after a primary episode of AP, the risk of future development of PDAC was 0.7%, after median of 55 months of follow-up. The study also showed that the risk of PC was 9 times higher in patients who developed CP (8). Another study among 790 patients with histologically confirmed PC, 114 (14.4%) had a history of pancreatitis (AP within 2 years of PC diagnosis in 69 [8.7%], remote history of AP in 28[3.5%], CP in 4[0.5%], and unknown duration of pancreatitis in 13[1.6%]. After controlling for sex, age, body mass index, smoking, alcohol history, and diabetic status at diagnosis, patients with a remote history of AP were diagnosed on average 4.7 years earlier with PC compared with PC patients without history of AP (9).

CP is a refractory disorder characteristic of progressive inflammation and fibrosis with irreversible damage to both exocrine and endocrine function of the pancreas. Acinar cell atrophy, pancreatic fibrosis, leukocytes infiltration, fatty replacement and blocked ducts represent the typical histopathologic features of CP (10). Both CP and PDAC include massive immune cell infiltration and intensive fibrosis (1).

A meta-analysis among patients with CP has shown a relative risk of 13.3% for developing PC (2). Chronic inflammation associated with CP facilitates the progression to cancer, resulting in the occurrence of three types of precancerous lesions: pancreatic intraepithelial neoplasia (PanINs), intraductal papillary mucinous neoplasms (IPMN) and mucinous cystic neoplasms (MCN). Subsequent evolution of these precursor lesions into PDAC ultimately involves a number of various molecular changes (11). Despite the strong link between CP and PC, less than 5% of patients with CP go on to develop the disease (2).

Among the non-modifiable risk factors are diabetes mellitus, age, genetic factors and gender. PC is more common in males than in females, possibly due to lifestyle (higher alcohol and tobacco consumption) (12).

Genetic factors contribute to increase risk of PC and the most important are genetic variation or mutation of BRCA1 (Breast cancer type 1 susceptibility protein), BRCA2, PALB2 (Partner and localizer of BRCA2, also known as PALB2), ATM (ataxia telangiectasia mutated), CDKN2A (cyclin-dependent kinase Inhibitor 2A) , APC (Adenomatous polyposis coli), MLH1 (MutL homolog 1, colon cancer, nonpolyposis type 2 E. coli), MSH2 (DNA mismatch repair protein Msh2), MSH6, PMS2(Mismatch repair endonuclease), PRSS1 (Mutations of Human Cationic Trypsinogen) and STK11 (Serine/ threonine kinase 11). 5-17% of families with familial PC have been identified with Germ-line BRCA2 gene mutations, the highest proportion of known causes of inherited PC. Also, familial cancer syndromes (hereditary non-polyposis colon cancer, Peutz-Jeghers syndrome, hereditary breast and ovarian cancer syndrome, familial adenomatous polyposis and Li-Fraumeni syndrome) found to be associated with PC (13).

LABORATORY INVESTIGATIONS

Serum bilirubin levels are greater than 10 mg/dL in CP, with a peak followed by a fall as inflammation decreases (14). In PDAC, bilirubin progressively increases till biliary decompression is done (15).When serum bilirubin >5.8 mg/dL, MPD> 11.5 mm, CBD>14.5 and CA 19-9 >127 UI/mL, the sensitivity for suspecting malignancy is 100% (16). Ca 19-9, the gold standard PDAC biomarker, is the single most important predictive biomarker of malignancy in mass forming CP with a sensitivity and specificity of 77% and 86% (17). However, the value can be elevated in obstructive jaundice, cholangitis, cirrhosis and CP in absence of malignancy (18). A proinflammatory marker of CXC chemokine family, interleukin 8, is found to be commonly expressed in PDAC (55.6%) and it can predict the prognosis (19).

Methylated biomarkers such as KRAS are found to be 95% specific and 75% sensitive for PDAC in comparison with normal controls and CP. A metabolic signature to distinguish PDAC from CP, with a diagnostic accuracy of 90%, contains CA 19-9 along with certain metabolites such as proline, 2 sphingomyelin, phosphatidylcholine, pyruvate, ceramide, histidine, sphingosine-1-phosphate and isocitrate, with an 85% sensitivity and 94,9% specificity (20).

The biomarker combination which is found to be very promising is CA 19-9 along with MUC5AC, a mucin family member produced by both well differentiated and poorly differentiated PDAC (17). MUC5AC is undetectable in normal pancreas, islet cell tumors and CP (21). Its levels are affected by elevated bilirubin levels. Serum levels can be measured by enzyme-linked immunosorbent assay, whereas tissue levels can be measured by immunohistochemistry (17).

A monoclonal antibody with high specificity for MUC1 **(**Mucin 1, cell surface associated) is PAM4, found to be expressed in PDAC as compared to normal pancreatic tissue and pancreatitis. It has a better sensitivity and specificity than CA 19-9 (22).

A recent study made by Lukasiewicz-Zajac *et al.* showed that matrix metaloproteinase-2 (MMP-2) and its tissue inhibitor (TIMP-2) are highly useful as potential biomarkers in the diagnosis of PC. MMP-2 is a proteolytic enzyme responsible for the degradation of the basal membrane and extracellular matrix. Imbalance between MMP-2 and TIMP-2 have a significant role

in invasion, migration, metastasis of tumoral cell and neoangiogenesis (23).

The study of Zhai *et al.* proved, by immunohistochemical methods, that the expression of MMP-2 was higher in PC tissue, fact that was positively correlated with higher CA 19-9 serum levels, with poor histological grade, advanced stage, perineural invasion, lymph node and distant metastasis (24).

Another study revealed a new combination of CA 19-9, thrombospondin-2 (THBS2) and circulating cell-free DNA (cfDNA) as a composite liquid biomarker for the diagnosis of early-stage PDAC. This panel showed a 92% sensitivity in discriminating PDAC from CP or IPMN (25).

IMAGING

Abdominal X-ray, transabdominal ultrasound (USG), contrast enhanced computed tomography (CECT) or magnetic resonance imaging (MRI) can be used to detect morphological changes in the pancreas, ductal findings, metastatic lesions, ascites, lymphadenopathy and vascular involvement (26, 27).

Radiologically, a malignancy in CP may appear like a lesion in diffuse CP or a mass forming focal CP (28).

USG can be useful especially when the patient has liver metastatic disease and/or ascites. In those cases, an USG-guided biopsy or fluid cytology can establish the diagnosis. A contrast-enhanced USG in arterial and venous phases is useful only when the malignancy is advanced with vascular involvement (29).

A triple-phase pancreas protocol CECT scan represents one of the main investigations. Frequently, PDAC is seen as a gradual, progressive hypo enhancing lesion with a delayed peak in the portal phase without washout and pancreatic parenchymal atrophy/PDAC, often localized in the head of pancreas, is associated with dilated CBD and MPD (double duct sign), with an abrupt cutoff at the mass. Vascular sheathing at origin of celiac artery and superior mesenteric artery by a hypodense mass is suggestive for malignancy (26). However, 23% of small PC (<20 mm) cannot be detected (30). Another causes of this low sensitivity may be represented by some pathological characteristics of the tumor such as lower tumor cellularity, less prominent tumor necrosis and more frequent intratumorally islet cells and acinar tissues (31).

MRI has a stronger contrast enhancement and a superior ability to detect PDAC when compared with CT (32). Diffusion-weighted MRI (DWI) can help detect pancreatic neoplasms with extracellular fibrosis or extremely dense cellularity, but it cannot definitively characterize solid lesions as neoplastic or inflammatory (33).

Positron emission tomography with 2-deoxy - 2[fluorine-18] fluoro-D-glucose (FDG-PET) is superior to MRI and CT, with 89% sensitivity and 88% specificity, being able to detect PC < 20 mm. For that matter, it is used for cancer staging and revealing recurrent lesions (34).

Endoscopic ultrasonography (EUS) become the most sensitive examination for PDAC in patients with high-risk factors for PDAC or with manifestations suggesting PC (35).

The conventional B-mode EUS imaging was complemented by ultrasound contrast agents (UCAs), which allows the evaluation of vascular structures. Contrast-enhanced high mechanical index (CEHMI) EUS and contrast-enhanced low mechanical index (CELMI) are helpful for detecting

macro vessels, respectively capillary vascularization patterns inside the area of interest. Endoscopic son elastography (ESE) tests the hardness of tissue and benign tissue is more pliant compared to a malignant tumor. A recent study made by Finn-Jorn *et al*. showed that CEHMI-EUS can detect PDAC and differentiate it from CP more effectively than CT, conventional EUS, ESE and CELMI-EUS (36).

ERCP has a low sensitivity for diagnosing PC, being considered to be inadequate for examination. Besides, post-ERCP pancreatitis is a very unpleasant adverse event (37). Serial pancreatic juice aspiration cytologic examination (SPACE) was recently developed. It proved to have a high sensitivity and specificity even for pancreatic carcinoma in situ or small lesions (38).

CONCLUSIONS

Pancreatic cancer is a major health problem, being one of the most aggressive forms of cancer.

Acute and chronic inflammation associated with CP facilitates the progression to cancer, resulting in the occurrence of precancerous lesions.

CA 19-9 with MUC5AC combination may become an ideal investigation in PC detection strategies (39).

FDG-PET is superior to MRI and CT, with high sensitivity and specificity, being able to detect PC < 2 cm.

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NEWS

POLYMERIC NANOPARTICLES FOR ORAL DELIVERY OF INSULIN

Biomacromolecules have transformed our capacity to effectively treat diseases; however, their rapid degradation and poor absorption in the gastrointestinal (GI) tract generally limit their administration to parenteral routes. An oral biologic delivery system must aid in both localization and permeation to achieve systemic drug uptake. Inspired by the leopard tortoise's ability to passively reorient, we developed an ingestible self-orienting millimeter-scale applicator (SOMA) that autonomously positions itself to engage with GI tissue. Gastrointestinal responsive polymeric nanospheres (NPs) based on hydroxypropyl methylcellulose phthalate (HPMCP) were prepared using spontaneous emulsification solvent diffusion method for improved oral administration of insulin. The NPs were prepared under optimized conditions have an encapsulation efficiency of 90% and a particle size of about 200 nm. In vitro drug release experiments demonstrated that the NPs exhibited a gradient release profile of loaded drug when the pH value gradually increased from 3.0 to 7.4. Enzyme resistance experiments showed that under simulated gastrointestinal conditions, the NPs protected more than 60% of the drug from being degraded by trypsin. The oral hypoglycemic experiments revealed that insulin-loaded NPs could significantly reduce blood glucose levels in diabetic rats with a relative bioavailability of 8.6%. *Ex vivo* imaging investigation of rat tissues showed that the drug-loaded NPs could promote the absorption of insulin in the ileum and colon. The work described here suggests that the gastrointestinal responsive polymeric NPs may be promising candidates for improving gastrointestinal tract delivery of hydrophilic biomacromolecules. Accordingly, the results indicated that HPMCP NPs with gastrointestinal stimuli responsiveness could be a promising candidate for oral insulin delivery (Y. Fang, Q Wang, Lin X, *et al.* Gastrointestinal responsive polymeric nanoparticles for oral delivery of insulin: optimized preparation, characterization and *in vivo* evaluation. *J Pahrm Sci* 2019; 108(9): 2994-3002).

TRATAMENTUL MEDICAMENTOS AL POLINEUROPATIEI DIABETICE PERIFERICE

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DRUG TREATMENT OF DIABETIC PERIPHERIC POLYNEUROPATHY (Abstract): Neuropathy is one of the many complications of diabetes mellitus, along with micro- and macroangiopathy. Chronic sensorimotor distal symmetric polyneuropathy is the most common form between neuropathies; more than 30% of the diabetic patients are affected by this complication. Treatment is based on three cornerstones: 1) multifactorial intervention aimed at normoglycemia; 2) drugs that target pathogenic mechanisms and 3) symptomatic treatment. Among pathogenic treatments, alpha-lipoic acid and benfotiamine are available in several countries. Neuropathic pain, which affects 8–26% of diabetic patients, exerts a substantial impact on the quality of life. Among the centrally acting analgesic drugs, tricyclic antidepressants, carbamazepine, gabapentin and opioids have been mainly used to treat neuropathic pain. More recently, significant pain relief has been reported using agents such as duloxetine, a dual selective serotonin noradrenaline reuptake inhibitor, and pregabalin, an anticonvulsant, a specific modulator of the $\alpha_2\delta$ subunit of the voltage-dependent calcium channels. Until now, at least 50 new molecular entities have reached clinical stage of development. Strategies that may show promise over existing treatments include topical therapies, analgesic combinations and, in future, gene-related therapies. **Key words**: DIABETIC POLYNEUROPATHY, NEURO--PATHIC PAIN, DULOXETINE, PREGABALIN

GOOD NEWS: COFFEE IN LIVER DISEASE

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GOOD NEWS: COFFEE IN LIVER DISEASE (Abstract): Each day more than 2.25 billion cups of coffee are consumed, requiring over 7 million tons of coffee beans. Roasted coffee brew contains more than 1,000 bioactive substances that provide substantial human health benefits. The micronutrient content of coffee depends on chemical transformation, type of bean, degree of roast, preparation method, while bioavailability is influenced by the individual genotype and gut microbiota. Coffee has proven bioactivities including antiproliferative effect, neuroprotective function, antioxidant activities, antidiabetic effect, and hypolipidemic effects. Coffee intake is associated with a reduced risk of hepatocellular carcinoma, liver-associated enzyme elevations, hospitalizations and mortality from cirrhosis and with a reduced replication capacity of the hepatitis C virus, thus improving the response to antiviral therapy. "A coffee prescription" can be a practical prophylactic and therapeutic approach. Randomized control trials are required to standardize the type, dose and method of preparation. **Keywords**: COFFEE, LIVER, ANTI-FIBROTIC, BIOACTIVE.

Coffee, known as "the wine of Arabia", is the second world's most traded commodity after petroleum and its rich history dates back more than one thousand years. The coffee plant first grew wild in the cloud forests in Kaffa, in the ancient Abyssinia (Ethiopia). According to "One Thousand and One Nights", a collection of Middle Eastern and South Asian folk tales published during the Islamic Golden Age, the brewing of coffee beans began after a goat herder named Kaldi noticed that his goats became energetic after eating a certain type of wild red berries. The first reliable evidence of coffee drinking is from the early 15th century, in the Sufi monasteries of Yemen (1). Through trade, the cultivation of coffee plant spread to Arabian Peninsula and later, to India, Europe and Latin America. Nowadays, Brazil is the world's largest coffee producer and exporter, followed by Vietnam and Colombia. Coffee, once thought to be a devilish drink, forged a social revolution all over the world, including among the great composers like Bach, who deliberately composed the "Coffee Cantata" (2).

Roasted coffee brew contains more than 1,000 bioactive compounds that provide substantial human health benefits. Some of them are represented by mineral ingredi-

ents such as Ca, K, Fe, P, Ni, Mg and Cr, polyphenols, caffeine, melanoidins, lipids and carbohydrates (3). The final coffee micronutrient content depends on chemical transformation, type of bean, degree of roast, preparation method, while bioavailability is influenced by the individual genotype and gut microbiota (4).

Coffee has proven bioactivities, including antiproliferative effect against some cancer cell lines, neuroprotective function by inhibiting acetylcholinesterase, playing a potential role in the treatment of Alzheimer's disease, antioxidant activities against ischemia-reperfusion injury, antidiabetic effect, each cup of coffee leading to a 7% reduction in the risk of type 2 diabetes, hypolipidemic effects, leading to weight reduction in experimental mice and antimicrobial activities, including anti-HIV activity (5).

Coffee consumption is consistently associated for about 20 years with a decreased risk of liver disease, but the first a histopathological evidence of a reduction of fibrosis occurred several years ago. Coffee intake is associated with a decreased risk of hepatocellular carcinoma (HCC), liver-associated enzyme elevations, hospitalizations and mortality from cirrhosis, and with a reduction in the replication capacity of hepatitis C virus, thus improving the response to antiviral therapy (6, 7).

Bioactive compounds of coffee with potential benefits in liver disease

The most representative and studied compounds are caffeine, chlorogenic acid, diterpenes and trigonelline (3). Caffeine is a purine methylxanthine alkaloid found in many products like *Caffea arabica*, *Caffea canephora* (*Robusta*), green tea, mate tea, chocolate, cola and energy drinks.

Experimental data revealed that caffeine has antioxidant activity and that its administration can block the elevation of TGF-β, a profibrogenic cytokine highly expressed in deregulation of physiological tissue remodeling processes (8,9). Other experimental studies suggested that caffeine induces the expression of uridine diphosphate-glucuronyltransferases in liver, the most important proteins involved in phase II drug metabolism (10,11).

Chlorogenic acid, a phenolic compound isolated from green coffee beans, is supposed to play an important anti-fibrotic role by blocking the miR-21-regulated TGF-β1/Smad7 signaling pathway (12).

The fat soluble diterpenes cafestol and kahweol are typically removed from coffee by paper filters. Cafestol can be found in both *robusta* and *arabica* beans while kahweol can be found only in the unfiltered major strain arabica (13). These compounds are believed to protect against oxidative stress by stimulating the NF-E2-related factor-2 (Nrf2)-ARE (antioxidant responsive element) pathway. Activation of Nrf2-ARE pathway ameliorates alcoholic liver disease, protects HCC cells and viral hepatitis-infected cells against oxidative injury and inhibits the c-Jun N-terminal kinases (JNK) pathway involved in the pathogenesis of nonalcoholic fatty liver disease (14).

Trigonelline, a pyridine alkaloid with antioxidant properties found in large amounts in *Caffea arabica*, prevents hepatic lipid accumulation in experimental models of nonalcoholic fatty liver disease by restoring hepatic autophagy (15). Quercetin is another coffee compound from the flavonoid group of polyphenols that has been found to have antioxidant properties due to intracellular free radical scavenging capacities, leading to improvement of liver inflammation and fibrosis (16).

Impact of coffee on liver enzymes

Inverse associations of coffee with liver enzyme gamma-glutamyltransferase (GGT) were first mentioned in "The Tromso heart study" published by Arnesen in 1986 (17). Many other population-based studies published in the last years noticed a dose-dependent decrease in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in individuals drinking > 3 cups of coffee daily (6). The outcome was more obvious in patients with diseases or conditions that were likely to cause liver injury (obesity, alcohol consumption of more than two alcoholic beverages daily, transferrin saturation >50%, impaired glucose tolerance and viral hepatitis) (6, 18). The enzyme-lowering effect was less marked in women aged over 50 years (6).

Nonalcoholic fatty liver disease

Current epidemiological and clinical evidence suggests that regular caffeine consumption, defined as the ingestion of caffeine only from regular coffee, excluding espresso, tea and cola, plays a protective role in the development of nonalcoholic fatty liver disease by decreasing the activity of connective tissue growth factor (CTGF), a central promoter of liver fibrosis, and by modifying effects on the metabolic syndrome (decrease in proinflammatory cytokines, change in adipose tissue gene expression, decrease of liver fat and collagen deposition) (6,18-20).

Chronic hepatitis B and C

Coffee intake is associated with lower rates of liver disease progression in patients with chronic hepatitis C (21). *In vitro* studies demonstrated that caffeine was able to inhibit viral replication in a dose-dependent manner at noncytotoxic concentrations (18,22). High coffee intake (more than 3 cups a day) in naive patients treated with peg-interferon and ribavirin resulted in increased tolerability of treatment (50.4-60.6%) and improvement of sustained virological response (SVR).

Reduction of side effects was also demonstrated in HIV-HCV-coinfected patients treated with peg-interferon and ribavirin (23).

Although *in vitro* studies have shown a quantitative reduction of HBsAg levels and a low level of HBV DNA in the HEPG2 cell lines, clinical evidence has not shown that coffee consumption could reduce liver fibrosis (18).

Liver cirrhosis

Coffee consumption was associated with lower risk of mortality from chronic liver disease (CLD) in a dose dependent manner (1 cup of coffee daily was associated with a 15% reduction in the risk of death from CLD while 4 cups daily resulted in a 71% reduction in risk of death from CLD). The same association was not statistically significant when black tea, green tea and other non-alcoholic caffeinated drinks were consumed.

In experimental rat models, coffee administration suppressed the activation of profibrogenic cytokines including TGF-β, PDGF and CTGF and protected the depletion of glutathione, superoxide dismutase and catalase in liver tissue, defending stellate cells from ROS (reactive oxygen species)-induced necrosis (19).

Hepatocellular carcinoma

Coffee consumption reduces the risk of HCC, irrespective of the etiology of liver disease, by 40% in coffee drinkers versus

non-drinkers, directly proportional with the amount and duration of coffee consumption.

Both *in vitro* and *in vivo* studies have demonstrated that chlorogenic acid has anticarcinogenic effects. Recent evidence suggests that chlorogenic acid improves the effects of Regorafenib in human hepatocellular carcinoma cells (24).

CONCLUSIONS

Our review suggests that no other available antifibrotic agent for liver disease is cheaper, more accessible irrespective of the socioeconomic status, pleasant to consume and effective regardless of the underlying liver disease than coffee. Randomized control trials are needed to standardize the coffee types, dose and method of preparation.

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NEWS

HYPERHOMOCYSTEINEMIA IS AN INDEPENDENT RISK FACTOR FOR INTRACRANIAL ATHEROSCLEROTIC STENOSIS

Stroke has become one of the main causes of disability and death among the population. Intracranial atherosclerotic stenosis (ICAS) is the most common cause of stroke. It is essential to identify, prevent and treatment he modifiable risk factors of ICAS. Hyperhomocysteinemia (HHcy) is considered a risk factor for cardiovascular disease and atherosclerosis. In addition, the exiting data in literature on the existing literature on the relationship between serum homocysteine level and stroke is still controversial. A new study published in *AGING* examines the association of homocysteine concentration with ICAS. According to statistical and processing data, HHcy could be an important marker of vascular injury and a potential target for the prevention of vascular injury, especially for intracranial artery. Therefore, the research group´s conclusion was that increased homocysteine may be an independent risk factor for intracranial atherosclerotic stenosis (Ying Liu,Jing-Hui Song, Xiao-He Hou et al. Elevated homocysteine as an independent risk for intracranial atherosclerotic stenosis. *AGING* 2019; 11(11)).

WHICH PATIENTS WITH SUSPECTED MYOCARDIAL ISCHEMIA AND BUNDLE BRANCH BLOCK SHOULD RECEIVE EMERGENCY REPERFUSION THERAPY ?

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WHICH PATIENTS WITH SUSPECTED MYOCARDIAL ISCHEMIA AND BUNDLE BRANCH BLOCK SHOULD RECEIVE EMERGENCY REPERFUSION THERAPY ? (Abstract): The electrocardiogram is one of the first test directing reperfusion therapy for patients with acute myocardial infarction, depending on the presence or absence of ST-segment elevation. The presence of left or right bundle branch block on an electrocardiogram is frequently encountered in our clinical practice and their hemodynamic implications are increasingly being studied. However, the presence of left bundle block is usually associated with a more reserved prognosis compared to normal intraventricular conduction. In the same time, the presence of right bundle branch block is often considered benign in the absence of a cardiac pathology. Also, left bundle branch block has historically prevented accurate recognition of acute myocardial infarction. This may lead to a poor allocation of reperfusion therapy and secondary to a suboptimal patient outcome. Over the past 20 years, the management guidelines for these patients have shifted from one extreme to the other, considering the limited knowledge on this topic. In this context, the new European Society of Cardiology guideline recommends for patients with persistent ischemic symptoms and right bundle-branch block, a primary percutaneous coronary intervention strategy, but the level of evidence is not high. Considering that new-onset right bundle branch block may indicate the proximal occlusion of the left anterior descendent artery and large infarction, which may cause severe heart failure, malignant arrhythmias, complete atrioventricular block, an emergency reperfusion therapy may prevent all these. This is probably the reason why the current reperfusion therapy has changed the overall incidence and significance of right bundle branch block in acute myocardial infarction. In this clinical and epidemiological context, we consider justified to reanalyze and review the significance of left and right bundle branch block in the reperfusion therapy era. **Keywords:** BUNDLE BRANCH BLOCK, ACUTE MYOCARDIAL INFACTION, CORONARY ARTERY DISEASE, MORTALITY, ELECTROCARDIOGRAM.

The electrocardiogram (ECG) is an integral part of the diagnostic workup of patients with suspected myocardial infarction and should be acquired and interpreted promptly (i.e. target within 10 min) after first medical contact (1, 2). Patients with

acute myocardial ischemia have often dynamic changes in ECG waveform and serial ECG acquisition can provide critical information, especially if the ECG at initial presentation is non-diagnostic. Sometimes, patients may have other ECG signs that indicate acute myocardial ischemia, such as: intraventricular bundle branch block, cardiac arrhythmias, atrioventricular conduction delays or loss of precordial R wave amplitude, a less specific modification. However, in some cases like, acute pericarditis, left ventricular hypertrophy, left bundle branch block (LBBB), Brugada syndrome or early repolarization patterns, the ECG by itself is insufficient to diagnose acute myocardial ischemia or infarction, because in these conditions ST deviation may also be observed (3). Ischemic symptoms and presumed new left or right bundle branch block (RBBB) are associated with an adverse prognosis (4).

Left bundle branch block is traditionally regarded as the equivalent of an acute myocardial infarction with ST segment elevation (STEMI), but also it can be a marker of an ischemic or non-ischemic disease progression, affecting not only the cardiac conduction system but also the myocardium. The presence of marked ST-segment abnormalities may indicate an acute myocardial infarction, even if in the presence of LBBB, the ECG diagnosis of acute myocardial infarction (AMI) is difficult. Complex algorithms have been offered to assist the diagnosis, but they do not provide diagnostic certainty (5, 6). The presence of concordant ST-segment elevation $\geq$ 1 mm (i.e. in leads with positive QRS deflections) in patients with LBBB appears to be one of the best indicators of ongoing MI with an occluded infarct artery (7). Regardless the moment of occurrence of the LBBB, patients with a clinical suspicion of ongoing myocardial ischaemia and LBBB should be managed in a similar way as STEMI patients. It is also important to mention that the presence of a (presumed) new LBBB does not predict an AMI per se (4, 8).

Patients with myocardial infarction and RBBB have a poor prognosis (9,10) and it may be difficult to detect transmural ischemia in these patients. In this cases, new or presumed new ST-segment elevation $\geq$ 1 mm, or ST-segment or T wave abnormalities (excluding leads V1-V4), may indicate acute myocardial ischemia. New, or presumed new, RBBB without associated ST-segment or T wave changes is associated with thrombolysis in myocardial infarction (TIMI) 0-2 flow in as many as 66% of patients (compared with > 90% in those with ST-segment or T wave changes) (11). Considering that the blood supply of the right bundle-branch is mainly provided by left anterior descending artery (LAD) or the proximal septal perforator branch separated from LAD, new-onset RBBB may indicate the proximal occlusion of the LAD and large infarction. This can lead to severe heart failure, malignant arrhythmias, complete AV block and also a high mortality. But reperfusion therapy may prevent the appearance or limit the duration of bundle branch block, confirmed by studies that have reported the relationship between the reversibility of conduction disturbances and coronary reperfusion (12).

Given the poor prognosis of AMI patients with RBBB and the difficulty of determining the transmurality of an infarct in the setting of RBBB (13), the new European Society of Cardiology guideline suggests a primary percutaneous coronary intervention strategy when persistent ischemic symptoms occur in patients with

right bundle-branch block, but the level of evidence is not high (4).

In this context, the aim of our study was to assess the current data regarding which patients with suspected myocardial ischemia and bundle branch block should receive emergency reperfusion therapy.

LEFT BUNDLE BRANCH BLOCK

Patients with left bundle branch block and suspected acute coronary syndrome represent a diagnostic and therapeutic challenge for the clinician. For over 60 years, clinicians recognized the challenge of diagnosing acute myocardial infarction in the presence of the left bundle branch block (14). Since left ventricular activation in LBBB is delayed and initial activation of the interventricular septum is performed from right to left (contrary to the normal situation), Q waves suggestive of acute myocardial infarction are missing. Also, the secondary modifications of ST segment and T wave occurring in LBBB do not allow the recognition of signs of myocardial ischemia (15, 16). The electrocardiographic diagnosis of acute myocardial infarction is therefore difficult for these patients due to the masking of typical electrocardiographic changes (17).

Patients with acute ischemic symptoms and LBBB pose a unique diagnostic challenge, as it is frequently difficult to determine quickly whether the LBBB is new or old. Primary percutaneous intervention (PCI) is, according to the currently guidelines, the preferred method of reperfusion (4), but data regarding patients with new LBBB are limited. Even if these patients are less likely to have an acute culprit lesion at cardiac catheterization, they represent a high-risk population (18).

There is still an ambiguity regarding the management of patients with ischemic symptoms and new or presumed new LBBB. On the one hand, if these patients do not benefit from angiographic evaluation in an emergency, this will likely reduce the incidence of false activation of the catheterization laboratory and inappropriate fibrinolytic therapy. On the other hand, with holding appropriate reperfusion therapy in patients with a true coronary artery occlusion or acute coronary syndrome has the potential to increase morbidity and mortality (18).

Since the optimal use of myocardial reperfusion strategies is based on the rapid diagnosis of acute myocardial infarction, for most patients with angina, the electrocardiogram is an important element for assessing the cause of chest pain and for selecting the appropriate therapeutic management.

In the last decades, various electrocardiographic criteria have been proposed for the diagnosis of acute myocardial infarction in the presence of left bundle branch block (19,20). Of these, the Sgarbossa criteria are the most used (6,14). Sgarbossa *et al*. evaluated the role of the electrocardiogram in the diagnosis of acute myocardial infarction in patients with left bundle branch block and AMI documented based on the increase of myocardial cytolysis enzymes.

Three criteria are included in Sgarbossa's criteria (6):

- ST elevation ≥ 1 mm in a lead with a positive QRS complex (i.e. concordance) 5 points;
- concordant ST depression ≥1 mm in lead V1, V2, or V3 - 3 points;
- ST elevation ≥ 5 mm in a lead with a negative (discordant) QRS complex - 2 points.
- ≥ 3 points = 90% specificity of STEMI (sensitivity of 36%).

Although the original Sgarbossa criteria were an important first step in correcting this problem, the study had 3 important limitations. First, they depend on a point system that relied on 3 findings, only 2 of which would provide enough points to make the diagnosis of AMI.

Second, Sgarbossa *et al*. used the outcome of acute myocardial infarction (diagnosed by creatine kinase-MB elevations) instead of angiographic evidence of acute coronary occlusion, which limits the sensitivity of the rule because it combines non-ST segment elevation myocardial infarction and ST-segment elevation myocardial infarction. Third, Sgarbossa *et al*. used an absolute criterion (5 mm) rather than a proportional criterion for excessively discordant ST elevation (in the opposite direction from the QRS complex). Not surprisingly, attempts to validate electrocardiographic criteria for AMI in LBBB have shown useful specificity but poor sensitivity (21, 22, 23).

In order to improve accuracy, Smith *et al*. derived the "modified Sgarbossa criteria", in which the absolute 5-mm criterion for excessively discordant ST elevation is replaced with a proportion (ST elevation/S-wave amplitude ≤ -0.25). Smith *et al*. used angiographic outcomes to define acute coronary occlusion (thereby excluding NSTEMI). This outcome was more relevant to the reperfusion decision. They reported superior diagnostic properties of the modified criteria compared to the original criteria in their derivation study (7).

Mayers *et al*. performed an external validation of the modified Sgarbossa criteria by comparing the diagnostic accuracy (in terms of sensitivity and specificity) of the original and modified Sgarbossa criteria for the identification of acute coronary occlusion in patients with LBBB. They concluded that the modified criteria were superior to the original Sgarbossa criteria for the diagnosis of acute coronary occlusion in LBBB. They believe that it is currently reasonable to begin in corporation the modified Sgarbossa criteria into practice as a supplementary clinical judgment (23).

The diagnostic approach and management strategy for STEMI have evolved in the last decade with primary PCI currently the preferred reperfusion therapy (4). This has produced abundant angiographic evidence about false catheterization laboratory activation. One of the most important study was conducted by Larson *et al*. The aim of the study was to determine the prevalence, etiology and outcomes of false-positive cardiac catheterization laboratory activation in patients with a suspected STEMI by three criteria: no culprit coronary artery, no significant coronary artery disease and negative cardiac biomarker results. Of the 36 patients who presented with a new or presumably new LBBB from a regional STEMI registry, 16 (44%) did not have a culprit coronary artery, 10 (27 %) had no significant coronary artery disease and 13 (36%) had negative cardiac biomarker results. Thirty-day mortality of these patients was 8.3% (24).

Chang *et al*. analyzed a cohort of patients who presented to emergency departments with suspected acute coronary syndromes. According to the electrocardiograms, patients were classified in LBBB not known to be old (new or presumed new LBBB), LBBB known to be old or no LBBB. From all 7937 patients included in the study, 55 had new or presumed new LBBB, 136 had old LBBB and 7746 had no LBBB. They did not find a difference in the rate of clinical AMI between patients

with a new or presumably new LBBB, old LBBB, or without an LBBB (7.3% *vs.* 5.2% *vs.* 6.1%, P = 0.75). Only 19.2% of patients with new or presumably new LBBB had documented coronary disease by angiography and 7.8% underwent coronary revascularization, at 30 days of follow-up. The conclusion of this study was that the presence of LBBB, whether new or old, did not predict AMI, but coronary artery disease (19.2% *vs.* 11.9% *vs.* 10.1%, p = 0.0004) and revascularization (7.8% *vs.* 5.2% *vs.* 4.3%, p = 0.04) were more common in patients with new or presumed new LBBB (25).

More recently, Jain *et al*. evaluated the hypothesis that new or presumably new LBBB in symptomatic patients frequently leads to an overdiagnosis of acute myocardial infarction. A total of 892 patients from a single-center STEMI registry were admitted with initial diagnoses of STEMI and only 36 patients (4%) had new or presumably new LBBB on their presenting electrocardiograms. Only 14 patients (39%) had final diagnoses of acute coronary syndromes, of which 12 were AMI, while 13 (36%) had cardiac diagnoses other than acute coronary syndrome and 9 (25%) had noncardiac diagnoses (16).

The angiographic studies mentioned before, suggest that new or presumably new LBBB provides limited diagnostic value in identifying patients with acute coronary occlusion and should not be considered a reliable indicator for STEMI. In this case, in the early fibrinolytic trials it is logical to postulate that a significant proportion of those patients with LBBB and suspected AMI did not have an occluded culprit artery and thus received inappropriate fibrinolysis. Such false catheterization laboratory activation exposes patients to unnecessary procedure-related risks, prolongs hospital stay, increases health care expenses, and imposes a burden on limited resources.

Between January 2011 and December 2013, we conducted a study at the Cardiology Clinic of the Cardiovascular Disease Institute "Prof. Dr. George I.M. Georgescu", Iaşi, which included 477 patients with the left bundle branch block, from which 319 patients had a new or presumably new left bundle branch block, and 158 had an old left bundle branch block. If coronary angiography was performed in 80.56% of patients with new LBBB, only 18.35% of patients with old LBBB were evaluated by coronary angiography. Nearly half of the patients with new LBBB had significant angiographic coronary lesions, most commonly single- or bicoronary lesions (15.67% and 12.22%), with frequent localization at the left anterior descending artery (32.91%). However, nearly 80% of patients with old LBBB (79.11%) did not have significant angiographic coronary lesions, and when they were present, they most frequently involved all three main coronary arteries (10.76%) (26,27).

In another study, Pera *et al*. enrolled a total of 3903 consecutive patients with suspected STEMI who presented to a PCI center, including 131 patients (3.3%) with new LBBB. Patients with new LBBB were older, more commonly women, with a history of previous MI or coronary artery bypass grafting (CABG) and more frequently presented with cardiac arrest or heart failure. A culprit lesion was present less frequently in patients with new LBBB compared to those without (54.2% *vs.* 83.3%; $P<0.001$), consistent with the finding of more patients with TIMI 2 or 3 flow pre-PCI (79.5% *vs.* 51.9%; $P<0.001$) and fewer having PCI performed (51.2%

*vs.*79.0%; $p<0.001$). They concluded that patients with acute ischemic symptoms and new LBBB represent a high-risk patient population with unique clinical challenges, and emergent angiography and primary PCI should be considered the optimal strategy. Delaying reperfusion therapy for patients with suspected STEMI and new LBBB is likely to reduce the benefits of revascularization in patients with an acute culprit lesion (18). This was also the conclusion of another prospective study published by Farre *et al*. They evaluated the incidence and outcome of patients with LBBB referred to primary angioplasty and compare them with patients with ST segment elevation. From 4593 patients included in the study, only 2.6% (120 patients) had LBBB. Only 35.8% (44 patients) with LBBB underwent a primary percutaneous coronary intervention for thrombotic coronary artery occlusion, in opposite to 85.8% of patients with STEMI, $p < 0.0001$. Also, there was a statistically significant difference between the final diagnosis of patients included in the study, because 89% of patients with ST-elevation had the final diagnosis of acute coronary syndrome, compared to only 44% in LBBB, $p< 0.001$ (28).

Evaluating the implications of left bundle branch block in acute cardiac events based on a 23-year registry, Alkindi *et al*. concluded that patients with LBBB were less likely to have acute myocardial infarction regardless of the presenting symptoms, compared to patients without LBBB. The most frequent cause of admission with LBBB was congestive heart failure (54.2%), followed by ST-segment elevation myocardial infarction (13.3%). Overall, acute myocardial infarction was diagnosed in 32% of non-LBBB patients *vs.* 21% of LBBB patients, with a statistically significant difference between these groups ($p = 0.001$) (29).

One of the most recent and important study regarding the diagnosis of acute myocardial infarction in the presence of left bundle branch block was published by Nestelberger *et al*. They prospectively evaluated the incidence of acute myocardial infarction in patients presenting with chest pain to 26 emergency departments, a total of 8830 patients, 2.8% (247 patients) with LBBB. They found no difference in the incidence of acute myocardial infarction between patients with known LBBB *vs.* those with presumably new LBBB (29% *vs.* 35%, $p = 0,42$), MI being the final diagnosis in 30% of patients with LBBB (30).

RIGHT BUNDLE BRANCH BLOCK

It is known that both patients with left ventricular conduction disorder and patients with right ventricular conduction disorder in the context of acute myocardial infarction have a poor prognosis and increased mortality (31,32,33). However, there are only a few studies that compared the prognosis of patients with suspected acute myocardial infarction and left and right bundle branch block.

For example, in a retrospective study involving 6742 patients with acute myocardial infarction, Widimsky *et al*. analyzed the angiographic characteristics of patients with acute myocardial infarction and right bundle branch block and compared them with those presenting with LBBB or with other electrocardiographic pattern (11). Of these, 3447 patients were diagnosed with STEMI and 907 with NSTEMI. Only 291 patients had LBBB, 427 patients had a right bundle branch block, the remaining 1670 patients had acute myocardial infarction with other electrocardiographic changes.

Transluminal primary percutaneous coronary angioplasty was performed in 80.1% of patients with RBBB *vs.*. 68.3% of patients with LBBB, with statistically significant differences between the two groups of patients, p = 0.004. The morbidity of patients with RBBB was more than double compared to STEMI mortality, respectively 14.3% for patients with RBBB, 13.1% for LBBB patients and 5.4% for patients with STEMI (p = 0.004). The conclusion of this study was that RBBB should strongly be considered as a standard indication for reperfusion therapy, in the same way as LBBB.

Recent, Wang *et al*. conducted a meta-analysis to evaluate the prognostic value of RBBB in AMI patients (13). They identified five studies of 874 patients and evaluated the prognostic value of new-onset RBBB in AMI. The results show that new-onset RBBB is associated with an increased long-term mortality (RR, 1.66, 95% CI [1.31-2.09], p = 0.000), and higher risk of ventricular arrhythmia (RR, 4.86, 95% CI [2.10-11.27], p = 0.000), and cardiogenic shock (RR, 2.76, 95% CI [1.66-4.59], p = 0.000), but not heart failure in patients with AMI. Previous RBBB may have more relations with heart failure than new-onset RBBB in AMI patients. Based on the basic characteristics of the included studies, patients in previous RBBB group have a higher median age and more comorbidities (hypertension, diabetes mellitus, etc.), especially previous myocardial infarction and previous angina. It may explain the higher proportion of heart failure in previous RBBB group (13). They concluded that revascularization therapies should be considered when persistent ischemic symptoms occur in patients with RBBB, especially with new-onset RBBB (13).

Other studies (11,34,35) have shown that new-onset RBBB in AMI subjects often suggest LAD lesion, proximal occlusion of coronary artery and large infarct size. It is also associated with more complications including heart failure, arrhythmias, and increasing mortality compared with non-RBBB group.

In another recent study, Li *et al*. (36) evaluated the clinical characteristics and the value of new onset right bundle branch block in the early reperfusion therapy for patients with acute myocardial infarction. They included a total of 845 patients with AMI subjected to primary coronary angiography, seventy patients (8.28%) presented RBBB and 56 (6.63%) presented LBBB. Compared with non-BBB patients, RBBB patients had a higher incidence of smoking, hypertension and diabetes, hyperlipidemia, worse heart function and higher peak enzyme level (P<0.05). Compared with LBBB patients, the RBBB patients were almost male, and have a lower incidence of smoking, hypertension and diabetes, and a lower peak enzyme level (P<0.05). In 54.29% of RBBB patients and 42.86% of LBBB patients (54.29 *vs.* 42.86%, p<0.001) the infarct related artery was anterior descending artery, while the right coronary artery was responsible for 38.57% of myocardial infarction in RBBB patients and 32.14% in LBBB patients. Most of the patients with new RBBB and new LBBB had anterior and/or high-lateral wall myocardial infarction (58.57% *vs.*. 55.36%), and the second localization of myocardial infarction was inferior and/or right ventricular for patients with RBBB and inferior and/or posterior wall for patients with LBBB (34.29% *vs.* 28.57%, p = 0.032). The cumulative one-year survival rate in RBBB patients was significantly lower than

those in the no BBB group ($p = 0.046$) and the LBBB patients ($p < 0.001$). As RBBB is associated with more severe symptoms, a higher incidence of complete occlusion of infarct related artery and a lower one-year survival rate, the conclusion of this study was that new onset RBBB should be considered as an indication of emergency revascularization (36).

The new European Society of Cardiology guideline for ST-segment elevation myocardial infarction recommends that left and right bundle branch block should be considered equal for recommending urgent angiography in patients with suspected myocardial infarction, but the level of evidence is not high. In fact, the presence of RBBB may lead to the misdiagnosis of transmural ischemia and mask the early diagnosis of ST-elevation myocardial infarction. Also, new-onset RBBB is occasionally caused by AMI (4). Moreover, they consider that in patients with LBBB, ST-segment elevation $\geq$ 1mm concordant with the QRS complex in any lead may be an indicator of acute myocardial ischemia. In patients with RBBB, new or presumed new ST-segment elevation $\geq$ 1mm, or ST-segment or T wave abnormalities (excluding leads V1-V4), may indicate acute myocardial ischemia (4). This ESC recommendation is based on a sole publication by Widimsky *et al*., which reported an association of RBBB and coronary artery occlusion.

To evaluate this novel recommendation of urgent angiography in patients with left or right bundle branch block and suspected myocardial infarction, Newmann *et al*. (37) conducted a prospective study and included a number of 4067 patients presenting to the emergency department with suspected myocardial infarction. In the overall study population 846 of 4,067 (20.8%) patients were adjudicated as having acute MI. In the ECG analyses RBBB was observed in 125 (3.1%), LBBB in 281 (6.9%) and bifascicular block in 60 (1.5%) of all patients, while 3601 patients had no complete bundle branch block. The final diagnosis of myocardial infarction was adjudicated in 20.8% (RBBB), 28.5% (LBBB), 23.3% (bifascicular block) and 21.6% (no complete block) of patients. To assess mortality, all patients were followed for up to two years, and they observed that the mortality rate after one year was 10.7% (RBBB), 7% (LBBB), 17.5% (bifascicular block) and 3.2% (no complete block). Patients having a RBBB were equally likely to have a final diagnosis of MI and to receive revascularization as those without bundle branch block. In contrast, patients with LBBB had a higher rate of MI diagnoses. Nevertheless, patients with RBBB represent a high-risk population with a high mortality rate. The clinical implications of this study are significant, as the rate of RBBB among patients with suspected MI is low, the final diagnosis of MI in patients with RBBB is equally likely when compared to patients without bundle branch block and the presence of RBBB is associated with adverse outcomes, but they disappear after adjustment for confounders (37).

There are also other studies that highlighted the association between new onset RBBB and acute coronary syndrome.

Timoteo *et al*. evaluated the differential impact of LBBB and RBBB in prognosis at one-year follow-up, after an acute coronary syndrome. From a total of 3990 patients included in the study, 3.4% had LBBB and 4.3% RBBB. Even if the medical treatment was similar, patients with bundle branch block were less often submitted to angioplasty. Bundle branch block patients had worse outcome ($p < 0.001$), but similar in

LBBB and RBBB (p = 0.597). Also, both LBBB and RBBB were predictors of all-cause mortality at one-year follow up in a univariate analysis, and after adjustment only RBBB remains an important predictor (HR 1.66, p = 0.007). The results of this study support the European guideline recommendation that we should pay special attention to patients with bundle branch block and treat them as least as aggressively as patients with a normal QRS duration (38).

In a recent article, Shaikh *et al*. (39) presented four cases of new RBBB associated with chest pain and critical lesions of the left anterior descending artery, highlighting the need to consider new RBBB in the setting of acute chest pain, as an indication for acute reperfusion therapy also in the American guidelines, as the European Society of Cardiology guideline recently implemented.

CONCLUSIONS

In conclusion, we consider that patients with persistent ischemic symptoms, and presumed new LBBB or RBBB that is not rate-related, have an adverse prognosis and they should be evaluated by emergency angiography.

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NEWS

CHEST COMPUTED TOMOGRAPHY AND COVID -19 DISEASE

COVID-19 (coronavirus disease 2019) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2. Definitive diagnosis of COVID-19 requires a positive PCR test. Multiple radiological organizations have stated that computed tomography (CT) should not be relied upon as a diagnostic or screening tool for COVID-19. Current best practice advises that chest computed tomography is not used to diagnose COVID-19, but maybe helpful in assessing for complications. The non-specific imaging findings are most commonly of atypical or organizing pneumonia, often with a bilateral, peripheral, and basal predominant distribution. According to Fleischner Society (April 2020) CT imaging is not indicated in patients with suspected COVID-19 and mild clinical features unless they are at risk for disease progression and is indicated in a patient with COVID-19 and worsening respiratory status. Patients requiring CT should receive a non-contrast chest CT (unless iodinated contrast medium is indicated). If iodinated contrast medium is indicated, for example a CT pulmonary angiogram (CTPA), a non-contrast scan should be considered prior to contrast administration, as contrast may impact the interpretation of ground-glass opacification patterns. The most frequent findings on CT are bilateral, subpleural, peripheral ground glass opacities, crazy paving appearance, air space consolidation, bronchovascular thickening in the lesion, traction bronchiectasis. (D. Bell *et al*., COVID-19. *Radiology Reference Article*, *Radiopaedia.org*, https://radiopaedia. org/articles/ covid-19).

A SYSTEMATIC REVIEW OF THE INCIDENCE AND RISK FACTORS FOR HEPATITIS E INFECTION IN ROMANIA

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A SYSTEMATIC REVIEW OF THE INCIDENCE AND RISK FACTORS FOR HEPATITIS E INFECTION IN ROMANIA (Abstract): The incidence of viral hepatitis E (HEV) has taken shape in European countries, economically developed areas and with a high level of hygiene. For the occurrence of this disease was initially incriminated the consumption of properly unprocessed pork or travels to endemic areas. Over time, other ways of transmission were discovered. The spread of the virus also included Romania. In this context, we aim to analyze data from the literature on the incidence and risk factors for HEV. Recent studies conducted in Romania have shown a seroprevalence between 9.6% and 50% in farms, highlighting a zoonotic transmission. Another study shown that the risk factors associated with HEV infections were previous blood transfusions, immunosuppressive disorders, or hepatitis B virus co-infection. In conclusion, HVE infection remains an undeclared disease, possible due to misdiagnosis, subclinical infection, or co-infection with another hepatitis virus. **Keywords**: HEPATITIS, ZOONOTIC TRANSMISSION, ENDEMIC DISEASE.

Hepatitis E (HEV) is an international public health problem (1). The infection is caused by hepatitis E virus (HEV), single-stranded, single-serotype, RNA virus belonging to the *Herpesviridae* family. Five HEV genotypes are known to cause human disease (2). Genotypes 1, 2, 4 and 5 are associated with illness in developing countries, genotype 1 and to some extent genotype 2 being associated with large waterborne outbreaks. HEV genotype 3 has been implicated in the occurrence of hepatitis E in developed countries (3).

The disease was identified in the 1980s as a result of outbreaks of non-A, non-B hepatitis in Africa and Mexico. The outbreaks were caused by a disorder with clinical and paraclinical manifestations of an acute hepatitis. Thus, appears HEV, a new type of hepatitis with enteral transmission. Subsequently, the infection broadens its horizons over time, appearing in regions with a high level of hygiene (Europe, North America, Australia), economically developed countries (4).

From the beginning, the route of transmission of the virus has been considered the fecal-oral route, through the consumption of drinking water and contaminated food, in less developed geographical regions, such as South-east Asia, Africa and Mexico, where it continues to evolve to a

high endemic level (5). The appearance of the infection in high-income countries, led to numerous hypotheses regarding the mode of transmission. Initially, travel to endemic areas and ingestion of contaminated food and zoonotic transmission was criminalized (6). Subsequently, it was found cases in non-endemic areas unrelated to the two factors mentioned above. Blood transfusions, organ transplants and contact with infected animals have also been reported (7). Thus, further research was needed to discover the risk factors associated with hepatitis E in non-endemic areas.

In most patients, hepatitis E causes a self-limiting illness that lasts for several weeks. Acute infection is generally asymptomatic. Regarding clinical hepatitis E infections and severe forms, it has been observed that they are more common in middle-aged and elderly men, who often have other coexisting diseases (8).

Although there are data in the literature describing and analyzing hepatitis E virus infection among different population groups, little is known about the actual sources of infection, routes of transmission and risk factors for the host (such as associated diseases and chronic treatment of the patient) regarding acute hepatitis E in Romania.

MATERIAL AND METHODS

This study is a retrospective analysis of the scientific literature regarding HEV. We included in the study articles published in medical journals that focus on the incidence and risk factors associated with HEV. We focused our attention on articles made in Romania, excluding those made in other geographical parts of the world.

We searched on electronic databases for articles on HEV incidence, seroprevalence and risk factors in Romania. The most used online databases were *PubMed, Scopus, ISI Web of Science, Google* research with no limitations placed on year of publication and language restriction. We used keywords like hepatitis E virus, HEV RNA, genotype, zoonotic transmission, enterically transmission, seroprevalence, Romania. And critically reviewed appropriate publications to extract consistent findings, identify knowledge gaps, and suggest future studies.

RESULTS AND DISCUSSION

Hepatitis E virus infection is not very common in our clinical practice, but the incidence is increasing. In Romania, the first research about HEV infection appeared in the 2000s and was limited to seroprevalence studies of specific anti-hepatitis E IgG. The earliest results shown a seroprevalence rate of 5.9% in the general population (9) and of 12% among patients with hepatitis B or C (10).

In 2010, in a report of the results of a study performed on students (mean age: 22.91 ± 0.56 years) and medical staff (mean age: 36.71 ± 8.88 years), the prevalence of these antibodies was 12.5%, respectively 14% (1,11).

Subsequently, several research were carried out by veterinarians on the evidence of hepatitis E virus infection in pigs and the population in the northeastern part of Romania. The results showed a seroprevalence of 17.14% (12/70) and 12.82% (10/78) in the tested human sera. In 2011, the youngest age group (9-20 years) tested negative, whereas as older age groups (> 20 and > 40 years) had seroprevalence of 28.6% and 12.9%, respectively. In 2012, the youngest age group (18-45 years) had seroprevalence of 6.25%, whereas the older

groups had the seroprevalence up to 28%. In the same research, it was found that HEV infections were found in middle-aged adults exposed to the virus through contact with pig farms. This confirms the potential role of the animal reservoir in the occurrence of this zoonosis, while also suggesting that the hepatitis E virus is found in a ubiquitous manner in pig farms in north-eastern Romania. Moreover, anti-HEV IgG was more prevalent in women (15.78%) than in men (10%) (12-14).

Other recent research in Romania has found an anti-HEV Ac IgG seroprevalence between 9.6% and 50% in farms, in farm pigs and wild boars in which PCR (polymerase chain reaction) analysis in a small number of liver samples and the spleen found only genotype 3, similar to neighboring countries. Data (in small samples) on the seroprevalence of Ac IgG anti HEV in humans are rare and variable: general population (5.9% - 28%, higher in older participants), students and medical staff (12.5-13.98 %) and patients with hepatitis B or C (12%) (15).

Another study, published in April 2019 in the Romanian Journal of Laboratory Medicine, aimed to identify the risk factors associated with HEV seropositivity. The group consisted of patients admitted to the National Institute for Infectious Diseases "Prof. Dr. Matei Balș" from Bucharest, Romania and included 175 patients. Forty-six patients (23.3%) had positive anti-HEV IgG antibodies, with a mean age of 54.5 years. The associated risk factors were previous blood transfusions (22.7%), immunosuppressive disorders (40.9%) and hepatitis B virus co-infection (31.1%) (16).

A systematic review of the published literature on the distribution of hepatitis E in South-Eastern Europe has shown that anti-HEV seroprevalence varies widely. Statistical data in different population groups from Romania ranged from 5.9% to 17.1% (17).

In early 2020, a study on hepatitis with enteral transmission over a period of 2 and a half years was published. The results show that the incidence of HEV has increased compared to previous years. The number of people diagnosed with HEV was 48, the mean age was 50.6 years and two-thirds of the patients were men. Laboratory data showed that patients with hepatitis E had significantly less modified AST and ALT, bilirubin, prothrombin index and INR levels. Severe forms were found in 12 (25%) of hepatitis E patients, of whom 3 died. Ribavirin treatment was initiated in 9 patients with acute-chronic hepatitis E, immunosuppression, cancer, or neurological manifestations, with good results (15).

There are few studies on HEV in humans, and new data are needed to define the characteristics and the real incidence of HEV infection. Studies performed on animals show the presence of anti-HEV IgG in farm and backyard pigs with high seroprevalence rates.

CONCLUSIONS

Most of the studies we have found in the literature study the seroprevalence of the infection, showing the increase in the incidence in economically developed areas. Zoonotic transmission and its appearance are predominant in middle-aged males.

However, in clinical practice this pathology is not very common. The use of different methods for the diagnosis of hepatitis and the increased incidence of HVE among asymptomatic patients, can make this pathology go undiagnosed. This may also be possible due to misdiagnosis

or double hepatitis.

Raising awareness of the importance of thermal food preparation, boiling drinking water and washing vegetables is an effective intervention to prevent hepatitis E infection.

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TUBERCULOSIS AND BACTERIAL CO-INFECTIONS IN CHILDREN

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TUBERCULOSIS AND BACTERIAL CO-INFECTIONS IN CHILDREN (Abstract): Tuberculosis is a major public health problem nowadays without a change to be eliminated in many regions. The study aim was to evaluate the bacterial co-infections diagnosed in children with TB, in a hospital in the eastern part of Romania. **Material and methods**: The study group consisted of 37 children diagnosed with TB and various bacterial co-infections and admitted in a pediatric hospital in Galati County, Romania, between 2014 and 2017. **Results**: In our study group characterized by the mean age of 10.40, with a male/female report = 0.94, a predominance of children aged 12-17 (45.94%), and no cases were recorded in 0-1 year of age group, the most frequent localization of TB lesions was pulmonary TB primoinfection (45.86%), bacterial pneumonia was the main co-infection in children with TB (64.86%), then bacterial acute bronchitis (28.73%) and gastroenteritis (5.41%). **Conclusions**: Our study evidenced that bacterial pneumonia was the most frequently diagnosed co-infection in children with TB (64.86%). The most frequent bacterial co-infections pathogens were *E. coli* (32.43%), *S. pneumoniae* (27.03%), and *K. pneumoniae* (21.62%) and the length of stay between 4 days and 92 days. **Keywords**: TUBERCULOSIS, PEDIATRICS, BACTERIAL CO-INFECTION, PREVALENCE.

According to WHO reports on the *END TB* strategy, in the present worldwide, the average rate of decrease in the incidence of tuberculosis (TB) was 1.6% per year between 2000-2018, and 2.0% between 2017 and 2018. The cumulative decrease between 2015 and 2018 was only 6.3%, considerably lower than the targets specified by the WHO that stipulated reductions of 20% by 2020. TB mortality was reduced by 11% in the period 2015-2018, these figures representing less than a third of the strategy objectives, however, in the WHO European region, countries are close to achieving the goals of the phase of TB elimination in 2020, because the TB incidence decreased by 15% and mortality by 24% (1).

Recently, WHO published estimates of child-specific diseases for the first time in 2012, reporting approximately 500,000 cases of TB among children under the age of 15 years. These figures were based on extrapolations from adult data, which were heavily weighted on sputum smear positivity and did not incorporate sufficient adjustments to account for under-detection and under-reporting in pediatric populations. Similarly, the estimated pediatric mortality from TB is poorly quantified, and in 2015, the WHO estimated 210,000 deaths from TB among children. In many countries with high mortality, deaths from

TB are often reported to be caused by pneumonia or meningitis (2, 3, 4).

The risk of active TB is higher in people suffering from other diseases that affect the immune system. For example, children with malnutrition are three times more at risk, and in 2018, 2.3 million new cases of TB associated with malnutrition were reported worldwide (5).

Age such as very young children, male gender and low socio-economic status can be factors favoring infection, along with malnutrition, poor living conditions, active or passive smoking (in young children) or associated infections (HIV / AIDS) (6, 7).

The study aim was to highlight the bacterial co-infections diagnosed in children with TB, in a hospital in the eastern part of Romania.

MATERIAL AND METHIODS

The cross-sectional study was conducted on a group of 37 patients admitted in the pediatric unit of The Hospital of Pneumology, Galati, Romania, during January 1st, 2014 and December 31st, 2017. The total number of children admitted in the study interval was 208, our study group representing 17.78% of total TB admitted cases. The inclusion criteria were pediatric age groups and suspicion of diagnosis of TB and the association of at least one microbiologically confirmed bacterial co-infection. The exclusion criteria were patients over 18 years old and no suspicion of co-infection with TB.

Statistical analysis consisted in the calculation of frequency, mean, linear regression ANOVA regression, Pearson's correlation between variables. The *p* value was set <0.05.

RESULTS

Demographic characteristics. The study group of children diagnosed with TB was characterized demographically by a mean age of 10.40 (minimum = 4; maximum = 17), with a homogenous distribution regarding gender: 18 females (48.64%) and 19 males (51.36%); M/F = 0.94. Distribution by age groups showed a predominance of children aged 12-17 years old (17 cases - 45.94% of total), following by 6-11 age group (13 cases – 35.13%) and 2-5 age group (7 cases – 18.93%). No cases were recorded in 0-1 age group (fig. 1).

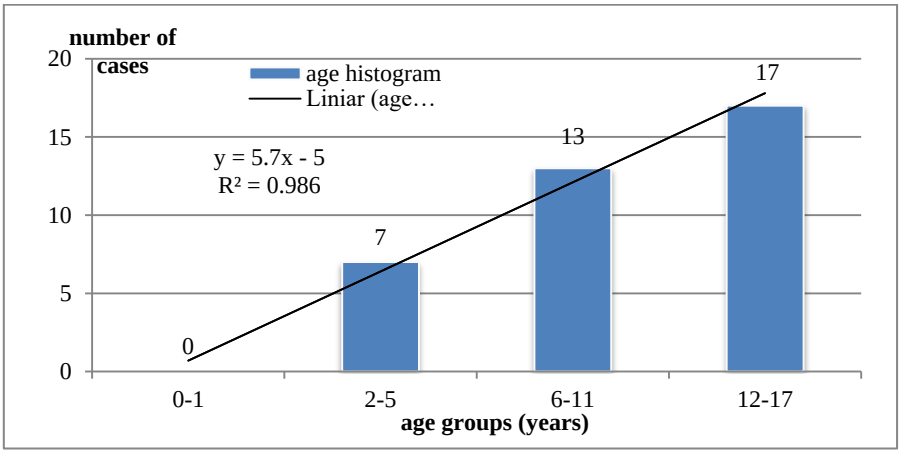

Fig. 1. Age histogram

Localization of TB lesions. The most frequent localization of TB lesions was pulmonary TB primoinfection (17 cases – 45.86%), then ganglionary infection (14 cases – 37.83%) and pleurisy (6 cases – 16.21%) (fig. 2).

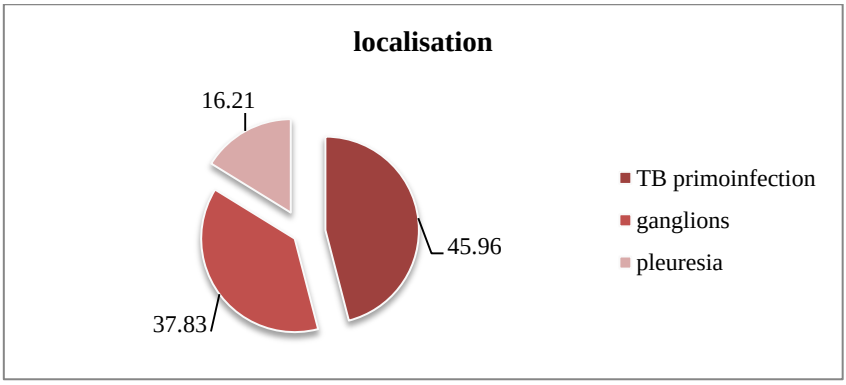

Fig. 2. TB localization

Bacterial co-infections. Bacterial pneumonia was the main co-infection with TB in children (24 cases – 64.86% of total), then bacterial acute bronchitis (11 cases – 28.73%) and gastroenteritis (2 cases – 5.41%) (fig. 3).

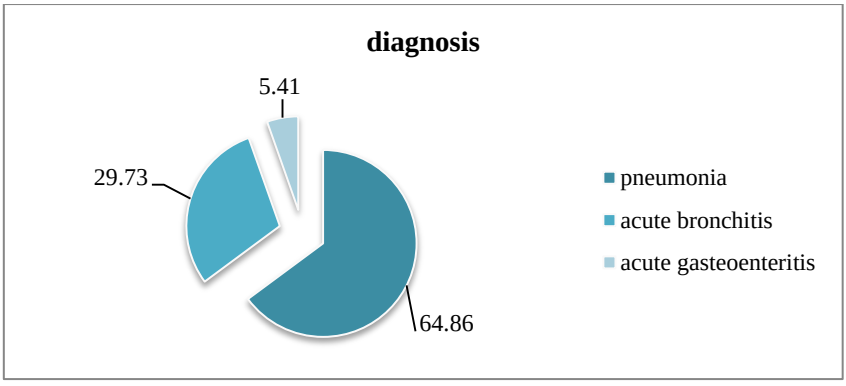

Fig. 3. Bacterial coinfections diagnosis

The bacterial co-infections pathogens were identified by microbiological methods as: *Escherichia coli* (12 cases – 32.43%); *Streptococcus pneumoniae* 10 cases – 27.03%); *Klebsiella pneumoniae* (8 cases – 21.62%); *Hemophilus influenzae* (5 cases – 13.51%); *Staphylococcus aureus* (2 cases – 5.41%) (fig. 4).

Hospitalization days. Pediatric patients with TB and bacterial co-infections were hospitalized a total number of 1567 days with a mean = 44.78 days, a minimum = 4 days (a 12-year female patient with pulmonary TB and acute bronchitis with *S. pneumoniae*) and a maximum of 92 days (a 11-year-old female patient with ganglionary TB and acute bronchitis with *H. influenzae*). Length of stay was 1-4 days in 1 case

(2.7% of total), 5-10 days in 6 cases (16.21%), 11-30 days in 5 cases (13.51%), 31-60 days in 14 cases (37.85%) and >60 – 92 days in 12 cases (32.42%) (fig. 5).

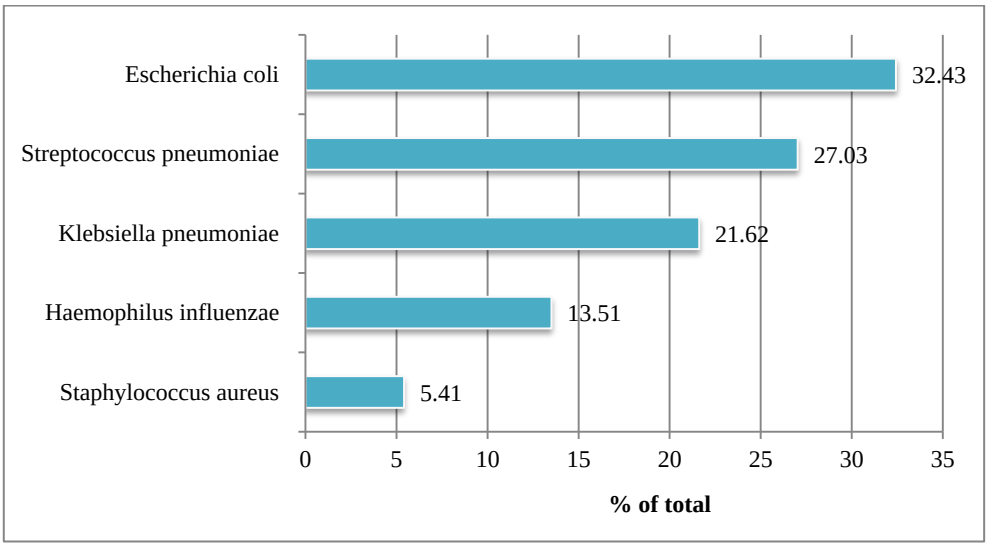

Fig. 4. Pathogens involved in bacterial coinfections in pediatric patients with TB

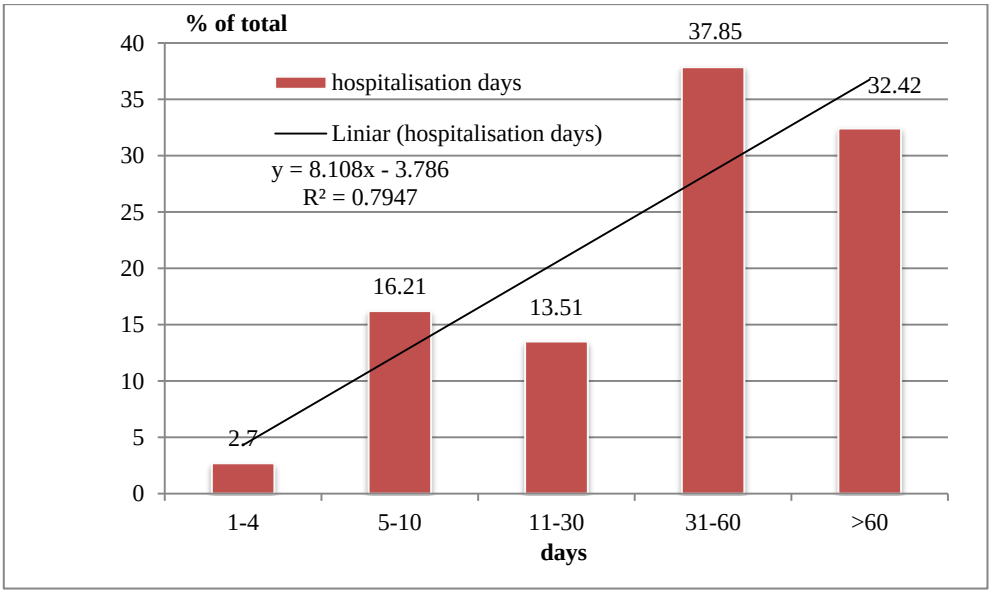

Fig. 5. Hospitalization days of patients with TB and bacterial coinfections

By applying *ANOVA linear regression*, a weak correlation between the age of the patients and the number of days of hospitalization (r = 0.1289) was revealed, but statistically non-significant (significance F = 0.44; p = 0.44).

DISCUSSION

TB occurs as a result of infection in previously uninfected people is called primary TB. Pulmonary TB in children, especially in young children, continues to be almost exclusively primary TB. A significant number of TB cases in adolescents and young adults are often the consequence of a recent infection, although the clinical and radiological picture suggests a secondary TB (primary and secondary tuberculosis). Primary occult TB infection, often ignored, is the most common of the clinical forms; the first manifest simple infection, in which the radiological examination identifies the primary TB complex (with satellite adenopathy). Primary infection with benign complications in which the primary complex is associated by local complications with benign evolution such as serofibrinous pleurisy and primary infection with serious complications, with a significant fatality rate such as bronchopneumonia are other important clinical forms. Secondary tuberculosis, specific to adulthood, is characterized by the predominance of cavitary forms and chronic clinical evolution. The clinical elements, regardless of location, only partially guide the diagnosis, in TB it is necessary to corroborate epidemiological, clinical and laboratory data (8).

Although TB is a preventable disease requiring coordinated systematic efforts, the prevalence is in fact the iceberg of the epidemiological burden and a child with active TB represents a sentinel event in the community, reflecting the intensive transmission among all the individuals. (2) Clinical symptoms of TB in children are often nonspecific, leading to a delayed diagnosis and a long period when the child is contagious and without specific treatment to prevent secondary transmission (9), and during this time the child, often immunosuppressed, is exposed to the pressure of the circulating community pathogenic flora, often aggressive and with a high level of antibiotic resistance (10).

There are few published studies on the burden of bacterial TB co-infection in the general populations of countries with a high prevalence of TB (11). Studies provided evidence that co-infection of other respiratory bacteria in children with TB was easily quantifiable, although the high rate of co-infection observed in people infected with TB might overestimate the real prevalence in all TB patients. The Gram-negative bacteria are, overall, the most commonly non-mycobacterial pathogens identified in patients with TB. Our data are consistent with published data of other studies (12, 13, 14).

Simultaneous occurrence of TB and bacterial co-infection in patients might be uncommon, according to a recent study. A triple infection of HIV/AIDS, TB, and pneumococcal pneumonia has been reported, the former being a crucial predisposing factor for the co-infection of TB and other bacteria, mainly pneumococcal pneumonia (15, 16, 17).

In populations where TB has a high prevalence, there is also co-infection of TB and bacterial pathogens. Differentiating TB from other lesions such as bacterial pneumonia is a clinical challenge, as it is difficult to diagnose TB in childhood. A study in Cambodia showed that 9% of all patients and 33% of patients with TB evaluated for respiratory symptoms, in whom sputum was collected and tested for *M. tuberculosis* and microbiological suffered from TB and bacterial co-infection, and *K. pneumoniae* and *Pseudomonas aeruginosa* were the main bacteria detected in the Asian study (12).

In contrast to the Asian study, we iden-

tified that *E. coli* and *S. pneumoniae* as the main bacterial co-infection pathogens involved in pediatric patients.

The strengths of our study are that we assessed bacterial co-infections associated with TB in children who come from open communities, where they come in contact with people with favorable factors, receptive to the disease. The weaknesses and the limitations of our study could be the small group of patients assessed for a few demographic variables and the non-inclusion into the evaluation the symptoms and other clinical features as well as the characteristics of bacterial antibiotic resistance.

CONCLUSIONS

Our study evidenced that bacterial pneumonia was the most frequently diagnosed co-infection in children with TB (64.86%). The bacterial co-infections pathogens were *E. coli* (32.43%), *S. pneumoniae* (27.03%), *K. pneumoniae* (21.62%), *H. influenzae* (13.51%), and *S. aureus* (5.41%).

The length of stay for pediatric patients with TB and bacterial co-infections consisted in a total number of 1567 days, with a minimum of 4 days and a maximum of 92 days.

The study team intends to continue the research on larger group of pediatric patients, considering the missing data exposed above. The results of further similar and improved studies could be extrapolated in the hospital management and community information regarding TB and bacterial co-infection prevention and best therapy protocol.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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NEWS

ATRIAL FIBRILLATION STRUCTURAL SUBSTRATES: AETIOLOGY, IDENTIFICATION AND IMPLICATIONS

AF is the most common sustained cardiac rhythm disorder and is associated with increased morbidity and mortality. Since the first description of AF initiation by triggers from pulmonary veins sleeves, pulmonary vein isolation (PVI) has become the standard ablation strategy in patients with AF. However, freedom from the arrhythmia, particularly in non-paroxysmal AF, remains suboptimal, and it is now clear that, in these patients, AF is maintained by an atrial substrate beyond the pulmonary veins. Although electrical remodeling may be reversible with termination of the arrhythmia, the development of atrial substrate due to fibrosis contributes to the progression of the AF phenotype from paroxysmal to persistent AF, leading to an arrhythmia that is more refractory to intervention. It is clear from animal and human studies that prolonged AF can cause this structural change. Moreover, it is also apparent that a range of risk factors associated with AF, including age, obesity, heart failure (HF), valvular heart disease, hypertension (HT), sleep apnea and alcohol intake, may also progress atrial remodeling. The rise in the prevalence of cardiovascular risk factors (particularly driven by ageing populations and the obesity epidemic) has been associated with an increase in the prevalence of AF and AF-related hospitalizations. In this review, the authors focus on insights from electrophysiological mapping studies in cohorts with AF risk factors. They also discuss substrate mapping and its implications for AF management and outcomes (Al-Kaisey AM, Parameswaran R and Kalman JM. Atrial Fibrillation Structural Substrates: Etiology, Identification and Implications. *AER* 2020; 9(3): 113-120).

EDITORIAL

ACTUAL STATUS IN BRAIN MAPPING

Starting from 1900 with the wide-known Broadmann mapping until present times with large-cohort functional studies, neuroscientists and neurosurgeons were looking for dividing the cortex in its areal components. The importance of this brain mapping is clearly stated: establishing the role and function of each brain area would facilitate diagnostic analysis, enhance comparisons between studies and people, improve quality of surgeries and facilitate statistical brain analysis.

In this editorial, authors have performed an analysis of the current status of brain mapping taking into consideration both general and particular data. Our aim was to provide the basic concepts to each of the aforementioned investigations and finally look at the present perspective on some of the wide-recognized brain mapping projects.

CYTOARCHITECTONIC – BROADMANN & MYELOARCHITECTONIC – VOGT

Cytoarchitectonic analysis is based on the different dimensions, distribution and shape of the cells that form the various aspects of the cerebral layers. Korbinian Broadmann may be one of the first most renowned scientist who attempted to divide the human cortex into areas based on the assumption that each of them has a different cytoarchitecture (1909). Even though his map was a pioneering approach in the domain at that time, now it is well known that it doesn't match the actual representation of brain mapping. Studies after Broadmann have changed completely the way to analyze brain. This included a completely different way of defining brain boundaries based on statistics and not on visual inspection, various staining and imaging techniques, larger samples analysis, 3D representation maps and probabilistic maps to analyze brain variability (1).

Another subdiscipline similar to the first one described is myeloarchitectonic which focuses on analyzing the distribution and orientation of single axons within the cerebral cortex. Cecile and Oskar Vogt (2) alongside their disciples published a lot of literature work in this domain. These data were recently gathered into a meta-analysis and transferred to their neuroanatomical sequence in a new representation (2). Individual mappings were superimposed, and the boundaries were averaged. Finally, their 180-areas-map exceeded the number of the areas of any other map that was done before: Brodmann (43), Von Economo and Koskinas (107), Sarkissov (71). Nowadays, myelin density is analyzed using longitudinal, relaxation rate, T1w/T2w mapping (3, 4). In these cases, the spatial difference in myelin content across brain areas becomes a measure for delimitating putative cortical boundaries. Age and localization of the cortical layer may affect myelin density. For example, people between 18-22 years have higher myelinated areas and inner layers showed higher T1w/T2w ratio (5).

TASK-BASED fMRI

Functional MRI (fMRI) imaging based its processing on the blood oxygen level dependent (BOLD) contrast mechanism. During fMRI scanning one subject could perform different tasks or rest. Task-based fMRI analysis concentrates its focus on brain activity during task performance. The

fMRI results depend on the relation between the level of attention of the subject to avoid motion that would increase the artifacts and achievement of statistically significant data for analysis (6). When analyzing brain activation, it should be considered that level of integration depends on the specific task demand. The strength of connections between core and peripheral areas within a network changes depending on the task. In stimulated areas functional connectivity patterns could be predictive factors for task identification (7). Other times, task-based fMRI showed to be useful in identification of altered brain areas, following mild brain injuries. Thus, Cook et al. demonstrated based on tfMRI that middle frontal gyrus neural activity alteration is responsible for loss of memory and attention (8).

Conventional, task-based fMRI (tfMRI) includes a single-task paradigm for each brain function, whose strategy varies: block design, event-related or mixed design. The first one uses equitemporal blocks and rests to elucidate the response, while in the second case, the events are presented randomly and focus on a specific cognitive event. Blocked design showed to be robust to the hemodynamic changes with great statistical power and to permit resting intervals yet may miss transiently activated regions. In contrast, event-related requires complex analysis and relies more on the linearity of BOLD responses, but allows shorter and randomly alternating trials, having a broader associative signal detection (9, 10).

RESTING-STATE fMRI

Resting-state fMRI (rsfMRI) assumes that in lack of any task, different connected regions may express similar patterns of BOLD signal following specific circuits, including somatosensory, visual and auditory networks. This specific particularity differentiates rsfMRI from tfMRI, in which different paradigms are designed for identification of each particular system. Moreover, rsfMRI has access to a broader range of patients and pathologies that cannot correctly perform the tfMRI (11). Back to the low-frequency signals, these are traditionally considered noises which are expressed under rest condition and may be dependent on the resting-state networks (RSNs) or may be independent of the neuronal activity. The argument in favor of the first statement is that these fluctuations recapitulate the functional track that is activated during specific task or appear in brain areas with activation relevance. Regarding the second option, BOLD-like signals may appear also due to head movement or vascular CO2 concentration fluctuation; specifically hypercapnia leading to an increase in BOLD-like signal, and hypocapnia to a decrease in BOLD-like signal (12).

DIFFUSE TENSOR IMAGING

While many brain investigations elucidate functions or different areal brain behavior, Diffuse Tensor Imaging (DTI) shows more accurate the actual relationship between different brain areas, providing a structural map of the brain tracks (13). Advancement in modern techniques has broaden the investigation of brain fibers.

Diffusion of water molecules is characterized by anisotropy (not the same movement in all directions of 3D space) dependent on the orientation of the fiber tracts modulated at a micro and macro level, specifically, being more hindered on the perpendicular axis rather than on the parallel axis (14). Density of the fibers, myelination, individual fiber diameter are important aspects to be considered. The orientation of the primary diffusion tensor within each voxel forms the primary vector (the eigenvector) of the voxel that by inter-voxel connectivity establishes the axis of

the tracked fibers (15).

BRAIN MAPPING AND NEUROSURGERY

Functional MRI has a lot of appliances in medicine, including neurosurgery. From the very beginning, fMRI can be used as a tool to prevent the injury of brain structures while removing tumors, especially in awake surgeries when the patient is asked to perform specific tasks that will activate the areas. By creating the functional brain map, neurosurgeons could determine how close the tumor is to the areas responsible for speech, movement, hearing.

Connectome analysis of resting-state fMRI could be used to determine the active areas in the brain, also called nodes, which can be find isolated or in segregation forming hubs and generally measures the intelligence index or how efficiently our brain integrates the information between areas. The existence of hubs demonstrates that selective small-nodes removal could be more efficient in preserving brain function during a tumor surgical resection than gross-total targeted removal. Another perspective is the "connection at risk" , or how much will affect an extended margin removal to the surrounding areas and to the patency of long connection within the hemispheres (16). Brain tumor resection has been demonstrated to trigger changes in the small-world distribution supported by rapid and real-time brain network integration. The degree of sparsity in the network has changed between pre- and postoperative group and decrease in the correlation degree was related to decrease in neurocognitive capacity (17).

In epileptic surgery, eloquent cortex responsible for motor, language and memory should be preserved as much as possible, thus, task-based fMRI is used to determine the active areas prior to surgery. Language fMRI could predict the decline in cognitive function after lobe excision and it is considered to be a better alternative to the classical Wada test (18). Beside the classical artifacts, preoperative fMRI could encounter some challenges as the anti-epileptic medication and long-term seizures could alter area activation during fMRI image acquisition and decrease Intelligence quotient (IQ). Another challenge is that seizures could alter localization and lateralization of language and memory function. Since language is a complex function of the brain, at least two block design paradigms should be addressed to the patient. Sentence Completion, Silent Word Generation, Rhyming, Object Naming, Antonym Generation and Passive Story Listening are some paradigms that activate middle frontal gyrus (the expressive region) and temporal lobe (the receptive region) recommended by the American Society of Neuroradiology (19). Regarding memory fMRI, left frontal and anterior medial temporal activations correlates with greater postoperatively verbal decline than bilateral posterior hippocampal activation considering the fact that left lateralization is more often encountered (20). In children, early onset of seizures modifies the normal lateralization, while the brain is focused on saving the vital cognitive functions. While memory fMRI was used frequently in adults, language fMRI was rarely used in children, based on the false assumption that either adults or children manifest co-lateralization of memory and language. However, recently, novel paradigms combining both block and event-related analyses are used to assess the memory outcome in pediatrics (21).

Increased neural adaptive plasticity has been observed in patient undergoing diffuse low-grade gliomas surgery (DLGG), leading to low neural disturbances prior and after surgery. Changes in functional activity patterns after surgery, even though

they are few in this case, were analyzed, anatomical and functional. Mean DO80 scores were similar in the pre- and postoperative group suggesting that each brain is responsible for its reorganization (22). Resting-state fMRI combined with direct electrical stimulation (DES) during awake surgery showed that brain areas affected by electrical stimulation during surgery are directly linked to areas responsible for cognitive functions, identified preoperative on fMRI. This combined approach reduces the restriction of each method and highlight the importance of functional mapping (23). MRIs were used for creating a probabilistic map that provides insights regarding the tumor behavior dependent on the location and the likelihood of a tumor to appear at some location (24).

CONCLUSIONS

From the first cytoarchitectonic maps of the 20th century until the large-scale functional MRI studies of the present times, brain mapping has encountered major changes in data acquiring and processing. As mentioned in this article, each of the investigation came with different pros and cons.

Resting-state connectivity which has been tested initially in predefined areas, currently is used for boundary mapping, allowing whole brain parcellation, control testing and identification of areas subdivisions. Graph theory integrates brain activity as a complementary tool for boundary mapping. Adaptation to the classical methods lead to more flexible thresholds for defining areas boundaries. While rfMRI and tfMRI demonstrate brain parcellation based on functional brain activation, DTI elucidates the actual relation between brain areas based on the underlying white matter structure.

Currently, by combining all of these methods into a multimodal approach, scientist have achieved the past century-objective obtaining an unprecedented representation of at least 180 brain areas per hemisphere. In this review, the authors aimed to provide an actual status of the brain mapping methods that would serve other scientists to understand better the basic concepts of this field.

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EDITORIAL

TRADITIONAL CARDIOVASCULAR RISK FACTORS AND CANCER - COINCIDENCE OR CORRELATION?

Cardiovascular and neoplastic pathologies are two entities that cause a huge "burden" for the public health system. It is estimated that there are currently 15 million people suffering from cardiovascular disease and 14 million patients with a history of neoplastic disease, with risk factors and common biological links underlying the pathophysiological mechanisms of both entities (1).

In recent decades, there has been a significant improvement in the survival rate of certain cancers and a steady increase in the number of survivors diagnosed with cancer. Cardiovascular complications encountered in these patients are common, having a profound impact on both morbidity and mortality, so that their identification and management have become important elements in the general care of cancer patients (1, 2). There has also been an overlap in the prevalence of cancer and cardiovascular diseases as well as the development of cancer therapies in many elderly patients, with a higher load of comorbidities, especially of a cardiovascular nature (3, 4). For this reason, the approach of the neoplastic patient as a whole requires a different level of complexity. Heart disease and cancer, although apparently two distinct conditions, share a number of common elements, including risk factors, genetic, metabolic and inflammatory components, as well as common prevention strategies. Together, they are the most common causes of death in the United States (5). In recent years, the synergistic link between cardiovascular diseases (CVD) and cancer has become increasingly evident through the existence of common risk factors, most of which are modifiable and traditional, such as smoking, obesity, diabetes, type of diet, sedentary lifestyle and alcohol. There are also studies that have highlighted potential common biological mechanisms (inflammation, oxidative stress and an increased release of oxygen free radicals, hormonal factors, cytokine release, growth factors, metabolic reactions), as well as the influence of active therapies for cancer on risk factors (6, 7).

It is admitted that even cardiovascular disease can itself predispose to the occurrence of some forms of cancer (e. g. heart failure) through much more complex mechanisms than the mere presence of traditional risk factors.

However, with the progress in oncology, the life expectancy of these patients increases with a simultaneous increase in the cardiovascular risk due to hypertension, dyslipidemia, metabolic syndrome, acute coronary syndromes, pathologies generally associated with old age. Thus, compared to the general population, a cancer survivor has a 7 times higher risk of CVD mortality, a 15 times higher risk of heart failure and a 10 times higher rate of CVD (6,7).

Smoking

It is one of the major cardiovascular risk factors, whose presence increases the risk of occurrence of CVD (especially ischemic) 2 to 4 times, the risk of stroke and peripheral arterial disease, but at the same time increases the risk of bronchopulmo-

nary cancer 25 times (1). To these is added the existence of a causal relationship with at least 11 other types of cancer (breast, stomach, pancreas, bladder) (1-5). Moreover, smoking cessation is associated with a 50% reduction in coronary heart disease compared to those who still smoke after only 1 year and a 50% reduction in the risk of cancer of the mouth, throat, esophagus and bladder after 5 years. After 10 years of smoking cessation, bronchopulmonary cancer mortality decreases by 50% compared to those who continue to smoke. The percentage of deaths caused by both cancer and coronary heart disease, which can be attributed to smoking and which has been reported in various studies in the United States, is about 30% (1, 6, 8). Beyond the cardiovascular and neoplastic risk, smoking is associated with a prothrombotic status that amplifies the risk of thromboembolic events and, on the other hand, obscures the prognosis of neoplasia.

Arterial Hypertension

Hypertension and dyslipidemia are two major risk factors for CVD; however, the results are somewhat divergent in their involvement as independent risk factors for cancer. Among patients with neoplasms, hypertension and dyslipidemia are common. Under the conditions of some common risk factors, the two pathologies show an increased co-prevalence, which makes it difficult to identify the sequence of their occurrence (1). A recent meta-analysis that included over 140 individual studies confirmed the existence of a causal relationship between hypertension and an increased risk for various cancers, including: kidney, colorectal, breast, endometrial, liver and esophageal cancer (9). The reverse relationship is also known. Cervical radiotherapy for certain types of cancer, through the injury of carotid baroreceptors and the secondary increase in sympathetic activity may be associated with hypertension. At the same time, chest radiotherapy for various cancers is associated with an increased risk of coronary heart disease; this risk increased fourfold when a history of hypertension was included (10).

An increase in the prevalence of hypertension has been reported in relation to the use of chemotherapy and adjuvant medication in cancer. Angiogenesis inhibitors (bevacizumab, sorafenib and sunitinib), corticosteroids, erythropoietin as well as non-steroidal anti-inflammatory medication are associated with an increase in blood pressure (1, 9, 10). Similarly, adult patients with autologous hematopoietic stem cell transplants with a history of hypertension who received high-dose anthracycline therapy (≥ 250 mg/m^2) had a 35-fold increased risk of heart failure (12).

Increasing the life expectancy of neoplastic patients frequently implies an increase in the incidence of hypertension, which draws attention to the importance of early detection and treatment, both as a risk factor for cancer and as an adverse effect of the associated treatments.

Dyslipidemia

The important role of cholesterol and triglycerides in maintaining the structural and functional integrity of cell membranes is known, while participating in the development, cell multiplication and stability of DNA structures. Thus, the role of dyslipidemia in cancer was studied, either as a predisposing factor for neoplasia or as a side effect. A significant association was identified between elevated LDL-cholesterol (LDL-c) levels and an increased risk for prostate cancer, with elevated HDL-cholesterol (HDL-c) values proving

to be a protective effect (13). The same lipid profile was correlated with the risk of breast cancer (14).

LDL-c is the fraction most prone to oxidation under pathological conditions, with oxidative stress resulting in increased levels of lipid peroxidation products that can alter the structure of cellular DNA. At the same time, HDL-c is a powerful antioxidant by counterbalancing the oxidative harmful effect of LDL-c, thus potentially becoming an anticarcinogenic. At the same time, it has been shown that elevated serum triglycerides are associated with decreased ligands for sex hormones, resulting in increased serum levels of free estradiol involved in breast cancer (15).

On the other hand, cases of cancer associated with hypolipidemia have also been reported, either by the secondary impairment of the lipid metabolism, or by the direct hypolipidemic effect of tumor cells, or by the treatments with antioxidant vitamins used in cancer (16). This inverse relationship between the lipid profile and neoplastic risk has been proven especially for head and neck cancers, with mortality being significantly higher in patients with low cholesterol (17). Also, cancers in the gynecological sphere and especially the ovarian one is mentioned as being associated with decreases in lipid values (18). The importance of these data comes from the usefulness of the lipid profile as an early diagnostic marker in neoplasms. In the relationship between dyslipidemia and neoplasms, it is worth mentioning the impact of cancer therapy on the lipid profile, with the concomitant influence on the cardiovascular risk. Thus, antiandrogen therapy in prostate cancer has been shown to be associated with significant increases in cholesterol and triglycerides (18,19). Also, some medications (bexarotene, asparaginase, corticosteroids) used in hematological neoplasms can significantly alter the lipid profile, these patients even needing plasmapheresis to avoid severe complications (acute pancreatitis). Hormone therapy such as tamoxifen may have a mixed effect on the lipidogram, with a decrease in total cholesterol and LDL-c levels, but with an increase in the values of triglycerides, which may contribute to more frequent thromboembolic events (20).

Statins are the basic treatment in dyslipidemia, with a proven role in the primary and secondary prevention of cardiovascular diseases (acute coronary syndromes, strokes, thromboembolic events). At the same time, however, due to their anti-inflammatory properties, induction of apoptosis, inhibition of tumor growth, angiogenesis and metastases, these drugs seem to have a synergistic action with chemotherapy in improving the prognosis in cancer (7). Statins have been reported to have a role in preventing breast cancer recurrence due in part to the reduced levels of an estrogen receptor ligand (27-hydroxycholesterol), which has been shown to regulate tumor growth dependent on this type of receptor (21). At the same time, in a study of over 12,000 participants, statins were shown to be useful in reducing mortality in patients with bronchopulmonary cancer (22). Moreover, recent studies are focused on the effects of statins in reducing the incidence of cancer. The results in this direction are contradictory. However, large meta-analysis studies have not yet shown statistically significant results regarding the role of statins in the prevention of neoplasms (23).

The study of the lipidogram in neoplastic patients also acquires a prognostic role. A 2017 meta-analysis of the prognostic significance of lipids in cancer identi-

fies elevated HDL-c and total cholesterol as positive prognostic markers for the survival of these patients (24). In the study of breast cancer, elevated LDL-c values correlated with disease progression, being a negative prognostic factor (25, 26). The negative prognostic value of LDL-c has been demonstrated for small cell lung cancer, prostate cancer, colorectal cancer and esophageal cancer (27-30). Triglycerides have also been shown to be an independent prognostic factor in the progression of hepatocellular carcinoma (31).

Diabetes mellitus

Diabetes is another well-established risk factor for CVD. In a meta-analysis that included 820,900 people from 97 prospective studies, diabetes was associated with an increase in cardiovascular mortality, but also in that caused by cancer, especially liver, pancreatic, ovarian, colorectal, pulmonary, bladder and breast (1, 32). Although individuals with diabetes appear to be at an increased risk of developing more than one type of cancer, it is unclear whether this link is causal or through common risk factors, including obesity, via insulin resistance as an intermediate pathophysiological mechanism. The strongest epidemiological data for a direct causal link between diabetes and cancer have been observed in breast cancer, intrahepatic cholangiocarcinoma, colorectal cancer and endometrial cancer (33). An increased prevalence of type 2 diabetes has been found, especially in patients with colonic polyposis located in the proximal colon and, especially in male patients (34).

Obesity

Obesity is an independent risk factor for CVD, cancer and diabetes, and is usually associated with other risk factors including high blood pressure and dyslipidemia (7). In 2012, increased body mass index (BMI) values were associated with approximately 500,000 new cancer cases worldwide, becoming the second risk factor after smoking in most Western populations (1, 35). Obesity has been associated with an increased prevalence of digestive (esophageal, colorectal, biliary tract, pancreatic), hormonal (breast, endometrial), renal and multiple myeloma neoplasms, data confirmed by the study of 204 meta-analyzes (1,36). The pathophysiological relationship between cancer and obesity is mainly explained by several mechanisms: insulin resistance associated with obesity, increased bioavailability of insulin-like growth factor (IGF-1) and elevated levels of endogenous estrogen (1, 37). Overweight and obesity at the time of cancer diagnosis appear to increase the risk of cancer reoccurrence and reduce the survival duration (38). An important thing to remember is the association of obesity with a prothrombotic status which in turn is responsible for the occurrence of thromboembolic events (especially pulmonary thromboembolism) in the postoperative period. The predisposition for a status of hypercoagulability in the postoperative period is enhanced in the neoplastic patient who also associates obesity.

Sedentarism

Physical activity (especially isotonic exercise) reduces the risk of CVD, all-cause mortality and certain types of cancer, including colon, breast and endometrial cancers (39). In a recent analysis that included 1.44 million participants, increased physical activity and smoking cessation were associated with a significantly lower risk of developing several types of cancer, including leukemia, esophageal, lung, kid-

ney, myeloma, rectum and bladder (39). Physical activity after the diagnosis of cancer has also been associated with a lower recurrence of cancer (40).

Malnutrition

Unhealthy eating habits have a significant impact on both cardiovascular risk factors and the appearance of various forms of cancer (especially digestive) (1, 41). The PREDIMED study (*Prevención con Dieta Mediterránea*) was a randomized controlled study that confirmed the observations of previous studies by demonstrating a 30% reduction in the risk of stroke, myocardial infarction and death from cardiovascular causes in those patients undergoing a Mediterranean diet, rich in extra virgin olive oil or walnuts (1, 42). A reduction in the incidence of breast cancer has also been observed in patients receiving a Mediterranean diet (42). Meta-analyzes of prospective cohort studies have repeatedly shown that consumption of red meat and processed meat increases the risk of cancers of the digestive tract, especially the colorectal tract, by 20-30% (43). A 2010 study found that inadequate eating habits were the leading cause of both mortality and decreased quality of life in patients with neoplasms (44).

Alcohol

The relationship between alcohol consumption and CVD is somewhat controversial due to a J-shaped "historical association" in observational studies in which light alcohol consumption appears to have a protective role on the occurrence of ischemic heart disease (especially red wine by vasodilation and growth of HDL-cholesterol levels) (1). However, the overall impact of alcohol consumption on CVD is still detrimental, with around 780,000 deaths from CVD due to global alcohol consumption (1, 45). The main harmful effects of alcohol consumption are more clearly defined as a causal relationship with at least seven types of cancer: oropharynx, larynx, esophagus, liver, colon, rectum and pre- and post-menopausal breast cancer. The combined consumption of tobacco and alcohol has a marked multiplier effect on cancers of the oropharynx, larynx and esophagus (45). Cardiac damage, even subclinical, due to alcohol may become manifest at the time of chemotherapy, accentuating its cardiotoxic effects (tachycardia, ventricular arrhythmias). Also, in the presence of more severe heart damage due to alcohol, such as dilated cardiomyopathy, an initially low left ventricular ejection fraction may make it difficult or even contraindicate the administration of chemotherapy.

The risk of cardiovascular disease in cancer survivors

Due to the increase in cancer survival, as a result of the emergence of modern therapies, but also their cardiotoxic potential, CVD occurs with increasing frequency among patients with neoplasms. Traditional individual cardiovascular risk factors, such as high blood pressure in a cancer survivor, appear to pose a higher risk than high blood pressure in a person with no history of cancer. In addition, each additional cardiovascular risk factor encountered in a cancer survivor appears to increase the risk of CVD to a greater extent than a simple additive effect (11).

Cancer survivors are prone to have an increased risk of CV morbidity and mortality in all age groups and have a higher percentage of traditional CV risk factors compared to age-appropriate control groups (47, 48). The Ontario Cancer Registry

evaluated 98,999 women with early-stage breast cancer and found that the risk of death at 10 years was higher in people with a history of CVD (1, 47, 48).

In conclusion, traditional individual cardiovascular risk factors in a cancer survivor amplify the overall risk. Cancer survivors are more likely to have an increased risk of CV morbidity and mortality in all age groups and have a higher percentage of traditional CV risk factors compared to the age-appropriate control groups.

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EDITORIAL

OLD AND NEW CHALLENGES FOR THE SCIENCE OF NUTRITION

At a quick search in a dictionary, one can find that nutrition is "the biochemical and physiological process by which an organism uses food to sustain its life; it includes ingestion, absorption, assimilation, biosynthesis, catabolism and excretion" (*Encyclopedia Britannica*) (1). However, the term nutrition also means "the science that studies the physiological process of nutrition (primarily human nutrition), interpreting the nutrients and other substances in food in relation to maintenance, growth, reproduction, health and disease of an organism" (2).

Is the science of nutrition the result and/or a component of a multidisciplinary, interdisciplinary or transdisciplinary system?

Today, teamwork is required in any medical, educational, social, or political field. This means common or compatible objectives, working methods and evaluation methods that ultimately prove their benefits and effectiveness. In research, multiple disciplinary approaches on the same continuum are encouraged to create hypotheses, develop studies that provide accurate perspectives on real-life issues, and possibly consensus. Many epistemologically distant disciplines can together provide answers to a complex issue. The terms interdisciplinary, multidisciplinary and trans-disciplinary, although they seem interchangeable terms, have their own definitions.

Multidisciplinary relies on knowledge from different disciplines, which remain within their limits. Professionals in these fields work independently on a common research issue from the perspective of their own discipline, each offering their own point of view. The conclusions are *additive* to each other. Consulting *Oxforddictionaries.com* we find that the adjective multidisciplinary describes "combining or involving several academic disciplines or professional specializations in approach to a topic or problem".

Interdisciplinarity analyzes, synthesizes, combines and harmonizes the links between disciplines in a coordinated and coherent whole. The conclusions are *interactive*, each discipline being able to affect the research results of the other, creating a new level of integration. According to *Oxforddictionaries.com* the adjective interdisciplinary describes "of or relating to more than one branch of knowledge".

Transdisciplinary *holistically* integrates the natural, social and health sciences in a humanistic context that transcends their traditional boundaries. The result can be a kind of xenogenesis in which the new creation of the integration of disciplines can be something completely new.

From these interactions new domains can crystallize. We can refer here to biochemistry, nanotechnologies, etc. The science of nutrition itself has needed, and still benefits from, the contribution of physiology, biophysics, genetics, and other basic sciences to understand the mechanisms, metabolic pathways, and their responses to malnutrition or overeating (3). The term "nutrition" encompasses biological as well as sociological aspects of how the human body accesses the substrates and cofactors necessary for normal conception, growth, development and aging.

In recent years, the term "convergence research" has also been introduced, which is a new term that integrates fields that have historically been distinct scientific and technological disciplines. "Multidisciplinary

research is the foundation of interdisciplinary and convergence research" (4).

Nutrition as an interdisciplinary field could also be included as an example of translational medicine. "Translational medical science, preclinical research, evidence-based research, or disease-targeted research, [is the] area of research that aims to improve human health and longevity by determining the relevance to human disease of novel discoveries in the biological sciences" (5).

Nutrition includes the two areas of translation, namely applying the findings of laboratory studies to the development of human studies and contributing to research aimed at improving the adoption of best practices in the community (guidelines) (6).

Nutrition has been called multi-, inter- or trans-disciplinary in numerous scientific articles and even in documents disseminated in the social media. It must be seen as a scientific culture of great complexity that faces problems that transcend the paradigm of disciplines (7).

As a science, nutrition is "young" (8). The first visible advances were seen at the end of the 18th century, when the laboratory methods of the "chemistry revolution" were applied to build new knowledge. For example, Antoine Lavoisier and Pierre-Simon Laplace showed that the slow combustion of organic compounds in tissues produced heat. The first controlled interventional clinical study was performed in 1746 by James Lind, on sailors suffering from scurvy. Twelve sailors, with a similar severity of the disease, were divided into pairs and, for 2 weeks, each pair was offered one of the recommended treatments at that time. The sailors who received lemons and oranges were almost healed after only 6 days. However, the cure for scurvy was not in fact found for many years, because it was not possible to store either lemons/oranges or their juice for such a long time on ships (to be given as a preventive food) and it was wrongly assumed that citric acid was the cure (9).

It was the era when attempts were made to understand the causes/ways of transmitting of diseases and to comprehend the ability to predict, control and manage life. The pursue for detailed knowledge of macronutrients and energy balance began. It was soon showed that it was not enough to meet the caloric requirements without considering the sources. The experiment of François Magendie at the beginning of the 19th century remains famous: he fed dogs with a single food considered "preeminently nutritious" which covered the energy needs. Whether it was sugar, olive oil or butter, the animals invariably died after a month. His conclusion, still valid today, was that "diversity and multiplicity of foods is an important rule of hygiene; which is, moreover, indicated to us by our instincts" (10).

Later, remedies for other diseases with a single nutrient deficiency such as pellagra, scurvy, beriberi, goiter, xerophthalmia, rickets or nutritional anemia were sought. In the first half of the 20th century, the role of vitamins and minerals was discovered and described, and the notion of *Recommended Daily Intake* (RDI) was established. The name *vitamin* is attributed to Casimir Funk, who in 1913 found that some foods contained a "vital amine" (11). Nutritional strategies were established to prevent/combat micronutrient deficiencies. A whole industry of synthetic vitamins, multivitamins, fortified foods, and food supplements was developed. Their marketing and use to protect against deficiency were successful, especially in rich countries. Unfortunately, this led to a reductionist model of scientific research based on addressing unique/singular nutrients, on identifying and isolating the element involved, on evaluating its physiological effect and quantifying the optimal level of intake to prevent

the disease. Nutritional guidelines were similarly focused on the benefits of unique nutrients.

After World War II, the gap between high-income and poor/developing countries widened. If in the former chronic diseases of the plethora began to appear, in the latter malnutrition and diseases of deficit remained predominant. For these countries, projects continued (scientifically unsustainable today) such as providing caloric foods and covering the "protein gap". Starting from the legitimate desire to produce more basic, energy-dense and affordable foods, some agricultural crops (wheat, rice and corn), farms (cattle) and industries (fortification of foods or even milk formulas) were subsidized (12). A nutritional imbalance was thus produced, with the consumption of mainly cheap food, but the global "hunger" was still not possible to combat. Many nutritional problems were still unresolved with important clinical implications. Poor nutrition has led to poor health, and this has led to poor nutrition.

If the prevalence of diseases of deficit has decreased, it has been observed that excess calories and dietary imbalance have led to increased cardiometabolic risk and the development of atherosclerotic pathology, type 2 diabetes and cancer. The growing burden of non-communicable nutrition-related diseases has begun to worry, which has led to new research directions such as dietary fats and sugar. Unfortunately, the current which concluded that protective diets should be *low-fat* has won and it has taken 3-4 decades to prove the futility and even danger of such recommendations (13). This mistake also stemmed from the reductionist view and the desire to find a unique nutrient, guilty of illness. Subsequently, nutritional study models were sought to be adapted to the research of chronic non-communicable diseases. Research into the complex, synergistic actions between nutrients and other food constituents has led to the study of food patterns. For this, modern statistical methods have been used (principal component analysis, cluster analysis, reduced rank regression). It should also be noted that many statistical tools are experiments from the field of nutrition (e.g., Fisher's exact test, known as "lady tasting tea" or student test – Gosset's from the Guinness Brewery). The study of food patterns has shown strong associations between prudent diets and health and between "Western" diets and atherogenesis. The study of factors associated with food patterns with risk can provide arguments for decision makers in medicine/society to implement successful nutritional interventions.

The number of population studies have multiplied in search for the links between diet and disease, the interactions of food consumption with the human body. Even a new branch of epidemiology has been developed -nutritional epidemiology- which seeks a thorough understanding of the science of nutrition in order to identify dietary risk factors for disease and provide specialists with reasoned targets for preventive measures (14).

Nutritional epidemiology faces many challenges and problems, some of which are methodological (study design, data collection and processing) and interpretation (statistical difficulties/lack of validated statistical methods, the risk of creating false cause-effect relationships).

Randomized nutritional intervention studies are not as common in research because diet is closely linked to multiple aspects of life, exposure time is long, there are practical and ethical obstacles in selecting and assigning subjects to specific diets, and there are many confounding factors in interpreting the relationship between eating habits, health and lifestyle (15).

Simultaneously with the multiplication and scale of nutritional studies (especially observational), their reporting to the public increased, even before the verification of the rigor of some scientific conclusions.

Thus, a binary characterization of foods into "good" and "bad" with adverse implications has been created, instead of considering the dietary needs of populations of different cultures. This leads to misconceptions, myths and fad diets.

The burden of nutritional diseases is on the shoulders of health systems, populations and governments. Diseases and suffering caused by a combination of declining physical activity and unbalanced, low-nutritional density diets are prevalent worldwide. The double burden of malnutrition refers to the coexistence of malnutrition and overweight/obesity, but in fact there are three dimensions of malnutrition: malnutrition, overnutrition and micronutrient deficiencies, with individual, family, national or global impact (16). Mapping the current state of malnutrition, which is complex and multidimensional, must be correlated with contextual determinants such as health and education systems or socio-economic status in order to have any chance of reducing the severity and duration of this burden. The lives of malnourished children, whether with stature hypotrophy ("stunting"), weight hypotrophy ("wasting") or micronutrient deficiencies, are characterized by a reduced ability to resist disease, to study and to progress throughout life. All forms of malnutrition throughout life or for generations impose increased metabolic burdens on limited homeostatic capacity (16).

Non-communicable diet-related chronic diseases have become endemic, being identified in the *Global Burden of Disease 2017* study as being responsible for 73% of the world's deaths (17). The double burden of malnutrition, as well as the spread of non-communicable chronic diseases, can be explained by the nutritional transition (but also the demographic and epidemiological transitions) related to biology and the obesogenic environment (food, economic and socio-cultural) (18). Consumption of ultra-processed, energy-dense, hyper-palatable foods and sugary drinks are among the causes of weight gain (19). The approach should be twofold: historical and biological, and the solution should be political and through structured programs.

Most countries face multiple disease burdens: the persistence of infectious diseases, malnutrition and maternal mortality, the challenges of chronic non-communicable diseases, and issues directly related to globalization, such as pandemics and the consequences of climate change on health (20). Especially on low- and middle-income countries, there is a threefold burden of: communicable diseases, non-communicable diseases and road accidents. Three other pandemics - "obesity, undernutrition, and climate change - represent The Global Syndemic that affects most people in every country and region worldwide. They constitute a syndemic, or synergy of epidemics, because they co-occur in time and place, interact with each other to produce complex sequelae, and share common underlying societal drivers" (21).

The science of nutrition must be concerned with promoting and maintaining the nutritional health of individuals, families, and communities. The mission of nutrition is research, education, and contribution / involvement in supporting the health and food security of the population, as fundamental human rights. It involves the acquisition and synthesis of knowledge in the field of nutrition but also its dissemination to other professionals and to the population.

Educational strategies for healthy eating and nutrition promote the creation, accumu-

lation, and dissemination of the values of nutrition science, the initiation of evidence-based programs and policies to encourage society's organized efforts to use resources wisely, to enable sustainable access to nutritious and safe food (22). Unfortunately, today we have to fight with a lack of confidence in politics, in our economic way of life and even in science and medicine (23). There are also optimistic messages. Two of the United Nations' 17 Sustainable Development Goals for 2030 are zero hunger and good health, and others address extremely relevant nutrition indicators: improving sanitation and access to clean water, reducing poverty, inequity, and inequality; increasing access to education, productive work, and health services (24).

In a world that is undergoing radical transformations, new challenges and opportunities appear for the science of nutrition. The responsibility for making efforts lies not only with the small group of nutrition professionals, but with society as a whole. Food remains the strongest lever for optimizing health and environmental sustainability. Creating and managing a complex program for achieving/maintaining health means using all available evidence in developing common local, regional, and global strategies and partnerships for implementation, not only between scientists and specialized agencies but also between countries with different levels of development.

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NEWS

VITAMIN D AND RESPIRATORY VIRAL INFECTIONS INCLUDING COVID-19

Vitamin D helps in decreasing the pro-inflammatory cytokines in the lungs. Due to its immunomodulatory function, it increases the anti-inflammatory and antiviral responses of the respiratory epithelial cells during an infection. Now more than ever, treating patients with Vitamin D is vital, mainly because of its various beneficial effects (innate immunity boosts) against viral diseases and potential role in decreasing the mortality associated with the cytokine storm in COVID-19 patients. Giving Vitamin D supplements in the autumn and winter months to high risk patients could add an extra layer of protection against the development of respiratory tract infections, especially during the COVID-19 pandemic. Even though the evidence suggests that vitamin D has anti-inflammatory and antiviral properties, further investigation is needed (such as randomized double-blinded controlled trials) in order to better understand the relation between Vitamin D and COVID-19. (Balla M, Merugu GP, Konala VM, Sangani V, Kondakindi H, Pokal M et al. Back to basics: review on vitamin D and respiratory viral infections including COVID-19. *J Community Hosp Intern Med Perspect* 2020;10(6):529-36).

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EDITORIAL

METABOLIC EVALUATION OF THE KIDNEY STONE PATIENT - THE KEY FOR PERSONALISED METAPHYLAXIS

Kidney stones represent one of the "oldest" diseases and have affected normal people but also great historical characters starting from Ramses II to George W. Bush or Lionel Messi. Despite progress in understanding the pathophysiology of kidney stones, we are witnessing an increase in the incidence and prevalence worldwide. The spread of urinary stones disease is not homogeneous on the planet, its epidemiology is influenced, among other things, by external factors such as geo-climatic conditions. In the United States, the prevalence between 1976-1980 was 3.8%, it increased to 5.7% between 1988-1994, while in late 2010 already reached 8.8% (1). Meanwhile in Europe, given that there are climatic differences, but also in terms of eating habits between different areas, it is expected that the prevalence of urinary stones will differ between countries or regions. In a relatively hot region like Andalusia, according to Arias Vega *et al*., in people aged 40 to 65 years old, the prevalence of lithiasis in 2016 was 16.4%, while in Europe's northern regions like Iceland the incidence between 2005-2008 was 138/100.000 according to Edvardsson *et al.* (2, 3).

However, climatic conditions alone cannot be blamed for increasing the number of lithiasis patients in the Eurasian area. According to Novikov *et al.,* after the collapse of the Soviet Union, the transition to a Western-style diet rich in sodium and proteins, as well as access to modern diagnostic methods, led to a dramatic increase in the incidence and prevalence of urinary stones in the current Russian Federation (4). The authors note that from 1980 to 2008 the incidence of urolithiasis increased almost 12 times from an average of 53/100,000 inhabitants (even 17 / 100,000 inhabitants in the Moscow area) to 609.3/100,000 inhabitants. However, the distribution of cases is quite heterogeneous. The highest increase in incidence was in the Russian far East between 2002 and 2008 from 529.9 to 714.7/100,000 inhabitants and the lowest in 2002 in the Ural Federal District (Okrug), respectively 360.6/100,000 inhabitants. The increase in the frequency of lithiasis also affected the former Soviet republics, from an average of 53/100,000 inhabitants in the USSR in 1980, in 2005 the incidence was 103/10,000 inhabitants in Belarus, 256/10,000 inhabitants in Tajikistan, and 658/10,000 inhabitants in Ukraine (4). We can say that this is also true in our country because in the last 30 years we have witnessed a radical change in the lifestyle of young people in Romania. Thus, the diet tends to copy the western one, rich in animal protein, salt and low in vegetable fiber. Fast food restaurants have seen an explosive expansion in our country. These foods are "seasoned" in abundance with carbonated beverages. If we consider the degree of the sedentary lifestyle of the young population, we will be able to draw a favorable picture of the appearance of urinary stones. The appearance of nephrolithi-

asis is not only the result of the presence of certain favorable factors (salt, animal proteins, refined carbohydrates, etc.) in these foods but also indirectly through changes in the body such as the development of diabetes and in some cases metabolic syndrome.

Unfortunately, one of the characteristics of urinary stones that makes it necessary especially in young patients for strategies of etiological evaluation, prophylaxis and metaphylaxis is the potential for recurrence. The relapse rate is variable depending on the geographical area, lifestyle, eating habits, etc. In a meta-analysis conducted in the late 1980s by Uribarri *et al.,* a "cumulative natural recurrence rate" of 14% at 1 year, 35% at 5 years, and 52% at 10 years were found (5). 25 years later, Rule *et al*. Found a recurrence rate in Olmsted County, Minnesota, the USA of 11% at 2 years, 20% at 5 years, 31% at 10 years, and 39% at 15 years (6). According to Osther, lithiasis has a high potential for recurrence among Europeans. According to the author, in general, when surveyed, 60% of German or Danish patients will report a single lithiasis episode, 18% two, 10% three, 2% four, and the rest more than four episodes. The chemical composition of the stones seems to play a very important role in recurrence (7). Thus, Levi *et al*., in a group of approximately 450 patients, found a 3-year recurrence rate of 24%, but patients who had uric acid or struvite stones were 2.4 times or 1.9 times higher show greater recurrence than those that formed calcium oxalate stones (8). Fortunately, knowing the potential for reversal by implementing prophylactic measures, according to Borghi *et al*., the recurrence rate can be reduced by up to 50% (9).

The purpose of clinical and metabolic evaluation of lithiasis patients is to prevent lithiasis recurrence, to stop the growth of existing stones and to reduce the need for surgery. Although it is a disease that mainly affects relatively young people, with an increasing incidence and prevalence all over the world, the investigation of the etiology, from the point of view of some authors, still has a controversial utility.

In terms of cost-effectiveness, Chandhoke suggested that in patients with the first episode of lithiasis, metabolic assessment is not warranted (10). The author indicated that only at a rate of 0.3-4 lithiasis episodes/year metabolic assessment and treatment is equivalent to the management of lithiasis recurrence. On the other hand, according to Chapple *et al.,* In patients with a first lithiasis episode, the recurrence rate after conservative treatment is 0.07 stones/patient/year. Because conservative treatment is at least as expensive for those with lithiasis recurrence, first-time patients should not receive special treatment (11). Therefore, it could be argued that while 24-hour urine analysis may not be considered a practical and sometimes uninformative tool, according to Hill *et al.,* It is a personalized impulse of the diagnostic pathway that will determine consideration of subsequent genetic investigations (12). The authors cite the situation where a 24-hour urine test or a cystine urine test (cystine/creatinine ratio) is a valid screening test for lithic patients to identify cystinuria. Moreover, the authors consider that even the analysis of the composition of the calculation provides only one temporary reference and can be misleading. For example, it has been found that some patients with cystinuria form calcium oxalate stones before the formation/identific-

ation of cysteine.

Most authors agree that in the case of patients on the first presentation but who may be included in a high-risk group, metabolic assessment is necessary. These high-risk groups include hereditary history, intestinal disorders, and chronic diarrhea, urinary tract infections, gout, osteoporosis, or bone fractures. To this could be added patients who are highly motivated to change their eating and health habits, those with single kidneys, those with multiple stones, or subjects with multiple and severe comorbidities to whom possible urological surgery would be at risk. grown. Although some authors argue that patients with uric acid, cystine, and struvite stones require metabolic evaluation, according to Chapple *et al*., These categories of patients were used to identify those who did not need metabolic evaluation (11). A special category of patients for whom the metabolic assessment is warranted is those whose lithiasis is difficult to treat, those with single kidney disease, and those with nephrocalcinosis. According to Chapple *et al.,* Children with urinary stones should be evaluated metabolically (11). Adolescents with lithiasis have the same risk of metabolic disorders as adults. The risk of ureterohydronephrosis, urinary tract infections, and an increased need for surgery further support metabolic assessment in children and adolescents.

The European Urology Association (EAU) guidelines recommend that after stone passage, every patient should be assigned to a low- or high-risk group for stone formation. To classify the patient, we need to perform the stone analysis through X-ray diffraction or infrared spectroscopy and metabolism-related laboratory analysis like 24-hour urinalysis (13). However, for some patients, 24-hour urine collection may not be an easy task, and in the end, compliance may be low. We, therefore, wonder if patients really want to be metabolically evaluated. A high level of interest for first-time patients is likely due to the severity of pain associated with renal colic and significant time lost from work or other activities. Once the patient's symptoms disappear, he is increasingly likely to lose interest in metabolic assessment. Given these disadvantages, we ask ourselves if one 24-hour urine sample is enough. According to Williams et al. one sample may not be enough, the authors recommend that at least two 24-h urine analyses for initial evaluation of stone patients. One collection should be made on a workday and the other should be made during a non-workday (14).

Once it is established that the patient needs a 24-hour urine test, the question naturally arises: when is the best time to affect this investigation? At the first presentation, the acute complications of lithiasis should be treated first and the symptoms that caused the patient to see a doctor should be alleviated. After clearing the pyeloureteral system, the etiological treatment will be applied: expulsion or surgical treatment. Once these goals are met, Chapple *et al.* Recommend that metabolic evaluation be performed at least one month after removal of the stone to allow the body to return to its original status (11). By taking blood or urine during the acute lithiasis episode (obstruction, infection, renal colic) irrelevant information is obtained. However, this opinion is not shared by Chandhoke, who considers that the patient doesn't need to be stone-free at the time of evaluation, the only precondition is

the absence of macroscopic hematuria and renal obstruction (15). In practical terms, 2-4 weeks wait after ESWL or another endourological procedure is usually sufficient before collecting urine specimens for metabolic analysis.

Evaluation of patients with an isolated lithiasis episode is still a matter of debate. Because they noted low morbidity associated with the first episode of lithiasis and a relatively high incidence of side effects of drug metaphylaxis, some authors do not recommend evaluating this category of patients. However, it has been shown that patients with an isolated lithiasis episode may have the same metabolic disorders as those with recurrent lithiasis, so they have the same risk of recurrence as those with multiple histories of lithiasis.

In addition to a detailed history, patients presenting for the first time with urinary stones should have a urinalysis and urine culture, ionogram, calcium, uricemia, to detect renal tubular acidosis, hyperparathyroidism, or gout, and 24-hour urine analysis. They should also be evaluated for imaging for outstanding lithiasis and possibly urinary tract malformations.

The chemical composition of stones has a crucial role in therapeutic conduct. In this way, patients who are candidates for lytic treatment and those who will undergo surgery can be selected. It can also guide treatment for metaphylaxis of lithiasis recurrence. There are currently two methods of analysis commonly used for stone analysis, namely X-ray powder diffraction crystallography (XRD) and Fourier transform infrared spectroscopy (FTIR). The classical technique of chemical analysis, the so-called “wet analysis”, is no longer recommended by the EAU due to its poor reliability. These two methods of analysis differentiate the crystalline composition based either on the uniqueness of the crystalline structures or the subtle energetic differences associated with the movement of atoms in chemical bonds found in the various stone components. The first results of the application of FTIR microscopy (mFTIR) to the urinary crystals of calculating stones were published in 1991 by Daudon *et al.* In subsequent research, the same group expanded studies combining FTIR with a pellet of potassium bromide (KBr) instead of FTIR microscopy. Combining the spectral results for urinary sediments and kidney stones obtained by the KBr pellet technique with the results of optical sediment microscopy, the authors found a 97.3% correlation between the type of calculation and the type of crystals in urine sediments. These studies have been restricted because urinary crystals can be investigated by the KBr pellet technique only when a significant number of precipitated crystals (16).

We consider that the most comprehensive and at the same time precise synthesis of the research in the field of the biochemical composition of lithiasis was made by Daudon *et al*.

This classification, which favors an easy communication between biochemists, nutritionists, and urologists, brings into question the following possible etiopathogenic situations (tab. I).

The morphological types of stones in urinary lithiasis are divided into Calcium and Non-calcium. Calcium-type stones are made up of either oxalate or calcium phosphate. Non-calcium urolithiasis is due to accumulations of uric acid, infections, or genetic causes (cystine).

TABLE I

Calcic		Non-calcic		Calcic		Aminoacids
Type I	**Type II**	**Type III**	**Type IV c**	**Type IV d**	**Type IV a**	**Type V**
Calcium oxalate monohydrate	Calcium oxalate dihydrate	Uric acid	Struvite (ammonium magnesium phosphate)	Brushite (calcium hydrogen phosphate dihydrate)	Carbonate apatite (calcium phosphate)	Cystine (L-cystine)
-Hyperoxaluria -Calcium/oxalate low ratio -Sponge kidney -Randall plaques -Urinary stasis	-Hypercalciuria -Calcium / oxalate increased ratio -Randall plaques	Hyperuricosuria -Low urinary PH -Insulin resistance / Type 2 diabetes -Urinary stasis	-Infections related to the production of urea by microorganisms -Presence of Struvite -a sign of an infection with urea splitting bacteria	-Hypercalciuria -Primary hyperthyroidism	-Hypercalciuria -Urinary infections -Sponge kidney -Renal tubular acidosis and Sjogren Syndrome	Cystinuria (autosomal-recessive disease)

The morphological types of stones in urinary lithiasis are divided into Calcium and Non-calcium. Calcium-type stones are made up of either oxalate or calcium phosphate. Non-calcium urolithiasis is due to accumulations of uric acid, infections, or genetic causes (cystine).

Calcium lithiasis

- Type I - calcium oxalate monohydrate occurs as a result of: hyperoxaluria, low calcium/oxalate ratio, sponge kidney, Randall plates formation, urinary stasis;
- Type II - calcium oxalate dihydrate, due to: hypercalciuria, increased calcium/oxalate ratio, Randall plaques formation;
- Type IV d - brushite (dicalcium hydric phosphate) - occurs in: hypercalciuria, primary hyperthyroidism;
- Type IV a - apatite carbonate (calcium phosphate): hypercalciuria, urinary infections, sponge kidney, renal tubular acidosis, Sjogren Syndrome.

Non-calcium lithiasis

- Type III - uric acid - occurs by precipitation of uric acid crystals in the following situations: hyperuricosuria, low urinary pH, insulin resistance / type 2 diabetes, urinary stasis, benign prostatic hyperplasia;
- Type IV c - struvite (ammonia-magnesium phosphate) is due to: urea splitting micro-organisms that cause urinary tract infections, the presence of struvite in the urine - a sign of urease-producing bacteria involved in urinary tract infections;
- Type V - cystine (L-cystine) - caused by autosomal recessive genetic disease that causes cystinuria.

Luckily, the progress made in determining the composition has helped us to be in the position to intervene decisively in cor-

recting important factors that cause the recurrence of kidney stones. We must mention the correction of urinary pH first, knowing that 76.3% of kidney stones are formed in either alkaline or acidic pH (17). This therapy must be integrated with the specific dietary guidelines for each biochemical type.

Uric acid lithiasis, which is formed in acid urinary pH, benefits from chemolysis, alkalinization being performed under control with preparations containing K and Mg citrate (e.g., ALKASOLV® or ALKALIUM®). Daily monitoring of urinary pH causes must lead to an ideal therapeutic pH of 6.8- 7.0.

Phosphate lithiasis that occurs at an alkaline pH may benefit from effective metaphylaxis with drugs containing L-methionine, which manage to acidify the urine (e.g., ACIDURIN®). In this way, careful monitoring of pH is the key to determining the right dose for the intended purpose (ideal treatment should lead to a urinary pH of 5.5-5.8). The medication will accompany an adequate diet and abundant consumption of water or mineral water (recommended for each biochemical type).

In conclusion, the correct metaphylaxis cannot be done without knowing the metabolic disorders that led to the appearance of the stones and without knowing the precise composition of the stones, performed by Fourier transform infrared spectroscopy (FTIR). Unfortunately, in about one to five patients the lithiasis recurrence has a different composition, which requires careful long-term monitoring and, in our opinion, a complex both morphological and spectroscopic analyze for each recurrent stone.

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NEWS

SIGNIFICANCE OF NON-HLA ANTIBODIES IN LOW-RISK KIDNEY TRANSPLANT RECIPENTS

This prospective study included total 94 recipients among 201 consecutive recipients who underwent an ABO-compatible living donor kidney transplant (LDKT). Angiotensin II type 1 receptor antibodies (anti-AT1R) and anti-endothelial cell antibodies (AECA) were measured before, during and after LDKT. The prevalence of acute rejection (AR) within 12 months post-transplant did not differ significantly based on anti-AT1R levels and AECA results. However, when a cut-off value of 11.5 U/mL was applied, it independently predicted a higher risk for AR. AECA (+) recipients had a higher risk for AR or microvascular inflammation (MVI) only during the follow-up period and also showed a rapid increase in creatinine and decrease in eGFR at approximately three months; after this point, they showed persistently higher creatinine and lower eGFR until 20 months. Pre-transplant anti-AT1R level was a significant risk factor for the development of AR, while AECA status was associated with impaired renal function regardless of AR. (Shinae Yu, *et al.*, Pre-Transplant Angiotensin II Type 1 Receptor Antibodies and Anti-Endothelial Cell Antibodies Predict Graft Function and Allograft Rejection in a Low-Risk Kidney Transplantation Setting. *Ann Lab Med* 2020; 40(5): 398-408).

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EDITORIAL

DERMATOVENEROLOGY CLINIC OF "SF. SPIRIDON" HOSPITAL IAȘI AT 140 YEARS

The Dermatovenerology Clinic of the Saint Spiridon Hospital Iași was established in 1849 by G. Cuciureanu as a Section for Syphilis of the hospital, and in 1882 it became a Dermatological Clinic. The first lecture in dermatology was given by Dr. Gh. Iuliano - May 20, 1882, and he taught until 01.02.1883. For almost 20 years, until 1900, the filling of the chair was done by professors from other departments: Prof. V. Negel, Prof. Russ Jr., Prof. Gh. Bogdan, and in the period 1900-1901 this activity of was assigned by Dr. I. Ţăranu, secondary doctor of the department. In 1901, Dr. Gh. Demetriade managed to obtain the position of full professor, following the organized competition.

Prof. Gh. Demetriade (1860-1923)

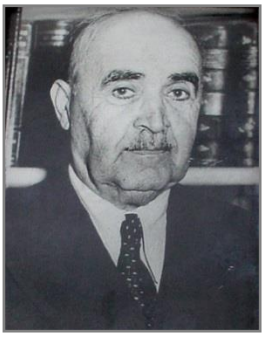
Prof. Gh. Demetriade (1860-1923), a student of Prof. Petrini-Galaţi, trained in Paris and Berlin: he was concerned with teaching, organizing the museum of molds and the library (hires as molds technicians the medical student E. Mironescu, then I. Rotaru). He was concerned with scientific research, introducing ultramicroscopy and serodiagnosis for syphilis. He was in charge of increasing the number of beds of the clinic, consultations and outpatient treatments for syphilis, soft canker, etc.

Prof. Dr. Eugen Mironescu (1883-1939)

From July 1923, Prof. Dr. Eugen Mironescu (1883-1939), occupies the position of head of department by transfer from pharmacology, contributing to the further development of education, renovating and modernizing the clinic, expanding the library and the mold museum (1).

I.I. Mironescu, as he signed as author and writer, is the pseudonym of professor and doctor Eugen I. Mironescu. He studied medicine in Berlin; he was the head of the Dermatology Clinic and a professor at the Faculty of Medicine in Iași; published valuable scientific papers; held numerous popularization conferences in the country; he ran a SPA and built a sanatorium out of his own money; initiated a medical publica-

tion; set up an antiparasitic treatment service and an office for treatment with therapeutic baths and free medication; supported medical students; he was a respected medical educator, gave numerous public lectures on family hygiene and the prevention of venereal diseases; led an intense anti-venereal campaign, writing and printing numerous brochures with explanations and advice for the population. I.I. Mironescu was a doctor on the front lines of the Balkan War; he was involved in stopping epidemics in the Great War - for his activity he was later decorated with high distinctions of the Romanian and French State (2, 3).



Prof. Dr. Gheorghe Năstase (1887-1975)

Since December 1st,1942, Prof. Dr. Gheorghe Năstase (1887-1975) is a full professor, head of department till 1956, consulting professor till 1962. He developed courses in dermato-syphilography, publishes under his editorship the *Treaty on dermato-venereology* unique in the country. He was a good organizer, renowned dermatologist, elite researcher (over 300 scientific papers, participation in congresses, description of new entities, formulation of etiological hypotheses, studies in mycoses, pyoderma, cutaneous tuberculosis, occupational dermatoses, skin cancer) (4, 5).

Prof. Dr. Doc. Virgil Costea (1906-1990)

Prof. Dr. doc. Virgil Costea (1906-1990) goes through the usual stages in his teaching activity, being the head of the discipline since 1961. He collaborates in writing the dermatology course of Prof. Gh. Năstase and writes a dermatology course adapted for pediatrics. He became a specialist in dermato-venereology and then in hygiene, following the courses he took; is in charge of organizing the dermato-venereology clinic and network in Iași and the Iași Region. Introduces for the first time in the world the treatment with corticosteroids in inflammatory trichophytosis, together with griseofulvin, obtaining a prompt reduction of the inflammatory phenomena and a faster healing (6).

Prof. Dr. Doc. Mircea Munteanu (1913-2002)

Prof. Dr. Doc. Mircea Munteanu (1913-2002) went through the usual stages in his teaching activity, becoming a professor since 1967. In his research activity he has over 300 scientific papers (more than 200 published); participates in scientific meetings in the country and abroad, in the organization of the Conference "Gh. Năstase Days" in Iasi. Obtain a certificate of innovator for an antiparasitic, antimicrobial and fungicidal ointment; He is a contributor to the International Contact Dermatosis Research Group and the International Psoriasis Research Group. He contributes to the organization of the Centenary of U.M.Ph. (1979) and the Centenary of the Dermatological Clinic (1982), of symposia to celebrate 100 years since the birth of Prof. Dr. E. I. Mironescu, then 100 years since the birth of Prof. Dr. Gh. Năstase (6).

Prof. Dr. Al. Dobrescu (1923-1999)

Prof. Dr. Al. Dobrescu (1923-1999) went through the usual stages in teaching, becoming a teacher in 1991. He is the head of the discipline and Dermatology Clinic from 1980 to 1994, when he became a consulting professor; He organizes together with Prof. M. Munteanu the Centenary of the Dermatological Clinic, of the Commemoration of the 100th anniversary of the birth of Prof. E. I. Mironescu and Prof. Gh. Năstase; Organizes and participates in the organization of the Conference "Gh. Nastase Days", in Iasi (6).

In his medical activity, he was concerned with organizing the activity in the new headquarters in the Tătărași neighborhood, in order to ensure the specialized assistance in accordance with the requirements of the Ministry of Health and Family.

Prof. Dr. Zenaida Petrescu has been the head of the Discipline and Clinic since 1993. He collaborated in the realization of a "Course in Semiology and Medical Pathology, Endocrinology, Dermatology and Phtiziology", Balan M., Zbranca E., Petrescu Zenaida, Mihăescu T. (1987), the treatise "Human Physiology" (Skin Physiology Chapter) edited by I. Hăulică (1996), the volume "Family Planning" (Chapter Sexually Transmitted Diseases) - Florentina Pricop and collaborators (1997); she organized the Dermatology Conference "Gh. Nastase Days". She obtained a certificate of innovator for a drug used in the treatment of pyoderma; training from a professional point of view under the guidance of the masters of the Clinic (Prof. Dr. Gh. Năstase, Prof. Dr. Doc. V. Costea, Prof. Dr. Doc. M. Munteanu, Prof. Dr. Al. Dobrescu) and advanced training in the service of Prof. J. Civatte, Prof. S. Belaïch, Prof. L. Dubertret, Prof. Camille Frances - Paris.

Currently, the Dermatovenerology Clinic of "Sf. Spiridon" Hospital is the largest in Iași and in the country with 40 beds and has been run since 2008 by Prof. Dr. Laura Gheucă Solovăstru, primary dermatologist, Doctor of Medical Sciences and coordinator of teaching activity, Dermatology Discipline, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy from Iasi. She was Vice Dean of the Faculty of Medicine from 2009 to 2012. She organizes the Dermatology Conference

"Gh. Năstase Days", now in its 28th edition, being the President and Executive Director of the Conference. She is the Director of the Department of Medical Specialties (III), Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy from Iasi and member of the University Senate.

In 2015, the headquarters of the Dermatological Clinic of the "Sf. Spiridon" Iași County Clinical Emergency Hospital returned from Tătărași in the center, to the Hospital Ambulatory where it occupies the right wing of the 3rd and 4th floors, with a practical work room for medical students in the basement of the building. and two consulting rooms on the 1st floor.

Today, ten dermatovenerologists work in the Dermatology Clinic together with fifteen nurses and ten caregivers. The department has a medical profile and a consistent surgical component, with great addressability. It ensures the diagnosis and treatment for the dermatological pathology of the patients from Iași, as well as from the counties from the northeast region of the country. The population served is numerous, and the medical act is characterized by a high professional, scientific and ethical responsibility.

The Dermatovenerology department also carries out clinical activity within a national program: *The program dedicated to patients with Bullous Epidermolysis*, carried out in collaboration with the Ministry of Health under the coordination of Prof. Dr. Laura Gheucă Solovăstru.

The clinic operates in an ANMCS accredited hospital - ISO certified - with high addressability. The investigations performed in the Clinic are processed in modern internal laboratories RENAR accredited. The activity takes place in a completely sanitized and modernized new space, ensuring superior hotel conditions to many Dermatovenerology clinics in the country. The clinic benefits from human resources with recognized responsibility and professional ethics, in assuming and carrying out professional tasks, helped by specialized equipment for services in the field of dermatology. The medical team consists of young physicians, academics, residents and registered nurses.

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COMORBIDITIES IN PATIENTS WITH POST-OPERATIVE MENINGITIS IN NEUROSURGERY

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COMORBIDITIES IN PATIENTS WITH POST-OPERATIVE MENINGITIS IN NEUROSURGERY (Abstract): Meningitis is a serious condition that affects the central nervous system and can be caused by bacterial, viral, or fungal infection . Postoperative meningitis is a post procedural complication and disorder of the nervous system. **Material and methods**: We performed a retrospective study between 2016 and 2019 including 627 patients who underwent neurosurgical interventions. Of these, 32 presented postoperative meningitis being admitted between 2016 and 2019 to the "Sf. Parascheva" Clinical Hospital of Infectious Diseases, Iasi, in order to assess the impact of comorbidities among these patients. **Results**: From 24 patients with comorbidities, we evaluated a percentage of 45.83% patients with ischemic heart disease. We identified a number of 15 patients (62.5%) with arterial hypertension, which demonstrates the high prevalence of vascular diseases among these cases. Also, we found 4 cases with metabolic disorders which means 16.66% from all the patients with associated pathology. On the other hand, we analyzed the prevalence of other important associated comorbidities which can increase the mortality and morbidity associated risk. **Conclusions**: The impact of multiple comorbidities on the management and evolution of infectious diseases, especially in postoperative meningitis, is complex and needs collaboration with colleagues from all the specialties involved. **Keywords**: POST-OPERATIVE MENINGITIS, COMORBIDITIES, NEUROSURGICAL COMPLICATION.

Meningitis is a serious condition that affects the central nervous system. It is an inflammation of the meninges, which is the membrane that surrounds both the brain and the spinal cord. Meningitis can be caused by bacterial, viral, or fungal infection (1). Postoperative meningitis is a post procedural complication and disorder of the nervous system. Measurement of cerebrospinal fluid (CSF) cytokine and lactate levels in conjunction with meningitis diagnostic procedures may contribute to the early and precise detection of post-neurosurgical meningitis. Measuring CSF lactate is affordable and cost-effective, especially in patients who have just had

neurosurgery (2). Molecular techniques, such as polymerase chain reactions that target 16S ribosomal RNA, can help with diagnosis, direct therapy, and pinpoint microbes that can't be cultivated. Recent studies recommends combining approaches for patients with bacterial meningitis suspected following neurosurgery, particularly when the culture is negative (3). A recent study showed *Klebsiella pneumoniae* (25.6%) was the most prevalent isolate in patients with community-acquired meningitis, followed by *Streptococcus pneumoniae* (18.6%) and *Listeria monocytogenes* (11.6%). Ceftriaxone, cefepime, and meropenem each had susceptibility rates of 85.7%, 81.3%, and 100% for *K. pneumoniae*, correspondingly. Patients who had meningitis while receiving neurological treatment most frequently had coagulase-negative staphylococci (28.0%), followed by *Staphylococcus aureus* (16.1%) and *Enterobacter* spp (13.6%). About 39% of patients experienced neurological problems, and 14.8% of patients died within three months. After controlling for confounders, older age, neurological issues, and an initial Glasgow coma rating of less than eight were all strongly linked to a poor outcome (4). It was concluded that intrathecal administration of antibiotics such as gentamicin, amikacin, and colistin with low blood-brain barrier transition in intravenous treatment will enhance survival in post neurosurgical meningitis caused on by carbapenem-resistant agents. In order to prevent nosocomial meningitis, intrathecal antibiotic treatment should be considered standard practice (5). In a sample group of patients with post-neurosurgical meningitis, diabetes mellitus (15.4%), chronic lung disease (12.8%), renal disease (6.0%), and cerebrovascular disorders (5.8%) were the most prevalent comorbidities. There were 1,120 (4.2%) cases of AIDS and HIV infection (6). Among patients with postoperative meningitis, the presence of comorbidities has been shown to be an important risk factor as in the other types of bacterial meningitis. According to research on patients with meningococcal meningitis over a forty-year period, there is a 2- to 40-fold higher chance of developing kidney disease, immunological problems, liver disease, cancer, and organ transplantation (7). According to cohort research from Spain, people with diabetes mellitus with bacterial meningitis tend to be older, have more comorbid conditions, and have higher fatality rates. The most common infections are *S. pneumoniae* and *Listeria monocytogenes*, with *L. monocytogenes* being more prevalent. *Neisseria meningitidis* is much less common in diabetics than in non-diabetics (8).

MATERIAL AND METHODS

We performed a retrospective study between 2016 and 2019 including 627 patients who underwent neurosurgical interventions. Of these, 32 presented postoperative meningitis being admitted between 2016 and 2019 to the “Sf. Parascheva” Clinical Hospital of Infectious Diseases, Iasi, in order to assess the impact of comorbidities among these patients. Statistical analysis was performed in *SPSS 27.0* using Pearson Chi-squared test.

RESULTS

The average length of hospital stay was 20.37 days. There were 8 patients who also associated sepsis. We also evaluated the length of intervention: 10 cases with more than 180 minutes, 10 cases with more than 240 minutes and 12 cases with more than 280 minutes (fig.1).

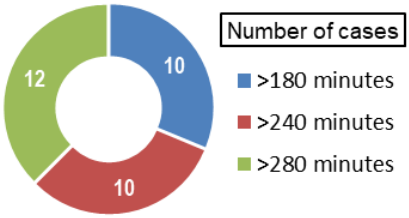

Fig. 1. Distribution of cases by intervention length

From a total of 32 cases with postoperative meningitis, 8 (25%) were complicated with sepsis. During hospitalization, we also assessed qSOFA score and Glasgow Coma Scale (GCS) (tab. I).

The patients management was individualized according to the specific profile of their comorbidities. From all the patients included in this study, 75 % had comorbidities (fig. 2).

From 24 patients with comorbidities, we evaluated a percentage of 45.83% patients with ischemic heart disease. Also, we identified a number of 15 patients (62.5%) with arterial hypertension, which demonstrates the high prevalence of vascular diseases among these cases (fig. 3).

From this point of view, we can also suggest that the double association ischemic heart disease-arterial hypertension was prevalent in elderly patients (7 patients from a total of 24 with comorbidities), with a median age of 63 years old (fig. 4).

We identified 4 cases with metabolic disorders which means 16.60% from all the patients with associated pathology (fig. 5).

On the other hand, we analyzed the prevalence of other important associated comorbidities which can increase the mortality and morbidity associated risk. We calculated the same percentage for pulmonary disorders and neoplasia (20.88%), both of them with important repercussion in patient's evolution and prognosis (fig. 6).

TABLE I.

qSOFA and GCS scores during hospitalization

Patient	qSOFA	Glasgow Coma Scale
1	1	6
2	2	9
3	0	13
4	1	13
5	2	14
6	2	13
7	1	child
8	1	child
9	1	15
10	1	15
11	1	10
12	0	15
13	0	12
14	1	child
15	1	10
16	3	5
17	3	5
18	3	6
19	0	13
20	2	12
21	1	13
22	2	8
23	1	10
24	0	11
25	1	11
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27	1	12
28	0	10
29	1	14
30	0	14
31	1	12
32	2	10

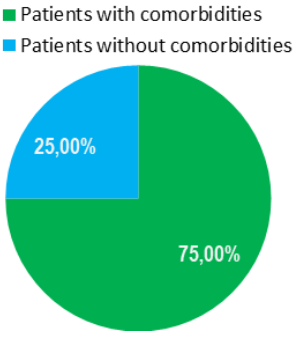

Fig. 2. Percentage of patients with comorbidities

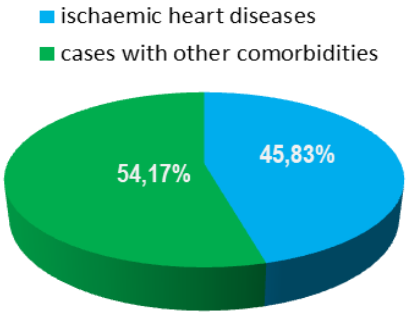

Fig. 3. Percentage of patients with ischemic heart disease from all cases with comorbidities

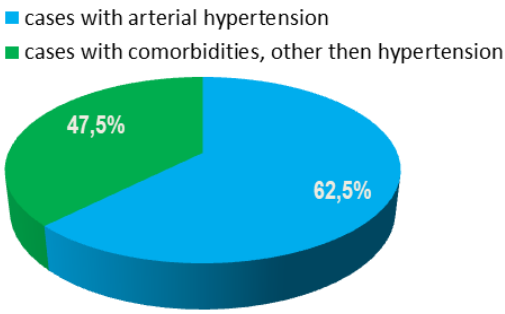

Fig. 4. Percentage of patients with arterial hypertension from all cases with comorbidities

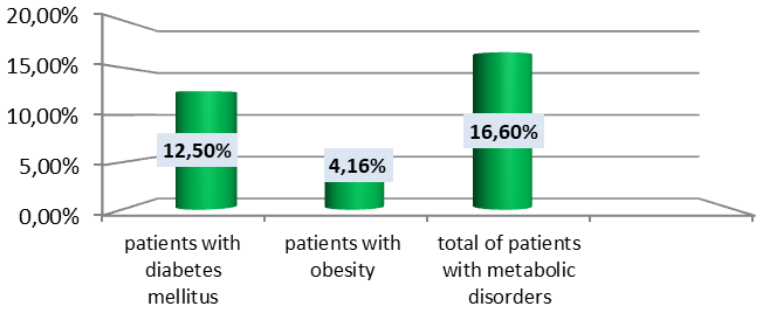

Fig. 5. Percentage of patients with metabolic disorders from all patients with comorbidities

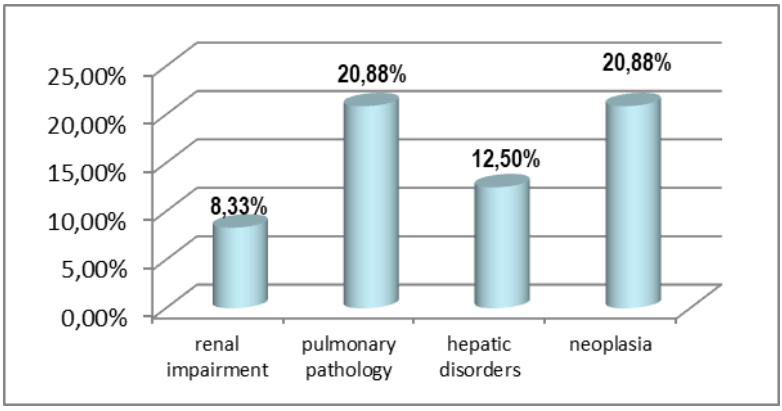

Fig. 6. Percentage of cases with other comorbidities than metabolic and cardio vascular

From the total number of cases with associated comorbidities 6 patients (25% from all cases with comorbidities) presented 3 or more associated pathology (fig. 7).

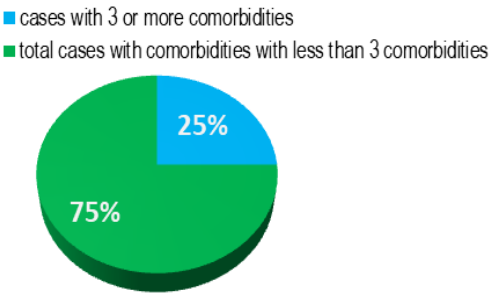

Fig. 7. Percentage of patients with 3 or more comorbidities from all patients with associated pathology

Taking into account the group of 28 adult patients who had a favorable evolution, in which no mortality was recorded, we evaluated the relationship between patients' age groups, the relationship between GCS score, as well as the relationship between systemic pathology and the prevalence of postoperative meningitis.

Statistical analysis. Table II shows the relation between patients' age groups and the prevalence of post-operative meningitis (POPM).

TABLE II.

Pearson Chi-squared test for prevalence of POPM related to gender

Pearson Chi-squared = 0.252 p = 0.616		POPM				Total	
		POPM+		POPM-			
		N	%	N	%	N	%
GENDER	F	10	35.7%	238	40.5%	248	40,3%
	M	18	64.3%	350	59.5%	368	59,7%
Total		28	100.0%	588	100.0%	616	100.0%

In patients with POPM the share of men is higher (64.3%, compared to 59.5% in males' group without POPM), but without statistical significance. In patients with POPM the share of patients with age <=60 years is significantly higher (67.9%) comparing with the same share in patients without POPM (2.4%) (tab. III).

TABLE III.

Pearson Chi-squared test for prevalence of POPM related to age group (<60 *vs.* ≥60)

Pearson Chi-squared = 225.996 p = 0.000** / OR = 86.556; 95%CI OR = 33.341 ÷ 224.702		POPM				Total	
		POPM+		POPM-			
		N	%	N	%	N	%
AGE GROUP	<= 60 years	19	67.9%	14	2.4%	33	5,4%
	> 60 years	9	32.1%	574	97.6%	583	94,6%
Total		28	100.0%	588	100.0%	616	100.0%

Table IV shows the relation between patients' GCS score and the prevalence of post-operative meningitis. In patients with POPM the share is significantly higher for patients with GCS 12-15 (53.6%) comparing to patients with GCS scores 10-11 (25%) and <10 (21.4%). This discrepancy is bigger in patients without POPM, among which the share of patients with GCS 12-15 is 81.0%, comparing to patients with GCS scores 10-11 (8.3%) and <10 (10.7%).

TABLE IV.

Pearson Chi-squared test for prevalence of POPM related to age group (<60 *vs.* ≥60)

Pearson Chi-squared = 13.420 p = 0.001**		POPM				Total	
		POPM+		POPM-			
		N	%	N	%	N	%
GCS	<10	6	21.4%	63	10.7%	69	11,2%
	10-11	7	25.0%	49	8.3%	56	9,1%
	12-15	15	53.6%	476	81.0%	491	79,7%
Total		28	100.0%	588	100.0%	616	100.0%

Table V shows the relation between patients' systemic pathology and the prevalence of post-operative meningitis. The share of patients with lung diseases is significantly bigger among those with POPM compared with those without POPM (21.4% *vs.* 6%); the same phenomenon is reported in the case of patients with liver diseases (10.7% from those with POPM *vs.* 0% from those without POPM).

TABLE V.

Pearson Chi-squared test for prevalence of POPM related to systemic pathology

SYSTEMIC PATHOLOGY		POPM				Total		Pearson Chi-squared	OR / RR*	95% CI OR / RR*
		POPM+		POPM-						
		N	%	N	%	N	%			
IM	YES	9	32.1%	154	26.2%	163	26.5%	Chi2 = .487	-	-
	NO	19	67.9%	434	73.8%	453	73.5%	p = .485		
LUNG DISEASES	YES	6	21.4%	35	6.0%	41	6.7%	Chi2 = 10.304	4.309	1.641 ÷ 11.312
	NO	22	78.6%	553	94.0%	575	93.3%	p = .001**		
DIABETES	YES	3	10.7%	147	25.0%	150	24.4%	Chi2 = 2.961	-	-
	NO	25	89.3%	441	75.0%	466	75.6%	p = .085		
CHRONIC RENAL INSUFFICIENCY	YES	2	7.1%	42	7.1%	44	7.1%	Chi2 = 0.000	-	-
	NO	26	92.9%	546	92.9%	572	92.9%	p = 1.000		
LIVER DISEASES	YES	3	10.7%	0	0.0%	3	0.5%	Chi2 = 63.308	24.520*	16.703÷ 35.996*
	NO	25	89.3%	588	100.0%	613	99.5%	p = .000**		
CANCER	YES	5	17.9%	147	25.0%	152	24.7%	Chi2 = .734	-	-
	NO	23	82.1%	441	75.0%	464	75.3%	p = .392		
Total		28	100.0%	588	100.0%	616	100.0%			

In which concerns the incidence of other systemic diseases, Pearson Chi-squared test did not find statistically significant differences between patients with POPM and patients without POPM.

CONCLUSIONS

The study confirmed the impact of comorbidities on the evolution of patients with postoperative meningitis. In our cohort it was a fatality of 12.5 % and 28 cases with a positive evolution. Deaths were reported predominantly among men (3 cases) aged 33 to 77 years. All the cases had associated pathologies and we observed the presence of an association of

cardiovascular and metabolic comorbidities. The management of all patients was daily revised and optimized with multidisciplinary approach which was individualized for every single case. The impact of multiple comorbidities on the management and evolution of infectious diseases, especially in postoperative meningitis, is complex and needs collaboration with colleagues from all the specialties involved. The positive evolution of postoperative meningitis needs an early diagnosis and a good partnership with other specialties with daily adaptive treatment depending on the particularities of the patient.

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EDITORIAL

SMALL DICTIONARY AT THE IT / MEDICINE INTERFACE

The immense and continuous progress that Information Technology (IT) has had in recent years and continues to have, inevitably, a major impact on the medical field as well. The benefit it can bring to medicine and the patient is indisputable, but it depends equally on the understanding on that one hand of the needs that the medical sector has to be solved by IT techniques by IT specialists. On the other hand, the interpretation of the data resulting from the application of IT techniques by IT professionals and doctors is for the final benefit of the patient. That is the existence of a common language that allows communication between these two domains. New terms appeared and continue to appear, most often neologisms from the English language. We proposed to bring together in this editorial a series of terms widely used at the IT/medicine interface, with the aim of facilitating communication in research carried out in mixed collectives. We did not intend to exhaust the terms that would be necessary to define, the scope of the subject would have exceeded the scope of an editorial. We only hope that this editorial will be a good starting point for other materials of this kind.

A: Artificial Intelligence (**AI**) - a general term used to describe algorithms designed for recognition, problem solving and reasoning, generally associated with human intelligence, essentially imitating some aspects of intelligent human behavior (1). The term artificial intelligence first appeared in an application for a 6-wk workshop entitled Dartmouth Summer Research Project on Artificial Intelligence at Dartmouth College in Hanover, New Hampshire, and is often defined as "intelligence demonstrated by machines, in contrast to the natural intelligence displayed by humans and animals" (2). Since its first appearance, the term has undergone a constant redefinition against the background of what is technically feasible. On the one hand, the definition per se is already vague, because the partial term intelligence is not itself well defined. On the other hand, it depends directly on human perception and evaluation, which change constantly (3).

Artificial Neural Network (**ANN**) - a subset of AI; in medical imaging, an ANN is an image analysis algorithm composed of layers of connected nodes that simulate the neuronal connections of the human brain (1).

B: Big Data - term defined based on the 4 Vs: variety, velocity, volume, and variety. Traditionally, data are considered big when they rank high on several of these dimensions (4).

C: Classification - term use in IT to define a process related to categorization, the process in which ideas and objects are recognized, differentiated and understood (5).

Computer-Aided Design (CAD) software - the use of computers (or workstations) to aid in the creation, modification, analysis, or optimization of a design. Today, CAD tools are being employed to bring about exciting new advances in biological and medical progress, as they are tackling challenges that in the past decades

could have only been dreamed of being conquered (6).

Computed Tomography (CT) - *in vivo* structural imaging acquisition technique, giving high resolution images. Usually, CT refer to the technique used in Radiology, where X-radiation is the physical agent, and the image is the expression of the degree of attenuation of this radiation in relation to the tissue density. A narrow beam of x-rays is aimed at a patient and quickly rotated around the body, producing signals that are processed by the machine's computer to generate cross-sectional images, or "slices". But, in fact, referring to the acquisition modality, there are also CT with other physical agents, as in SPECT (with gamma radiation) (7).

D: Decision Tree - represent a computerized decision support tool that uses a tree-like model of decisions and their possible consequences, including chance event outcomes, resource costs, and utility. It is one way to display an algorithm that only contains conditional control statements. Decision trees are commonly used in operations research, specifically in decision analysis, to help identify a strategy most likely to reach a goal but are also a popular tool in ML (6).

Deep Learning (DL) - a sub-type of ML that adds a number of processing layers (depth) to detect complex features in an image typical of a certain type of ANN (1).

Digital Imaging and Communications in Medicine (**DICOM**) - the international standard to transmit, store, retrieve, print, process, and display medical imaging information (8).

Digital Health - refer to the technologies use computing platforms, connectivity, software, and sensors for health care and related uses. These technologies span a wide range of uses, from applications in general wellness to applications as a medical device. They include technologies intended for use as a medical product, in a medical product, as companion diagnostics, or as an adjunct to other medical products (devices, drugs, and biologics). They may also be used to develop or study medical products (9).

Digital Technology - encompasses digital devices, systems, and resources that help create, store, and manage data (10).

E: e-health - refer to the use of information and communication technology to support health and healthcare. Three major predicted areas of disruption are telemedicine, smartphone Apps, and wearable sensors. Increasingly, citizens are using digital technologies to support their own health and healthcare decision making (11).

F: Fused image - image that is the result of superposing a functional image (image that is related to the function/phenotype characteristics of the tissue) with a structural one (image that depend only on the structural characteristics of the tissue, but not directly on the nature/phenotype markers of the cells). Give the possibility to accurate localize a specifical functional image. It represents, nowadays, the most advanced part of imaging, with clear advantages for the surgical approach of tumoral processes. Examples: SPECT-CT, PET-CT, PET-MRI (12).

G: Gamma camera - detection device in nuclear medicine, for *in vivo* imaging. Detects the gamma radiation emitted by the radioisotope within the specifically administered radiotracer (technique known as scintigraphy). Depending on the type of the device and the type of radioisotope/ radiotracer used or acquisition technique, can be distinguished SPECT, PET, whole body or

planar imaging techniques. Also called **scintillation camera** or **Anger camera** (7).

I: Image processing - The action of analyzing images acquired *in vivo* or *in vitro*, for the purpose of qualitative and quantitative evaluation and imaging diagnosis, using computer software, in order to get an enhanced image or to extract some useful information from it. It is a type of signal processing in which input is an image and output may be image or characteristics/features associated with that image. Image processing depends on the computer's software and IT capabilities, evolving along with them (13).

Information Technology (IT) - the use of computers to create, process, store, retrieve, and exchange all kinds of data and information (8).

Internet of Things (IoT) - a network of physical objects that are fitted with sensors, software and other technologies. Connected to the Internet, these "things" are able to exchange real time data with other connected devices and systems over networks. These connected devices combine with automated systems to gather IoT data that can be analyzed to assist with tasks or learn how to improve a process (14).

M: Machine Learning (ML) - a subtype of AI that employs algorithms through data analysis without being explicitly programmed. ML tends to be associated with solving problems of logic after learning from human-defined teaching cases typical of an ANN (1).

Magnetic Resonance Imaging (MRI) - a complex medical technique that can also be used for *in vivo* medical imaging, to form images of the body's anatomy and physiological processes. In essence, it can be considered to be based on a "magnet-like" behavior of stable atomic nuclei with an odd number of protons in a magnetic field. The simplest such nucleus is the hydrogen nucleus in the constitution of water – which is, in fact, a proton, and will behave like a small magnet, participating in "resonance" and then returning to the initial state, through "relaxation". As a result, MRI images will be able to provide information about the proton density in the body, which will be higher where the amount of water is higher. So, information will be related to the liquid structure of the examined formations. Complex definition can be found in (15).

Mobile Health (mHealth) - is the practice of medicine and health care over mobile computer devices. As an industry, the mHealth field has seen exponential growth in recent years thanks to widespread use all over the world and increasingly accessible mobile technology (16).

N: Natural Language Processing (NLP) - a subfield of AI. It helps computers process and understand the human language so that they can automatically perform repetitive tasks. Examples include soft translation, summarization, ticket classification or spell check (8).

Next Generation Data - rely on software-defined technologies as their logical layer, allowing for better control of physical and virtual resources. One of the biggest priorities of a next generation data center is automation-streamlining workflows and reducing the manual upkeep (17).

P: Personalized medicine (PM) - seek to improve stratification and timing of health care by utilizing biological information and biomarkers on the level of molecular disease pathways, genetics, proteomics as well as metabolomics (18).

PET - acronym widely used to mean

Positron Emission Tomography, an *in vivo* technique in the imaging field of nuclear medicine that provides functional images by using radiotracers with positron-emitting radioisotopes and tomographic acquisition, with the reconstruction of images in sections. Because PET radiotracers cellular localization is related to the metabolic activity of the cells, PET brought functional images at the new level of molecular imaging (19).

PET-CT - *in vivo*, fused, functional (PET) and structural (CT) imaging.

PET-MRI - *in vivo*, fused, functional (PET) and structural (MRI) imaging.

Podcast - is a broadcast that is placed on the Internet for anyone who wants to listen to it or watch it. Some radio stations already are posting their talk shows on websites to reach podcast audiences. Podcasts and their use in medical education is growing and becoming more popular, all while not knowing what podcasts are available for each specialty (8).

Precision medicine - aims to improve patient outcomes by stratifying or individualizing diagnosis and treatment decisions. The term relies on genetic, phenotypic, or psychosocial characteristics to provide precise identification of patient subsets for treatment targeting (20).

R: Radiopharmaceutical - also named **Radiotracer**, the substance that is administered to the patient in nuclear medicine techniques. There are two categories of radiopharmaceuticals: radioisotope alone (examples 131I, 201Tl) or radioisotope + vector molecule (in this chemical compound one or more atoms are replaced by a suitable radioisotope). The radioisotope is a gamma (in the case of SPECT) or positron (in the case of PET) emitter. The radiopharmaceutical is administered intravenously orally or by inhalation, depending on the type of investigation, circulates into the blood and is captured cellularly through an uptake mechanism in relation to the phenotypic characteristics of the visualized cells/tissues. A gamma radiation will be emitted from the uptake structures and will be detected by a gamma camera type device (19).

Radiography - an *in vivo* imaging technique from the field of **Radiology** (medical imaging domain that uses *in vivo* medical X-ray imaging to diagnose diseases and guide their treatment). It uses X-rays that will be attenuated differently in relation to the tissue density, making evidence of the structural characteristics of the internal tissues/organs. The images are recorded nowadays be the mean of a computer and can be delivered on an information support (electronic, currently) (9).

S: Smart health (sHealth) - a healthcare system that enables patients and doctors to communicate with each other and remotely exchange information monitored, collected, and analyzed from patients' daily activities via the IoT (21, 22).

Smart healthcare - can be defined as an integration of patients and doctors onto a common electronic platform for intelligent health monitoring by analyzing day-to-day human activities.

Smart data - data from which signals and patterns can be extracted by intelligent computer algorithms (8).

Smart patient care - system that includes electronic devices, being needed to help nurses make more informed prioritization decisions between responding to alarms and ongoing tasks and, finally, assist them in adjusting their work priorities in various situations (23).

SPECT - acronym for **Single Photon**

Emission Computed Tomography. A nuclear medicine procedure in which images are acquired through the rotation of a gamma camera around the patient and takes images from different angles, which a computer then uses to form a tomographic (cross-sectional) image, within different sections (classical sagittal, transversal, coronal but sometimes also, dedicated). The images are the result of the emission of gamma radiation, after the administration of a radiotracer that includes a gamma emitter radioisotope (2).

SPECT-CT - acronym for **Single Photon Emission Computed Tomography–Computed Tomography**. Fused image technique that superposes functional (SPECT) image with anatomical (CT) image (24).

Support vector machines (SVMs) - a set of supervised computers learning methods used for classification, regression and outliers' detection (25).

Synthetic intelligence (SI) - provides authentic higher-order reasoning using technology like quantum logic, while AI simply imitates human thought (1).

T: Telehealth - the delivery of health care services, where distance is a critical factor, by all health care professionals using information and communication technologies for the exchange of valid information for diagnosis, treatment and prevention of disease and injuries, research and evaluation, and for the continuing education of health care providers, all in the interests of advancing the health of individuals and their communities - the World Health Organization definition. Support and promote long-distance clinical health care, patient and professional health-related education, and public health and administration (26, 27).

Telemedicine - Telemedicine is the use of telecommunications technology to deliver health care to populations with limited access to care (28). Also, can be defined as the two-way, real time interactive communication between the patient and the physician or practitioner at distant site. Starting from this basic definition, new terms continue to appear, in relation to the scope of telemedicine, for example: telepathology (remote viewing of microscopic images), telerehabilitation, tele-oncology, tele-education and the list rest open.

Theragnostic - a term that refer to a treatment strategy that combines therapeutics with diagnostics. It associates both a diagnostic test that identifies patients most likely to be helped or harmed by a certain medication, and a targeted drug therapy based on the diagnostic test results. Usually is developing in oncology (29). Both parts rely on the same tissular principle through the same specific phenotype markers, but the physical used agent must be no harmful for diagnostic and destroy the identified tumoral tissue, in the therapy part.

U: US - Abbreviation that stands for **ultrasound imaging** (or **echography**, **sonography**). This *in vivo* imaging technique uses high-frequency sound waves to view inside the body. Because ultrasound images are captured in real-time, they can also show movement of the body's internal organs as well as blood flowing through the blood vessels, without any invasiveness (9).

V: Vector molecule - a molecule (chemical compound) that can be labeled with a radioisotope suitable for the diagnostic detection/treatment in nuclear medicine and is used to direct this radioisotope to a tissue/organ that will capture it preferentially, sometimes specifically, in relation

to its phenotypic characteristics. In this way, by detecting the emitted gamma radiation, it is possible to visualize the tissue in relation to its functional characteristics, acquiring a map of distribution of the radiotracer in the explored organ (30).

No doubt, the list can go on. We hope that through this introductory material we have helped to clarify some notions for all those involved in IT software development collaborations for medical use, and, above all, that we have opened the readers' appetite to develop and clarify as many terms as possible from the avalanche of neologisms appearing at the IT / Medicine interface, for the benefit of the development of the medical fields and, not lately, of the patient.

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NEWS

DIAGNOSTIC ACCURACY OF POINT-OF-CARE LUNG ULTRASOUND IN COVID-19

The aim of this single-center, prospective, diagnostic accuracy study was to determine the diagnostic accuracy of Point-of-care Ultrasound **(**POCUS) for the diagnosis of pulmonary manifestations of COVID-19 in patients presenting to the ED. A convenience sample of 100 patients with suspected COVID-19 was enrolled between 14 April and 22 April 2020. POCUS had a sensitivity of 89%, specificity of 59% and a NPV of 93% compared with PCR (or CT scanning if negative PCR) in diagnosing pulmonary manifestations of COVID-19. Pulmonary manifestations of COVID-19 may be excluded by POCUS in patients without previous cardiac or pulmonary medical history. In conclusion, POCUS of the lungs could serve as a valuable, easily accessible and radiation-free tool for excluding pulmonary manifestations of COVID-19 in patients in the ED at the point of assessment, especially in patients without previous cardiopulmonary disease (Haak SL, Renken IJ, Jager LC, Lameijer H, van der Kolk BBY. Diagnostic accuracy of point-of-care lung ultrasound in COVID-19. *Emerg Med J* 2021; 38(2): 94-99 / doi: 10.1136/emermed-2020-210125)

EDITORIAL

ARTERIAL STUMP MEASUREMENT: QUALITY INDICATOR IN COLORECTAL SURGERY

Measuring quality of surgery in colorectal resections for cancer has become an important subject of research since the principles of total mesorectal excision (TME) for rectal cancer and complete mesocolic excision (CME) for colon cancer have been brought forward. CME surgery proved to decrease local recurrence in colon cancer to 3.3% (1, 2). Similarly, TME was shown to drastically improve surgical outcomes in rectal cancer.

CME as described firstly by Hohenberger *et al*.(1) is based on three principles: (1), mobilizing the colon in the natural, embryological planes, maintaining an intact, spillage-free mesocolon; (2), ligation of the main vascular pedicle at its origin (i.e., central vascular ligation - CVL), ensuring removing the entire mesenteric root along with the central lymph nodes and (3), adequate distal and proximal margins of at least 5 cm.

While the intact dissection of the mesocolon and ensuring negative longitudinal margins has become well standardized and adopted by most surgeons, controversies still exist on the ideal location of CVL and whether CVL does actually impact survival. Adequate trials to define the ideal CVL point at which recurrence rate is improved compared to conventional surgery are lacking mainly because measurable indicators are not well standardized. One may predict the point of CVL indirectly by measuring the distance from the margin of the tumor to the apex of the specimen, but we must be aware of the natural anatomical variability in mesocolic area and pedicle length among patients, which will bias results.

One simple, non-invasive way of measuring the point of CVL and thus predicting the extent of apical lymph node dissection is to measure the length of the arterial stump on postoperative contrast enhanced computed-tomography (CT) scans. This is the most direct way of comparing oncological outcomes between CVL and non-CVL colectomies and can be done even in a retrospective manner. Both the ileocolic artery (ICA) and inferior mesenteric artery (IMA) stumps are readily visible on standard CT scans which are part of the routine colorectal cancer follow-up. Although it seems an innovative, simple technique, and we have tested its feasibility in our own previous studies (3, 4), it has yet to reach significance in clinical practice. Currently on PubMed there are seven original articles (3, 5-10) which tested the value of arterial stump measurement as a surgical quality marker.

Stump measurement: is it difficult?

The most valued trait of arterial stump measurement it`s the simplicity of assessing the length on routine postoperative CT's, which are already done as a standard cancer follow-up, thus measurements can be done retrospectively, and lengths can be easily linked to survival of patients. While some studies performed mesenteric enhanced CT's, in our experience this is not

required. Apart from the visible contrast, arterial stumps can be identified due to tip granuloma which appears at the ligation point, or, if clips are used, then measurement becomes even easier, by visualizing the radiopaque clip at the tip of the contrast enhanced stump. Most studies used experienced radiologists to perform stump measurements, mainly to improve reliability of results, but this can be done by surgeons as well if they are used to analyzing CT images of colonic anatomy in their practice. In general, surgeons are able to identify the correct stump length in at least 80% of cases when the radiologists' measurement is considered the benchmark length. On the right side, the superior mesenteric vein (SMV) and superior mesenteric artery (SMA) are identified. The ICA stump is identified as the last vessel arising from the SMA on the right side. On the left side, the inferior mesenteric artery (IMA) can be identified at the level of the 3rd lumbar vertebrae with a left trajectory. The stumps are measured from their origin to the clip or, if a clip was not used, a suture granuloma is usually visible. Overall, none of the included studies had issues or discussed technical difficulties in measuring the arterial stump both at the right and left colon.

Arterial stump:
marker of surgical quality?

Correct reporting on surgical specimens has become a requisite in any cancer centre. The advent of CME and TME and their impact on recurrence rates in colorectal cancer has made institutions to audit their specimens and ensure an adequate standard is met. Currently, measurements on fresh specimens are the norm and proved to predict outcomes. In our practice, among other institutions, stump measurement has become, so far, a complementary indicator of extent of resection. It is logical to assume that stump length is inversely proportional to all other macroscopic pathological measurements on the specimen, as the stump is anatomically part of the specimen, representing the tip of the pyramid both on the right and left colon, thus a shorter stump will naturally lead to a longer and wider specimen, a longer distance from the tumor to the apical vascular tie and a higher lymph node yield. All these pathology measurements proved to have prognostic implications and are regarded as standard indicators for quality of surgical specimens. For clinicians to embrace and use stump measurement as a quality marker, one must prove it has the same sensitivity and specificity while being easier to measure by non-pathologists.

Arterial stump: prognostic marker?

Hypothesizing that the stump length could correlate with the rate of local and/or systemic recurrence is an intriguing scenario. Increasing number of studies praise the importance of apical lymph node dissection especially in cN+ tumors, where the rate of central lymph node metastasis is higher11-15. In patients with positive central lymph nodes leaving a short arterial stump (less than 1.5cm) is recommended in avoiding local and systemic recurrence (17, 18, 19). More so, only by leaving a short stump, surgeons can manage to dissect the mesocolon fully intact up to its root. Some argue that leaving a longer stump while still performing central lymphadenectomy is and adequate replacement (20, 21, 22). This holds true for early stage cN0 cancers, where positive lymph nodes at the edge of the mesentery are very unlikely; however, in more advanced tumors complete meso-

colic excision up to the root of the peritoneum is the standard procedure with the best recurrence free survival rates and this can be achievable only by ligating the main pedicle at its origin leaving a small stump. Thus, arterial stump length may be regarded as an indirect marker of CME and CVL. Despite this, no study in current literature analyzed the prognostic implications stump length and this should be addressed. If the stump length proves to predict survival, then it would be a simple adjunct tool to assess whether a patient would benefit from adjuvant treatment.

In conclusion, postoperative arterial stump measurement after colorectal resections seems to be a simple tool to assess surgical quality, however the evidence is poor. Institutions that practice stump measurement should retrospectively compare the stump length with other pathology measurements and, more importantly, to assess whether leaving a shorter stump has an impact on recurrence free survival and, if it does, then a minimal accepted length should be brought forward.

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EDITORIAL

PALLIATIVE MEDICINE - BEYOND ADVANCED CANCER CARE

Nowadays, medicine has the same value and meaning, and is answerable to the same formidable requirements it has always been. Medicine must soothe, heal, prevent and limit the spread of disease. A physician has the opportunity to live inspiring moments while brings the very sick patients again to life and these rare moments will uplift him in the most difficult situations. In some specialties, such moments could repeat more often as in surgery, emergency medicine and sometimes in cardiology or neurology. In the majority of time, the practicing physician has to deal with chronic diseases, some of them which aggravate and complicate itself, ultimately conducting to organ failures and a very poor quality of life. Moreover, due to the success of medicine in preventing some diseases, in treatment of infectious diseases and in effective treatment of ischemic heart disease, diabetes mellitus etc. people live much longer, and experience new problems related to advanced age (1). Death, the termination of life is seen as a disease and drained by any spiritual meaning. Twenty-one century medicine is dealing with termination of life problems, with the emerging "assisted suicide" solution for the difficulties related to advanced diseases and advanced age and with a huge pressure on health system done by chronic advanced diseases with low quality of life and disability (2). New rules, new laws, new concepts are required to respond to new approaches and needs. A different health system organization is needed.

An example of a disease which needs a new approach is decompensated liver cirrhosis.

Cirrhosis represents the final irreversible stage of chronic progressive liver disease. The patients' prognosis depends on etiology, comorbidities, and especially stage and complications (3). Cirrhosis stages are classically seen as: compensated and decompensated, the last one being defined by the occurrence of complications as: ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, jaundice, upper gastrointestinal bleeding by variceal effraction, hepatic encephalopathy (4). Other complication which may darken the prognosis of cirrhotic patients is hepatocellular carcinoma, often diagnosed in advanced stage. „End-stage liver disease" is another term used to describe advanced liver diseases stage, characterized by chronic liver failure, and decompensated cirrhosis, just to emphasize the irrevocable severe course of the illness and the inevitable somber lethal outcome.

However, liver transplantation emerged as an outstanding cure for patients with advanced cirrhosis, ensuring lifesaving, life prolongation and also regain of life quality. There are well-defined classification systems, which aim to objectively establish the indication for liver transplantation for each individual patient with cirrhosis. The MELD („*Model for End-Stage Liver Disease*") score, able to predict prognosis of cirrhotic patients, has been officially adopted by United Network for Organ Sharing and Eurotransplant as a tool for

prioritizing patients awaiting liver transplantation (5). Using MELD score (which takes into account serum bilirubin, serum creatinine, and the international normalized ratio for prothrombin time), patients are assigned a score from 6 to 40, which correspond to estimated 3-months survival rate of 90% to 7%, respectively (6). Patients with MELD scores 15 or greater are considered candidates for liver transplantation, while patients with lower values are considered as having a better prognosis without. Still, patients with cirrhosis may present important serious complications, which are not always reflected by a high MELD score, but which equally negatively affect their survival rate. For instance, spontaneous bacterial peritonitis or variceal hemorrhage due to portal hypertension, are poor prognostic indicators.

Undoubtedly, liver transplantation is a potential definitive curative treatment for advanced liver disease. However, decompensated cirrhosis remains a disease with variable prognosis and unpredictable outcome. Even with the hope of liver transplantation, patients are at risk for adverse outcome, with, generally, an average survival without liver transplantation of approximately two years (7). Many patients on the waiting list die, with an estimated percentage of 15% annually (8), while, usually, another 10% are delisted in the same year due to worsening of their condition (9). Additionally, liver grafts are limited, and the number of patients needing liver transplantation exceeds the organ availability. Moreover, liver transplantation is not a *panacea*, as many patients are not candidates, due to comorbidities, or being "too sick" to be transplanted, or precluded by social factors.

Thus, palliation should not be limited to end-of-life care but provided throughout the entire trajectory of illness. Palliation should not be understood (only) as care measures offered to patients at the end of life, having as main aim the sufferance relief, but as maximal life-prolonging therapeutic actions, including curative approach of complications.

Contrary to appearances, palliative care and liver transplantation are not mutually exclusive. In fact, keeping the end-stage liver disease patient alive enough and well enough to be transplanted, is the key for cure. Providing supportive care for patients with complications needing transplantation may be the redeeming quality to survive until liver transplantation.

However, hospital readmissions in patients with decompensated cirrhosis, as well as their hospital lengths of stay are challenging matters. Readmission rates of cirrhotic patients vary depending on the follow-up duration and the studied population, different studies showing early readmission rates, defined by another hospitalization due to an acute situation within 30 days of the index hospitalization, from 20% to 50% (10,11). Readmission rates increase with the duration of the follow-up period, and the main reasons for early readmission appear to be specific complications as hepatic encephalopathy, variceal bleeding, or infections, as showed by one of our studies regarding predictive factors for the prognosis of alcoholic liver cirrhosis (11). Prolonged length of stay is also an issue in cirrhotic patients' care, representing a supplementary burden for hospitals, especially for those designated to function as emergency units.

Nonetheless, for patients in terminal end-of-life stage, as advanced end-stage liver disease and hepatocellular carcinoma with Barcelona Clinic Liver Cancer stage D

tumor (12), the care will be primarily focused on comfort and alleviating suffering and pain. Hospices are dedicated institutions aiming to assist terminal life stages, targeting comfort and quality of life.

Thus, throughout the entire trajectory of the illness, a dynamic process of identification of patient-centered goals of care is compulsory and of utmost importance. Along evolution, as long as liver transplantation is still an option, management must be centered on life-prolongation measures, complication treatment and preventions strategies for further decompensation, while comfort ensuring will be prioritized as strategy of end-of-life care. However, there are still gaps in defining end-of-life criteria, as long as prognosis and life expectancy in cirrhosis are not precisely predictable.

As physical deterioration and psychological suffering coexist in advanced liver disease, symptoms control and psychical needs are both equally important targets to consider. Healthcare services should offer not only physical medical care, but also mental and social support, towards patients and also their families.

Appropriate palliative care in advanced liver disease is a decisive determinative factor for patients' outcome, in terms of life and death, literally. This fact, as well the need to offer the optimal tailored assistance, was understood properly in the recent years by all the caregivers, and the interest in palliation in cirrhosis is growing.

Therefore, adapted strategies should be wisely adopted for every patient with liver cirrhosis, in order to obtain both prolongation and quality of life. Understanding each individual cirrhotic patient's needs, rethinking care strategy and, if needed, restructuration of hospitals, defining special care units, might be the key issues to ensure the best end-stage liver disease care.

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EDITORIAL

THE IMPACT OF CHRONIC DISEASE ON STUDENTS' QUALITY OF LIFE

In 2020, there were about 18 million tertiary students in Europe (1). The average of those reporting chronic health conditions was around 18% in Switzerland, with the highest level of 39% in Iceland and the lowest of 7% in Romania, Albania and Serbia (2). The health status of students should be a concern for health professionals, alongside academics, as higher education plays an important role in society, increasing people's well-being, fostering development and economic growth. However, our knowledge of the impact of chronic illness on university life is limited (3), with minimal research on the differences and similarities of the academic experience of students with chronic illness compared to students without health difficulties (4) but also how universities can support these students (5). As a result, through this exploratory narrative review we aim to synthesize some key ideas about the impact of chronic illness on students' quality of life and formulate a series of recommendations that can be followed by university support services to encourage quality of life and academic adjustment for this group of students.

Chronic diseases are medical conditions with implications on the main dimensions of a person's life, felt on a long-term or even lifelong basis, accompanied by a series of emotional, social and lifestyle challenges (6). Young people with a chronic illness often report feeling that their chronic illness prevents them from living a normal life (7).

Chronic diseases are diverse and can cause varying degrees of impairment, but thanks to advances in medicine, increasingly young adults are pursuing higher education and can even achieve very good health outcomes (8). However, unlike students without significant health problems, students with chronic conditions have lower graduation rates and quality of life (9). All students, especially first-year (10) and final-year (11) students, experience challenges in adjusting to the academic environment, such as difficulties with sleep, learning, engagement, homesickness, and difficulties with roommates (12), but for those with chronic illnesses, health care challenges are also added (13). In general, students with chronic illnesses may face more difficulties in pursuing and completing higher education (3), experience stress related to adapting to academic and social demands, and also feelings of loneliness and isolation (9).

Despite the well-known idea that student hood is a very challenging time, and the fact that quality of life assessment has been evolving since the 1970s, too little attention has been paid to the quality of students' lives (14). The World Health Organization (15) defines quality of life as "people's perception of their position in life in the context of the culture and value systems in which they live and in relation

to their goals, expectations, standards and concerns". It is, therefore, a subjective perception of the degree of contentment, satisfaction, towards four domains: physical, psychological, social and environmental, not just the absence of illness or disability (16). According to Li and Zhong (17), factors that can negatively impact on quality of life in university students can be grouped into six categories. The above authors conducted a narrative review and identified the following variables for the six categories of factors: (a) demographic factors: gender, age, religion, ethnicity, urban/rural origin, family income, only-child status, family type and parent-child relationship; (b) physiological factors: musculoskeletal pain and chronic diseases; (c) psychological factors: anxiety/depression, social phobia, attention deficit disorder, and childhood neglect/abuse; (d) social factors: experiences of neglect; (e) lifestyle and behavioral factors: physical activity, sedentariness, and exercise; and (f) academically relevant factors: major, interest in learning, academic stress, and academic performance.

In general, the support provided by universities is appropriate for good or permanent, predictable and stable health conditions, which is at odds with the unpredictability of chronic diseases. Diseases such as asthma, lupus and multiple sclerosis are notorious for periods of crisis, when symptoms are actively manifest, followed by periods of remission, for example. Such changes, amidst the rigidity of educational institutions, lead to reduced employability and reduced chances of completion for students (18). Moreover, there are also situations under which educational institutions may be rigid, undesirable or unable to adapt services to the specifics of chronic illness and the needs arising from it (3). In university settings, according to the study by Hamilton *et al.* (4), students face misconceptions about chronic illness, feelings of inequality and being undervalued. They have the perception that many staff members do not understand them and therefore 'politicize' academic regulations rather than adapt them for their chronic illnesses. Toller and Farrimond (3) identified three main difficulties faced by chronically ill students in their undergraduate studies, namely the reorganization of daily activities according to the difficulties of the sick body, institutional support that is not adapted to the unpredictability of the demands of a sick body, and poor awareness of chronic diseases. Added to all of these there are also financial difficulties, as some chronically ill students are dependent on loans specifically for students (7). Thus, it appears that the challenges faced by students with chronic diseases in the university environment are particular, with the need for knowledge and adaptation on the part of universities being a pressing one, especially amidst the increasing prevalence of chronic diseases among students (19, 20).

The aim of the review is to synthesize a range of relevant information about students' quality of life, with a focus on the quality of life and university experience of those with chronic diseases.

QUALITY OF LIFE OF STUDENTS VERSUS QUALITY OF LIFE OF THE GENERAL POPULATION BEFORE AND AFTER COVID-19 PANDEMIC

Compared to the general population, both before and during the COVID-19 pandemic, students had a lower quality of life (21), being extremely vulnerable to

mental health problems (22). Interestingly, this result is concluded even though they associate university studies with improving their quality of life in the future. One possible explanation may be that during their years of study they are stressed and anxious because of academic obligations, their own and their parents' high expectations of performance and their desire to meet them (23).

According to the survey conducted by UNESCO (24), in April 2020, as a result of the COVID-19 pandemic, 1.5 billion learners, a large proportion of them students (25), had to stay at home and take online courses as educational institutions were closed. This, along with other restrictions, led to reduced or even no social interaction, worries about finances, fear, the need to adapt to online learning and new ways of assessment, frustration and boredom (26, 27). Cheah *et al.* (25) identified that the psychological dimension of students' quality of life suffered most as a result of movement restrictions in the pandemic. Although, the social dimension was also affected as a result of social distancing, communication via technology was a buffering coping (25).

Leong Bin Abdullah *et al.* (28) assessed students' quality of life and associated factors during the COVID-19 pandemic and identified that student scores on the psychological and social dimensions of quality of life were lower than non-pandemic norms of the general population, while scores on the physical health and environmental dimensions of quality of life were comparable. A positive effect on the quality of life of the students investigated by the previously mentioned authors was the higher number of hours of classes attended online, along with higher social support from significant others when demographic, personal, and clinical variables were controlled. For the same students, quality of life was negatively impacted by the high prevalence of COVID-19, high levels of depression, stress, and frustration arising from study discontinuation (28). Other factors that could explain the impairment of students' quality of life during the pandemic were high levels of distress, anxiety, depression and suicidal thoughts (22, 29).

On a more specific level, both relative to the general population and to other categories of students, medical students appear to have a worse quality of life, mainly due to lack of leisure time and fatigue (14, 30). At the same time, there are also research studies in which medical students have better, or comparable, quality of life outcomes to other categories of students (31). These differences could be explained by some specific characteristics of the investigated populations (14). Although no clear trend can be identified in this regard, it is certain that, regardless of their major, students have a lower quality of life than the general population.

QUALITY OF LIFE OF CHRONICALLY ILL STUDENTS COMPARED TO STUDENTS WITHOUT SIGNIFICANT HEALTH DIFFICULTIES

According to the scientific literature (21, 30), students have a lower quality of life than the general population. Those with chronic illnesses even have significantly lower quality of life and higher levels of anxiety and experiential avoidance than students without significant health difficulties (32). One explanation may be the ma-

jor changes that occur in a young person's life when they acquire student status. They either start commuting or move away from home and have to take care of themselves, from housework to paying the bills, they have to adapt to a new city, a new social and educational environment. All these challenges mobilize a person's resources and can be associated with significant distress, which explains the lower quality of life. On top of all this, for people diagnosed with a chronic illness, the demands and restrictions of experienced medical conditions are added, leading to an even more noticeable decrease in quality of life (33).

Chronic diseases are diverse and can have varying degrees of impairment, which will be further associated with different levels of quality of life and, in general, school absenteeism. Wikel and Markelz (8) argue that the more a student misses classes, the more their academic performance will be affected, with this correlation being particularly common among students with chronic illnesses. Chronic illness predominantly causes a negative impact on students' quality of life, with acute feelings of loneliness and poorer academic performance (34), compared to students without chronic medical conditions, who perform better on both variables (9). Thus, chronic illness may also have significant effects at the academic level, in addition to those determined at the physiological level, as sick students have lower retention and graduation rates (9), while students without chronic illness and with a better quality of life are able to access resources and services available at the university, thus having a better integration into the social and academic environment. Gazibara *et al.* (35) also found that students with chronic diseases have a worse quality of life than healthy students, with female students scoring lower on the physical and mental functioning dimensions compared to male students. Similarly, students with irritable bowel syndrome have lower quality of life than students without this chronic health condition (14, 36).

Concluding, regardless of the health status of students, universities could design and implement health promotion and prevention activities. These institutions should ensure that they use useful channels of information so that the whole academic community is made aware of the resources available for students with chronic diseases on campus and in the community. Better screening to identify students with chronic illnesses in their first year of study and including them in a mentoring programme where students with experience of managing chronic illnesses support new ones can improve their sense of belonging and integration into the academic community. Universities may consider including in their academic regulations some flexibility in academic work, such as extending deadlines for projects and exams, to allow students with chronic illnesses to better manage their medical needs and meet their academic obligations at the same time. Last but not least, financial support is important, given that some students are dependent on loans specifically targeted for them in order to support themselves and cover the costs of treatments.

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CONSIDERATIONS REGARDING TUBERCULOSIS AND VIRAL INFECTIONS IN CHILDHOOD

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CONSIDERATIONS REGARDING TUBERCULOSIS AND VIRAL INFECTIONS IN CHILDHOOD (Abstract): Tuberculosis is a major public health problem worldwide, with mortality rates that have not decreased significantly in many regions. **Aim**: to evidence a correlation between antecedents of varicella and other infectious diseases caused by viruses in childhood and the subsequent diagnosis of tuberculosis in children and young adults. **Materials and methods**: a descriptive study was conducted on a group of 507 children and young adults, hospitalized for a period of 5 years, in a pneumology hospital from Eastern Romania. **Results**: The frequency ratio indicated a predominance of females (53.8%), from the urban environment (52%), mean age = 11.40 years, close to the median value. Infectious diseases were identified in 38.7% of patients. The most common infections were varicella (28.8% of total; 44.5% aged 8-14 years, p=0.004) and mumps (15.4% of total; 52.6% aged 15-23 years, p=0.001), but rubella (4.7%) and measles (4.1%) in antecedents were also identified. **Conclusions**: Our study highlighted the hypothesis that, by frequency, infectious diseases in antecedents, especially varicella, could predispose to a later TB infection, in children aged of 8-14 years. **Keywords**: TUBERCULOSIS, CHILDREN, VARICELLA, INFECTIOUS DISEASES.

Tuberculosis (TB) is a major public health problem worldwide, with mortality rates that have not decreased significantly, and in some regions are increasing (1). The recent ECDC rapport showed that more than 230,000 people were diagnosticated with TB in 2021, with an incidence of 25/100,000 inhabitants. For the first time in two decades, the incidence rate is estimated to have increased by 1.2% from 2020, reflecting the impact of the disruption of TB surveillance caused by the COVID-19 pandemic (2).

The TB mortality rate at the regional level decreased cumulatively by 52%, from 4.4 to 2.1 deaths per 100,000/inhabitants, in the last decade, when the average annual decline in TB incidence rate was 4.6%, which is significantly higher than the global rate of decline in TB incidence (2%) and the fastest decline in the world compared with other regions. Romania (with -7.9%) was among countries with the highest annual decline rate (2).

TB leads to a latent, asymptomatic infectious process and children under the age of 5 years are more exposed due to an immature immune system. In children with direct and

chronic contact with a carrier, TB can progress rapidly, with respiratory symptoms, but sometimes with miliary tuberculosis and tuberculous meningitis. Diagnosing, treating and preventing TB in children can be difficult, and failure to diagnose can have devastating consequences both for the child and the family, as well as for the community and health services (3).

In areas with at least 100 new and relapsing TB cases, the proportion of TB in children (0-14 years) varied between 1-3%. The difference in childhood TB case rates between regions may reflect differences in case-finding practices (such as contact tracing), the age structure of the population, and the underdiagnosis of pediatric TB. The average percentage of new and relapsing TB patients under the age of 15 in the EU was 4.1% (2).

The evolution of latent infection to active TB is influenced by a series of risk factors, such as age, respectively children under 5 years old, or teenagers (4). Certain comorbidities, especially those that induce a degree of immunosuppression of cellular immunity (5), diabetes, malnutrition, immunosuppressive treatments, chronic renal failure, HIV/AIDS infection or childhood viral infectious diseases (6) are considered important risk factors, which leads to the progression of a latent tuberculosis infection to the disease.

The study aim was to highlight a correlation between antecedents of varicella and other infectious diseases caused by viruses in childhood and the subsequent diagnosis of TB in children and young adults, in an Eastern region of Romania, during a transition period, when varicella vaccination had just entered (7) the National Immunization Programs in European Union.

MATERIALS AND METHODS

A descriptive study was conducted on a group of 507 patients, hospitalized for a period of 5 years (2005-2009), in a pneumology hospital from Eastern Romania. The *inclusion criteria* in the study were the clinical diagnosis of TB, the pediatric age groups and the young adult up to 24 years, and the *exclusion criteria* were the invalidation of TB and age over 24 years.

Statistical methods

The data were centralized in an SPSS 18.0 database and processed with the statistical functions to which they lend themselves, at the significance threshold of 95%. The linear trend is a method of predicting the evolution of the disease in the following period.

In the calculation of the significant difference between two or more groups, depending on the distribution of the value series, at the significance threshold of 95%, for the quantitative variables, we applied: a) *t-Student test* - parametric test that compares the mean values recorded in two groups with normal distributions; The test calculates a *t* based on the mean and standard deviation for each batch, depending on the number of degrees of freedom (df). Starting from the "null hypothesis", which assumes that there is no difference between the studied groups, calculating the value of t and comparing it with the tabular *t*, if the calculated value is greater than the tabular one, then the null hypothesis is rejected, and the difference is declared "statistically significant". The unanimously accepted significance threshold is 95%, (p=0.05). The smaller the value of p is than this value, the stronger the signifi-

cance; b) *F-test (ANOVA)* used when comparing three or more groups with normal distributions, combined with the application of the Bonferroni correction (Bonferroni post-hoc) to reduce the error rate when testing several hypotheses.

The χ^2 test is a non-parametric test that compares two or more frequency distributions from the same population, it is applied when the expected events are excluded. Sometimes when a frequency in the calculation formula is small, Yates is applied to correct the formula, to get a higher estimate of the difference. If the obtained *p* is lower than the tabular one at the 95% significance threshold, the events are not excluded - in this case, the question arises as to whether they are dependent.

RESULTS

The highest number of children diagnosed with TB was highlighted in 2008 (n=172), representing 33.9% of the total study group, predicting an increasing trend in the following period (y = 87 + 4 .8 x) (fig. 1).

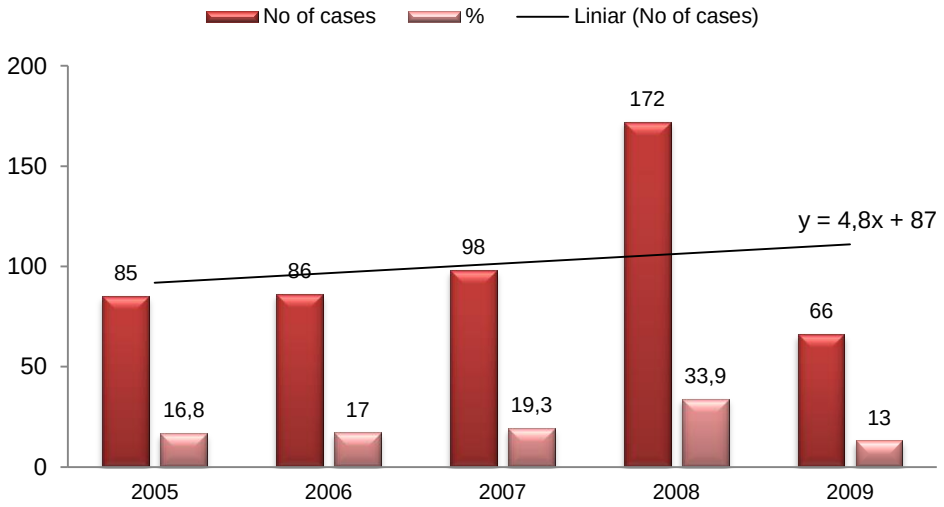

Fig. 1. Number of TB cases in study group

The frequency ratio indicates a predominance of females 1.2 times higher compared to males (53.8% *vs* 46.2%). The frequency of cases from urban environment was slightly higher compared to those from rural areas (52% *vs* 48%) (tab. I).

The age of the children varied from 1 month to 23 years, registering an average age of 11.40 years ± 5, close to the median value of the study group (tab. I, fig. 2).

Antecedents of infectious pathology was identified in 196 children (38.7% of the total study group) (tab. I), from which 41.3% were diagnosed with TB in 2008 (p=0.015) (fig. 3).

Among the 196 cases with TB and *infectious pathology in antecedents*, 104 patients (53.1%; p=0.778) were females, 82 children aged between 8-14 years (41.8%; p=0.001), and 118 cases (60.2% p=0.003) from urban areas (fig. 4).

TABLE I.

Descriptive statistics of age (years)

N	Valid	505
	Missing	2
Mean		11.40
Median		12.00
Std. Deviation		5.00
Variance		25.00
Skewness		-0.310
Std. Error of Skewness		0.109
Minimum		1 month
Maximum		23 years
Percentiles	25	7.00
	50	12.00
	75	16.00

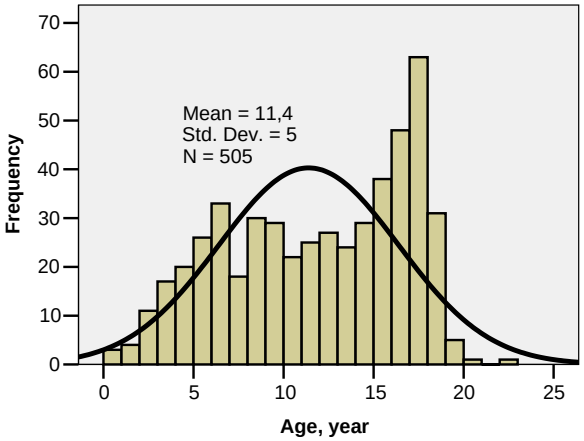

Fig. 2. Age histogram

TABLE II.

Baseline characteristics of the total study group

Parameters		n	%
Demographic data			
Sex	Male	234	46.2
Age (y)	mean±SD	11.40 ± 5.0	
Area	Urban	263	52.0
History of infectious diseases			
Infectious pathology		196	38.7
Varicella		146	28.8
Measles		21	4.1
Rubella		24	4.7
Mumps		78	15.4
HIV		7	1.4
Other		2	0.4

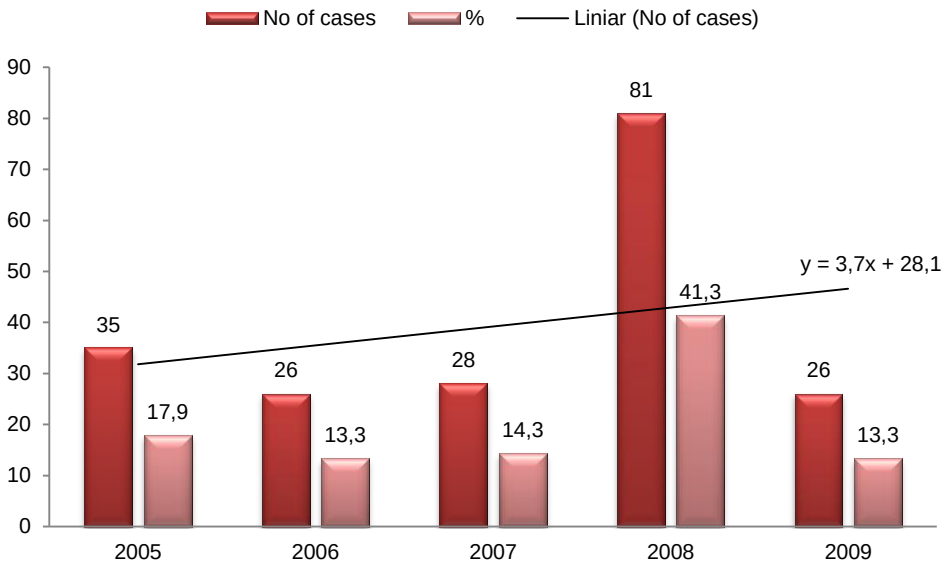

Fig. 3. Distribution of TB cases with infectious pathology in antecedents

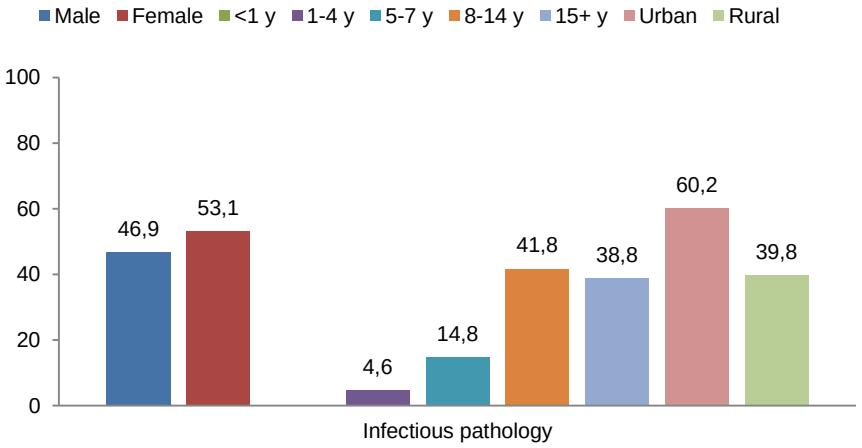

Fig. 4. Distribution of cases with TB and infectious pathology according to demographic characteristics

The most frequent infections were varicella (28.8%) and mumps (15.4%), but a percentage of 4.7% cases of rubella and 4.1% cases of measles in antecedents were also registered (tab. I).

Among the 146 cases with TB and *varicella in antecedents*, 82 children (56.2%; p=0.505) were females, 65 children aged between 8-14 years (44.5%; p=0.004), 89 cases (61%; p= 0.01) from the urban environment (fig. 5).

Among the 78 cases with TB and *mumps*

in antecedents, 40 children (51.3%; p=0.324) were males, 41 patients over 15 years old (52.6%; p=0.001), and 50 children (64.1%; p=0.019) from the urban areas (fig. 6).

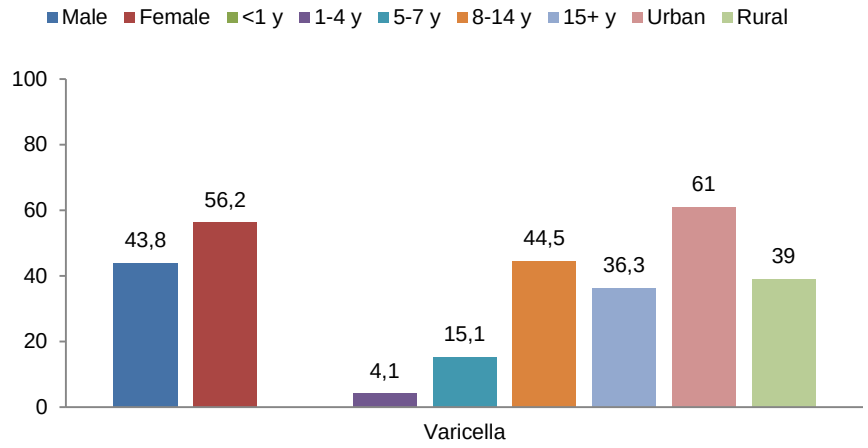

Fig. 5. Distribution of TB and varicella cases by demographic characteristics

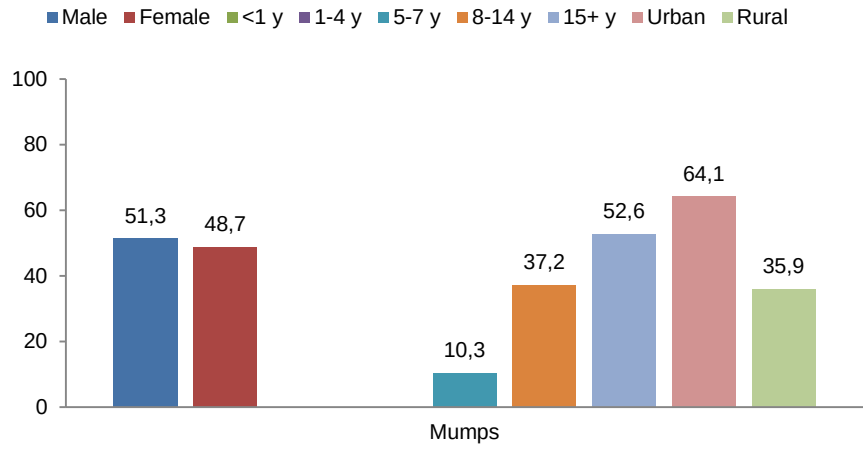

Fig. 6. Distribution of TB and mumps cases by demographic characteristics

Among the 24 cases with TB and *rubella in antecedents*, 16 children (66.7%; p=0.191) were females, 10 children aged between 8-14 years (41.7%; p=0.698), and 19 patients (79.1%; p= 0.005) from urban environment (fig. 7).

Among the 21 cases with TB and *measles in antecedents*, 12 children (57.1%; p=0.303) were males, 11 patients over 15 years old (52.4%; p=0.152), and 13 children (61.9%; p=0.350) from the urban areas (fig. 8).

The Skewness test suggested that the *monitored laboratory parameters* did not have homogeneous values; therefore, the significance tests for continuous variables could not be applied on the study group (tab. IV).

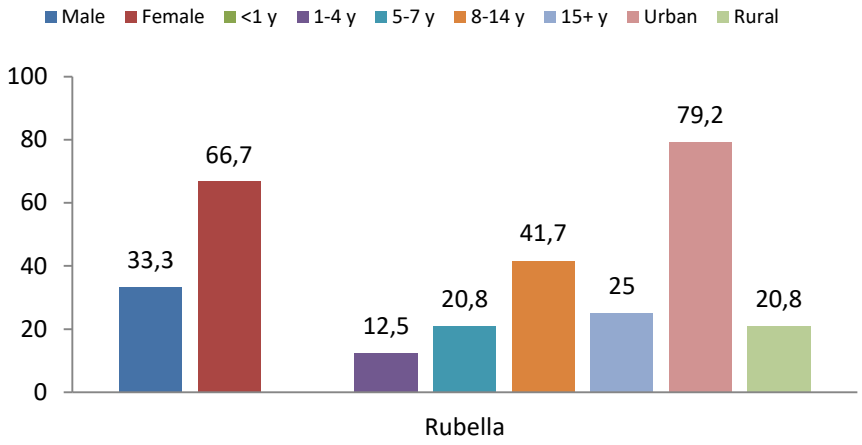

Fig. 7. Distribution of TB and rubella cases by demographic characteristics

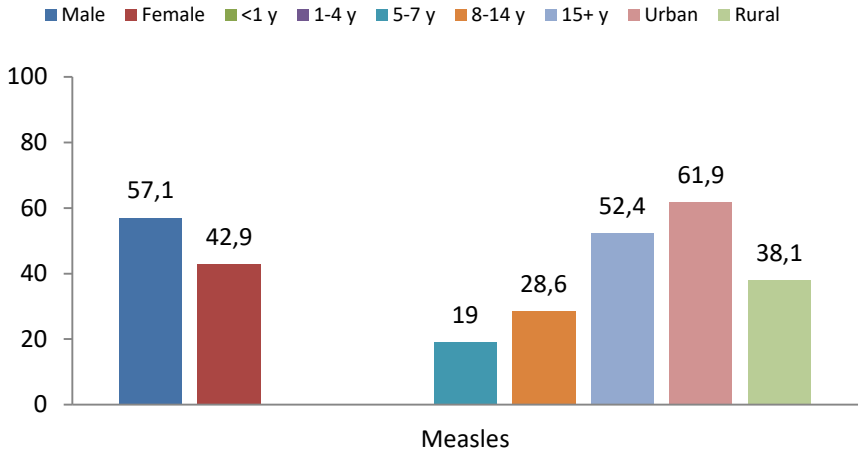

Fig. 8. Distribution of TB and rubella cases by demographic characteristics

TABLE III.

Average age according to infectious pathology

Infectious pathology	n	Mean Age ± DS		P values for *t*-Student test
		With infectious pathology	Without infectious pathology	
Measles	21	13.05±4.33	11.32±5.02	0.122
Varicella	146	11.84±4.30	11.22±5.25	0.201
Rubella	24	10.08±4.83	11.46±5.00	0.188
Mumps	78	13.60±3.75	10.99±5.10	0.001
HIV	7	14.86±4.85	11.35±4.99	0.050
Other	2	13.50±0.71	11.39±5.01	0.552

TABLE IV.
Descriptive statistics of clinical parameters

		Hb	Ht	ESR	Lc	ALT	AST	Creatinine
N	Valid	486	481	471	463	490	489	441
	Missing	21	26	36	44	17	18	66
Mean		12.26	36.85	24.73	8.33	23.71	26.31	0.92
Median		12.40	37.50	13.00	7.80	21.00	24.00	0.83
Std. Deviation		1.76	5.97	28.09	3.28	16.28	12.20	1.14
Variance		3.09	35.68	789.32	10.78	264.95	148.90	1,30
Skewness		-2.665	-3.054	2.154	2.661	6.089	1.746	18.884
Std. Error of Skewness		.111	.111	.113	.113	.110	.110	0.116
Minimum		2.00	2.00	2	2.00	2	2	0.18
Maximum		18.10	64.40	225	41.00	228	98	24.00
Percentiles	25	11.60	35.00	6.00	6.40	16.00	19.00	0.75
	50	12.40	37.50	13.00	7.80	21.00	24.00	0.83
	75	13.20	39.70	35.00	10.00	28.00	31.00	0.91

HB-hemoglobin; HT - hematocrit; ESR - erythrocyte sedimentation rate;
Lc- leucocyte count; ALT - alanine aminotransaminase; AST - aspartate aminotransferase.

DISCUSSION

Our study showed that in the group of pediatric patients, 38.7% of children had a history of infectious diseases, of which 28.8% mentioned varicella, 15.4% - mumps, but there were patients who also suffered from rubella - 4.7%, measles - 4.1%. HIV/AIDS was reported in 7 cases (1.4%).

The immune system in children might remain modified after an infectious disease, so that an exposure to *M. tuberculosis* could lead later to TB, especially on this modified immune background. Although several reports have been published, the correlation between infections with other pathogens and TB has not yet been well deciphered (6). A significant epidemiological overlap has been found between certain infectious diseases and TB in children, such as the geographical distribution of an increased prevalence of TB in children and adolescents and a high prevalence of infections with herpes viruses, Ebstein-Barr virus, malaria or HIV/AIDS (8).

A case report of primary cutaneous TB with *M. tuberculosis* was published by researchers in Turkey. The pediatric patient was diagnosed with varicella infection in antecedents. The authors considered that this case could be explained by cellular immunosuppression mediated by the varicella-zoster virus and its involvement in a subsequent TB immune response (9).

Varicella can lead to decreased cellular immunity by decreasing CD4+ T lymphocytes, increasing suppressor CD8+ T-lymphocytes, previous studies showing significant changes in T lymphocyte subsets during the acute phase of infection, including a decrease in CD4+ T cells and an increase in CD8+ T cells (10). Depletion of CD4+ T cells results in higher viral loads, prolonged viremia, and more severe clinical forms (11). CD4+ T cells depletion is accompanied by delayed and reduced varicella-specific antibody and CD8+ T cells responses (12).

Other studies carried out in various regions, have mentioned a correlation between antecedents of infectious diseases such as measles (13) or cytomegalovirus infection (14) and subsequent TB in children or young adults.

The *limitations of our study* referred to a short period of time, the inclusion in the study of certain cases from a specific region of the country, and also to the lack of a control group, in order to be compared

with the cases, for the development of statistically more rigorous conclusions. Moreover, the period of time between the antecedents of infectious diseases and the diagnosis of TB was not mentioned in the medical records from where the data were collected, and this period is useful and important in understanding this correlation attributed to the immune system disturbed (6) by viral diseases.

The *perspective of our study* is related to further research regarding the relationship between TB in children and adolescents and viral infections in childhood, by collecting more details about their epidemiology and demographic data of the study groups. Such information would bring additional data in the diagnosis and treatment of children with TB and would prove useful in vaccination (15) strategies.

CONCLUSIONS

Our descriptive study highlighted the hypothesis that, by frequency, infectious diseases in antecedents, especially varicella, could influence a later TB diagnosis, in children aged of 8-14 years from urban areas.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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CLINICAL, PATHOLOGICAL AND MOLECULAR INSIGHTS IN METASTATIC COLORECTAL CANCER WITH BONE METASTASES: A RETROSPECTIVE STUDY

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CLINICAL, PATHOLOGICAL AND MOLECULAR INSIGHTS IN METASTATIC COLORECTAL CANCER WITH BONE METASTASES: A RETROSPECTIVE STUDY (Abstract): Metastatic colorectal cancer (mCRC) represents a complex disease entity characterized by diverse evolutionary trajectories stemming from its inherent heterogeneity, influenced by a myriad of clinical, pathological, and molecular factors. Among the spectrum of mCRC cases, those harboring bone metastases constitute a rare subset. **Materials and methods:** This retrospective study investigated 334 patients diagnosed with mCRC and treated at the Regional Institute of Oncology Iași between 2012 and 2019, of whom 35 manifested bone metastases. Comprehensive characterization encompassing clinical, pathological, molecular features, and prognostic determinants was undertaken for this specific cohort. **Results:** The incidence of bone metastases was recorded at 10.5%, with a median age at diagnosis of 65 years, predominantly affecting males (60%), and demonstrating a predilection for primary tumors located in the left colon (57.1%). Peritoneal (26.5%) and hepatic (17.1%) metastases emerged as frequently associated secondary sites. Histologically, 73.5% of subjects presented with grade 2 differentiated tumors, with KRAS mutations identified in 37.1% of cases, while NRAS and BRAF mutations were detected in 2.8% only. The treatment most commonly used by clinicians was a chemotherapy doublet consisting of a fluoropyrimidine (5-fluorouracil or capecitabine) combined with oxaliplatin in 85.6% of cases, with the addition of an angiogenesis inhibitor (Bevacizumab) in 77.1% of cases. Prognostic indicators revealed a median progression-free survival of 10.7 months and an overall survival of 17.3 months. First line anti-EGFR biological treatment was associated with increased survival, meanwhile peritoneal metastases and NRAS mutation were unfavorable prognostic factors. Despite enriching the understanding of this rare cohort of mCRC patients, the study underscores the imperative for prospective investigations with augmented sample sizes to delineate better the characteristics of this specific subset. **Conclusions:** Patients exhibited worse PFS and OS compared to those described in the literature for other metastatic sites. Among the identified prognostic factors, anti-EGFR treatment was associated with a favorable outcome, while the presence of peritoneal metastases and NRAS mutation were indicative of a poorer prognosis. **Keywords:** METASTATIC COLORECTAL CANCER,

BONE METASTASES, CLINICAL, PATHOLOGICAL AND MOLECULAR FEATURES, PROGNOSTIC FACTORS.

Colorectal cancer (CRC) ranks as the third most prevalent neoplasm worldwide. In its metastatic phase, the median survival typically ranges from 24 to 30 months (1). However, clinical observations have revealed diverse patterns of disease progression that often diverge from established prognostic factors (2).

Metastatic colorectal cancer (mCRC) frequently disseminates to the liver (70%) and lungs (37%), while bone metastases occur less frequently, affecting roughly 3-7% of CRC patients (3, 4). Over the past decade, the incidence of secondary bone lesions has risen due to enhanced survival rates among mCRC patients and advancements in medical imaging technology (5). These patients constitute a distinct subgroup with an unfavorable prognosis, as evidenced by a median overall survival (OS) of approximately 10 months. Prognostic factors for patients with bone metastases remain uncertain, and the development of a well-defined prognostic model is yet to be realized. Furthermore, the role of palliative chemotherapy in CRC patients with bone metastases has received limited investigation (6-8).

This study aims to describe the clinical, histopathological, molecular characteristics, and prognosis of patients with mCRC identified in a tertiary center in Romania to better understand the peculiarities of this type of mCRC and to complement the scarce literature data. Additionally, we attempted to identify prognostic factors for this patient category.

MATERIALS AND METHODS

Aim of the study. This retrospective study aimed to investigate the clinical, pathological, and molecular characteristics of patients diagnosed with mCRC presenting with bone metastases and additionally to assess the prognostic significance of various parameters routinely used in the clinical practice.

Study population and data collection. The study cohort consisted of patients treated at the Regional Institute of Oncology Iasi between 2012 and 2019. Inclusion criteria encompassed patients aged 18 or older, histopathological diagnosis of colorectal carcinoma, imaging confirmation of bone metastases through CT and/or PET-CT and/or MRI and/or bone scintigraphy, Eastern Cooperative Oncology Group (ECOG) performance status of 0-2, and a minimum of 6 months of systemic treatment. Patients who received adjuvant systemic treatment for colorectal cancer CRC in stages I-III were excluded.

Ethical approval was obtained from the Ethics Committee of the Regional Institute of Oncology Iasi and the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy of Iasi.

Data were extracted from the Hippocrate electronic database of the Regional Institute of Oncology Iasi. Various parameters were evaluated in the database, including socio-demographic factors (age, sex), clinical characteristics (ECOG performance status, primary tumor localization, metastasis type), surgical interventions (primary tumor surgery and metastasis excision), pathological features (grading), molecular markers (KRAS, NRAS, BRAF), and survival parameters (PFS and OS).

Statistical analysis. Was performed using *SPSS software version 25.0*. Univariate analysis with the log-rank test and Cox regression were utilized to identify prognostic factors and construct a multivariate model. The study aimed to delineate the clinical, pathological, and molecular profiles of patients with bone metastases and mCRC, while also assessing the prognostic significance of these parameters.

RESULTS

The characteristics of the study population can be seen in detail in tab. I. The median age at diagnosis was 65 years, with 60% (n=21) of patients being male. 88.5% (n=30) had an ECOG performance status of 1. The primary tumor was more frequently located in the left colon, accounting for 57.1% (n=20) of cases. Peritoneal metastases were occurring in 26.5% (n=9) of cases, while 73.5% (n=25) of subjects had G2 tumors. Primary tumor resection was performed in 51.4% (n=18) of patients. Looking at the molecular landscape, KRAS mutation was detected in 37.1% (n=13) of patients, while NRAS and BRAF mutations were each found in 2.8% (n=1) of patients.

TABLE I.
Characteristics of the study population

No. of cases	35 (100%)	
Median age (years)	65	
Gender	Male	21 (60%)
	Female	14 (40%)
ECOG PS	0	1 (2.8%)
	1	30 (85.7%)
	2	2 (5.8%)
Primary tumor location	Left colon	20 (57.1 %)
	Right colon	15 (42.9 %)
Metastases	Pulmonary	2 (5.7%)
	Hepatic	6 (17.1%)
	Peritoneal	9 (26.5%)
	Cerebral	1 (2.9%)
Grading	G1	4 (11.8%)
	G2	25 (73.5%)
	G3	5 (14.3%)
Primary tumor surgery	No	17 (48.6%)
	Yes	18 (51.4%)
Familial history of cancer	No	33 (94.2%)
	Yes	2 (5.8%)
Personal history of cancer	No	31 (88.5%)
	Yes	4 (11.8%)

Metastases surgery	No	31 (88.6%)
	Yes	4 (11.4%)
KRAS mutation	No	22 (62.9%)
	Yes	13 (37.1%)
NRAS mutation	No	1 (2.8%)
	Yes	34 (97.1%)
BRAF mutation	No	1 (2.8%)
	Yes	34 (97.1%)
RAS wild type	No	19 (54.3%)
	Yes	16 (45.7%)
Surgery of primary tumor	No	17 (48.6%)
	Yes	18 (51.4%)
Chemotherapy protocol	FOLFOX	18 (51.4%)
	CAPEOX	12 (34.2%)
	Capecitabine	5 (14.2 %)
Biological treatment	Bevacizumab	27 (77.1%)
	Anti-EGFR	8 (22.8%)

ECOG PS, Eastern Cooperative Oncology Group performance status; KRAS, Kirsten rat sarcoma viral oncogene homolog; NRAS, neuroblastoma ras viral oncogene homolog;

BRAF, serine/threonine-protein kinase B-raf.

The median progression-free survival (PFS) in patients with mCRC and bone metastases in the studied cohort was 10.8 months (7.6-13.9), while the median OS was 17.3 months (15.3-19.3) (figs. 1, 2).

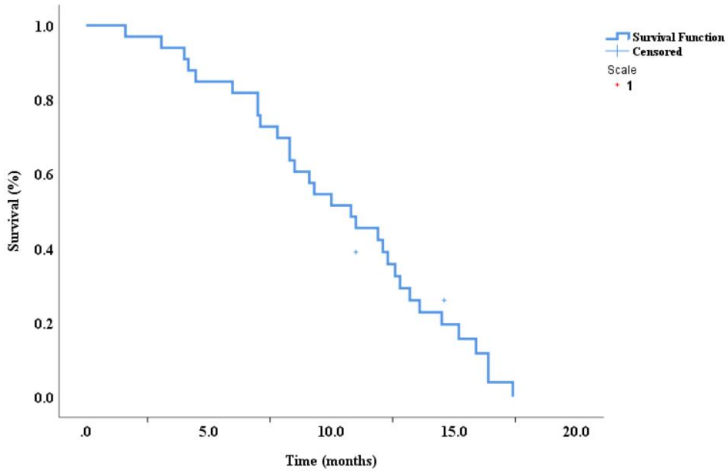

Fig. 1. Progression free survival in patients with CRC and bone metastases

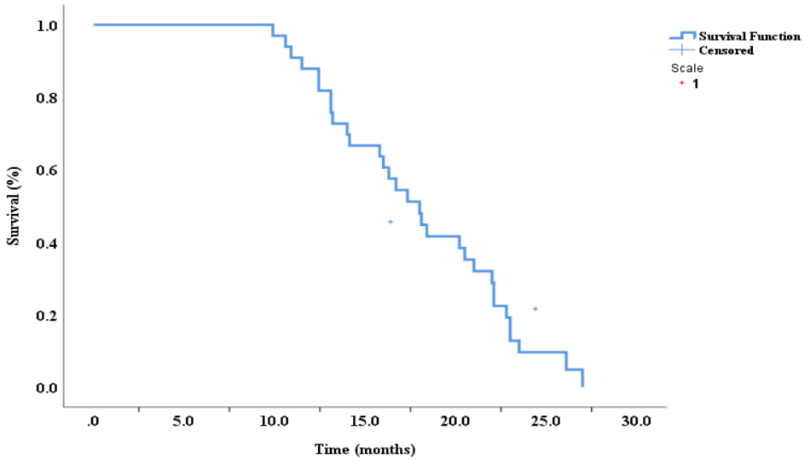

Fig. 2. Overall survival in patients with CRC and bone metastases

We performed univariate analysis to identify prognostic factors for PFS in patients with mCRC and bone metastases. No factors showed prognostic significance (tab. II). Regarding OS, factors identified as prognostic in univariate analysis included sex, peritoneal metastases, NRAS mutations and biological treatment. These factors were subsequently confirmed in multivariate analysis (tab. III, tab. IV).

TABLE II.

Univariate analysis for multiple factors regarding PFS

Z	p value	Hazard ratio (95% C.I.)
Sex	0.03	2.27 (1.04-4.97)
Age	0.78	0.89 (0.42-1.91)
Familial history of CCR	0.24	2.47 (0.54-11.21)
Personal history of CCR	0.07	0.25 (0.95-1.16)
ECOG PS	0.43	0.62 (0.18-2.1)
Primary tumor location	0.98	1.1 (0.48-2.09)
Liver metastases	0.96	1 (0.3-3.5)
Peritoneal metastases	0.06	2.27 (0.95-5.4)
Pulmonary metastases	0.1	5.88 (0.68-50.38)
Grading	0.17	1.74 (0.78-3.8)
Primary tumor surgery	0.66	1.17 (0.57-2.41)
KRAS mutation	0.99	1.01 (0.45-2.2)
NRAS mutation	0.34	2.67 (0.34-20.9)
BRAF mutation	0.4	2.3 (0.3-18.4)
RAS wild type	0.17	0.58 (0.26-1.27)
Chemotherapy protocol	0.86	1.03 (0.69-1.54)
Biological treatment	0.67	0.67 (0.21-2.14)

CCR, colorectal cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; KRAS, Kirsten rat sarcoma viral oncogene homolog; NRAS, neuroblastoma ras viral oncogene homolog; BRAF, serine/threonine-protein kinase B-raf.

TABLE III.

Univariate analysis for multiple factors regarding OS

Parameter	p value	Hazard ratio (95% C.I.)
Sex	0.04	2.21 (1.1-4.75
Age	0.18	1.61 (0.79-3.2)
Familial history of CCR	0.57	1.53 (0.34-6.77)
Personal history of CCR	0.1	2.6 (0.81-8.3)
ECOG PS	0.39	1.68 (0.5-5.69)
Primary tumor location	0.41	1.34 (0.65-2.72)
Liver metastases	0.39	1.6 (0.54-4.7)
Peritoneal metastases	0.02	2.7 (1.15-6.51)
Pulmonary metastases	0.72	1.44 (0.19-10.92)
Grading	0.83	1.09 (0.48-2.46)
Primary tumor surgery	0.65	0.85 (0.41-1.72)
KRAS mutation	0.44	0.75 (0.36-1.55)
NRAS mutation	0.04	10.15 (1-97.66)
BRAF mutation	0.4	2.38 (0.3-18.5)
RAS wild type	0.57	0.81 (0.39-1.66)
Chemotherapy protocol	0.93	1.01 (0.64-1.61)
Biological treatment	0.05	0.27 (0.07-1)

CCR, colorectal cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; KRAS, Kirsten rat sarcoma viral oncogene homolog; NRAS, neuroblastoma ras viral oncogene homolog; BRAF, serine/threonine-protein kinase B-raf.

TABLE IV.

Multivariate analysis for OS

Parameter	p value	Hazard ratio (95% C.I.)
Sex	0.23	1.73 (0.7-4.27)
Peritoneal metastases	0.005	3.66 (1.47-9.08)
NRAS mutation	0.05	11.61 (0.99-31.9)
Biological treatment	0.04	0.26 (0.07-0.98)

NRAS, neuroblastoma ras viral oncogene homolog;

Peritoneal metastases were identified as a negative prognostic factor, with a median OS of 16.4 months compared to 18.1 months in subjects without this type of secondary lesions (P=0.005, HR=3.66, 95% CI, 1.47-9.08) (fig. 3). NRAS mutation was associated with a median OS of 11.5 months, which was 5.8 months less than in patients without NRAS mutation (P=0.006, HR=29.2, 95% CI, 2.68-31.9) (fig. 4.). Anti-EGFR treatment increased OS, with patients receiving Bevacizumab having an OS of 29.2 months compared to 47.8 months in those treated with Cetuximab or Panitumumab (P=0.04, HR=0.26, 95% CI 0.07-0.98) (fig. 5).

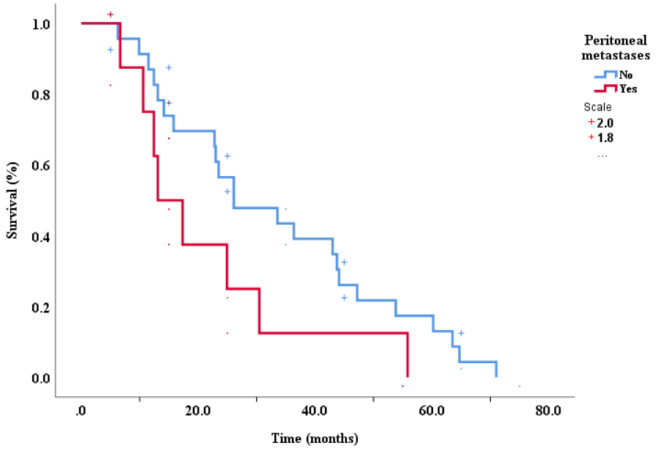

Fig. 3. Peritoneal metastases and OS in patients with mCRC and bone metastases

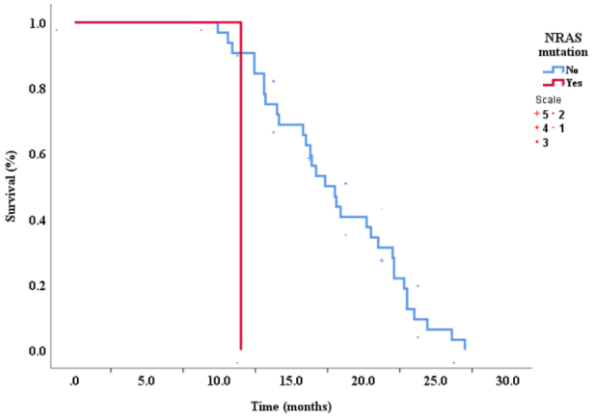

Fig. 4. NRAS mutations and OS in patients with CRC and bone metastases

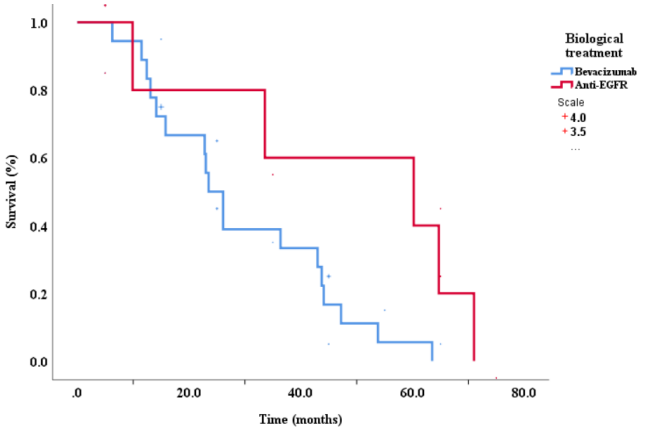

Fig. 5. Biological treatment and OS in patients with CRC and bone metastases

DISCUSSION

Regarding the conducted study, we have discussed the most significant clinical, pathological and molecular aspects for patients with CRC and secondary bone lesions.

Incidence. In the current study, we assessed the incidence of secondary bone lesions in 334 patients with mCRC treated at IRO Iași between 2012 and 2019. The overall incidence was 10.5% when considering all types of secondary lesions and 5.1% in patients with only bone lesions.

Bone metastases are exceedingly rare, particularly when occurring as the sole site of metastasis. Existing literature reports a frequency ranging from 5% to 11% (9,10). CRC demonstrates a distinctive metastatic pattern, with the liver and lungs being the predominant sites, while metastases to the adrenal glands, bones, brain, and skin are infrequently documented (3). In a study encompassing 5352 patients over a 25-year span, Kanthan *et al.* identified a frequency of 6.6%, with only 17% of these cases presenting isolated bone metastases, resulting in a frequency of 1.1%. Additionally, 83% of patients with bone metastases were concomitantly associated with other metastatic sites (11). The rarity of such occurrences suggests a distinct biological behavior in metastatic dissemination.

Numerous studies have suggested that the presence of portal circulation significantly predisposes the development of hepatic and pulmonary secondary lesions in mCRC. This predilection may be attributed, as posited by earlier research, to the dense capillary networks present in the lungs and liver, facilitating the lodging and subsequent metastatic growth of CRC tumor cells (7). Furthermore, alongside the hemodynamic theory of metastasis, the theory of organ selectivity also assumes importance. It has been elucidated that each organ harbors a unique tumor microenvironment, inclusive of blood vessels that modulate tumor cell behavior through specific signaling pathways, thereby influencing adhesion molecules crucial for tumor dissemination and implantation (12,13).

Sex. The male gender may potentially correlate with a heightened susceptibility to bone metastases although findings in the literature are inconclusive. A study conducted in China by Liu reported a greater incidence of bone metastases among men. One plausible rationale for this observation could be that men more frequently present primary tumors in the rectum, a site commonly associated with bone metastases (5). Similarly, a study by Riihimaki *et al.* demonstrated a significantly elevated risk of bone metastases in men compared to women within the population of patients diagnosed with colon cancer (3). However, several smaller-scale investigations have failed to establish a conclusive association between gender and the manifestation of metastases in mCRC (14,15). In the present study, 60% of the subjects were male and 40% were female, aligning with the existing literature findings.

Age. In Riihimaki's investigation, a correlation was observed between younger age (under 60 years old) and a heightened occurrence of bone metastases. Patients aged over 79 years exhibited an odds ratio for developing bone metastases of 0.4 in comparison to those under 60 years old (3). Typically, younger age is indicative of a more biologically aggressive disease profile (16). In our study, the median age of 65 years was consistent with findings reported in existing literature.

Primary tumor location. The prevalence of bone metastases was notably higher in the left colon (57.1%, n=20). A systematic review revealed an association between the distal localization of the primary tumor and the occurrence of bone metastases. Eight studies reported an elevated risk of

bone metastases development for tumors situated in the rectum (3, 5, 14, 15, 17-20), with an odds ratio (OR) of 1.5 according to Riihimaki's investigation (3). Additionally, Chiang *et al*. observed a heightened risk for distal rectal tumors compared to proximal rectal tumors (20). The underlying pathophysiological mechanism may be attributed to the presence of the Batson venous plexus, a network of valveless veins connecting the thoracic and deep pelvic veins to the internal vertebral venous system (21).

Other types of metastases. The study cohort predominantly exhibited peritoneal (26.5%), hepatic (26.5%), and pulmonary (5.7%) secondary lesions. In contrast to our findings, metastases to lymph nodes post-primary surgery and pulmonary metastases at any point were identified as potential risk factors for bone metastases development (22). All studies indicated an increased risk association between bone metastases and pulmonary metastases (19), with reported odds ratios ranging between 2.5-4.8 in patients with lung secondary lesions (10,14). While some studies suggested an association between liver metastases and bone metastases, the data remain inconclusive due to conflicting findings (23). Notably, our study reported a higher frequency of peritoneal metastases alongside bone metastases; however, it's essential to acknowledge the limited size of the study cohort, and statistical significance was not established in this context.

Primary tumor surgery. Remains a subject of ambiguity in mCRC management, often recommended for patients with advanced tumors causing symptomatic presentations (24). In our examined cohort, 51.4% of patients underwent primary tumor resection, indicating a significant proportion of cases with symptomatic and advanced tumors. Notably, conclusions regarding the potential association between T stage and the risk of bone metastases could not be definitively drawn. Sundermeyer's study suggested a correlation between early-stage tumors and bone metastases, while conflicting evidence from another study proposed an association between advanced tumors and bone metastases (19).

Regarding **pathological subtypes,** signet ring cell carcinoma demonstrates a potential association with increased susceptibility to bone metastases, although this relationship remains underexplored. Conversely, other histological subtypes do not seem to influence the likelihood of bone metastases (25). Two studies noted an association with the mucinous subtype among patients with metastases (23,26). However, in our investigation, information regarding tumor pathological subtypes was not available.

Degree of differentiation did not exhibit an association with bone metastases in our study. The majority of patients presented with moderately differentiated (G2) tumors, accounting for 73.5%. Although four studies did not identify associations with poorly differentiated tumors, high-grade tumors were linked to a shorter duration before the onset of bone metastases (7, 27).

KRAS, NRAS, and BRAF genetic mutations were detected in 37.1% (n=13) of patients, with NRAS and BRAF mutations each present in 2.8% (n=1) of patients. Hypotheses suggest that distinct molecular profiles of cancer may elucidate metastatic patterns, potentially increasing tumor propensity for seeding specific organs such as bone, brain, or lung. RAS mutations are recognized predictors of unfavorable responses to anti-EGFR treatment and are associated with aggressive biological profiles (28-30).

Of the mutations evaluated for association with bone metastases, only RAS mutations have been identified as potentially favorable factors, albeit with limited and conflicting evidence. Four studies investi-

gated the relationship between RAS mutations and bone metastases (18, 31, 32). Yaeger's study revealed a significant association between RAS mutations and bone metastases in multivariate analysis, reporting a hazard ratio (HR) of 1.62 (18,28). Kemeny *et al.* focused solely on KRAS mutations in exon 2 but observed a significant incidence of bone metastases in patients with mutations compared to wild type (28). Conversely, two other studies did not establish a significant association between RAS mutations and bone metastases (31,32).

The potential association between RAS mutations and bone metastases may arise from differential gene expression and protein activation, including variations in integrin expression between RAS wild-type and RAS-mutated colon cancer cells (33). Studies have suggested an organ-specific exosomal protein expression pattern influencing the creation of a premetastatic niche (34). Specifically, exosomal integrin has been implicated in directing metastatic dissemination to bone (35).

Treatment modalities for bone metastases have been documented in three studies, encompassing bisphosphonates, chemotherapy, radiotherapy, surgery, and analgesic therapy. The efficacy of bisphosphonates was statistically assessed across all three studies (5, 7, 36). However, the randomized trials were characterized by small sample sizes and divergent methodologies, yielding low-level evidence regarding the potential of bisphosphonates to delay adverse skeletal events such as pathological fractures, spinal cord compression, and the need for bone radiation therapy, as well as alterations in antineoplastic therapies and bone surgery. Notably, no significant impact on OS was observed (8). Denosumab's efficacy in CRC bone metastases remains unexplored, consistent with existing research on antiresorptive treatment effects in bone metastases of lung, breast, and prostate cancers, which have demonstrated a reduction in skeletal events. Investigations into the effects of other therapeutic modalities on CRC bone metastases have been limited. The impact of chemotherapy and radiotherapy on OS following bone metastases diagnosis was examined solely in a retrospective cohort study. Systemic chemotherapy demonstrated an improvement in OS in patients with bone metastases based on univariate analysis; however, it's noteworthy that the majority of patients also presented with visceral metastases, potentially influencing the observed increase in OS (37). In our study, a high majority of patients received FOLFOX or CAPEOX (85.6%) associated with Bevacizumab (77.1%).

Prognosis. In this study, the median OS was 17.3 months, with a PFS of 10.7 months, representing a superior outcome compared to the majority of previous investigations. Analysis of survival among patients with bone metastases was referenced in 16 studies, revealing a range of median OS durations from 5 to 21 months (8). Khattak and Riihimaki reported a median survival ranging between 5.1 and 5.5 months. Notably, various studies have explored the prognostic outlook of patients with bone metastases in comparison to those with metastases at alternate sites, yielding conflicting results. Khattak and Riihimaki observed that patients with mCRC and exclusive bone metastases exhibited shorter survival durations than those with metastases confined to a single site or affecting other sites such as the liver or lungs (38,39). In the same direction, Qui *et al.* identified a one-year OS rate of 29.6% for patients with bone-only metastases, compared to 60.2% for those with hepatic metastases and 60.2% for individuals with pulmonary metastases (17). Three additional studies reported a more favorable median survival for patients

with solitary bone metastases in comparison to those with concurrent bone and visceral metastases. Several prognostic variables were associated with an unfavorable prognosis, including osteolytic lesions, multiple bone lesions, metastases to the ribs, elevated levels of carcinoembryonic antigen (CEA), presence of other metastatic sites, pulmonary metastases, and bone metastases at initial diagnosis. Nonetheless, variations in study designs, cohorts, and inclusion of different variables rendered direct comparisons challenging. Furthermore, only a minority of studies incorporated multiple variables in their analyses across disparate datasets (7, 40, 41).

In our study, in multivariate analysis for OS, anti-EGFR treatment demonstrated a positive association with increased survival of 17.6 months. Although patients with left-sided mCRC may derive benefits from chemotherapy and anti-EGFR treatment compared to Bevacizumab as first-line therapy, the differences observed in randomized clinical trials were typically around 3 to 7 months (42-44). It is probable that due to the small sample size in our study, these survival benefits were amplified; however, they should be considered more as a point for further hypothesis within this patient subset. Conversely, peritoneal metastases and NRAS mutation emerged as negative prognostic factors for survival, leading to reductions of 1.7 months and 5.8 months, respectively. Notably, these prognostic factors were not previously associated with survival in patients with CRC and bone metastases according to existing literature. Therefore, these findings represent a novel contribution, first described in our study for patients with CRC and bone metastases.

Study limitations. The conducted study provides insights into the clinical, pathological, and molecular characteristics of patients with CRC and bone metastases, along with certain prognostic factors. However, it is crucial to interpret the presented information cautiously as this is a retrospective, single-center study with a small sample size and limited statistical power. Nevertheless, it offers preliminary data regarding this patient subset within a population from Romania for the first time and could serve as a foundational framework for further investigation in prospective, multicenter studies on this topic.

CONCLUSIONS

We investigated the clinical, pathological, and molecular characteristics of a highly rare subset of patients, specifically those diagnosed with mCRC presenting with bone metastases and treated at a tertiary center in Northeast Romania. Subsequently, the observed incidence of bone metastases stood at 10.5%, with a median age of onset being 65 years. These metastases displayed a higher prevalence among males, with primary tumors predominantly localized in the left colon. Tumors were frequently classified as grade 2 differentiation (G2), with KRAS mutations being the most prevalent, while NRAS and BRAF mutations were exceedingly rare occurrences. Patients exhibited worse PFS and OS compared to those described in the literature for other metastatic sites. Among the identified prognostic factors, anti-EGFR treatment was associated with a favorable outcome, while the presence of peritoneal metastases and NRAS mutation were indicative of a poorer prognosis.

CONFLICT OF INTEREST AND FUNDING

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ANTIBIOTIC USE AMONG PATIENTS WITH ACUTE EXACERBATION OF COPD IN PULMONARY DISEASES UNIVERSITY CLINICAL HOSPITAL FROM NORTH-EASTERN ROMANIA

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ANTIBIOTIC USE AMONG PATIENTS WITH ACUTE EXACERBATION OF COPD IN PULMONARY DISEASES UNIVERSITY CLINICAL HOSPITAL FROM NORTH-EASTERN ROMANIA (Abstract): **Aims:** Acute exacerbations of Chronic Obstructive Pulmonary Disease (AECOPD) need sometimes hospitalizations and requires five to seven days of oral antibiotics, according to GOLD recommendations. The aim of our study was to find out the most common used antibiotics in patients with AECOPD admitted in a University Clinical Hospital of Pulmonary Diseases from North-Eastern Romania, from June 2014 to May 2019. **Materials and methods:** A descriptive cross-sectional, single-center, retrospective study, conducted among 460 admissions in hospital with J44.0 (COPD with lower airway acute infection) and J44.1 (COPD with acute, nonspecific exacerbation) diagnostics. **Results:** The majority (354; 77%) of admissions were for male; average age was 66 ± 9 years. The preferred antibiotic for first use was ceftriaxone (51%), but the patients were treated also with ciprofloxacin (13%), levofloxacin (12%), ceftazidime, amoxicillin + clavulanic acid or other antibiotics according to antibiograms, with a medium of 10 days treatment. Most of the patients received one antibiotic during hospitalization, 23.9% received two antibiotics and 7.6% of patients received 3 antibiotics. Hospitalization average length was 11 ± 5 days. **Conclusions:** Ceftriaxone was the preferred drug for acute COPD exacerbation treatment for hospitalized patients, while the average hospital length in our study was 11 days. **Keywords**: ANTIBIOTIC USE, ACUTE EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE, LENGTH OF HOSPITAL STAY.

INTRODUCTION

Antimicrobial chemotherapy choice is a challenge in patients with Chronic Obstructive Pulmonary Disease (COPD), due to both bacterial and viral causes of acute exacerbation. COPD, one important cause of mortality and morbidity worldwide (1), had a hospital in-patient admission rate of 4.70% in Romania in 2017. The most frequent bacteria causing infections in COPD patients are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, but other bacteria such as Enterobacteriaceae and *Chlamydia pneumoni-*

ae might also be involved (2). Acute exacerbations of COPD (AECOPD) need sometimes hospitalizations and have socio-economic impact through severe deterioration of general health status, the progression to complications of disease, and increase use of antibiotic consumption (3-5).

COPD exacerbations require five to seven days of oral antibiotics, according to GOLD recommendations. The initial therapy of AECOPD is with an association of aminopenicillins and clavulanic acid, tetracyclines (doxycycline) or macrolides (azithromycin), in selected patients quinolone (5) and identification of the bacteria and antibiogram are needed. A judicious use of antibiotics administration for AECOPD decreases the risk of treatment failure and early relapse.

There are very few studies on the use of antibiotics in AECOPD in North-East Romania, therefore the aim of our study was to find out the most common used antibiotics in patients with AECOPD admitted in a University Clinical Hospital of Pulmonary Diseases from North-Eastern Romania, from June 2014 to May 2019.

MATERIALS AND METHODS

Study design

This is a descriptive cross-sectional, single-center, retrospective study, conducted in Iasi University Clinical Hospital of Pulmonary Diseases from North-Eastern Romania (340 beds- adult and pediatric pulmonology, thoracic surgery and intensive care). The study received the approval from the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, Romania, and the Ethics Committee of Clinical Hospital of Pulmonary Diseases of Iasi, Romania (approval date 04.06.2020, respectively 22.01.2021).

The inclusion criteria were: 1) Patients of more than 40 years, with diagnosis of COPD, based on spirometry (with the ratio post-bronchodilator forced expiratory volume in one second (FEV1)/ forced vital capacity (FVC) < 0.70),admitted in the hospital, 2) specific J44diagnosis codes of COPD established by International Classification of Diseases 10thRevision (ICD-10): J44.0 - COPD with lower airway acute infection, J44.1 - COPD with acute, nonspecific exacerbation; 3) more than 24 h of hospital length; 4)received at least one antibiotic during hospitalization. There were excluded: patients admitted to the intensive care unit (ICU), other chronic pulmonary diseases (asthma, pulmonary interstitial disease, and pulmonary embolism), any type of cancer disease, tuberculosis under treatment and incomplete data in clinical record file.

There were extracted the following data from electronic and paper medical clinical record files: date of admission and discharge from hospital, length of hospitalization, age, gender, administered antibiotics, bacterial pathogen from antibiogram.

Data Analysis

Results are presented as mean ± standard deviation (SD) for continuous variables and as absolute values and percentages for categorical variables.

RESULTS

A total of 460 admissions in hospital with J44.0 - COPD with lower airway acute infection and J44.1 - COPD with acute, nonspecific exacerbation, were included. They were 354 (77%) admissions for male and 106 (23%) admissions for female. The age of patients varied from 44 years to 88 years, average age was66 ±9 years.

The patients were treated with ceftriaxone, ciprofloxacin, levofloxacin, ceftazidime, amoxicillin + clavulanic acid or cefuroxime according to antibiograms, with a medium of 10 days treatment. In COPD

patients most of the time treatment was started with cephalosporins, rarely with amino-penicillins. Other antibiotic that was recommended in small proportion were clarithromycin, gentamicin, clindamycin, trimethoprim/sulfamethoxazole. The preferred antibiotic for first use was ceftriaxone (fig. 1). Most of the patients received one antibiotic during hospitalization, 23.9% received two antibiotics and 7.6% of patients received 3 antibiotics; the predominant route was intravenous. The predominant bacteria in the sputum were Gram Negative bacteria, followed by *Streptococcus spp.*, while MRSA (methicillin resistant *Staphylococcus aureus*) bacteria was not detected.

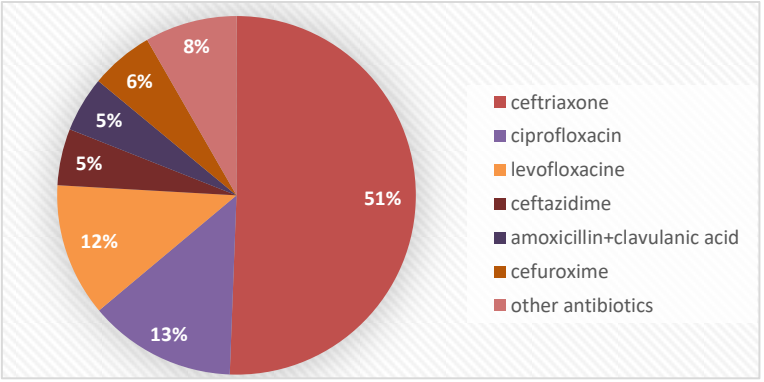

Fig. 1. Patients antibiotic therapy during hospitalization.

Trend of antibiotic use, depending on trimesters and year are depicted in figure 2. There was a higher use of ceftriaxone and ciprofloxacin in the third trimester of 2015.

Hospitalization average length was 11 ± 5 days (fig. 3), the longest duration of hospital stay being of 38 days due to comorbidities. Most of length of hospital stay was in the category of mid-stay: 60 brief-stay (2-6 days), 396 mid-stay (7-30 days) and 4 long-stay (>31 days).

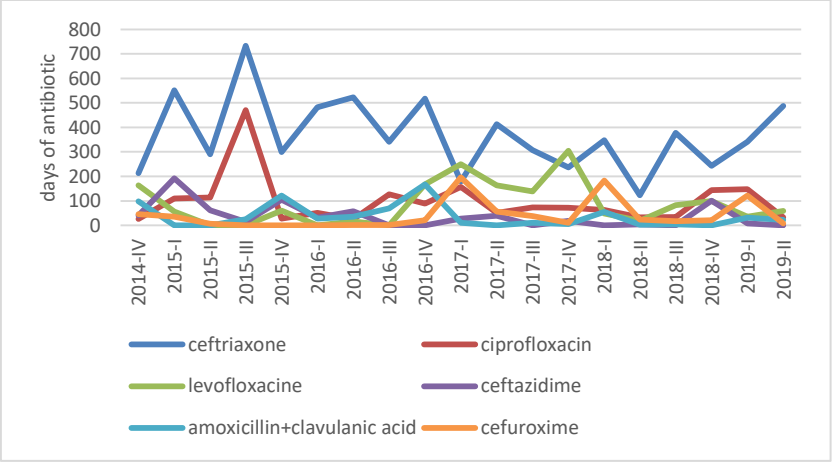

Fig. 2. Trend of antibiotic use (days of antibiotic).

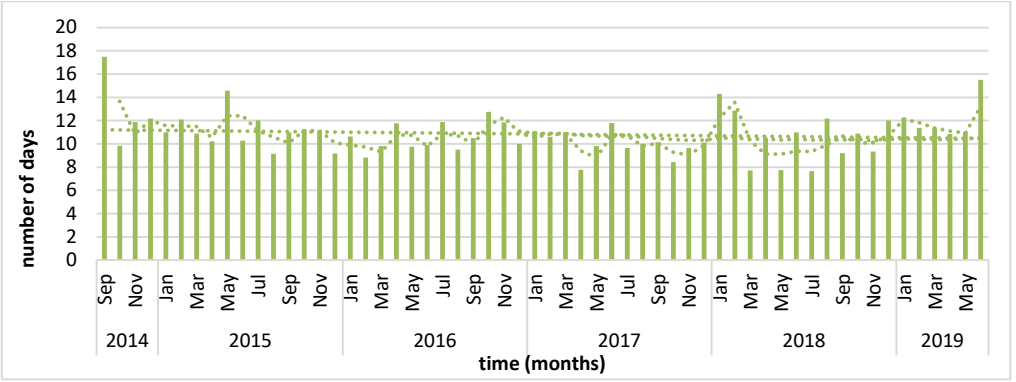

Fig. 3. Average of hospital day length (days)

DISCUSSION

Our findings showed that the preferred antibiotic to be used in COPD patients was ceftriaxone, followed by ciprofloxacin or levofloxacin. According to GOLD recommendations, the preferred route of administration is oral, but the intravenous and the duration of therapy can be longer in COPD exacerbations admitted in hospital. The choice of antibiotic should take into consideration also the local hospital/ region antimicrobial resistance map (5). The European Respiratory Society and the American Thoracic Society guidelines from 2017 recommend beta-lactams in COPD exacerbations to reduce the risk of treatment failure and increase the time between exacerbations according to local hospital guidelines and because the risk of co-infections are frequently reported with the increase in the severity of COPD (5, 6). The use of antibiotics during hospital stay reduce the risk of readmissions and the risk of mortality (7-10).

According to 2020 evaluation of World Health Organization, death due to COPD was on the third place among global death causes (6% of total deaths), due to comorbidities that lead to frequent exacerbations with or without hospitalizations (3). Data obtained from larger cohort might give details on difference on gender or smoking as factors that influence hospitalization length. A recent study on AECOPD inpatients, found that females and males had similar in-hospital and long-term survival and that female smokers had significantly worse outcomes (7).

The use of macrolides in our study was low, even macrolides might be recommended as an adjunctive treatment AECOPD. Since this practice becomes increasingly common, there are studies that found the importance to monitor its benefits on AECOPD, the adverse events and antimicrobial resistance patterns (11).

The average hospital length in our study was 11 days, which is longer in contrast to a cohort study where the mean was 6-7 days in AECOPD patients (12), but among the values from studies that mention a variable average of the length of stay for AECOPD, ranging from 5 to 12 days (13, 14).

Limitations of the study

There are some limitations in our study: it was a single center study with a small number of hospitalized COPD patients; therefore, our findings might not be generalized.

CONCLUSIONS

Ceftriaxone was the preferred drug for AECOPD treatment for hospitalized patients, followed by quinolones (ciprofloxacin or

levofloxacin), while the average hospital length in our study was 11 days. It is difficult to differentiate between bacterial or viral cause of infection; therefore, clinicians are tempted to recommend antibiotics, that might lead to overuse of antibiotics.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest.

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PRELIMINARY DATA ON THE LINK BETWEEN THE INTESTINAL MICROBIOME AND AUTISM SPECTRUM DISORDERS

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PRELIMINARY DATA ON THE LINK BETWEEN THE INTESTINAL MICROBIOME AND AUTISM SPECTRUM DISORDERS (Abstract): Today autism is an extremely serious problem. Although the number of the children born with this pervasive developmental disorder has increased, the causes of its occurrence have not yet been identified. **Aim:** We want to demonstrate that the severity of gastrointestinal disorders would be related to the worsening of symptoms in people diagnosed with autism spectrum disorders (ASD). Analyzing the intestinal microbiome is particularly important for people with autism because an imbalance in the intestinal microbiome affects their good physical and mental development. **Materials and methods:** In our study, we highlighted the importance of a suitable diet for a better development of children with autism. Children with ASD are at a significantly increased risk of chronic gastrointestinal problems from an early age. The underlying mechanism to explain how gastrointestinal dysfunction can affect autism spectrum disorder behaviors has led to the idea that the microbiome-gut-brain axis contributes to behavioral and cognitive disorders. **Results:** These children have a high prevalence of intestinal transit disorders such as constipation, a fact that influences the severity of symptoms in ASD. The results of our research indicate an increased prevalence of gastrointestinal symptoms among children with ASD compared to the control group. **Conclusions:** Intestinal dysbiosis can influence the severity of symptoms in autism. An understanding of the mechanisms by which the microbiome-gut-brain axis acts will lead to the use of therapeutic strategies. **Keywords:** INTESTINAL MICROBIOME, DYSBIOSIS, AUTISM SPECTRUM DISORDERS.

The specificity of children with autism spectrum disorders (ASD) is the way in which they manifest behaviorally, the withdrawal from social life or even from reality, but also the fact that autism affects such young and innocent children, depriving them of the opportunity to enjoy life. The development of children with autism, later of adults, is affected by this isolation from the environment in which they live. Although these people live in society and have families, they fail to integrate properly. The destructive character of autism is mainly the detachment from the social environment. Fecal analysis can provide clues about intestinal microbial metabolism. However, most studies focus on metabolites in urine and blood. Only a few studies have examined the gut microbiota and the metabolic products of the microbiota and attempted to correlate them with ASD. Wang *et al*. have observed increased concentrations of short-chain fatty acids (SCFA) and ammonia in the feces of children with ASD (1). The gut microbiota plays an essential role in human health and in various disorders such as ASD. A growing body of preclinical research suggests that gut dysbiosis may influence brain function and social behavior, but little is known about the mechanisms underlying these interrelationships and how they may influence pathogenesis or severity in ASD (2). In their research, Angelis *et al.* reported a decrease in total SCFA in children with ASD (3). They also observed altered levels of the neurotransmitter's glutamate and GABA in feces samples. Glutamate was highest among children with autism and GABA was lowest among children with pervasive developmental disorder not otherwise specified (PDD-NOS). In addition, phenolics, including p-cresol, were higher in fecal samples from children with autism and PDD-NOS, but phenolic and p-cresol levels were similar between children with ASD and children from control group (3). ASD patients with gastrointestinal symptoms have changes in microbial composition that are likely related to digestive enzyme deficiencies, carbohydrate malabsorption, selective eating, bacterial toxins, serotonin metabolism, and inflammation (4). The high prevalence of gastrointestinal disorders among ASD patients has led researchers to consider the gut microbiota as a possible factor in the pathogenesis of ASD. Thus, many studies have linked gut dysbiosis, which is frequently observed in patients with autism spectrum disorders, to the modulation of brain function and social behavior, but little is known about this relationship and contribution to the etiology of ASD (5, 6, 7). Hill *et al*. noted that people with ASD prefer energy-dense, nutrient-deficient foods and reject fruits, vegetables, and whole grains (8). The introduction of a new food or physical activity can often cause disruptive behavior that ultimately leads parents to give in to their children's demands (9). Therefore, new foods should be introduced to these children gradually to ensure familiarity with the taste and texture of the food (10).

Parents should teach babies good eating habits from the time the babies start to eat solid food. Autism spectrum disorders can have various social implications, both for people diagnosed with this developmental disorder, as well as for their families and society in general. The literature reports that parents of these children experience increased psychological distresses, including depression, anxiety, interpersonal sensitivity, hostility, schizoid traits, paranoia, and schizophrenia (11). Stressors relate to both

family and social life and include the emotional aspects of a disabled child, understanding the child's needs, various aspects of primary care, medical and educational services, and financial difficulties (12,13).

Due to the high incidence of ASD, as well as the large number of medical services that affected children need, the impact of ASD on the family and the state budget becomes increasingly important. Financial resources allow services to be planned, resources to be allocated and policies to be modified accordingly (14). Medical costs for children with ASD average 3 to 9 times higher than for other children (15).

Therefore, further elucidation of microbiota composition will provide a novel and promising therapeutic approach to control or prevent symptomatology exacerbation in autism. There is growing evidence that the microbiota-gut-brain axis can influence gastrointestinal health and modify those behaviors associated with ASD. Gut microbiota and the metabolic products of microbial populations may be linked not only to gastrointestinal problems but also to behavioral symptoms in autism. In particular, we focus on how gut dysbiosis may affect intestinal epithelial permeability, immune function and microbial metabolites in individuals with autism. To better understand the structure of the gut microbiota in children with autism spectrum disorders of different ages, as well as the relationship between the gut microbiota and fecal metabolites, we need to assess the gut microbial population.

The purpose of this research is to identify if there are significant differences between the intestinal microbiome analysis in the case of children with ASD compared to the control group consisting of neurotypical children.

MATERIALS AND METHODS

We aimed to identify if there is a causal link between intestinal transit disorders such as chronic constipation, the increased level of psychomotor agitation, frequent colds and the presence of an imbalance in the intestinal microbiome bioindicators. We also wanted to observe if there are differences between the values of bioindicators from the intestinal microbiome obtained by the participants from the two research groups.

Objectives

O1. Carrying out the analysis of the microbiome from fecal matter in subjects with ASD and those in the control group.

O2. Establishing how the intestinal imbalance influences the severity of symptoms.

O3. Identification the sort of enterotype of the research participants.

Hypotheses

HS1.It is assumed that there are statistically significant differences between the results of bioindicators from the intestinal microbiome in children with ASD and in neurotypical children.

Hs2. It is assumed that the presence of transit disorders such as constipation can be a cause of imbalances in the intestinal microbiome.

Hs3. It is assumed that the disturbance of the profile of intestinal bacteria can influence the behavioral manifestations.

Ethics approval. In order to participate in this research, the parents of children with ASD and those in the control group have signed a consent agreement.

Study participants. A number of 16 children have participated in this study, 8 of whom have been diagnosed with ASD,

have an increased psychomotor agitation, frequently catch colds due to low immunity and have intestinal transit disorders such as constipation, and 8 are children without disabilities. The children from the first group have been diagnosed with infantile autism and severe developmental delay before the age of 3 and have started psychological and speech therapy immediately after diagnosis at the Psychological Centers in the city of Constanţa.

The ages of the participants in this study are between 3 and 17 years. The groups are similar in terms of age and gender and all live in Constanţa.

Inclusion and exclusion criteria

Group 1. ***Inclusion criteria:*** diagnosis of ASD; informed consent signed by the parents; constipation – defined as the condition of eliminating bowel contents with a frequency less than 3 stools per week (16); increased psychomotor agitation; frequent colds due to low immunity. ***Exclusion criteria***: absence of autism diagnosis; lack of transit disorders; absence of consent agreement.

Group 2. ***Inclusion criteria:*** children without disabilities; informed consent signed by the parents. ***Exclusion criteria:*** presence of ASD diagnosis; absence of consent agreement.

Methods. The participants from both groups have been analyzed for the microbiome from fecal matter, analysis carried out at the *Bioclinica laboratories*.

All the data obtained from the analysis of the bioindicators of the intestinal microbiome were statistically analyzed with the *SPSS version 24 program*. The statistical results will be presented in the tables of the paper.

RESULTS

Enterotypes distribution

In group 1, four participants (50%) had enterotype 1, two participants (25%) had undetermined enterotype, one participant (13%) had enterotype 2 and one participant (13%)had enterotype 3 (fig. 1). In group 2, all eight subjects (100%) had enterotype 1 (fig. 2).

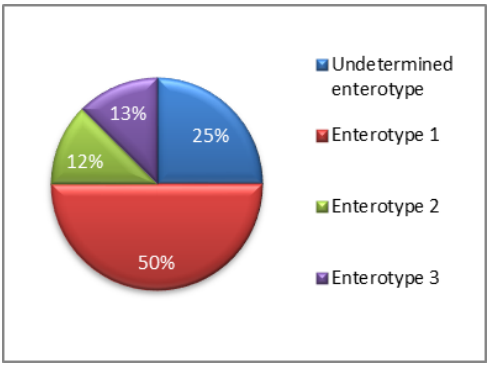

Fig. 1. Distribution of enterotypes group 1

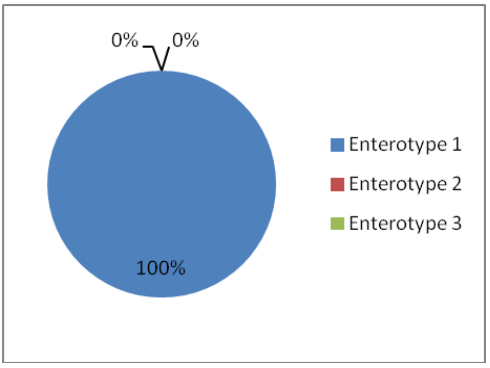

Fig. 2. Distribution of enterotypes group 2

Bioindicators analysis

Fecal pH

In group 1, three participants (38%) had high values, and five participants (62%) presented normal values (fig. 3). None of the participants had low values. In group 2, one participant (13%) had high value, seven participants (87%) had normal values. None of

the subjects presented low values (fig. 4).

Biodiversity (Shannon index)

In group 1, five participants (62%) had low values, three participants (38%) had normal values, while none of the participants presented high values (fig. 5). In group 2, seven participants (87%) had normal values, one participant (13%) had high values, while none of the subjects presented low values (fig. 6).

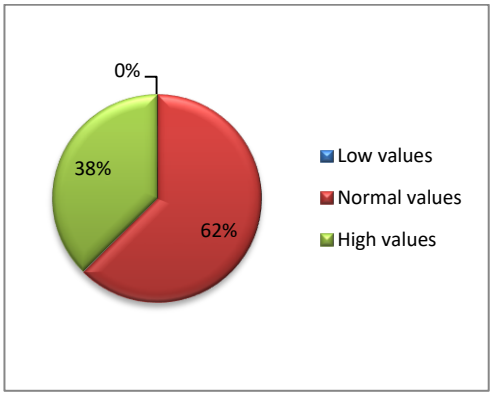

Fig. 3. Fecal pH – group 1

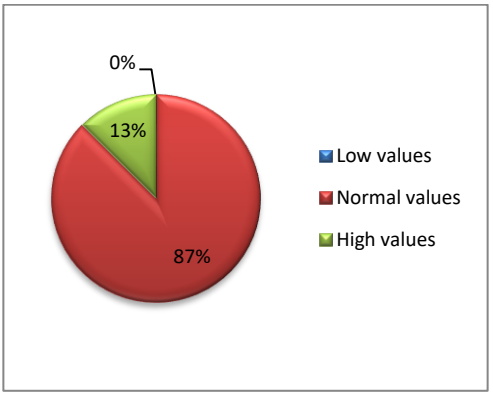

Fig. 4. Fecal pH – group 2

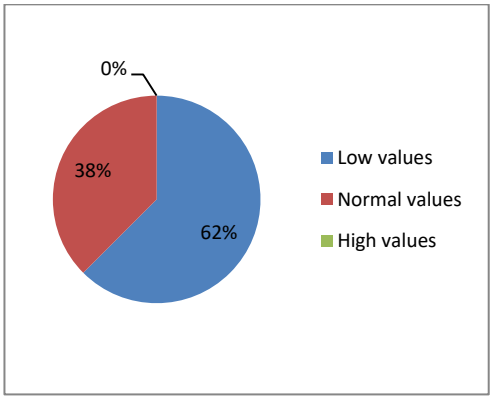

Fig. 5. Biodiversity (Shannon index) group 1

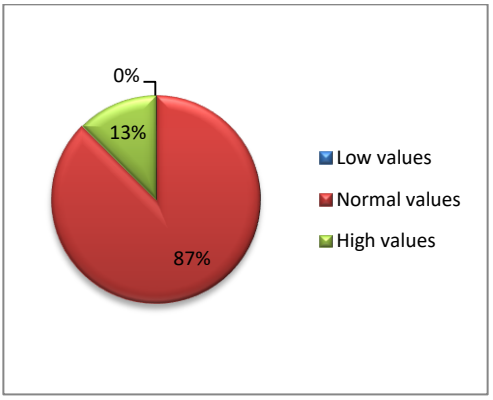

Fig. 6. Biodiversity (Shannon index) group 2

Firmicutes/Bacteroidetes Report

All the participants in group 1 and 2 had normal values.

Butyrate production

In group 1, two participants (25%) had low values, while six participants (75%) had normal values (fig. 7). In group 2, seven participants (87%) had normal values, and one participant (13%) had high values (fig. 8).

Lactate production. In group 1, four participants (50%) had normal values, while the other four (50%) had low values (fig. 9). In group 2, six patients (25%) had normal values, while two subjects (25%) presented high values (fig. 10).

Acetate production. In group 1, five patients (62%) had normal values, while three patients (38%) had high values (fig. 11). In group 2, all subjects had normal values (fig. 12).

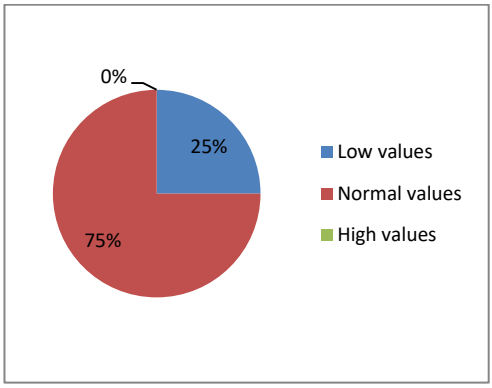

Fig.7. Butyrate production – group 1

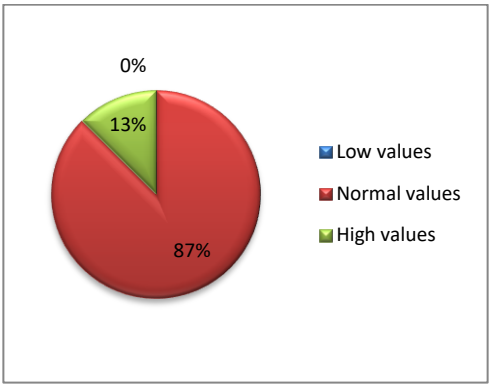

Fig. 8. Butyrate production – group 2

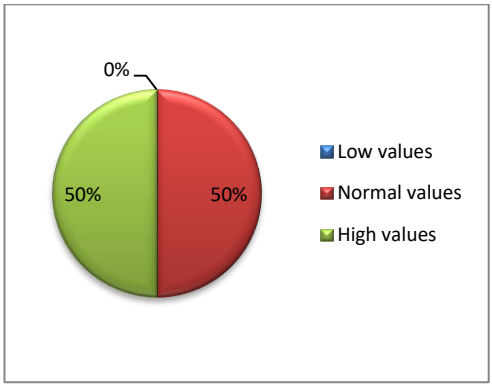

Fig. 9. Lactate production - group 1

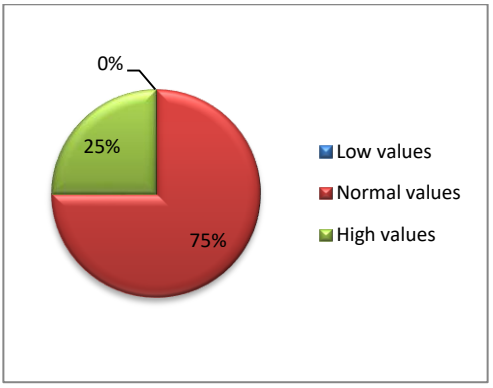

Fig. 10. Lactate production – group 2

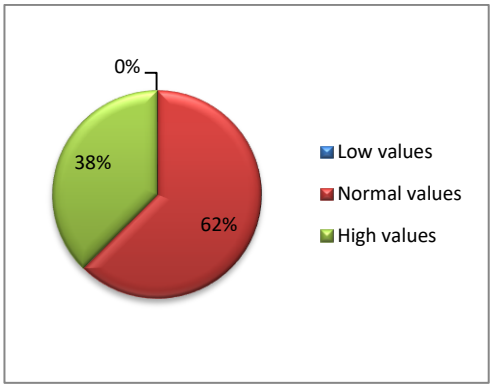

Fig. 11. Acetate production – group 1

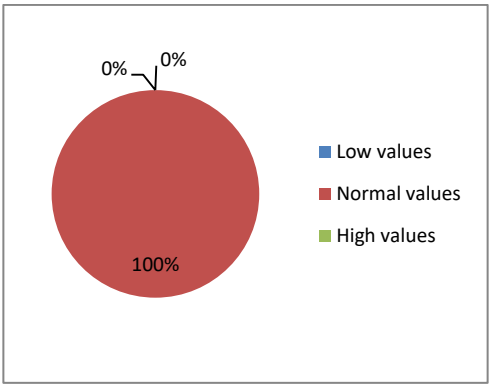

Fig. 12. Acetate production – group 2

Mucin degradation. In group 1, one patient (12%) had low values, six participants (75%) had normal values, while one participant (13%) had high values (fig. 13). In group 2, seven subjects (87%) had normal values, and one (13%) had low value (fig. 14).

Lipopolysaccharide (LPS)-positive bacteria

In group 1, four participants (50%) had normal values, while other four (50%) had high values (fig. 15). In group 2, seven subjects (87%) had normal values, while one (13%) had high values (fig. 16).

Statistics

To test the normality of the distribution, we will apply the Kolmogorov-Smirnov test and the Shapiro-Wilk test and we will interpret the results according to the statistical significance. Conform to the results obtained by the goup 1 in the Kolmogorov-Smirnov test we have an abnormal distribution for Lactate production because $p = 0.001 < 0.05$, for Mucin degradation $p = 0.007 < 0.05$, for Butyrate production $p = 0.001 < 0.05$, and for the rest of the bioindicators the distribution is normal because $p > 0.05$. In the case of the Shapiro–Wilk test, we have an abnormal distribution for Lactate production because $p = 0.004 < 0.05$, for Mucin degradation $p = 0.006 < 0.05$, for Butyrate production $p = 0.003 < 0.05$, and for the rest of the bioindicators the distribution is normal. Since we have a small number of participants, the Shapiro–Wilk test is relevant (tab. I).

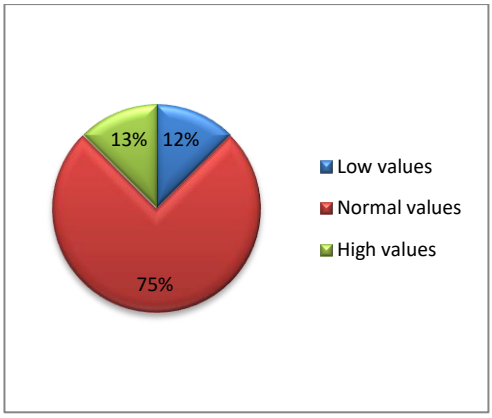

Fig. 13. Mucin degradation – group 1

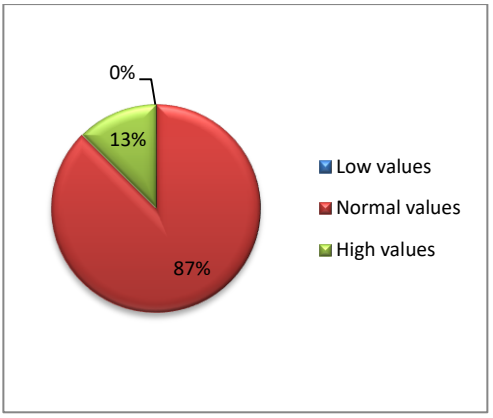

Fig. 14. Mucin degradation – group 2

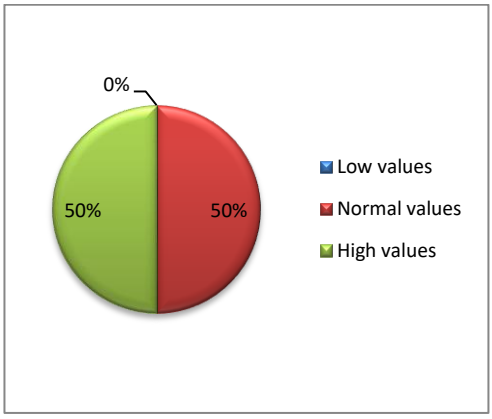

Fig. 15. LPS-positive bacteria – group 1

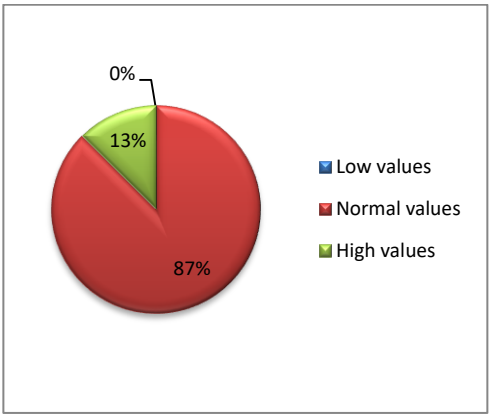

Fig. 16. LPS-positive bacteria – group 2

TABLE I.

Kolmogorov-Smirnov and Shapiro-Wilk normality tests for group1

Tests of Normality						
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Fecal pH	.301	8	.051	.782	8	.058
LPS-positive bacteria	.262	8	.114	.840	8	.076
Mucin degradation	.339	8	.007	.736	8	.006
Lactate production	.381	8	.001	.726	8	.004
Acetate production	.262	8	.114	.888	8	.225
Butyrate production	.379	8	.001	.716	8	.003
Firmicutes/Bacteroidetes Report	.162	8	.200*	.897	8	.274
Biodiversity (Shannon index)	.256	8	.130	.886	8	.213
*. This is a lower bound of the true significance. a. Lilliefors Significance Correction						

In the case of Kolmogorov-Smirnov test for second group we have an abnormal distribution for Lactate Production because $p = 0.000 < 0.05$ and for the rest of the bioindicators the distribution is normal because $p > 0.05$. In the case of the Shapiro-Wilk test, we have an abnormal distribution for Lactate production because $p = 0.000 < 0.05$, and for the rest of the bioindicators, the distribution is normal because $p > 0.05$. Since we have a small number of participants, the Shapiro–Wilk test is relevant (tab. II).

After analyzing the Shapiro-Wilk normality test from both groups, we notice that we have a normal distribution for: Acetate production, LPS-positive bacteria, Faecal Ph, Shannon Biodiversity and Firmicutes/Bacteroidetes report and in the case of bioindicators: Lactate production, Mucin degradation and Butyrate production we have an abnormal distribution.

TABLE II.

Kolmogorov-Smirnov and Shapiro-Wilk normality tests for group 2

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Acetate production	.197	8	.200*	.930	8	.520
LPS-positive bacteria	.325	8	.063	.707	8	.053
Mucin degradation	.261	8	.116	.843	8	.082
Firmicutes/Bacteroidetes Report	.260	8	.118	.771	8	.054
Lactate production	.432	8	.000	.498	8	.000
Butyrate production	.168	8	.200*	.966	8	.862
Fecal pH	.250	8	.150	.849	8	.093
Biodiversity (Shannon index)	.125	8	.200*	.984	8	.978
*. This is a lower bound of the true significance. a. Lilliefors Significance Correction						

We will apply the T test in the case of bioindicators with normal distribution and the non-parametric Wilcoxon test in the case of abnormal distribution.

The descriptive statistics of the T test includes the statistical indicators Mean, Standard Deviation and Standard Error Mean (tab. III).

TABLE III.

T-test between group 1 and group 2

Group Statistics					
	Lot	No	Mean	Std. Deviation	Std. Error Mean
Fecal pH	1	8	6.375	.4432	.1567
	2	8	6.500	.3780	.1336
Biodiversity (Shannon index)	1	8	2.713	.6334	.2240
	2	8	2.900	.4899	.1732
LPS-positive bacteria	1	8	1.188	.4324	.1529
	2	8	1.650	2.0424	.7221
Acetate production	1	8	32.13	9.326	3.297
	2	8	31.25	9.192	3.250
Firmicutes/Bacteroidetes Report	1	8	1.350	.1195	.0423
	2	8	1.400	.1414	.0500

The Independent Samples Test compares differences in means of two independent groups in order to determine whether there is statistical evidence that the associated population means are significantly different. The Independent Samples Test is a parametric test (tab. IV).

Levene's subtest is applied to verify homogeneity of variance. The homogeneity of the variance is a basic condition of the T-test. We observe that $p > 0.05$ for all the values of the bioindicators, so the homogeneity of the variance is verified.

In the T-test, all values of $p > 0.05$, so there are no statistically significant differences between the two groups. The same thing can be observed from the values of t are small.

The effect size in the Independent T-test shows how strong are the differences between the groups. The values smaller than 0,20 indicates that the effect size is weak, the values situated between 0,20 and 0,60 indicate that size effect is medium, and the values bigger than 0.60 indicates that the effect size is strong.

For the bioindicators Fecal pH and Biodiversity (Shannon index) we have a medium effect size, in case of LPS-positive bacteria and Acetate production we have a strong effect side and for Firmicutes/Bacteroidetes Report we have a weak effect size (tab. V).

The non-parametric Wilcoxon test is a statistical test that compares two paired groups. The tests essentially calculate the difference between sets of pairs and analyze these differences to establish if they are statistically significantly different from one another. For Mucin degradation it can be seen that $p = 0.208 > 0.05$, so we have no statistical significance between the two groups and the null hypothesis will be retained (tab. VI).

TABLE IV.

Independent Samples Test

Independent Samples Test											
		Levene's Test for Equality of Variances		t-test for Equality of Means							
		F	Sig.	t	df	Significance		Mean Difference	Std. Error Difference	95% CI of the Difference	
						One-Sided p	Two-Sided p			Lower	Upper
Fecal pH	Equal variances assumed	1.167	.298	-.607	14	.277	.554	-.1250	.2059	-.5667	.3167
	Equal variances not assumed			-.607	13.659	.277	.554	-.1250	.2059	-.5677	.3177
Biodiversity (Shannon index)	Equal variances assumed	.895	.360	-.662	14	.259	.519	-.1875	.2831	-.7947	.4197
	Equal variances not assumed			-.662	13.167	.260	.519	-.1875	.2831	-.7984	.4234
LPS-positive bacteria	Equal variances assumed	4.288	.057	-.627	14	.271	.541	-.4625	.7381	-2.0456	1.1206
	Equal variances not assumed			-.627	7.626	.275	.549	-.4625	.7381	-2.1792	1.2542
Acetate production	Equal variances assumed	.006	.940	.189	14	.426	.853	.875	4.630	-9.055	10.805
	Equal variances not assumed			.189	13.997	.426	.853	.875	4.630	-9.055	10.805
Firmicutes/ Bacteroidetes Report	Equal variances assumed	.000	1.000	-.764	14	.229	.458	-.0500	.0655	-.1904	.0904
	Equal variances not assumed			-.764	13.622	.229	.458	-.0500	.0655	-.1908	.0908

TABLE V.

Independent Samples Effect Sizes

		Standardizer[a]	Point Estimate	95% Confidence Interval	
				Lower	Upper
Fecal pH	Cohen's d	.4119	-.303	-1.284	.688
	Hedges' correction	.4357	-.287	-1.214	.650
	Glass's delta	.3780	-.331	-1.314	.675
Biodiversity (Shannon index)	Cohen's d	.5662	-.331	-1.313	.662
	Hedges' correction	.5990	-.313	-1.241	.626
	Glass's delta	.4899	-.383	-1.369	.630
LPS-positive bacteria	Cohen's d	1.4762	-.313	-1.295	.679
	Hedges' correction	1.5617	-.296	-1.224	.642
	Glass's delta	2.0424	-.226	-1.206	.768
Acetate production	Cohen's d	9.260	.094	-.888	1.073
	Hedges' correction	9.796	.089	-.839	1.015
	Glass's delta	9.192	.095	-.889	1.073
Firmicutes/Bacteroidetes Report	Cohen's d	.1309	-.382	-1.365	.615
	Hedges' correction	.1385	-.361	-1.291	.581
	Glass's delta	.1414	-.354	-1.338	.655

a. The denominator used in estimating the effect sizes.
Cohen's d uses the pooled standard deviation. Hedges' correction uses the pooled standard deviation, plus a correction factor.
Glass's delta uses the sample standard deviation of the control group.

TABLE VI.

The non-parametric Wilcoxon test

Hypothesis Test Summary				
	Null Hypothesis	Test	Sig.[a,b]	Decision
1	The median of differences between Mucin degradation - group1 and Mucin degradation – group 2 equals 0.	Related-Samples Wilcoxon Signed Rank Test	.208	Retain the null hypothesis.
a. The significance level is .050. b. Asymptotic significance is displayed.				

For Lactate production the value of p = 0.866 > 0.05, so we have no statistical significance between the two groups, so the null hypothesis will be retained (tab. VII).

For Butyrate production, we see that value of p = 1.000 > 0.05, so we have no statistical significance between the two groups, so the null hypothesis will be retained (tab. VIII).

TABLE VII.

Wilcoxon Test

Hypothesis Test Summary				
	Null Hypothesis	Test	Sig.[a,b]	Decision
1	The median of differences between Lactate production - group 1 and Lactate production - group 2 equals 0.	Related-Samples Wilcoxon Signed Rank Test	.866	Retain the null hypothesis.
a. The significance level is .050. b. Asymptotic significance is displayed.				

TABLE VIII.

Non-parametric Wilcoxon test

Hypothesis Test Summary				
	Null Hypothesis	Test	Sig.[a,b]	Decision
1	The median of differences between Butyrate production - group1 and Butyrate production - group 2 equals 0.	Related-Samples Wilcoxon Signed Rank Test	1.000	Retain the null hypothesis.
a. The significance level is .050. b. Asymptotic significance is displayed.				

DISCUSSION

Because the children from different racial, socioeconomic, and ethnic backgrounds are affected by ASD and because of the high prevalence of ASD in the world, many of the problems associated with ASD cause challenges for society as a whole. Establishing the diagnosis before the age of two and starting psychological and communication therapy help to create quality and can significantly reduce the symptoms associated with autism, due to the plasticity of the brain, which is a characteristic of the early period of development. Balancing the body, especially from a biochemical point of view, can increase the chances of symptom relief and, implicitly, of improving the general state of health. In both groups there are children who present imbalances at the level of the microbiome. This may be influenced by diet and cause further research focusing on the link between diet and the microbiome.

Using a diet, specific nutrients im-

portant for making learning more efficient and increasing social integration cannot be missing. Children with autism spectrum disorder tend to have a limited food repertoire and a greater reluctance to eat food than neurotypical children. Modulation of the gut microbiota in individuals with ASD and gastrointestinal disorders appears to be a promising future medical target. A probiotic approach should act as a means of restoring a healthy microbiota in addition to reducing gut permeability. To confirm the efficiency of probiotic therapy in children with autism spectrum disorders, larger studies with laboratory analyzes of the microbiota are needed for better direction. Further research on the brain-gut-microbiome axis may lead to new methods of identifying gastrointestinal disorders in children with autism spectrum disorders and new treatments for autism spectrum disorder behavior.

Diet plays an important role in determining gut microbial composition and function; therefore, a selective diet can influence the gut microbial community. Overall, the approach to autism needs to be one based on respect, inclusion and support for people with autism and their families. By promoting awareness, understanding and acceptance, we can help create a more inclusive and supportive society for all its members.

CONCLUSIONS

Our research aimed to identify if there is a causal link between intestinal transit disorders such as chronic constipation, the increased level of psychomotor agitation, frequent colds and the presence of an imbalance in the intestinal microbiome bioindicators. After analyzing the values obtained for the bioindicators from the intestinal microbiome, we notice that all the participants in group 1 have values different from the normal ones for at least 1 analyzed indicator, and in the case of group 2, we note that a number of 4 participants have all normal values, and the other 4 participants have at least one value different from the normal one. It can be observed, following the statistical interpretation of the results from test T, that there are no statistically significant differences in terms of microbiome analysis between the two groups participating in the research, $p > 0.05$, a fact that refutes hypothesis 1, namely: *it is assumed that there are statistically significant differences between the results of bioindicators from the intestinal microbiome in children with ASD and in neurotypical children.*

Regarding the connection between constipation and the imbalance at the level of the microbiome, it can be observed that the participants from group 1 present more imbalances at the level of the intestinal microbiome than the participants from group 2, which shows us that hypothesis 2 is confirmed, namely: *it is assumed that the presence of transit disorders such as constipation can be a cause of imbalances in the intestinal microbiome.*

For hypothesis 3, it can be observed, after analyzing the data, that there is a close connection between the problems arising at the level of bioindicators of the intestinal microbiome and the increased psychomotor agitation present in the study participants from group 1, so the hypothesis is thus confirmed, namely: *it is assumed that the disturbance of the profile of intestinal bacteria can influence behavioral manifestations*.

For group 1, we notice that we have a percentage of 75% of participants with enterotype 1 and for group 2 we have a percentage of 100%, that is all participants, with enterotype 1. After the laboratory

analysis, the enterotype of each participant has been identified. In two of the children with ASD, those who are 3 years old, enterotype determination could not be performed, probably due to their young age. We recommend consulting a pediatric gastroenterologist for the implementation of microbiota-targeted therapies, such as probiotics, prebiotics, food supplements. New insights into potential therapeutic interventions that can be used to reduce and treat symptoms associated with autism spectrum disorders can be mentioned.

Our research has showed that people with ASD that have severe symptoms and also transit disorders such as constipation, have an imbalance in the intestinal microbiome.

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EDITORIAL

ESOPHAGEAL SURGERY AT THE CROSSROADS - PAST, PRESENT AND PERSPECTIVES - MINIMALLY INVASIVE ESOPHAGECTOMY

If the first author of the editorial represents the past (and perhaps the present), of this difficult type of surgery, the other author (Dr. Florin Achim, university Assistant Professor in the clinic), represents the future of this niche surgery, benefiting from a doctorate with the theme "Minimally invasive surgery of esophageal cancer", with serious training fellowships working directly with the greatest esophageal surgeons in Germany (Professor Arnulf H. Hölscher and Professor Karl-Hermann Fuchs - Fellowship Grant of The European Association of Endoscopic Surgery(EAES) in Klinik für Allgemein- Viszeral-, und Thoraxchirurgie Agaplesion Markus-Krankenhaus, Frankfurt am Main) with another fellowship in Dublin (Ireland) in St. James Hospital under Professor John Reynolds (President of the European Society of Diseases of the Esophagus-ESDE).

He recently completed a 3-month fellowship in a minimally invasive esophageal cancer surgery under Professor Koji Otsuka and Professor Haruhiro Inoue at the Esophageal Cancer Center, Showa University Hospital and Koto Toyosu Hospital, Tokyo, Japan; not to mention that he is also a promoter of minimally invasive esophageal surgery in our clinic. On the initiative of Dr. Florin Achim, a collaboration agreement was established between the Showa University Faculty of Medicine and the "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, a partnership that will benefit future generations of students, residents, young specialists and other researchers.

One of the most difficult esophageal pathologies to treat, esophageal cancer, currently benefits from a much more effective treatment through the multidisciplinary approach and the creation of centers of excellence with a high volume of esophageal pathology. Advances in staging, surgical technology, neoadjuvant therapy, and perioperative care have resulted in reduced morbidity and mortality. The current mainstay of curative treatment for localized disease is surgery, combined with neoadjuvant chemoradiotherapy for locally advanced stages.

The development of esophagectomy techniques initially went through two periods: that of the safety of the procedure, that of radicality, and currently it is in a new era of the minimally invasive approach that ensures a better quality of life for the patient.

To reduce postoperative morbidity, minimally invasive surgical techniques were introduced 32 years ago into the therapeutic arsenal of esophageal cancer. However, there are controversies related to the use of the minimally invasive approach in practice because it requires a demanding and difficult to access technical base, a laborious operative technique and has a long learning curve.

The first thoracoscopic esophagectomy was performed by Sir Alfred Cuschieri in 1992 (fig. 1) (1). Considering the high degree of complexity of minimally invasive esophagectomy (MIE) with esophageal reconstruction, 10 years followed in which the technique was viewed with reluctance by

teams of esophageal surgeons. At the same time, it was also a period of the beginning of the centralization of esophageal cancer cases, the results obtained by the specialists were good and many centers were not interested in introducing a new technique. Also, the immediate postoperative results were initially not up to expectations. Apart from a faster postoperative recovery and a lower rate of pulmonary complications, the minimally invasive esophagectomy failed to drastically influence the postoperative results. There were controversies in the literature regarding the quality of oncological resection.

Fig. 1. Sir Alfred Cuschieri

The results were also difficult to interpret due to the different types of approach used in the treatment of esophageal cancer and the complications associated with esophageal reconstruction. The imposition of minimally invasive techniques as standard treatment has come a long way in a difficult surgery like esophageal surgery, where excellence is achieved after a lifetime. Randomized clinical trials have confirmed the feasibility of minimally invasive esophagectomy, its role in reducing postoperative complications, especially pulmonary ones, and obtaining oncological results similar to the classic technique.

Surgeons familiar with video-assisted techniques continued to perform the operation observing a much better quality of life of the patients in the immediate postoperative period. All variants of the classic esophagectomy were reproduced through a minimally invasive approach including assisted by the da Vinci robot (RAMIE).

The minimally invasive esophagectomy was initially performed through a hybrid approach in several variants: thoracoscopic esophagectomy with laparotomy, *video-assisted thoracoscopic esophagectomy (VATE)* with laparotomy, laparoscopic mobilization of the stomach with right thoracotomy or thoracoscopic esophagectomy with manual assisted laparoscopic mobilization of the stomach (*hand-assisted laparoscopy*).

The first transhiatal esophagectomy through laparoscopic approach was performed by De Paula Al. in 1995 (2). Pneumoperitoneum facilitates the mediastinal transhiatal dissection, the direct esophageal branches of the aorta that are clearly identified and clipped, thus reducing bleeding risk. The thoracic duct can be viewed and clipped, reducing the risk of chylothorax.

Luketich has used minimally invasive esophagectomy in the treatment of the resected esophageal cancer in over 1,000 patients and found that the minimally invasive approach has appropriate results to the open esophagectomy according to the oncological resectability (3).

Watson DI improves the technique described by Ivor Lewis by introducing the minimally invasive approach (4). The technique allows an excellent view in both operative stages, the ability to perform lymphadenectomy on two fields and the avoidance of neck dissection of the esophagus in the 3-stage McKeown approach, with all the risks included, for example, the stenosis of the

esophagus anastomosis, the cervical anastomotic leakage or the injury of the recurrent left nerve (5).

Minimally invasive esophagectomy by 2-stage Ivor Lewis modified laparoscopic and thoracoscopic is a safe technique that has already passed the time trial and is effective in the surgical treatment of the esophageal cancer.

The thoracoscopic esophagectomy was initially performed in the left lateral decubitus similar to the classic technique, because in the event of some hemorrhagic incidents it was necessary to convert the case, performing the emergency thoracotomy is much easier.

Cuschieri *et al.* have improved the technique by changing the position from the left lateral decubitus to the procubitus which allowed the operation without a complete collapse of the lung aiming at the reducing of the postoperative lung complications that are the most rewarding (6).

Palanivelu *et al.* published a series of 130 patients who benefited from thoracoscopic esophagectomy with the patient in the prone position using a right posterior approach to which in addition to ventilation of the left lung, it was possible to partially ventilate the right lung. The average duration of the procedure was 220 minutes (160-450 minutes). The average hospitalization in the ATI section was 1 day (1-32 days). Pneumonia was one of the postoperative complications to 1.54% of patients (7).

The thoracoscopic esophagectomy performed in a left semi-prone position combines the advantages of the left lateral decubitus (allowing a rapid conversion) and prone position (which offers a more accurate view of the operating field and facilitates a complete mediastinal lymphadenectomy (fig. 2). In addition to mobilization of the conduit, celiac lymphadenectomy, pyloroplasty and jejunostomy performed using a laparoscopic approach represent the minimally invasive alternative for the radical treatment of esophageal cancer.

The first robotic esophagectomy was performed through transhiatal laparoscopic approach by Horgan S. in 2003 (8).

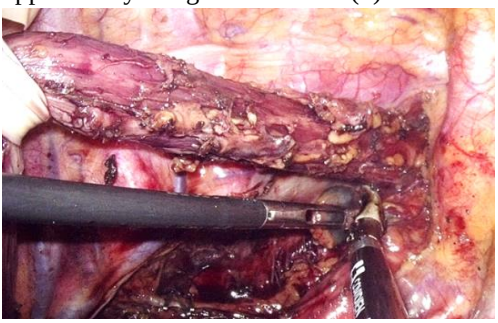

Fig. 2. Mediastinal lymphadenectomy

Some limitations of the standard minimally invasive esophagectomy which include bidimensional view, low grade of free mobility, narrow operating field of mediastinum and low coordination between eyes and surgeon's hand, have been eliminated by robotic surgery (fig. 3). Robotic platform reduces the complexity of thoracoscopic and laparoscopic maneuvers using the technology of endo-wrist da Vinci™ robotic upper arm (instrumental joint has 7 grades of mobility (9).

3D image of the highest augmented HD resolution, the ability of performing fine movements and filtering the tremor of the surgeon's hand are other advantages of robotic approach during esophageal dissection, allowing to perform an extended mediastinal lymphadenectomy and a "hand-made" esophago-gastric anastomosis (10).

Another unexpected advantage of robotic surgery is represented by the reducing of learning curve (20 procedures would be enough) in comparison to the standard MIE, so it could increase the number of specialists who could achieve adequate and specific

competences in a much shorter period. (11). In this way, Ivor-Lewis robotic assisted esophagectomy (RILE) has replaced the modified McKeown triple approach (RELA) many times through performing a qualitative intrathoracic anastomosis (12-14).

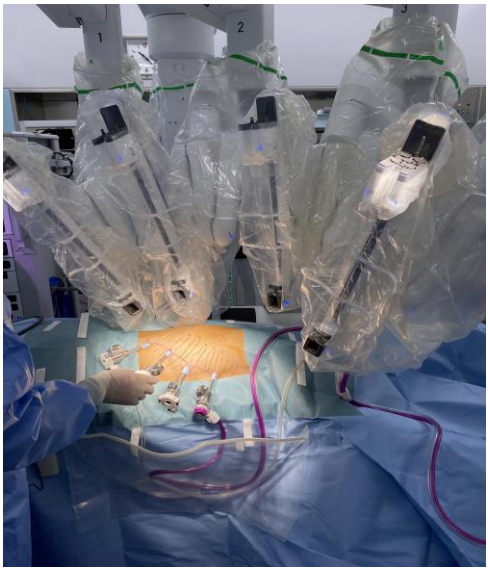

Fig. 3. Robotic esophagectomy

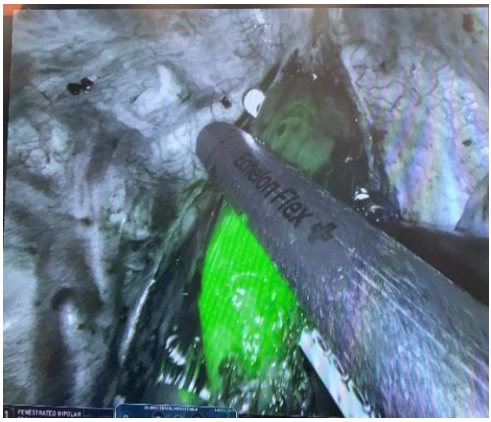

Fig. 4. ICG-enhanced robotic assisted surgery - DaVinci Firefly

The robotic platform has one or two DaVinci master units with a 3D HD camera system and near infrared imaging (fig. 4). The use of two consoles allows first assistant to perform surgical maneuvers and this assembly is useful for training.

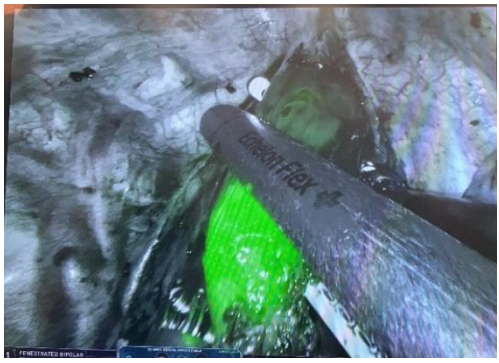

Fig. 4. ICG-enhanced robotic assisted surgery- DaVinci Firefly

Esophagectomy is associated with mortality, significant comorbidities and a long-term recovery and the results are based on the experience of surgical centers where the operation is performed, the surgeon and his team, and on involvement of an intensive care unit team which is ready for the treatment of non-surgical immediate or distant postoperative complications. MIE has the advantages of representing a less traumatic procedure with a short postoperative recovery and less pulmonary complications or infections of postoperative wounds.

Minimally invasive esophagectomy has long learning curve, but the expertise in open esophageal surgery is difficult to gain, and the knowledge about advanced techniques of minimally invasive surgery is mandatory. Technical difficulties in case of an esophageal tumor are similar to open surgery, thus, the enlarged image in the operating field could be an advantage for minimally invasive esophagectomy.

MIE enables the simplification of postoperative medical care (blood transfusions, postoperative treatment that relieves the pain), the decrease of the incidence for post-

operative complications, especially pulmonary complications and postoperative infections, deep veins thrombosis, shorter hospitalization and rapid social reinsertion.

The distant oncological results of minimally invasive esophagectomy analyzed in randomized clinical trials (TIME, MIRO and ROBOT) were recently published. Thus, patient survival at 3 years was 42.9% MIE vs 41.2% EC in the TIME trial, 67% MIE vs 55% EC in the MIRO trial, and overall survival at 5 years was 42% in the ROBOT study (15-17). The results of randomized clinical trials thus confirm the fact that minimally invasive esophagectomy reduces postoperative complications in resectable esophageal cancer with oncological results similar to open esophagectomy.

Summing up the expertise in open esophageal surgery and laparoscopic surgery of gastro-esophageal junction in the General and Esophageal Surgery Department, Sf. Maria Clinical Hospital, Bucharest, the minimally invasive esophagectomy has been performed using the modified 3-stage McKeown approach since 2015(the operative technique is described in *"Tratat de Patologie și Chirurgie Esofagiană"* edited by the Romanian Academy Publishing House in 2017 (fig. 5) and *"Surgery of the Esophagus"*, revised and added edition and translated into English published by the Romanian Academy Publishing House in 2019 (fig. 6) .

The 3-stage approach is performed in our Clinique because it does not imply performing an intrathoracic anastomosis which, in the occurrence of a mediastinal fistula, could lead to mediastinitis with significant postoperative morbidity and mortality. A cervical leak could benefit from conservative treatment, and an anastomotic stenosis is optimally resolved by performing endoscopic dilations (18).

Fig. 5. The Textbook *"Tratat de Patologie și Chirurgie Esofagiană"*

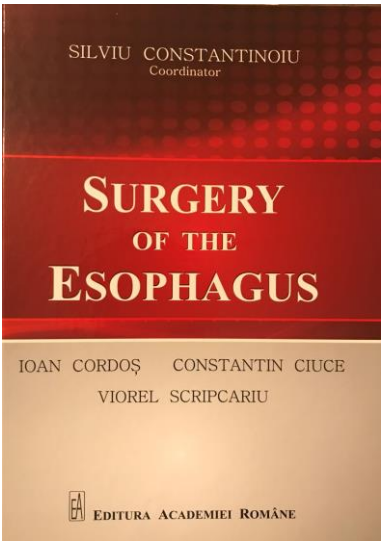

Fig. 6. The Textbook *"Surgery of the Esophagus"*

Technological advances in esophageal surgery

Recent technological advances of minimally invasive surgery focusing especially on the areas of **imaging, instrumentation, cameras and robotics**.

The augmented **3D High-Definition image, 4K Ultra HD** or **8K camera**, provides an excellent visual field, that allows accurate identification of dissection planes and extensive periesophageal and perigastric lymphadenectomy. Near-Infrared Fluorescence is available in multiple endoscopic platforms including the DaVinci Robot Firefly system. NIF allows intraoperative tumor localization and in the assessment of gastric conduit perfusion, but also in mediastinal lymphadenectomy. Single-port Video Assisted Surgery has proven its usefulness both in the abdominal approach and in the thoracic approach, being also available with the DaVinci robotic system. Tri-Staple™ Technology offers a safer anastomosis and is an essential element in esophageal surgery. Mini-thoracoscopy has not yet entered esophageal surgery, but it finds a very good use in pediatric surgery.

The EndoEye Flex 3D deflectable videoscope finds a very good use in the recently promoted and increasingly used approach, namely mediastinoscopically assisted transhiatal minimally invasive esophagectomy.

Intraoperative recurrent laryngeal nerve monitoring reduces the risk of recurrent nerve damage during a crucial time of esophagectomy, namely upper mediastinal lymphadenectomy (fig. 7).

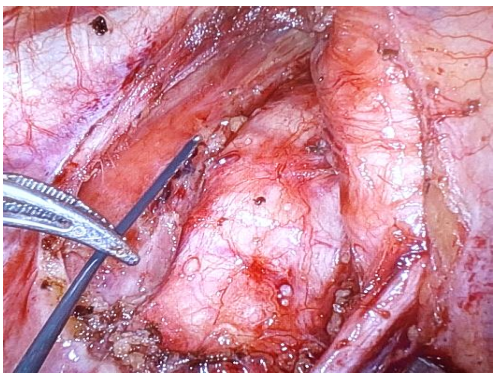

Fig. 7. Intraoperative recurrent laryngeal nerve monitoring

Surgical robotic systems will take off in the coming years with the expiration of the patents of the first DaVinci surgical robot. The first modular robotic systems have already appeared (Hugo from Medtronic, Versius (CMR Surgical Ltd. UK, the last one also available in the "Sf. Maria" Clinical Hospital Bucharest) which offer increased mobility of the robotic arms and reduce the conflict between instruments which is essential in small rigid spaces such as the ribcage.

Artificial intelligence has also begun to make its presence felt, advanced software programs have already been included in endoscopic platforms and are used in the detection of esophageal cancer through real-time analysis during endoscopy. Other applications with clinical utility are sentinel node navigation surgery, AI for learning esophagectomy, real-time detection of the recurrent nerve, predicting postoperative complications, machine learning for reducing readmissions after esophagectomy or AI for predicting esophageal cancer recurrence.

In the future, the following artificial intelligence technologies will probably be available in esophageal surgery: Surgical Training in Virtual Reality (VR) with realistic surgical simulations, AI as the Surgical Mentor, Personalized 3D Patient Models: 3D replicas of patient anatomy, The Infallible Robotic Aide: AI-empowered surgical robots, Holographic Surgical Visualization: interactive holograms, real-time 3D view or Postoperative Care Revolution: personalized rehabilitation after esophagectomy.

Artificial Intelligence for esophageal cancer patients is already a support tested by patients with esophageal pathology and which offers detailed information about the diagnosis and treatment of various esophageal diseases. AI will require the evolution of work practices, but will also enhance

patient safety and surgical quality outcomes, and provide significant value for surgeons and health systems. The use of AI in surgery is still evolving, and its implementation should always be supervised and guided by qualified healthcare professionals. As surgeons, we must not forget that the development in surgery was based on technological advances.

Advanced technology and optics of endoscopic surgery will allow better assessment of anatomical plans, a very good access to narrow spaces, and therefore a more precise dissection with lower trauma in esophageal cancer surgery.

The long-term outcomes of surgical treatment of esophageal cancer are an additional motivation for treatment orientation in order to obtain good results in quality of life of the patients in the first postoperative year, results in which minimally invasive technique is surpassing open technique.

The modern Upper Gastro-intestinal surgeon should offer to the patients the opportunity of benefit of the minimally invasive approach and he should perform an esophagectomy with low morbidity and mortality.

Evaluating the results of minimally invasive treatment of esophageal cancer is a constant concern worldwide, given that in some countries minimally invasive esophagectomy has become the standard indication, and open esophagectomy is performed only for the treatment of complex cases.

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A RETROSPECTIVE INVESTIGATION ON THE INCIDENCE, CLINICAL CHARACTERISTICS, AND OUTCOMES OF PNEUMOTHORAX IN HOSPITALIZED COVID-19 PATIENTS IN NORTH-EAST OF ROMANIA

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A RETROSPECTIVE INVESTIGATION ON THE INCIDENCE, CLINICAL CHARACTERISTICS, AND OUTCOMES OF PNEUMOTHORAX IN HOSPITALIZED COVID-19 PATIENTS IN NORTH-EAST OF ROMANIA (Abstract): The COVID-19 clinic ranges from asymptomatic to critical and fatal disease. Pneumothorax and pneumomediastinum are rare complications of COVID-19 infection but can occur at any time during the disease. The **aim** of this retrospective study was to analyze the impact of acute respiratory complications on patients diagnosed with SARS-CoV-2 infection. **Materials and methods:** 177 patients with COVID-19 were recruited to analyze the correlation between pneumothorax and demographics and general data of the patients. **Results:** In our study, pneumothorax or pneumomediastinum was much more frequent among younger, smoking individuals from urban environments. Moreover, the pneumothorax group had statistically significantly higher white blood cells and erythrocyte sedimentation rate values than the control group. The duration of hospitalization in patients who developed pneumothorax was similar to those who did not develop this complication. **Conclusions:** Patients experiencing this consequence had a significantly higher mortality rate compared to those in control groups. Although the precise origins of this complication remain unclear, individuals with preexisting pulmonary dysfunction appear to experience it more frequently. **Keywords:** COVID-19, COMPLICATION, PNEUMOTHORAX, PNEUMO-MEDIASTINUM, RETROSPECTIVE STUDY.

INTRODUCTION

In March 2020, the World Health Organization declared the COVID-19 pandemic. The Coronavirus disease 2019 (COVID-19) cause serious damage to the respiratory system. As a result of this damage air can pass into the intrapleural space or into the mediastinum and pneumothorax or pneumomediastinum can develop. Barotrauma, which includes pneumothorax or pneumomediastinum has been observed as an emerging complication in CODIV -19 patients (1, 2, 3). COVID-19 may cause spontaneous pneumothorax to the many progressive pathological changes in the lungs such as pneumonia, cystic changes or enlargement of blebs. Also, in clinically observed COVID-19 cases pneumothorax can develop without clinical or radiological parenchymal changes in the lung (4). Traumatic pneumo-

thorax results from injury to the pleura, as well iatrogenic pneumothorax result from injury to the pleura during medical procedures. Secondary spontaneous pneumothorax is a pneumothorax that occurs as a complications of underlying lung disease and the primary spontaneous pneumothorax is defined as pneumothorax in a healthy individual. Spontaneous pneumothorax and pneumomediastinum have been described as complications of COVID-19 with a wide incidence from 10% in patients with COVID-19-related acute respiratory distress syndrome, increasing to 24% in patients receiving mechanical ventilation (5). In another study by Geraci et al., the incidence of pneumothorax in COVID-19 was reported as 7.4% (6). Patients experiencing this consequence had a significantly higher mortality rate compared to those in control groups (7). Although the precise origins of this complication remain unclear, individuals with preexisting pulmonary dysfunction appear to experience it more frequently (7). In our study, we aimed to describe the clinical characteristics and outcomes of patients with pneumothorax or pneumomediastinum or both due to COVID-19 pneumonia.

MATERIALS AND METHODS

Study design. Patients were recruited for this retrospective study from the "Sfântul Ioan cel Nou" County Emergency Hospital from Suceava, designated for treating SARS-CoV-2 infections. The study included patients diagnosed with SARS-CoV-2 infection through PCR (polymerase chain reaction) testing from March 2020 to November 2020.

Ethical Approval. The study received approval from the Ethics Committee of the "Sfântul Ioan cel Nou" County Emergency Hospital from Suceava (No. 4/ 27.11.2020).

Patients. In the study interval, out of 28,678 admitted patients, 7,325 were confirmed with a diagnosis of Covid-19. Initially, the study included 113 patients confirmed with Covid-19 infection, pneumothorax, and/or pneumomediastinum. After excluding 7 patients with traumatic pneumothorax and 6 patients with iatrogenic pneumothorax, the study included 100 patients. The diagnosis of pneumothorax or pneumomediastinum was based on clinical symptomatology and confirmed by imaging. In the control group, we included only 77 patients out of 7212 who had a confirmed diagnosis of SARS-CoV-2 infection but no diagnosis of pneumothorax or pneumomediastinum. In the selection of patients for the control group several criteria of similarity were rigorously followed, such as hospital admission in the same period, data regarding the onset of COVID-19 disease, similar symptoms and demographic data such as environment of origin or smoking status. These patients also presented respiratory pathology in association such as chronic obstructive pulmonary disease, bronchiectasis, bronchial asthma or post-tuberculosis lung sequelae, so definite diagnosis of pre-existing lung damage. The inclusion criteria were age over 18 years, a diagnosis of SARS-CoV-2 infection, and pneumothorax. The exclusion criteria were age under 18, pregnant individuals, and the negation of a Covid-19 diagnosis.

Statistical analysis. The data analysis was realized using *SPSS version 23.0* (Statistical Package for the Social Sciences, Chicago, Illinois). For continuous variables, the data were presented as median with interquartile range or as mean ± standard deviation (SD). For categorical variables, the data were presented as the

number of cases with percent frequency. Continuous normally distributed variables were compared by Independent Samples t-Test in case of two samples, or by One-Way ANOVA, in case of comparisons of more than two samples. Categorical comparisons were performed by Fisher's exact test or by Chi-square test.

RESULTS

Thus, we have enrolled in our study 177 patients (100 in the pneumothorax / pneumo-mediastinum group and 77 in the control group). Regarding the general characteristics of the two populations, we have observed that the patients included were predominantly males from urban area. There were no significantly statistical differences regarding the age of the two groups. Regarding the educational status, we can observe that most of the included individuals had a medium level of education, followed by the ones with high education.

Regarding the employee status, again no statistically significant differences could be observed, but it worth mentioning that the majority of them were retired, followed by full-time workers. Moreover, we can observe that in the pneumothorax group there was a higher prevalence of smoking and a lower BMI, compared to the control group (tab. I).

TABLE I.

General characteristics of the study groups

General characteristics		Pneumothorax Group (n = 100)	Control Group (n = 77)	p
Gender n (%)				
	Male	56 (56.0%)	49 (63.6%)	0.355
	Female	44 (44.0%)	28 (36.4%)	
Living environment, n (%)				
	Rural	41 (41.0%)	25 (32.5%)	0.275
	Urban	59 (59.0%)	52 (67.5%)	
Age, years (mean ± DS)		59.98±13.95	64.11± 15. 18	0.066
Education status, n (%)				
	No education	10 (10.0%)	2 (2.6%)	0.070
	Medium level of education	40 (40.0%)	51 (66.2%)	0.001
	High level of education	32 (32.0%)	23 (29.9%)	0.870
	Unspecified	18 (18.0%)	1 (1.3%)	0.000
Employee status, n (%)				
	Household	8 (8.0%)	3 (3.9%)	0.353
	Full-time	43 (43%)	28 (36.4%)	0.440
	Retired	49 (49%)	46 (59.7%)	0.173
Alcohol consumption, n (%)		4 (4.0%)	1 (1.3%)	0.389
Smoking, n (%)		41 (41.0%)	23 (29.9%)	0.083
BMI (kg/m^2)		25.06 (22.40; 32.00)	27.98 ±5.14	0.157
Days of hospitalization (mean± SD)		14.00 (8.75; 18.25)	13.37±7.05	0.070

The prevalence of COVID-19 infection types within the pneumothorax group, where the moderate form exhibited the highest prevalence, succeeded by the severe form, and the mild type was the least frequent. In contrast, the control group showed the highest prevalence in the severe form, followed by the moderate and mild types (tab. II).

TABLE II.
Types of the COVID-19 infection within the study groups

Type of disease	Pneumothorax Group (n = 100)	Control Group (n = 77)	p
Mild	19	13	0.844
Moderate	42	30	0.758
Severe	39	34	0.539

When evaluating the symptomatology of the patients, we observed statistically significant differences regarding nausea, ageusia, anosmia, myalgia, and asthenia, these symptoms being more frequent in the pneumothorax group compared to the control group. Regarding the cough, we can observe that the dry one was more prevalent in the pneumothorax group compared to the control group. Statistical significance was also observed for almost all the classes of dyspnea (tab. III).

TABLE III.
Associated symptomatology in the two studied groups

Symptomatology		Pneumothorax Group (n = 100)	Control Group (n = 77)	p
Nausea		26 (26.0%)	7 (9.1%)	0.006
Vomiting		4 (4.0%)	5 (6.5%)	0.505
Ageusia		24 (24.0%)	3 (3.9%)	0.000
Anosmia		25 (25.0%)	6 (7.8%)	0.003
Myalgia		63 (63.0%)	24 (31.2%)	0.000
Headache		60 (60.0%)	16 (20.8%)	0.000
Asthenia		87 (87.0%)	42 (54.5%)	0.000
Thoracic pain		46 (46.0%)	30 (39.0)	0.363
Cough	Dry	64 (64.0%)	37 (48.1%)	0.401
	Productive	10 (10.0%)	17 (22.1%)	0.042
Dyspnea	MRC 0	17 (17.0%)	25 (32.5%)	0.021
	MRC 1	29 (29.0%)	9 (11.7%)	0.006
	MRC 2	25 (25.0%)	18 (23.4%)	0.861
	MRC 3	21 (21.0%)	8 (10.4%)	0.067
	MRC 4	8 (8.0%)	17 (22.1%)	0.009

We can observe that in the pneumothorax group there are statistically significant higher values of the while blood cells and erythrocyte sedimentation rate compared

to the control group. Moreover, it worth mentioning that the number of platelets, urea and creatinine were higher in the pneumothorax group compared to the control, but without statistical significance (tab. IV).

TABLE IV.

Laboratory tests in the two studied groups

Laboratory values	Pneumothorax Group (n = 100)	Control Group (n = 77)	p
WBC	10.52 ± 4.12×10^3/mm^3	7.77 ± 3.62 ×10^3/mm^3	0.000
PLT	256.66 ± 96.38	219.00 (161.50; 309.00)	0.318
ESR	99.50 (78.00; 124.00)	59.81 ± 39.35	0.000
CRP	11.64 (3.31; 24.15)	20.18 (6.64; 45.56)	0.422
Urea (mg/ dL)	45.00 (34.00; 62.75)	38.00 (29.30; 62.55)	0.606
Creatinine (mg/ dL)	0.90 (0.74; 1.24)	0.89 (0.68; 1.20)	0.101
GFR (ml/min/1.73 m^2)	78.86± 33.65	84.05 ± 28.14	0.315

WBC – white blood cells, PLT – platelets, ESR – erythrocyte sedimentation rate, CRP – C reactive protein, GFR – glomerular filtration rate

DISCUSSION

The study aimed to quantify the number of patients who developed pneumothorax following SARS-CoV-2 infection and identify the risk factors that caused the development of sars-cov-2. In our study, the incidence of SARS-CoV-2 infection was 25.46% among patients hospitalized at the "Sfântul Ioan cel Nou" County Emergency Hospital Suceava, from March 2020 to February 2021. In addition, the incidence of pneumothorax in patients with COVID-19 was 1.36%. Studies reveal that patients with this infection encounter pneumothorax much more frequently than those with other respiratory infections, with an incidence of 10% among those with severe forms of the disease (2). In the cases included in the study, the incidence of pneumothorax was approximately the same for both moderate (42%) and severe (39%) forms of the disease, but without significant statistical significance. On the other hand, data from the literature show strong correlations between the severe form of the disease and the occurrence of complications such as pneumothorax (9).

Moreover, a systematic review published in 2020, which included 9 observational studies, highlights that the rate of pneumothorax would be higher among men (10, 11, 12). This can also be observed among the patients included in our study (56% *vs.* 44%). One possible cause for this difference between sexes is that smoking is more common among men. Regarding the symptoms, patients most frequently presented to the hospital with dyspnea (83%), myalgia (63%), headache (60%), asthenia (87%), anterior chest pain (46%), and dry cough (64%).

Smoking is a well-known risk factor for respiratory diseases. The patients included in the study with pneumothorax had a much higher smoking rate than the control group (41% vs. 29.9%). Still, there was no statistically significant correlation between smoking and this complication arising from SARS-CoV-2 infection. Even though smoking is considered an emerging risk

factor for pulmonary pathologies such as chronic obstructive pulmonary disease, studies in the literature have failed to identify a correlation between this habit and the occurrence of pneumothorax in patients with COVID-19 (12, 13).

The urban environment is another risk factor involved in the development of pneumothorax in COVID-19 patients. It seems that existing pollution, lifestyle, sedentary behavior, and diet-factors predominant in cities-negatively influence the progression of COVID-19. From a future perspective, this idea should be further explored to see if the number of pneumothorax cases is higher in industrialized cities compared to smaller towns (14).

Increased white blood cell counts and inflammatory markers like CRP and ESR indicate an inflammatory status, which prolongs the healing period of lung infections and increases the risk of complications (15). In the case of patients monitored at the "Sfântul Ioan cel Nou" County Emergency Hospital Suceava, it is observed that both white blood cells and ESR have a much higher value among patients with pneumothorax, while the CRP value is lower in these patients.

Throughout the pandemic, several studies have emerged highlighting that obese patients were much more prone to complications associated with SARS-CoV-2 infection than normal-weight patients. Abdominal adipose tissue predominantly hinders respiratory movements, leading to greater respiratory effort. Furthermore, obesity is considered a factor that maintains the inflammatory status, promoting the occurrence of numerous complications (16). However, our study did not find a statistically significant correlation between BMI and the occurrence of pneumothorax.

Another particularity of our study would be the duration of hospitalization, although pneumothorax typically results in a higher number of hospitalization days, the two groups showed no statistically significant differences (10).

CONCLUSIONS

Since 2020, infection with the SARS-CoV-2 virus has caused numerous deaths due to associated complications. One of the most severe complications encountered in these patients was pneumothorax, which led to an increased length of hospitalization and, consequently, to exposure to the risk of developing superadded respiratory infections or even those associated with medical procedures. In our study, it seems that this complication was much more frequent among younger, smoking individuals from urban environments.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding.

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EDITORIAL

MINIMALLY INVASIVE APPROACH IN SURGERY OF INGUINAL HERNIA

The history of groin hernia repair dates back to the times of Ancient Egypt and Greece, including also Hippocrates reference, Galen anatomical insights and passing to Middle Ages in 13th Century when Ronaldo Parmensis in *Practica Chirurgiae* established a cornerstone with details of medical treatment of hernia with an astonishing picture.

Many other surgeons contributed to the development of groin hernia repair and split it into two major directions, both of which rely on the anatomical concept of the myopectineal orifice and the pathological concept of repairing the fasciascie transversalis (Bassani, Shouldice, 1953).

One direction is autologous repair, using the patient's own tissue without implanting foreign materials, with the advantages of no risk of immunological reaction to foreign bodies, lower cost as no mesh is required, but with important disadvantages of using weak patient tissue for repairing a weak parietal structure (like "poor tailoring patches"), more postoperative pain (2) but, mainly, not respecting the "tension free" concept introduced by Lichtenstein in 1986.

The other direction is heterologous repair started with Stoppa in 1975, using a foreign material to consolidate the weak inguinal region and with the advantages of respecting the tension-free concept with the consequences of less pain and recurrences, but with disadvantages of possible immunological and inflammatory reactions to foreign bodies, mesh infection, and higher costs because of the mesh requirement (3).

Currently, autologous techniques are used with good results in a few places, such as the Shouldice Center, hernias with contaminated tissues, or mesh contraindications.

Heterologous techniques are now the gold standard for most parietal defects made in an open approach, such as the *Lichtenstein procedure*, and in minimally invasive approach, like the transabdominal preperitoneal technique (TAPP) and total extraperitoneal technique (TEP) - intraoperative aspects of TEP repair. In the EHS guidelines, mesh-based techniques-particularly the *Lichtenstein technique* - and endoscopic methods are recommended for treatment of symptomatic primary inguinal hernia in adult men (strength of recommendation IA) (4). Robotic surgery as a minimally invasive approach has shown good results.

The competition in inguinal hernia repair lies between the *Lichtenstein procedure* and *minimally invasive surgery* (MIS). The *Lichtenstein procedure* is well standardized, has a shorter operative time, is less expensive, and requires local or regional anesthesia. MIS brings the benefits of laparoscopic procedures: short hospitalization, reduced return to work time, and fast recovery.

The answer to the question of which is the best approach in hernia repair was determined by a prospective randomized study in 2022 on 174 patients comparing the surgical outcomes of TEP and *Lichtenstein tension-free open mesh inguinal hernia repair* (5). The surgical outcomes evaluated were postoperative pain, recurrence rate, operative time, and complications including seroma, infection, and chronic groin pain.

In conclusion, although TEP provides evidence of a longer operative time and learning curve, advantages such as less

postoperative pain, early resumption of normal activities, less chronic groin pain, and comparable recurrence rate with *Lichtenstein repair* are significant. Therefore, TEP can be performed by experienced surgeons with acceptable outcomes and fewer postoperative complications. The indications for MIS are unilateral, bilateral, and incarcerated inguinal hernia (4). In experienced hands, even large and strangulated inguinal hernias can be considered, depending on the patient particularities and anesthesia tolerance. TAPP has the advantage of easy reduction of the hernia sac and treatment of its contents (epiploon resection, enterectomy with anastomosis, etc.). TEP is also possible, but it requires first, intraperitoneal entry for reduction and content treatment and then, followed by withdrawal of the camera in the proper space to continue the procedure. Between TAPP and TEP, we consider that TEP allows the use of a larger mesh and avoids the boring and time-consuming step of peritoneal suture, with a lower risk of peritoneal adherence, but we admit that our most experience is with TEP. Currently, glue is used instead of the peritoneal sutures. Another advantage of TEP is that it does not require mesh fixation to the Cooper's ligament.

Regarding mesh fixation, a systematic review and meta-analysis of randomized controlled trials included eight trials with 2,617 patients with 2,878 hernias (6). The non-fixation group was associated with lower postoperative pain scores at 24 hours and 6 months, shorter mean operative time, and lower incidence of urinary retention. There were no significant differences in the recurrence rates of patients with an inguinal orifice smaller than 4 cm.

Another point of interest is the necessity of controlling the exploration and repair of an eventually occult inguinal hernia. A systematic review of 13 studies (one randomized and 12 observational cohorts with 5,000 patients) concluded that immediate repair of occult contralateral inguinal hernias diagnosed at the time of elective hernia repair is not justified, and following intraoperative diagnosis of an occult contralateral hernia, more than 70% of the patients will not require treatment, without contralateral exploration less than 10%, are likely to present for contralateral repair. Immediate diagnosis and repair can result in more complications than expectant management (7).

What about the defect closure? We believe that a defect more than two centimeters should be laparoscopically closed, preferably with a V-loc suture to reduce seroma formation, or loop closure (8).

Regarding the learning curve there is data showing that TEP and TAP seems to be similarly, with 20-60 cases for a beginner in TEP and 20-65 for TAPP, as couple of studies present (9, 10).

There are some limitations of the minimally invasive approach in inguinal hernia: intolerance to pneumoperitoneum with necessity of conversion, reduced space of dissection, adherences, and poor knowledge of proper anatomy, mostly in TEP.

Another difference is the necessity of adequate instrumentation to enter the peritoneal space during TEP. With more experience, proper space entry could be done with the usual laparoscopic instrumentary.

Finally, I consider that each patient should have a personalized indication for TAPP, TEP, or an open approach, *Lichtenstein procedure*. TAPP and TEP in robotic surgery have more facilities but with higher costs (11).

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NAVIGATING GENDER DYSPHORIA THROUGH POLITICAL TURMOIL: A ROMANIAN PERSPECTIVE

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NAVIGATING GENDER DYSPHORIA THROUGH POLITICAL TURMOIL: A ROMANIAN PERSPECTIVE (Abstract): In the charged environment of the 2024 national and international legislative elections, this study explores how campaign rhetoric and media narratives reshaped Romanian adults' attitudes toward gender dysphoria, incongruence and minority identities. Its **aim** is to determine the extent to which evolving legislative debates influence public perceptions and individuals' self-assessed social acceptance of gender diversity. We compared acceptance levels before and during the elections, identified demographic and psychosocial factors driving respondent engagement and assessed the prevalence of perceived discrimination and institutional obstacles of adults with diverse gender identities. This study shows how political rhetoric, media and public attitudes either promote or impede gender inclusivity in Romania. **Materials and methods:** We conducted a comparative, exploratory, descriptive cross-sectional study before and during the 2024 elections among Romanian adults aged 18-65 y.o. **Results**: This study reveals that Romania's 2024 legislative election cycle, marked by polarized campaign rhetoric and intensified media narratives, coincided with a measurable decline in self-assessed social acceptance of gender diversity and a surge in perceived discrimination. Demographically, young, urban and highly educated women led participation, yet cisnormative attitudes continued to dominate across all groups. Although legislative frameworks remained largely neutral, aggressive political discourse and social media messaging amplified psychological distress, discouraged open dialogue about gender dysphoria and deterred engagement in research participation. **Conclusions:** These findings reveal that political and media climates shape public views of diverse identity and gender dysphoria, calling for education, policy, and community initiatives to foster inclusion. **Keywords:** IDENTITY, GENDER, DYSPHORIA, CISNORMATIVE, INCLUSIVITY.

INTRODUCTION

Gender dysphoria, as defined by DSM-V, is a significant incongruence between a person's experienced gender and their assigned gender at birth, lasting at least six months. Introduced in 2013, it shifted focus to incongruence itself, emphasizing distress as the key diagnostic criterion (1).

ICD-11 similarly includes "gender incongruence" to ensure healthcare access but classifies it under sexual health instead of mental disorders, using terms like "expe-

rienced gender" and "assigned sex" instead of "opposite sex" or "anatomical sex". While both DSM-V and ICD-11 highlight distress and dysfunction as diagnostic criteria, ICD-11 does not mandate these aspects for diagnosis (2).

The historical evolution of gender identity has transitioned from psychiatric diagnosis to depathologization, highlighting a distinctive feature of contemporary times and the focus of this study (3, 4). While references to gender change appear in Greco-Roman sources, individuals considered transgender today existed in premodern eras. By the mid-19th century, medical interest in this phenomenon emerged, and depathologization currently shapes official attitudes. Though the etiology of transgender behavior remains unclear, research suggests neurobiological underpinnings, dismissing the idea that it stems from social trends or should be subject to religious or political regulation.

Gender dysphoria frequently prompts discussions on the socio-cultural and political conditioning of its public recognition, tied to societal acceptance. Another perspective views these elements within gender ideology as a phenomenon of "social contagion", which is notably widespread in Western societies (3, 4, 5).

This research takes place during a tumultuous historical period marked by significant global changes that also resonate within Romanian society. Some authors highlight ideological clashes impacting traditionalist cultures, driven by themes like human-induced climate change and gender ideology, which have become prominent in political campaigns. In 2024, approximately 1.6 billion people worldwide elected new political leaders, reflecting ongoing ideological shifts between "globalism" and "sovereignism", reshaping citizens' lives. These societal tensions often foster intolerance and discrimination, including against nonconforming gender identities, regardless of whether individuals experience gender dysphoria.

The study analyzes data from specialized literature on changes in the "social acceptance" of gender nonconformity and dysphoria within the politically charged context of the 2024 legislative elections across various countries. International developments are seen as influencing Romanian society. In the U.S., gender diversity was historically promoted as an expression of personal authenticity, aligning inner experiences with self-perceived gender and sexuality. Gender was viewed as self-constructed, sexuality as fluid, and monogamy as a "cultural constraint".

Progressive policies in the U.S. and Canada have enabled gender self-identification from an early age and medical transition without requiring gender dysphoria, though phenomena like "rapid onset gender dysphoria" remain debated. Social media plays a significant role in influencing gender incongruence among youth, with global attitudes shifting from intolerance to greater acceptance. Studies document widespread "gender affirming" procedures, often undertaken without dysphoria as a criterion (6, 7, 8, 9, 10).

Acceptance of gender diversity is closely tied to political context. Liberal policies foster inclusivity, while conservative ideologies often correlate with trans prejudice. Recently, social acceptance in the U.S. and Western Europe has stagnated, partly due to negative political discourse, especially from right-wing parties. This shift affects healthcare, education, and overall societal attitudes, as seen in studies like D. Brokke's on LGBT+ acceptance in the Netherlands (11, 12).

Reports such as GLAAD's "Accelerating Acceptance Study" show that while

overall acceptance of LGBTQ individuals remains high, support has declined. In 2024, 80% of respondents supported equal rights for LGBTQ individuals, down from 84% a year prior. Additionally, 70% of Gen Z LGBTQ adults reported discrimination based on their sexual orientation (12, 13).

The influence of politics on social acceptance is evident in legislative decisions. The "2024 in LGBT Rights" report highlights 78 legislative changes globally in 2024 impacting LGBT individuals, with 123 changes in 2023 and 625 since 2020 (13). These changes affect civil rights, specialized healthcare for transition processes, legal document updates, and the transitioning of minors. While approaches vary, the scope of the "trans" phenomenon is widely recognized, especially in Western countries, where many aspects are legally regulated (13).

In 2024, gender-related rights varied significantly worldwide: In Canada, "Gender Affirming Care" was legal, with specific regulations differing by region, but funding was limited to individuals with gender dysphoria. It was also legal in Wales, UK, but banned in Iraq. Blood donation bans for LGBTQ+ individuals were lifted in Honduras, Denmark, Czechia, Bermuda, Estonia, and the Netherlands. Germany recognized nonbinary genders and allowed annual gender and name changes based on self-identification under the "Selbstbestimmungsgesetz". In the USA, "Gender Affirming Care" and gender transitions were restricted in states like Georgia, Kentucky and Idaho, while other states like Arizona and Florida permitted such rights, although shifts in policies reflected political changes. Tennessee enacted laws against LGBTQ+ couples and transgender individuals. Peru classified transgenderism as a mental disorder. Same-sex marriage became legal in Aruba, Curaçao, Greece and Estonia, while civil unions were recognized in Lithuania. Nepal legalized same-sex unions without granting full civil rights. Anti-LGBTQ propaganda laws were enacted in Bulgaria, South Carolina, El Salvador and Transnistria, the latter imposing imprisonment to "protect traditional values". Conversion therapy bans were introduced in Mexico, parts of Switzerland, Peru and Portugal. Namibia and the Dominican Republic decriminalized homosexuality, while Iraq maintained criminalization but allowed gender-confirmation surgeries for intersex children.

In Romania, a traditionally conservative society, gender nonconformity has gained visibility but faced resistance. While there is no specific legislation, a 2023 European Court of Human Rights ruling required Romania to recognize and protect LGBTQ families (14). Adherence to EU directives led to the acknowledgment of same-sex marriages performed in EU countries, though public acceptance remains low, with over 60% opposing LGBTQ rights according to surveys (15). Nevertheless, societal tolerance has grown incrementally, with nearly 40% expressing support or neutrality toward the LGBT community.

Efforts promoting diversity and inclusivity reflect global legislative shifts, but significant disparities remain across regions (15, 16).

Professor Daniel David highlights that Romanian psychology tends to resist change, favoring stability and continuity. He notes that Romania scores high in uncertainty avoidance (17), perceiving future changes as threats rather than development opportunities. In this context, gender nonconformity and dysphoria pose significant challenges for social acceptance.

Following major political shifts in the U.S. in January 2025, progressive gender ideology has been actively opposed by the

new administration. This shockwave is expected to influence Europe, including Romania, where transgender-related policies and healthcare interventions have not been widely implemented. In Western countries, many individuals who underwent gender transition procedures now face medical challenges, particularly in the U.S., where funding for specific healthcare has been halted. Similarly, progressive policies in Europe have faced increasing opposition, with legislative elections in 2024 bringing politically contrasting forces to power. Researchers have observed a decline in social acceptance of transgender individuals, a trend expected to continue into 2025 across Europe.

Future studies will shed light on how Romanian society, with its dominant cultural traditions, will adapt to and reposition itself regarding gender incongruence.

MATERIALS AND METHODS

This study examines the self-assessed social acceptability of gender incongruence and dysphoria among Romanian adults in 2024, amid limited awareness and heightened negative propaganda during electoral campaigns. While Romanian psychological traits suggest both tolerance and conservatism, public understanding of gender dysphoria remains low, often shaped by biased social media content. Political rhetoric in 2024 amplified intolerance, reflecting global tensions between progressive and conservative ideologies.

Using a comparative, exploratory and descriptive cross-sectional approach, the study distributed questionnaires twice monthly to Romanian adults (18-65 y.o.) via social media and messaging platforms. It analyzed social media posts, demographic data, and self-perceived discrimination during electoral campaigns. Data were collected across two periods: before (June 1st-July 1st, 2024) and during the elections (October 25th-November 24th, 2024) and correlated with in-depth interviews.

Findings indicate that aggressive political discourse negatively impacted social acceptance of gender identity. Challenges of the research included platform restrictions, misinterpretations and hostile responses. The study underscores the need for interdisciplinary collaboration, improved medical education and public health initiatives to enhance understanding and support for individuals with gender dysphoria.

Ethical guidelines ensured confidentiality and GDPR compliance, with data securely stored offline. The research adhered to European human rights standards, prioritizing human dignity. A total of 170 respondents participated, with significantly lower engagement during the campaign period, likely due to socio-political tensions.

RESULTS

The study highlights a noticeable decline in willingness to participate during the electoral period, potentially linked to heightened social tensions. Data collected included demographic details and responses to open-ended questions, complemented by in-depth interviews for a nuanced interpretation. The findings suggest a correlation between social tension and reduced engagement, emphasizing the impact of political propaganda on societal attitudes toward gender identity.

Young adults (18-29) showed the most interest, followed by those aged 30-39, with no participants over 60. Women were significantly more engaged, making up 85.88% of respondents. Gender identity was predominantly cisgender (81.1%), with

5.29% identifying as gender nonconforming and 2.94% choosing not to respond.

Most respondents lived in urban areas (79.41%) and had higher education (77.64%). Regarding marital status, 64.11% were in a relationship, with fewer married participants in phase II. Orthodox affiliation was 67.05%, lower than the national census (85%)

Professionally, 62.94% were unemployed (58.95% in phase I, 77.77% in phase II), with only 30.58% employed. Intellectual work was prevalent (51.49% in phase I, 33.33% in phase II, totaling 47.64%).

Most participants came from traditional family backgrounds. Some (13 total) reported parental preference for them to be the opposite sex, potentially influencing gender identity. Parental affection tied to gender was noted by 10 respondents.

A vulnerable psychological profile emerged, with 119 reported traumas. In phase I, serious illness of a loved one (36 cases), bullying (30), verbal abuse by fathers (20), family violence (19), and other parental abuse (neglect, withdrawal of affection) (18) were most common. In phase II, bullying (10), other parental abuse (7), family violence (5), and physical aggression by mothers (5) were frequently reported. Physical aggression by fathers was noted by 15 respondents across both phases.

Most participants did not seek psychiatric care (113 in phase I, 32 in phase II), while a small number did (19 in phase I, 4 in phase II). Two in phase I reported past psychiatric hospitalizations, and psychiatric treatment was mentioned by 14 respondents overall. Preference for alternative therapies was noted in 34 cases.

Self-harming behaviors were reported by 27 participants, with substance abuse including smoking (32 cases), alcohol (10 cases), and psychotropic medication use (7 cases). One respondent admitted to illegal drug use.

Eating disorders were confirmed by 12 respondents through specialist consultations. Suicide attempts, the most severe self-harming behavior, were acknowledged by 17 participants, with 4 declining to answer.

The DSM-V evaluation showed that most participants did not meet the criteria for gender dysphoria (90.29% in phase I, 86.11% in phase II). Among the 18 diagnosed, key issues included gender incongruence, distress and a desire for gender affirmation.

Of 170 respondents, only 7 considered medical affirming intervention, while 11 diagnosed individuals did not plan to transition. Four had already undergone gender affirmation procedures, one was in treatment, and three planned future interventions. A significant number (14 in each phase) declined to answer, reflecting reluctance to discuss sensitive topics.

Most respondents (91.76%) reported no legislative barriers to gender identity expression, while 3.52% faced obstacles, and 4.70% declined to answer. Workplace discrimination was noted by 3.52%, and 2.35% experienced healthcare discrimination, particularly among nonconforming individuals.

Overall, 8.23% perceived gender identity-based discrimination, with higher rates in phase II (16.66%) compared to phase I (5.97%), likely due to aggressive political discourse. Despite this, 88.23% did not perceive discrimination, reflecting Romania's traditionally tolerant traits, though phase II showed a decline in this perception.

Social media (47.05%) and television/mass media (26.47%) were key sources of gender identity information, with 73.52% acknowledging media influence. The study compared two periods: one

with low public interest and traditionalist views, and another during the electoral campaign, where gender identity was framed as a threat to family values and social morality.

Official data showed that 21.29% of Romania's 9.4 million presidential election voters supported conservative, traditionalist options, rejecting gender ideology. Among 711,067 diaspora voters, 55% favored sovereigntist views despite exposure to Western progressive ideologies.

In the parliamentary election, 30% backed conservative choices, with similar trends in urban and rural areas. Diaspora voters in the U.S. (69.64%), Australia (71.11%), Canada (75.60%), and Russia (58.59%) showed strong anti-progressive preferences, reflecting cultural resistance to gender ideology even abroad.

CONCLUSIONS

The socio-political climate in Romania mirrored the U.S. in 2024, where voters shifted toward conservatism, traditionalism, and intolerance of gender nonconformity, contrasting with the previous administration's progressive stance. Studies highlight the interplay between politics and religion, with Christian nationalism emerging as a key predictor of traditionalist gender ideologies, influencing broad support for the newly elected conservative U.S. president in 2024.

The study found that Romania's 2024 electoral campaigns negatively impacted participants' self-assessed social acceptance, regardless of gender identity, due to socio-political pressure. Participation declined during the campaign period, with most respondents being young, urban, highly educated women from traditional backgrounds. Cisgender identities dominated, with only 5.29% identifying as nonconforming and 2.94% opting not to disclose their gender identity, reflecting cultural conservatism.

Limited awareness of gender ideology was noted, with media-especially social media and television-shaping public perception. Psychological vulnerabilities, including parental divorce, bullying, and family violence, were common, with eating disorders and self-harm increasing in phase II. Psychiatric consultations were more frequent in phase I, while suicide attempts rose in phase II, possibly due to socio-political tensions.

Traditional cisgender identities remained dominant, with reported legislative barriers, workplace discrimination, and limited healthcare access. Social acceptance of gender diversity declined during the campaign, with fewer participants and increased reluctance to engage. The findings highlight the need for further research on societal attitudes and the influence of political and cultural factors on gender diversity in Romania.

The study highlights the impact of Romania's 2024 electoral campaigns on gender identity perceptions, with socio-political pressure reducing self-assessed social acceptance and participation. Traditional cisgender identities dominated, while nonconforming identities remained a minority, reflecting cultural conservatism.

Media, especially social media and television, shaped public perception. Psychological vulnerabilities, including trauma, bullying, and family violence, were common, with self-harming behaviors and eating disorders increasing during the campaign. Psychiatric consultations were more frequent in phase I, while suicide attempts rose in phase II, likely due to political tensions.

Despite some progress in tolerance, legislative barriers, workplace discrimination,

and limited healthcare access persist. The study underscores the need for further research, education, and policy development to foster gender inclusivity in Romania's evolving social landscape.

CONFLICT OF INTERESTS AND FUNDING

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OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA. A SINGLE-CENTER EXPERIENCE FROM THE IAȘI REGIONAL INSTITUTE OF ONCOLOGY

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OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA: A SINGLE-CENTER EXPERIENCE FROM THE IAȘI REGIONAL INSTITUTE OF ONCOLOGY (Abstract): Allogeneic stem cell transplantation (allo-SCT) remains the only curative treatment option for acute myeloid leukemia (AML) patients. We present the outcomes of 59 AML patients who underwent allo-SCT at our center. **Materials and methods**: We retrospectively analyzed patients transplanted between 2019 and 2024. Baseline characteristics included age, sex, ELN 2022 risk, disease status at transplant (CR1, CR2, active disease), donor type, GVHD prophylaxis. Outcomes evaluated were engraftment, acute and chronic GVHD, cumulative incidence of relapse (CIR), non-relapse mortality (NRM), overall survival (OS), disease-free survival (DFS). Survival was estimated by the Kaplan-Meier method. **Results**: The median age was 44 years. 84.7% of patients were transplanted in CR1. Donors included: matched sibling 42.4%, unrelated 49.2%, and haploidentical 8.5%. Incidence of grade II-IV acute GVHD was 16.9%, and chronic GVHD 25.4%. At a median follow-up of 26 months (95% CI: 32-44), OS and DFS were not reached. Patients transplanted in CR1 showed the best outcomes (mean OS 88.7 months; 2-year DFS: 74%), while CR2 patients had inferior results (mean OS 33.5 months; 2-year DFS: 40-45%). Differences were significant (OS p=0.004; DFS p=0.007). Measurable residual disease at day+90 was the single most robust factor for predicting outcome in both univariate and multivariable analysis. **Conclusions**: Our single-center experience confirms that allo-SCT is feasible and effective in AML, with outcomes comparable to international reports. The disease status at the moment of transplantation and measurable residual disease remain the most powerful prognostic factors to identify patients at high risk of relapse.
Keywords: ACUTE MYELOID LEUKEMIA, EUROPEAN LEUKEMIA NET, ALLOGENEIC STEM CELL TRANSPLANT, MEASURABLE RESIDUAL DISEASE.

INTRODUCTION

Acute myeloid leukemia (AML) is a highly aggressive hematologic malignancy defined by the proliferation of immature myeloid precursors and extensive bone marrow (BM) infiltration, ultimately leading to patient demise in the absence of therapy. AML is a rare form of cancer, with an incidence of 5 cases per 100000 healthy individuals in Europe (1).

The last decade has seen significant advances in the therapy of AML, with the advent of the novel targeted molecular therapies or immunotherapies, used either in association with standard intensive "3+7" chemotherapy in younger patients or alongside hypomethylating agents in cases deemed unfit for an intensive approach. Despite these advances, virtually all patients are at risk of relapse. Relapsed AML has a dismal prognosis, with 2-year survival rates as low as 7% (2, 3).

Allogeneic hematopoietic stem cell transplantation (allo-SCT) is a potentially curative therapy for AML, that relies on the recognition and elimination of leukemic cells by donor T cells, as part of the graft versus leukemia effect (GVL) (4). However, allo-SCT is complicated by graft versus host disease (GVHD), a pathological state characterized by donor T cell-mediated lysis of liver, gut and skin cells, which is a main driver post-allo-SCT morbidity and mortality, with transplant-related mortality reaching roughly 20% (5). Thus, allo-SCT is recommended only when the risk of disease relapse and leukemia-related mortality exceeds its inherent risks.

The European Leukemia Net (ELN) risk stratification system integrates cytogenetic and molecular abnormalities to classify AML patients into favorable, intermediate, or adverse categories. By linking specific genetic profiles to long-term outcomes, the system guides therapeutic decisions: patients with favorable-risk disease are managed with chemotherapy alone, while those in intermediate- and adverse-risk group are considered prime candidates for allogeneic transplantation in first remission. In this way, the ELN classification can identify patients who are most likely to benefit from allo-SCT and those in whom its risks can be safely avoided (6).

The year 2022 marked a turning point in the field of AML, with the simultaneous publication of the fifth edition of the WHO Classification of Hematolymphoid Tumors and the revised, 2022, ELN recommendations. Both classifications emphasize the central role of molecular genetics in disease definition, risk stratification, and therapeutic decision-making, drastically modifying the clinical approach to AML (6, 7).

The prognostic system proposed by the ELN 2022 classification has not yet been systematically validated in Romanian AML cohorts. Only in recent years has a national program provided consistent access to immunophenotyping, cytogenetic, and molecular testing, enabling clinicians to apply these risk stratification tools in routine practice. At the same time, data from the World Health Organization highlight that Romania continues to carry a disproportionate burden of comorbid conditions such as cardiovascular disease, chronic obstructive pulmonary disease, asthma, tuberculosis, and depression-each of which may adversely affect AML outcomes (8). In addition, broader determinants of health reported by the WHO, including disparities in education, uneven access to medical services in rural regions, deficiencies in primary care, and some of the lowest public health expenditures in the European Union, are likely to further influence prognosis in this population (9). In this particular context, real-world data remain essential to assess how these global advances in the molecular and genetic classification and risk prognostication of AML translate into local practice.

MATERIALS AND METHODS

In this retrospective study, we have investigated 59 patients diagnosed with *de*

novo AML who received an allo-SCT in the Bone Marrow Transplant Department of the Regional Institute of Oncology, Iasi, Romania, between 2019 and 2024. The diagnosis of AML was established based on the 2022 WHO classification of myeloid neoplasms. Furthermore, the patients were risk stratified according to the 2022 ELN recommendations. This study was approved by the institutional ethics committee. The diagnosis of AML was confirmed by immunophenotyping according to EuroFlow standards (10). Conventional cytogenetic G-banding was performed using standard procedures. Four recurrent fusion transcripts (BCR-ABL1 p210, PML-RARA, RUNX1-RUNX1T1, CBFB-MYH11) were assessed by reverse transcriptase quantitative PCR. Molecular testing included NPM1 mutation analysis and FLT3-ITD detection. Complete remission (CR) was defined as the presence of <5% blasts in the bone marrow, absence of circulating blasts or extramedullary disease and recovery of the peripheral blood counts with an absolute neutrophil count $\geq 1.0 \times 10^9$/L and a platelet count $\geq 100 \times 10^9$/L, and no transfusion requirement. Pre-transplant patient fitness was assessed using the Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI) score. Measurable residual disease (MRD) was evaluated by multiparameter flow cytometry and reverse-transcriptase polymerase chain reaction in patients in whom leukemia-associated phenotypes or traceable molecular anomalies were identified (10).

The statistical analysis was performed using the IBM SPSS Statistics 21.0 Software. The Chi square and Mann Whitney tests were used to analyze the associations between different variables. A p value <0.05 was considered to be statistically significant. Overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan Meier method. The log rank test was used for comparisons of Kaplan-Meier curves. OS was defined as the interval from the date of transplantation to death from any cause or to the date of last follow-up for surviving patients. DFS was defined as the time from transplant to relapse, death by any cause, or the date of the last follow-up.

RESULTS

We retrospectively analyzed 59 AML patients, diagnosed and classified as per the WHO 2022 and ELN 2022 classification and risk prognostication systems, who underwent allo-SCT at our center between 2019 and 2024. The baseline clinical and laboratory characteristics are presented in first table.

The median age at diagnosis was 44 years. The mean follow-up duration was 26 months. According to the 2022 WHO classification of myeloid neoplasms, the distribution of AML subtypes was as follows: AML with recurrent genetic anomalies- 19 cases (32.2%), AML with myelodysplasia related features- 8 cases (13.6%), AML defined by differentiation- 32 cases (54.2%). The detailed cytogenetic and molecular anomalies identified are presented in Table I. Notably, FLT3-ITD (Fms-like tyrosine kinase 3-internal tandem duplication) mutations were identified in 9 patients (15.3%), NPM1 (nucleophosmin 1) in 4 cases (6.8%), while the two mutations co-existed in 10 patients (16.9%). Based on the cytogenetic and molecular evaluation, patients were stratified according to the 2022 ELN classification into favorable (9 cases- 15.3%), intermediate (37 cases- 62.7%), and adverse risk groups (13 cases- 22%).

TABLE I.

Demographic, clinical, biological, cytogenetic, molecular and therapy response data of AML patients.

Patient characteristics	
Sex, n (%)	
Male	26 (44.1%)
Female	33 (55.9%)
Age	
Median (years)	44
<35/ >35yrs., n (%)	19 (32.2%)/ 40 (67.8%)
ECOG score at diagnosis	
0-1/2-3, n (%)	47 (79.6%)/12 (20.3%)
WBC at diagnosis	
Median	10300/μL
>100000/<100000, n (%)	7 (11.9%)/ 52 (88.1%)
AML type, n (%)	
De novo	48 (81.4%)
Therapy-related	2 (3.4%)
Secondary AML	9 (15.3%)
WHO 2022 classification, n (%)	
AML with recurrent genetic abnormalities	19 (32.2%)
AML with multilineage dysplasia	8 (13.6%)
AML, defined by differentiation	32 (54.2%)
AML, defined by differentiation, type, n (%)	
Minimally differentiated	1 (3.1%)
Without maturation	4 (12.5%)
With maturation	7 (21.8%)
Acute myelomonocytic leukemia	12 (37.5%)
Acute monoblastic leukemia	8 (25%)
AML therapy type, n (%)	
Intensive, "7+3" based	53 (89.8%)
Venetoclax+Azacitidine	6 (10.2%)

AML= Acute Myeloid Leukemia,
ECOG= Eastern Cooperative Oncology Group

TABLE II.

Transplant specific variables of AML patients undergoing allo-SCT

Variable	
Disease status at SCT	
CR1, n (%)	42 (71.2%)
CR1, MRD neg, n (%)	23 (46.9%)
CR2, n (%)	12 (20.3%)
Progressive disease, n (%)	5 (8.5%)
HCT-CI	
0-2, n (%)	50 (84.7%)
>2, n (%)	9 (15.3%)
Donor type	
MSD, n (%)	25 (42.4%)
MUD, n (%)	29 (49.2%)
Haplo, n (%)	5 (8.5%)
Conditioning regimen	
MAC, n (%)	30 (50.8%)
RIC, n (%)	29 (49.2%)
GVHD prophylaxis	
TACRO/MMF, n (%)	49 (83%)
PTCy, n (%)	40 (67.8%)
CSA/Metothrexate, n (%)	10 (17%).
GVHD (any type)	24 (40.7%)
Acute GVHD, (%)	10 (41.7%)
Skin	8 (33.3%)
GI tract	4 (16.7%)
Liver	3 (12.5%).
Chronic GVHD, (%)	12 (50%)

CR= complete remission, CSA= Cyclosporin A, Haplo= haploidentical donor, HCT-CI= hematopoietic stem cell transplantation comorbidity index, MAC= myeloablative conditioning, MRD= measurable residual disease, MSD= matched sibling donor, MUD= matched unrelated donor, N/A= not applicable, PTCy= post-transplant Cyclophosphamide, RIC= reduced intensity conditioning, SCT= stem cell transplant

89.8% of patients (n=53) received intensive chemotherapy with the standard "7+3" chemotherapy regimen as a backbone, while 10.2% (n=6) were treated with a less intensive induction regimen consisting of Venetoclax and Azacitidine. 84.7% (n=50) of patients achieved complete re-

mission following induction. Out of the patients achieving CR, 46.9% (n=23) presented MRD negativity after induction. Of the 59 patients, the majority (42 cases, 71.2%) underwent allo-SCT in CR1 (first CR), while 12 patients (20.3%) were transplanted in CR2. A smaller subset of 5 patients (8.5%) proceeded to transplant with progressive leukemia. The median time from diagnosis to transplant was 8 months.

All patients in our study group underwent allo-SCT at our center. The main transplant-related parameters are presented in second table. The CR rate at day+30 was 98.3%.

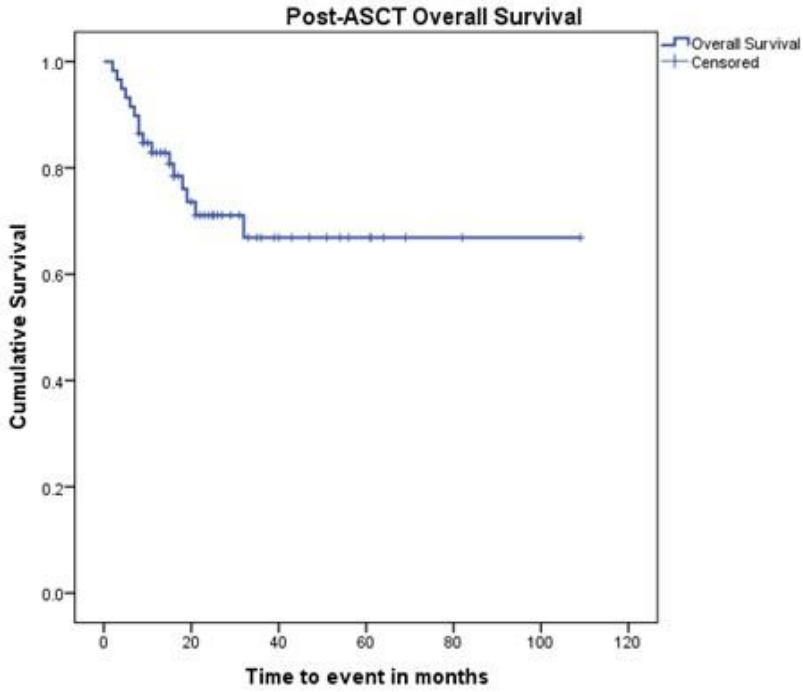

Fig. 1. Kaplan-Meier curve **for overall survival** in the entire study cohort.

At a median follow-up of 26 months, 43 patients (72.9%) were alive and 42 patients (71.2%) remained in remission. The median OS and DFS were not reached. The mean OS was 77.4 months (95% CI, 64.2-90.7), and the mean DFS was 77.6 months (95% CI, 64.9-90.2). The Kaplan-Meier estimated OS at 24 months was 74% (fig. 1), while the corresponding DFS rates were 74% at 12 months and 69% at 24 months (fig. 2).

Patients with post-transplant relapse (n = 17) had poor outcomes, but the occurrence of GVHD was strongly associated with improved survival. The median OS for patients without GVHD was 8 months (mean 11.2 months, 95% CI 6.1-16.3), with no long-term survivors. In contrast, patients who developed GVHD had a median OS of 18 months (mean 41.0 months, 95% CI 11.5-70.5), and two patients remained alive beyond 5 years.

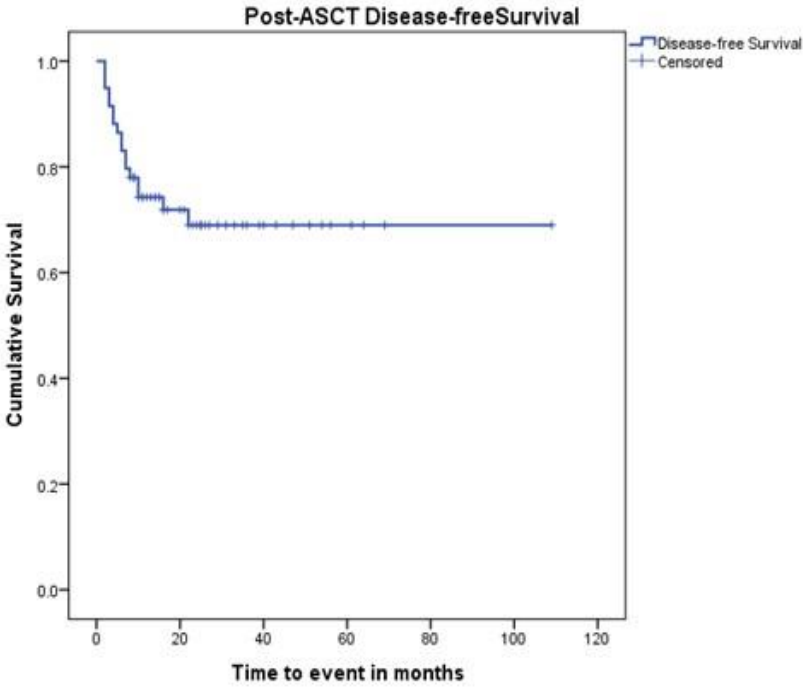

Fig. 2. Kaplan-Meier curve **for disease-free survival** in the entire study cohort.

On Kaplan-Meier analysis, OS and DFS differed significantly according to AML type, ELN 2022 risk, HCT-CI score, and disease status at transplantation (tab. III). Patients with *de novo* AML and those transplanted in CR achieved the most favorable outcomes, while secondary and therapy-related AML and active disease at transplant were associated with markedly inferior OS and DFS ($p < 0.05$ for all). OS also decreased significantly with increasing HCT-CI score (> 2; $p = 0.002$). MRD negativity, both pre-transplant and at days +30 and +90 post-SCT, correlated with improved OS, with the strongest effects observed at day +30 ($p = 0.007$) (fig. 3) and day +90 ($p = 0.014$) (fig. 4). DFS showed a similar but nonsignificant trend. GVHD occurrence was associated with longer OS ($p = 0.024$). No significant associations were found for age, WBC count, donor type, conditioning intensity, or molecular subtype (all $p > 0.05$). Other baseline parameters, including age at diagnosis, white blood cell count at presentation, the ECOG performance status, the type of AML according to the WHO classification, the NPM1 and FLT-3 status did not show a significant association with either OS or DFS in our cohort (all $p > 0.05$, log-rank test) (data not shown).

Out of the transplant-related parameters, we did not identify a significant association between OS or DFS and the donor type, the degree of HLA mismatch, the type of conditioning or immune suppressive regimen used (all $p > 0.05$, log-rank test) (data not shown).

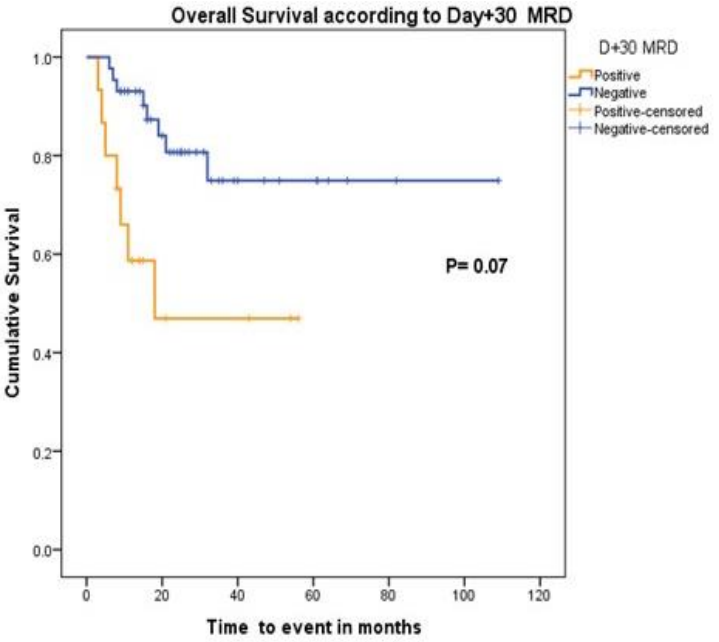

Fig. 3. Overall survival according to minimal residual disease status at day **+30** after allogeneic stem cell transplantation.

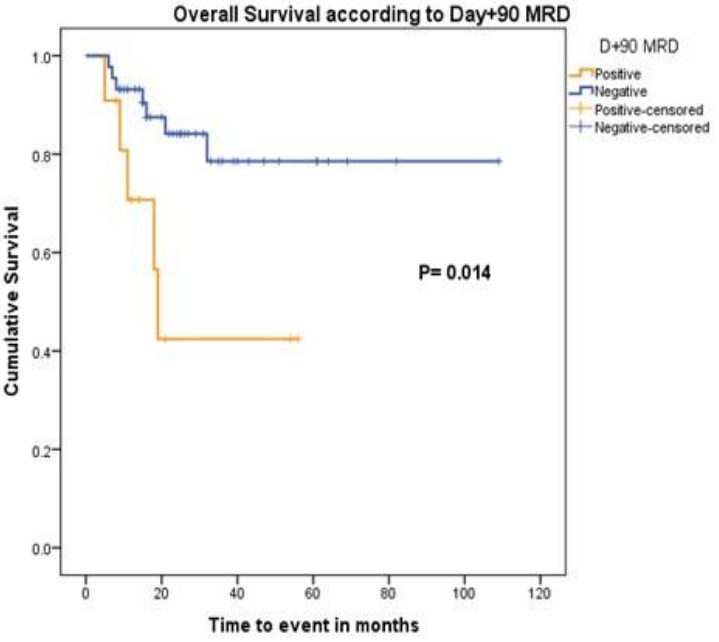

Fig. 4. Overall survival according to minimal residual disease status at day **+90** after allogeneic stem cell transplantation.

TABLE III.

Univariate analysis (Kaplan-Meier) of overall survival and disease-free survival

Variable	Subgroups	OS mean (mo) [95% CI]	DFS mean (mo) [95% CI]	Median OS/DFS	p (OS)	p (DFS)
AML ELN de-nominator	De novo (n=48)	82.9 [69.4-96.4]	82.2 [69.0-95.4]	NR/NR	0.036	0.038
	Secondary (n = 9)	29.8 [20.3-39.2]	28.6 [17.9-39.2]	NR/NR		
	Therapy-related (n = 2)	2.0 [-]	12.0 [0-31.6]	2/2		
ELN 2022 Risk	Favorable (n = 9)	97.8 [77.0-118.5]	97.2 [75.5-119.0]	NR/NR	0.032	0.159
	Intermediate (n = 37)	62.4 [50.5-74.3]	47.6 [38.9-56.3]	NR/NR		
	Adverse (n = 13)	33.6 [16.5-50.7]	37.8 [19.8-55.8]	32/22		
HCT-CI	0-2 (n=50)	83.3 [69.8-96.7]	-	NR/NR	0.002	-
	>2 (n=9)	13.5 [7.6-19.4]	-	11/-		
Disease status al allo-SCT	CR (n=49)	83.5 [69.4-97.6]	82.1 [68.8-95.4]	NR/NR	0.003	0.027
	Progressive (n=10)	9 [4.4-13.6]	4 [12.7-44.3]	9/4		
MRD pre-SCT	Negative (n=20)	95.8 [78.3-113.2]	-	NR/NR	0.074	-
	Positive (n=28)	48.7 [37.3-60.1]	-	-		
MRD D+30	Negative (n=43)	86.2 [71.9-100.6]	84.3 [70.8-97.7]	NR/NR	0.007	0.145
	Positive (n=15)	31.2 [18.0-44.4]	32.9 [18.7-47.2]	19/NR		
MRD D+90	Negative (n=44)	89.5 [76.0-102.9]	87.1 [74.4-99.9]	NR/NR	0.014	0.001
	Positive (n=11)	31.5 [16.9-46.1]	34.6 [18.1-51.2]	19/NR		
GVHD (any)	Present	94.4 [79.0-109.7]	86.5 [69.0-104.1]	NR/NR	0.024	0.186
	Absent	38.3 [28.6-48.0]	40.9 [31.7-50.1]	NR/NR		

AML= acute myeloid leukemia, CR= complete remission, D=day, DFS= disease-free survival, ELN= European Leukemia Net, GVHD= graft versus host disease, HCT-CI= hematopoietic stem cell transplantation comorbidity index, MRD= measurable residual disease, NR= not reached, SCT= stem cell transplant

To identify independent predictors of OS and DFS, we further performed multivariable Cox proportional hazards regression including variables with statistical significance in univariate analysis. In the multivariable model for OS, MRD positivi-

ty at day +90 post-transplant emerged as the only independent adverse prognostic factor (HR = 12.5, 95% CI 1.6-99.5, p = 0.017), after adjusting for MRD at day +30 and GVHD occurrence. MRD positivity at day +30 showed a nonsignificant trend toward inferior survival (HR = 7.4, 95% CI 0.7-74.6, p = 0.09). Neither acute nor chronic GVHD retained statistical significance after adjustment.

For DFS, no independent predictors reached statistical significance in multivariable analysis. MRD positivity at day +90 remained associated with a numerically higher relapse hazard (HR = 11.5, 95% CI 1.4-91.9, p = 0.021), but the overall model fit did not reach significance (LR χ^2 = 4.6, p = 0.20). Chronic GVHD and MRD at day +30 showed no significant effect.

The AML type, pre-transplant disease status, and HCT-CI score were not retained as significant predictors of either OS or DFS in the multivariable models.

At a median follow-up of 26 months, the CIR was 28%, while NRM remained low (2%). The majority of deaths were relapse-related, underscoring disease recurrence as the principal cause of post-transplant mortality in our study cohort.

DISCUSSION

Allo-SCT represents an essential tool in the management of AML, offering the only curative option for high-risk patients. The integration of dynamic prognostic markers such as MRD into pre-transplant individualized risk stratification and post-transplant monitoring strategies further reduces the risk of relapse, by facilitating pre-emptive, MRD-driven therapeutic interventions.

Nevertheless, several reports have questioned the prognostic applicability of cytogenetic and molecular risk factors in patients from developing countries (11,12), including Romania, where socio-economic disparities, uneven access to specialized care, and limited molecular diagnostic infrastructure may influence outcomes (9). In this context, we aimed to evaluate the relevance of the 2022 ELN risk classification system and the prognostic contribution of allo-SCT in a real-world cohort of AML patients treated at the Iași Regional Institute of Oncology.

In our study cohort, the AML type and the disease status at the moment of allo-SCT had a significant impact on post-transplant outcomes. According to the 2022 ELN denominators of disease ontogeny, patients with *de novo* AML achieved the best long-term outcomes. On the other hand, those diagnosed with secondary or therapy-related AML had markedly inferior OS and DFS despite undergoing allo-SCT. These findings evidence that the adverse biological features and treatment resistance associated with antecedent hematologic disorders such as myelodysplastic syndromes or therapy-related AML might not be fully overcome by the allo-SCT procedure. Similar findings have been reported in larger studies, where the disease ontogeny and pre-transplant remission status remained independent predictors of relapse and survival (13,14). Early referral and timely transplantation in molecular remission remain therefore very important, particularly for patients with secondary or therapy-related AML.

Regarding DFS, although a numerical gradient was observed across the ELN 2022 risk groups, the differences did not reach statistical significance. This is most likely explained by the relatively small number of patients in each subgroup, limiting the statistical power of the analysis.

Additionally, the HCT-CI score provided relevant prognostic information regarding survival in our study group, as patients with higher HCT-CI score had significantly inferior OS, confirming that comorbidity burden remains a major determinant of post-transplant mortality. This finding is consistent with prior studies showing the predictive value of HCT-CI for post-allo-SCT outcomes in AML (15,16).

Moreover, in our cohort, patients who developed chronic GVHD had superior OS compared to those with acute or overlap GVHD. This finding likely reflects the protective graft-versus-leukemia effect associated with chronic GVHD, which reduces relapse risk despite its associated morbidity. By contrast, acute and overlap GVHD contributed to inferior outcomes due to early transplant-related mortality and the combined toxicities of both entities.

While both MRD positivity at day +30 and day +90 were associated with inferior survival, persistence of MRD at day +90 identified a particularly poor-risk subset, reflecting failure of GVL activity to eradicate residual disease and indicating imminent relapse.

In multivariable analysis, only MRD positivity at day +90 remained an independent predictor of inferior OS. This finding is consistent with previous reports showing that later post-transplant MRD monitoring can predict relapse risk and long-term outcomes in AML, but also facilitate timely therapeutic interventions, such as donor lymphocyte infusions (DLI) (17,18). The absence of independent predictors for DFS likely reflects the limited event number and interdependence between relapse and transplant-related factors.

Finally, in our cohort, patients experiencing relapse after allo-SCT had a median OS of 11 months, a figure that compares favorably with international registry data. Large series from the EBMT and CIBMTR typically report median survivals of only 3-6 months following post-transplant relapse, with fewer than 15% of patients alive at 2 years despite salvage interventions such as DLI. While our results confirm that relapse after allo-SCT remains a severe event, the observed median OS suggests that intensive and structured management may extend survival even in resource-limited settings. This finding underscores the potential benefit of complex post-relapse therapeutic strategies and highlights that outcomes achieved in our center are not markedly inferior to those reported in higher-resource contexts.

CONCLUSIONS

Allo-SCT remains a curative therapy in AML, particularly for intermediate and adverse ELN risk patients. In our study, survival was strongly influenced by disease status and MRD at transplant, confirming the prognostic importance of achieving pre-allo-SCT deep remission. Secondary and therapy-related AML, high HCT-CI scores, and active disease at transplantation were associated with inferior outcomes. These findings emphasize the need for timely, MRD-driven transplant referral and for expanding transplant capacity in Romania to optimize long-term outcomes of leukemia patients.

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THE ORAL-SYSTEMIC INTERFACE IN SEVERE MENTAL ILLNESS: A COMPARATIVE ANALYSIS OF DIETARY PRO-OXIDANT PATTERNS AND BIO-CLINICAL INTERSECTIONS IN PATIENTS WITH SCHIZOPHRENIA

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THE ORAL-SYSTEMIC INTERFACE IN SEVERE MENTAL ILLNESS: A COMPARATIVE ANALYSIS OF DIETARY PRO-OXIDANT PATTERNS AND BIO-CLINICAL INTERSECTIONS IN PATIENTS WITH SCHIZOPHRENIA (Abstract): This study aims to investigate the nutritional pathways and lifestyle behaviors that bridge psychiatric symptomatology and oral pathological outcomes, specifically focusing on the role of dietary-induced oxidative stress in patients with schizophrenia compared to a healthy control group. **Materials and methods:** A comparative cross-sectional analysis was conducted on 40 participants (20 patients with schizophrenia and 20 demographically matched controls). Nutritional habits, antioxidant intake and pro-inflammatory food frequencies were assessed using validated questionnaires. Data were statistically analyzed to identify significant divergences in consumption patterns. **Results:** Patients with schizophrenia showed a significantly lower intake of dietary fiber compared to controls (1.65 ± 1.51 *vs*. 2.80 ± 1.92, $p = 0.041$). Significant increases were observed in the consumption of fried foods ($p = 0.029$) and processed items ($p = 0.048$). Although antioxidant and fruit intake followed a downward trend, they did not reach the significance threshold, contributing to a documented pro-oxidant dietary environment. **Conclusions:** The results confirm a compromised "gut-brain-mouth" axis in schizophrenia. The dietary-induced pro-oxidant environment serves as a systemic driver for oral degradation, necessitating integrated psychiatric and dental management. **Keywords:** SCHIZOPHRENIA, ORAL HEALTH, OXIDATIVE STRESS, NUTRITIONAL PSYCHIATRY, DIETARY PATTERNS.

INTRODUCTION

Schizophrenia remains one of the most challenging conditions in modern psychiatry, not only due to its debilitating cognitive and emotional symptoms but also because of the significant somatic burden in imposes patients.

Beyond the neurological manifestations, recent research has shifted the focus toward the "whole-body" impact of the disorder, highlighting a state of chronic, low-grade systemic inflammation (1). One of the most

critical yet frequently overlooked components of this physical decline is oral health, which acts as a bio-indicator for the overall psychological status of the patient (2).

The relationship between severe mental illness and dental pathology is multifaceted. Historically, poor oral outcomes in these patients were attributed solely to suboptimal hygiene or the side effects of antipsychotic medication, such as xerostomia (3). However, contemporary perspectives, including the "gut-brain axis" theory, suggest that the pathobiological intersections are much deeper. The nutritional status of patients with schizophrenia often reveals a profound imbalance, characterized by preference for high-caloric, nutrient-poor diets (4). This dietary pattern does not merely contribute to metabolic syndrome but also fuels a pro-oxidant environment that accelerates tissue destruction in the oral cavity.

The oral microenvironment is highly sensitive to the body's antioxidant defense mechanisms. When dietary intake of polyphenols and vitamins is replaced by refined sugars and saturated fats, the resulting oxidative stress triggers an inflammatory cascade in the periodontal tissues (5). Therefore, understanding the dietary choices of these patients is not just a matter of nutritional counseling, but a necessity for preventing irreversible dental damage.

Despite the clear clinical links, there is a scarcity of integrated data that correlate specific food frequency patterns in psychiatric patients with their systemic inflammatory risk. This study, conducted in the Iasi region, provides a foundation for a more holistic approach in both dental and psychiatric clinical practice. By examining these intersections, we aim to demonstrate that oral health management must become a core component of the integrative treatment of schizophrenia.

MATERIALS AND METHODS

This comprehensive, cross-sectional study was conducted between December 2024 and January 2025 in Iasi, Romania. The research focused on evaluating the nutritional architecture and lifestyle factors of patients with severe mental illness in relation to their potential impact on oral health. The study population consisted of two distinct cohorts: a clinical group (n=20) comprising patients diagnosed with schizophrenia according to the DSM-V criteria and a healthy control group (n=20) recruited from the general population with no history of psychiatric or significant systematic disorders.

Inclusion criteria for the clinical group were: a confirmed diagnosis of schizophrenia, age over 18 years and the cognitive ability to provide informed consent and complete the assessment tools. Exclusion criteria included the presence of acute medical emergencies, pregnancy or current participation in intensive nutritional intervention programs. The control group was age-matched to ensure demographic comparability across the 18-60 age range.

The study was conducted in accordance with the Declaration of Helsinki. All participants were informed about the nature of the research, the confidentiality of the data and their right to withdraw at any time. Written informed consent was obtained from each participant or, where applicable, their legal guardians.

A comprehensive digital survey was employed to gather data on demographics, clinical history and dietary habits. The nutritional assessment was based on a modified Food Frequency Questionnaire (FFQ),

specifically designed to capture the "patho-biological intersections" between dietary intake and systemic inflammation. Participants reported their consumption frequency for various food groups over the previous month using a Likert-type scale ranging from "Never" to "Daily".

The survey focused on three specific dietary domains:

1. Protective factors: Frequency of intake for fresh fruits, vegetables, dietary fibers and antioxidant-rich foods (e.g, berries, nuts, leafy greens).
2. Pro-inflammatory and cariogenic factors: Consumption of refined sugars (candies, pastries), carbonated beverages, fast food and deep-fried items.
3. Lifestyle and comorbid factors: Prevalence of smoking, alcohol consumption and the use of dietary supplements or probiotics.

Quantitative data derived from the frequency scales were converted into numerical values to facilitate comparative analysis. Frequency scores were assigned based on consumption regularly (e.g, Daily = 7; 2-3 times/week = 2.5; weekly = 1; Never = 0). Descriptive statistics, including means and percentages distributions were calculated for each dietary variable. The significance of differences between the schizophrenia group and the control group was evaluated using independent t-test or Mann-Whitney U test, depending on the data distribution. A *p-value* less than 0.05 was considered statistically significant. All analyses were performed using Microsoft Excel, ensuring a rigorous interpretation of the nutritional gaps identified.

RESULTS

The study cohort consisted of 40 participants, equally divided between patients with schizophrenia (n = 20) and healthy controls (n = 20). The demographic analysis showed that the psychiatric group was predominantly composed of individuals in the 31-60 age range (85%), with a higher prevalence of unemployment (90%), reflecting the social burden of the disease. In contrast, the control group was more diversely distributed across age groups and employment statuses.

Specifically, the intake of dietary fiber was significantly lower in patients compared to controls (1.65 ± 1.51 *vs.* 2.80 ± 1.92, p = 0.041), representing a 41% reduction. Similarly, consumption of fresh fruits, vegetables and antioxidant-rich foods showed a marked downward trend in the psychiatric cohort, although these did not reach statistical significance (tab. I).

TABLE I.

Comparative analysis of protective dietary factors

Nutritional Factor	Control Mean (± SD)	Schizophrenia Mean (± SD)	*p-value*
Fruits and Vegetables	2.65 (± 2.00)	1.67 (± 1.42)	0.083
Antioxidant-rich Foods	2.27 (± 1.77)	1.42 (± 1.47)	0.107
Dietary Fiber	2.80 (± 1.92)	1.65 (± 1.51)	0.041*

Note: Scores calculated on a scale of 0 (Never) to 7 (Daily); SD = Standard Deviation;
** indicates statistical significance (p < 0.05 based on Independent T-test).*

The nutritional gap is visually represented in the comparison of protective factors (fig. 1).

Consumption of fried foods and saturated fats was nearly double in patients with schizophrenia (2.45 ± 1.75) compared to control group (1.47 ± 0.80, p = 0.029). This pro-inflammatory trend was also evident in the consumption of refined sugars and carbonated beverages (fig. 2).

Processed food consumption was also significantly higher in the psychiatric group (1.25 ± 1.28 *vs.* 0.50 ± 1.03, p = 0.048), while the increased intake of refined sugars (2.35 *vs.* 1.82) contributes to the overall cariogenic potential (tab. II).

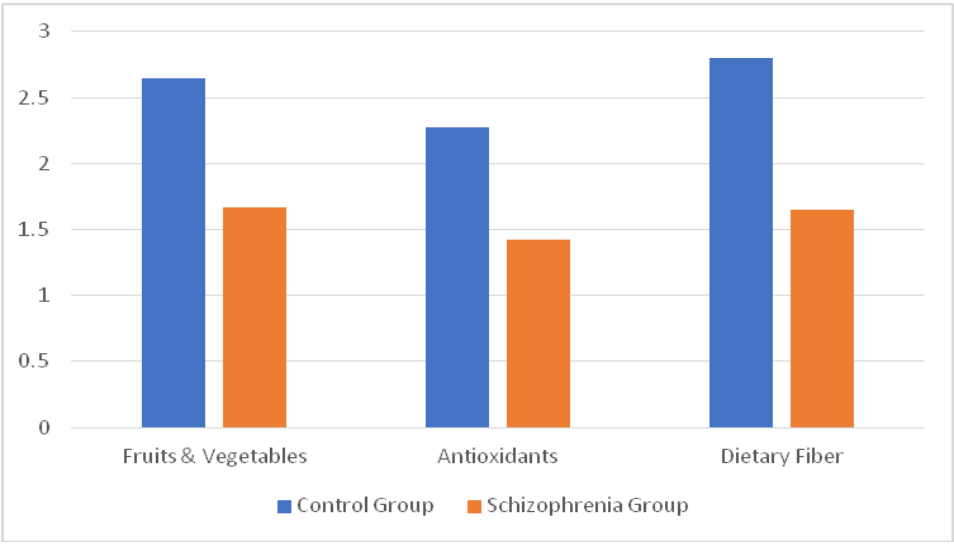

Fig. 1. Protective factors

TABLE II.

Pro-inflammatory and cariogenic risk profile

Risk Factor	Control Mean (± SD)	Schizophrenia Mean (± SD)	*p-value*
Fried Foods and Saturated Fats	1.47 (± 0.80)	2.45 (± 1.75)	0.029*
Refined Sugars (Sweets)	1.82 (± 1.55)	2.35 (± 1.28)	0.249
Carbonated Beverages	1.27 (± 1.01)	1.95 (± 1.91)	0.170
Processed Foods	0.50 (± 1.03)	1.25 (± 1.28)	0.048*

*Note: SD = Standard Deviation; * indicates statistical significance (p < 0.05 based on Independent T-test).*

The escalation of pro-inflammatory dietary choices in the psychiatric group is evident (fig. 2).

Lifestyle variables further compounded these risks. Although smoking frequency was high across both groups, alcohol consumption frequency was more than double in the schizophrenia group (0.90 *vs.* 0.42).

The cumulative data indicate that patients with schizophrenia live in a "pro-oxidant nutritional environment", which, in the absence of adequate antioxidant defense, serves as a primary driver for oral tissue degradation.

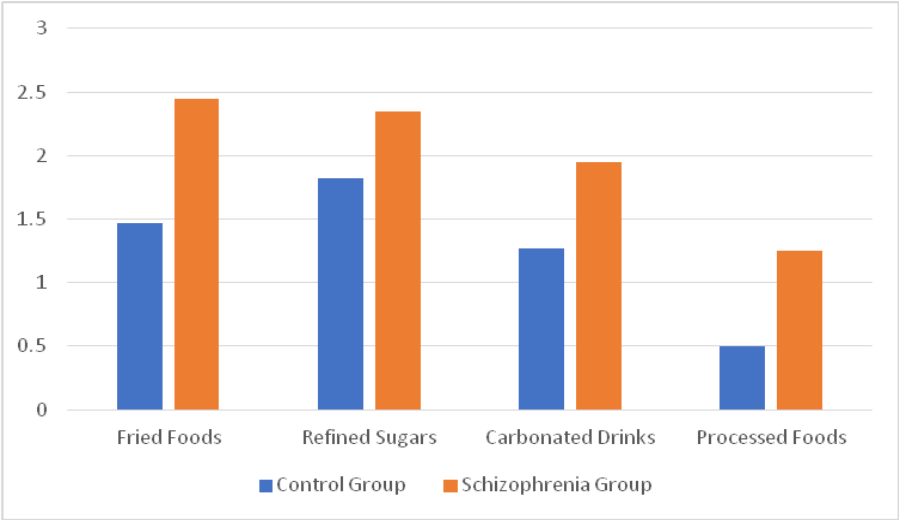

Fig. 2. Risk Factors

DISCUSSION

The nutritional architecture of patients with schizophrenia in the Iasi region reveals a significant systemic vulnerability. Our results demonstrate a profound "nutritional gap" characterized by a critical deficit in antioxidants and fiber ($p = 0.041$), coupled with a nearly twofold increase in the consumption of pro-inflammatory foods like fried fats ($p = 0.029$) and refined sugars (tabs. I, II). This dietary shift does not merely represent a lifestyle preference but serves as a primary driver for oral-systemic degradation.

The significant reduction in antioxidant intake observed in our cohort is a key finding that aligns with current models of "Nutritional Psychiatry". Previous work by our group has extensively examined the intersection of molecular markers and clinical outcomes, emphasizing that the gap between laboratory findings and patient care can only be bridged through an oxidative stress-centered approach (6). This is particularly relevant in oral medicine, as periodontal tissues are highly sensitive to the balance between reactive oxygen species (ROS) and antioxidant defenses. When protective factors are absent, as seen in our data, the oral mucosa becomes vulnerable to the same inflammatory cascades observed in other chronic conditions. For instance, the therapeutic potential of natural polyphenolic extracts has been documented in mitigating oxidative damage in gastrointestinal models (7), suggesting that the systematic absence of such compounds in the diet of psychiatric patients directly impairs their capacity to resolve periodontal inflammation.

Furthermore, the high prevalence of fried food and saturated fat consumption in the schizophrenia group (+0.98 difference, $p = 0.029$) points toward a systemic pro-inflammatory status. Research conducted by our team in related medical fields highlights that chronic systemic stressors often manifest through significant structural and functional changes in

organ systems, such as increased tissue stiffness in extrahepatic cholestasis (8) or the development of small bowel malignancies (9). While these studies involve different anatomical sites, they establish a critical principle of "tissue vulnerability" under chronic stress. In the context of dental medicine, this suggests that the oral environment in schizophrenia is not an isolated compartment but part of a wider pathobiological intersection where systemic inflammation, driven by poor nutrition, accelerates the breakdown of tooth-supporting structures.

This metabolic complexity is further highlighted when managing high-risk clinical populations. Our experience with complex chronic conditions, such as HCV cirrhosis in elderly patients (10), has taught us that therapeutic safety and efficacy are inextricably linked to the patient's underlying systemic resilience. Similarly, the dental management of a patient with schizophrenia cannot succeed through local hygiene alone. The high intake of carbonated beverages and refined sugars found in our study mirrors findings from international cohorts, where psychiatric patients are reported to have significantly higher rates of decayed, missing and filled teeth (DMFT) due to dietary cariogenicity (11, 12).

Our findings on the deficit of fruits and vegetables are consistent with studies by Vetrani *et al*. (13) and Dipasquale *et al*. (4), which identified that patients with severe mental illness often consume diet types that are high in energy but low in essential micronutrients. This nutritional pattern has been linked to increased levels of C-reactive protein (CRP) and other pro-inflammatory cytokines (14). In the oral cavity, this systemic inflammation is exacerbated by psychological stress, which has been shown to induce oxidative DNA damage in salivary glands (15).

Moreover, the "gut-brain-mouth" axis suggested by our data is supported by recent research into the oral microbiome. Studies by Comparelli *et al*. (16) and Gurbuz *et al*. (17) have demonstrated that the dietary habits of schizophrenic patients alter the oral microbial ecosystem, favoring the growth of periodontal pathogens. When combined with the high frequency of smoking observed in our study, this creates a "synergistic insult" to the oral tissues. The lack of dietary fiber found in our patients ($p = 0.041$) further complicates this, as fiber is known to modulate systemic inflammation through the production of short-chain fatty acids (18), which play a role in maintaining the integrity of both the gut and the oral mucosal barriers (19, 20).

Ultimately, the nutritional gap identified in this study advocates for a multidisciplinary clinical shift. Dental professionals must move beyond the "drilling and filling" paradigm and recognize the psychiatric patient's dietary status as a primary clinical marker. Integrating antioxidant monitoring and nutritional counseling into the standard psychiatric protocol is essential to mitigate the systemic and oral complications of the disease.

However, certain limitations of the present study must be acknowledged, such as the relatively small sample size and the reliance on self-reported dietary data, which may be subject to recall bias. Furthermore, while the identified nutritional patterns strongly suggest a pro-oxidant state, longitudinal studies incorporating

direct biochemical markers of oxidative stress are required to establish a definitive causal link between specific dietary deficits and the progression of periodontal destruction in this population.

CONCLUSIONS

The results of this study confirm a profound nutritional imbalance in patients with schizophrenia, characterized by a statistically significant deficit in dietary fiber ($p = 0.041$) and a marked escalation in the consumption of pro-inflammatory foods, such as fried fats ($p = 0.029$) and processed products ($p = 0.048$). This "nutritional gap" creates a systemic pro-oxidant environment that directly compromises oral mucosal integrity and periodontal health. Our findings suggest that the oral cavity acts as a clinical mirror for the metabolic disturbances associated with severe mental illness. Consequently, managing these patients requires a multidisciplinary shift that integrates nutritional counseling and antioxidant monitoring into standard psychiatric and dental care to mitigate the systemic drivers of oral degradation.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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THE ROLE OF SMOOTH MUSCLE AND SUBMUCOSAL GLANDS IN THE AIRWAY REMODELING IN ASTHMA

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THE ROLE OF SMOOTH MUSCLE AND SUBMUCOSAL GLANDS IN THE AIRWAY REMODELING IN ASTHMA (Abstract): **Aim:** Bronchoconstriction, chronic inflammation, and airway remodeling are the main factors associated with the airflow restriction in patients with asthma. Airway remodeling includes all morphological changes of the bronchial wall in asthma, contributing to clinical manifestations and progressive loss of lung function. The aim of this study was to evaluate the morphological changes associated with airway remodeling, being focused on submucosal glands and muscular tissue, in a group of asthmatic patients. **Materials and methods:** We performed a retrospective analysis on 38 patients with a history of bronchial asthma, with different degrees of severity. All patients were classified according to the latest Global Initiative for Asthma (GINA) criteria and underwent a fibro bronchoscopy examination associated with biopsy of tissue samples for microscopy. **Results:** The study included 38 asthmatic patients, with a female predominance (68.42%) and a mean patient age of 49±16 years. The microscopic exam revealed an increase of smooth muscle and submucosal glandular areas in asthmatic patients in comparison to healthy persons and a close correlation with the asthma severity, reflected by FEV1 values. The statistical analysis showed an inverse correlation between FEV1 (%) values and muscular ($r = -0.8997$, $p < 0.001$) and glandular area hypertrophy ($r=-0.887$, $p < 0.01$). **Conclusions:** The increase of muscle and submucosal glands areas, as part of the airway remodeling process, represents a specific pathological feature mainly associated with severe forms of asthma, being correlated with lung function restriction. **Keywords:** ASTHMA, BRONCHIAL SMOOTH MUSCLE, SUBMUCOSAL GLAND, FEV1.

INTRODUCTION

Airway hyper-responsiveness, added to chronic airway inflammation and reversible airflow limitation, are the most important features of asthma (1). These changes occur as a result of bronchial wall remodeling, leading to its thickening (1, 2). Morphologically, these features are represented by epithelial cell damage, goblet cell hyperplasia, basement membrane thickening with subepithelial fibrosis, edema and neoangiogenesis in the lamina propria, submucosal glandular hypertrophy, and smooth muscle hyperplasia or hypertrophy (2- 4).

Variations of the airway wall thickness have been correlated with lung function and they may therefore reflect the severity of asthma (5). Airway hyper-reactivity is also determined by the contractile activity of bronchial smooth muscle, which is the principal effector in bronchoconstriction. Notably, chronic inflammation of the bron-

chial wall plays a central role in modulating both bronchial smooth muscle activity and the other structural changes that characterize airway remodeling, including mucus hypersecretion (3).

In recent years, numerous studies have focused on the mechanisms underlying bronchial smooth muscle and submucosal glandular hypertrophy in asthmatic patients, with the aim of identifying novel therapeutic targets (1). Thus, increased bronchial wall thickness has been associated with impaired lung function and reduced spirometry parameters, such as Forced Expiratory Volume in 1 second (FEV1) and the FEV1/Forced vital capacity (FVC) ratio, particularly in uncontrolled (severe) asthma (6). Corticosteroid therapy in asthmatic patients has been also demonstrated to reduce the bronchial wall inflammation, with a limited effect on submucosal glandular hypertrophy and bronchial smooth muscle hypertrophy (6, 7). However, the molecular mechanisms underlying submucosal glandular and smooth muscle hypertrophy or hyperplasia associated with airway remodeling are extremely complex and remain only partially deciphered (6, 7).

In this context, this study aims to demonstrate the airway remodeling and its relationship with spirometry parameters in a group of asthmatic patients, being focused on bronchial glandular and smooth muscle hypertrophy or hyperplasia.

MATERIALS AND METODS

The study group included 38 adult asthmatic patients diagnosed in the Specialty Ambulatory of the Pneumology Clinic Hospital, Iasi, Romania. All patients were non-smokers (totally non-smoker or former occasional smoker), receiving treatment with inhaled corticosteroids, without other chronic or acute lung diseases, and able to adequately and correctly perform a spirometry test. The inability to adequately and correctly perform a spirometry test along with the presence of other chronic or acute lung diseases or the patient declaring himself a smoker were used as exclusion criteria from the study group. Patients' history, chest X-ray, and spirometry assessment using FEV1 were used to classify the asthmatic patients according to the last GINA classification (8). Subsequently, a fibro bronchoscopy exam was performed with the collection of five to seven tissue fragments for each case, to limit the risk of insufficient material or inconclusive results (2, 9). The collected tissue fragments were fixed in buffered formalin, processed for paraffin embedding, and stained with Hematoxylin & Eosin and Goldner-Szekely trichrome staining.

The thickness of the bronchial smooth muscle and submucosal glandular areas was assessed using a semi-quantitative scoring system [score 1 (+) = almost normal, score 2 (+) = moderate increase and score 3 (+++) = severe increase] as the distance between the epithelial layer/basal membrane and smooth muscle or submucosal glandular areas at 4 x magnification, by two independent pathologists (10, 11). The control group included two patients without asthma symptoms, which have been investigated for tumor suspicion.

The proliferation potential of bronchial muscle cells and submucosal glandular areas was evaluated by Ki-67 index, using immunohistochemistry (clone MIB-1; Dako), according to the manufacturer protocol.

The study was conducted in accordance with the Declaration of Helsinki for research involving human subjects and patients were included in the study group based on informed consent.

The statistical analysis was performed using *SPSS version 25* (IBM, Armonk, NY, USA). Associations between continuous variables were assessed using Pearson's and Spearman's correlation coefficients and linear regression analysis was performed to estimate the slope of the relationships. Statistical significance was set at $p < 0.05$.

RESULTS

Following clinical examination and spirometry testing, the 38 patients included in the study were classified into three severity degrees, according to the GINA 2025 criteria: 17 cases (44.73%) of well-controlled (intermittent/mild) asthma, 16 cases (42.10%) of partially controlled (moderate) asthma, and five cases (1.31%) of uncontrolled (severe) asthma. The mean value of FEV1 (% of predicted) was 78.3±22.6%, the lowest mean values being registered in the uncontrolled (severe) asthma cases (43.6±10.8), followed by partially controlled asthma (66.7±6.2%), and well-controlled asthma (100.4 +10.1%).

The median age of asthmatic patients was 49±16 years (range: 22-72), most of cases being included in the 6th and 7th decades of life (26.32% each), followed by the 3rd decade (21.05%), the 4th decade with 7.89% cases, and the 8th decade with 2.63% cases. The study population was predominantly female (n = 26; 68.42%) and the mean age was higher among women (52±15.6 years) than in men (42.4±15.5 years). The majority of patients were from urban areas (n = 28; 73.68%), with only 10 patients (26.32%) from rural areas, and most had a duration of bronchial asthma between 15 and 20 years.

A bronchial wall thickening due to smooth muscle hypertrophy or hyperplasia (score 3) was detected in four cases of uncontrolled asthma, while a moderate smooth muscle hypertrophy or hyperplasia has been registered in 16 cases (15 cases of partially uncontrolled asthma and one case of uncontrolled asthma). The remaining cases exhibited a near-normal appearance of the muscle layer (figs. 1 and 2). Ki-67 immunoexpression was negative in all cases, except in two cases of uncontrolled asthma, with weak and rare positivity (figs. 3 and 4).

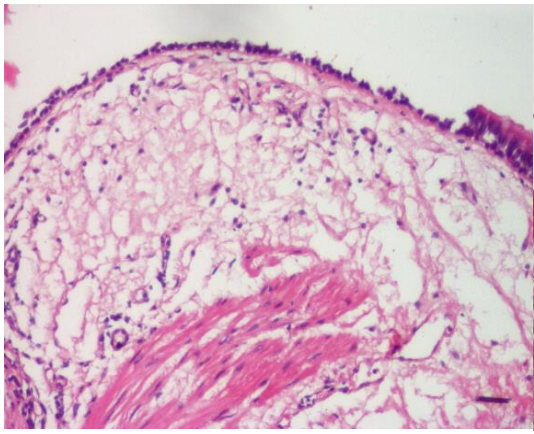

Fig. 1. Near-normal histologic features of the muscle layer in well-controlled asthma, H&E, 4x

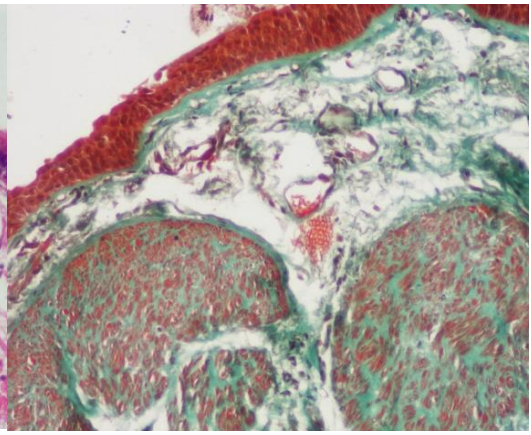

Fig. 2. Significant increase of smooth muscle surface area in uncontrolled asthma, Goldner-Szekely trichrome, 4x

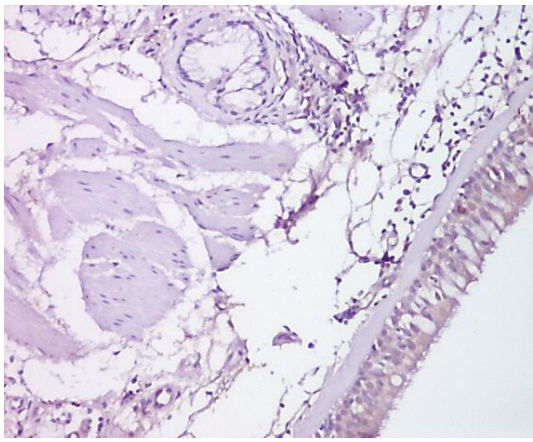

Fig. 3. Negative Ki-67 immunoexpression in bronchial muscle cells in partially controlled asthma, 10x

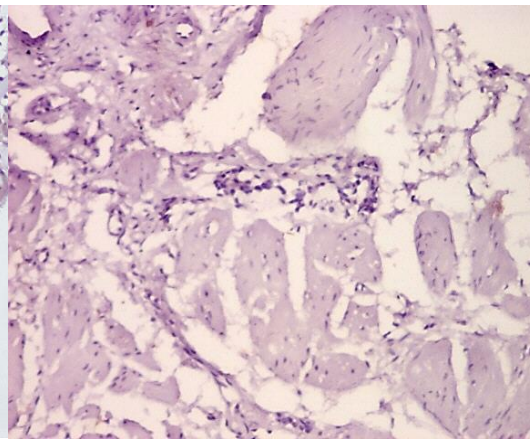

Fig. 4. Ki-67 immunoexpression: weak and rare positivity of bronchial muscle cells in uncontrolled asthma, 10x

A significant submucosal glandular hypertrophy has been also registered in all patients with uncontrolled asthma and in two cases of partially uncontrolled asthma. Near-normal glandular areas have been observed in 16 cases (42.10%) of well-controlled asthma, while a moderate increase of submucosal glandular area of the bronchial wall has been observed in 15 cases (39.47%) (14 cases of partially controlled asthma and one case of well-controlled asthma) (figs. 5 and 6). A negative Ki-67 immunoexpression of the bronchial glandular area has been observed in all biopsies of asthmatic patients (figs. 7 and 8).

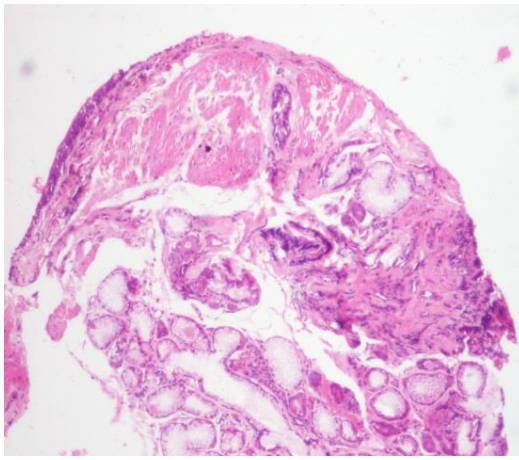

Fig. 5. A moderate submucosal glandular area hypertrophy in partially controlled asthma, H&E, 2x

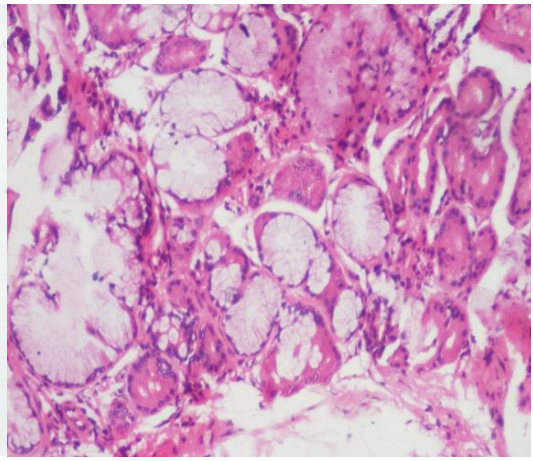

Fig. 6. A significant submucosal glandular area hypertrophy in uncontrolled asthma, H&E, 10x

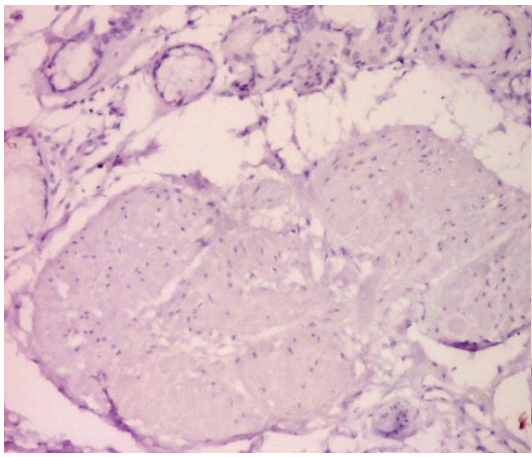

Fig.7. Negative Ki-67 immunoexpression in bronchial glands and muscular area in partially controlled asthma, 10x

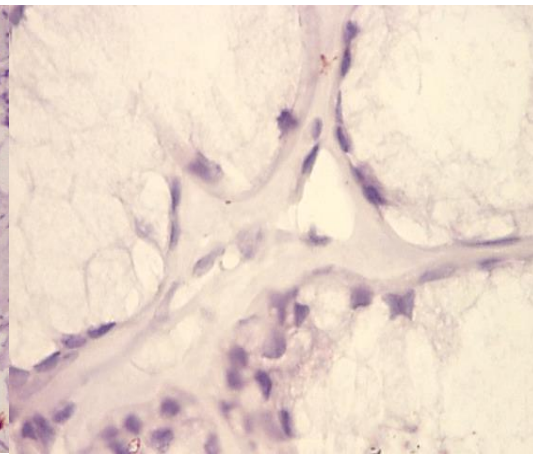

Fig. 8. Negative Ki-67 immunoexpression in bronchial glands in uncontrolled asthma, 40x

The statistical analysis applied to evaluate the association between muscle mass hypertrophy and bronchial asthma severity revealed a Spearman correlation coefficient of rho = 0.93, $p < 0.01$ (tab. I). Moreover, the bronchial wall muscular hypertrophy was mainly noted in patients with low FEV1 values, this inverse association between FEV1(%) values and muscle mass hypertrophy or hyperplasia of bronchial wall being supported by the high value of the correlation coefficient ($r = -0.8997$, $p < 0.001$) and confirmed by a significant negative regression slope (fig. 9).

Regarding submucosal glandular area hypertrophy, the statistical analysis revealed a strong positive correlation between asthma severity and the glandular area hypertrophy of the bronchial wall (Spearman's rho = 0.92, $p < 0.01$) (tab. II). A significant inverse correlation between FEV1 (%) values and the submucosal glandular area hypertrophy was observed ($r=-0.887$, $p < 0.01$), which was further supported by linear regression analysis (fig. 10).

Mean FEV1 (%) values were higher in asthmatic patients with a near-normal glandular area and lower in cases with marked submucosal glandular hypertrophy.

TABLE I.

Statistical parameters estimated in the testing of the association of muscular area hypertrophy or hyperplasia *vs.* bronchial asthma severity

	Pearson's chi-square test	**df**	***p* *95% CI***
Pearson's chi-square - χ^2	58.66844	df=6	0.000
M-LChi-square	62.82472	df=6	0.000
Correlation coefficient (Spearman Rank R)	0.9352601		0.000

CI - confidence interval; df - degrees of freedom; M-L Chi-square - Maximum Likelihood Chi-Square

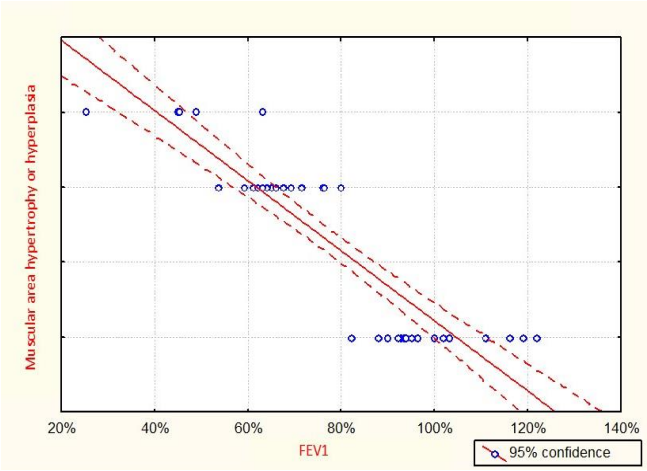

Fig. 9. The regression line showing a negative correlation FEV1 *vs.* bronchial wall muscular area hypertrophy or hyperplasia

TABLE II.

Statistical parameters estimated in testing the association of submucosal glandular area hypertrophy *vs.* bronchial asthma severity

	Pearson's chi-square test	**df**	***P* *95% CI***
Pearson's chi-square - χ^2	57.64917	df=6	0.000
M-L Chi-square	60.69115	df=6	0.000
Correlation coefficient (Spearman Rank R)	0.9211776		0.000

CI - confidence interval; df - degrees of freedom; M-L Chi-square - Maximum Likelihood Chi-Square

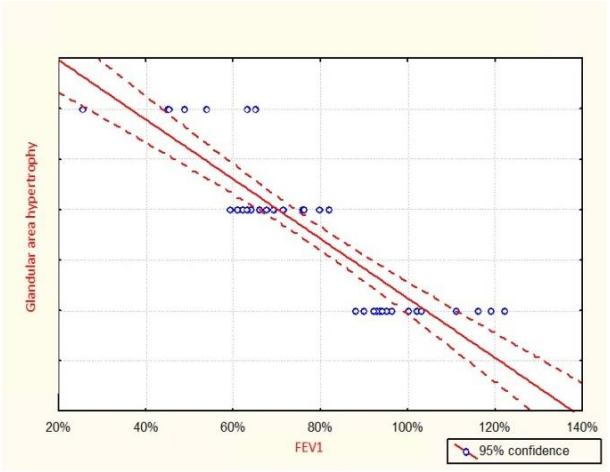

Fig. 10. The regression line showing a negative correlation FEV1 *vs.* bronchial wall submucosal glandular area hypertrophy

DISCUSSION

Bronchial wall remodeling is a consequence of chronic airway inflammation in asthma, a feature which was also observed in other chronic inflammatory diseases (12-15). As a part of the airway remodeling process, an abnormal increase of submucosal glandular and smooth muscle bronchial areas has been mainly detected in severe forms of asthma, features modulated by IL-5, IL-13, and other non-T2 cytokine-dependent-mechanisms (1, 16, 17). In this context, a 50-100% thickening of the bronchial wall was observed in patients with fatal asthma, compared to patients with non-fatal asthma, this increase being correlated with pulmonary functional status (1).

Although bronchial submucosal gland hypertrophy has been demonstrated as a key contributor to mucus hypersecretion, together with goblet cell hyperplasia of the respiratory epithelium (1), the origin and the molecular mechanisms underlying the thickening of the airway smooth muscle layer remain partially elucidated (6, 18).

In recent decades, different studies have explored the potential proliferation of muscle cells in the airway wall in asthmatic patients, with contradictory results. In this context, an increased proliferative capacity of airway muscle cells was observed in asthmatic patients compared to control individuals, especially in the case of partially uncontrolled or uncontrolled asthma (19, 20), while other studies suggest that the thickening of the asthmatic bronchial smooth muscle layer is not associated with active muscle cells proliferation (21, 22). A very limited proliferative activity of airway muscle cells was observed in uncontrolled asthmatic cases in our study, suggesting that the increase of muscle thickness involves other mechanisms, such as hypertrophy or cellular differentiation or trans differentiation. According to recent data, myoblasts, mesenchymal stem cells, epithelial cells, and pericytes may contribute to the thickening of the bronchial muscle mass by migration and trans differentiation into smooth muscle cells, mainly in severe asthma (23, 24). Furthermore, glucocorticoid administration may reduce the microvascularisation in the lamina propria and may decrease the basement membrane thickness in asthmatic cases (25, 26). However, their effect on the decrease of the size of the smooth muscle area associated with airway wall remodeling in asthmatic patients is limited (25, 26). Our results reinforce this data, showing that among asthmatic patients receiving chronic inhaled corticosteroid therapy, bronchial muscle area was significantly increased and correlated with asthma severity, as indicated by reduced FEV1 values. Furthermore, a significant increase of the smooth muscle area was observed in patients with uncontrolled asthma compared to partially controlled or well-controlled asthma patients, which is consistent with other studies (10, 27, 28). In this context, a mean bronchial muscle area of 0.24 ± 0.03 mm^2 was registered in patients with uncontrolled asthma compared to partially controlled patients (0.05 ± 0.01 mm^2) in a group of 28 asthma cases (10). In addition, smooth muscle cells accounted for 27% of the subepithelial tissue, in a group of 24 children with asthma (28), a proportion that can increase up to 40% in patients with uncontrolled asthma (27). All these data support the direct relationship between asthma severi-

ty, reflected by reduced spirometry parameters, and increased muscle area associated with the bronchial wall remodeling process that can occur in asthmatic patients, as also observed in our study.

Another morphologic component of the airway remodeling process in asthma is the submucosal glandular area hypertrophy (1). Goblet cell hyperplasia added to hypertrophy of bronchial glandular area may lead to a three-fold increase in mucin secretion in asthma (17, 29). Mucus hypersecretion induces "mucus plugs" occurrence that is associated with airflow obstruction (1, 29). Thus, a 30-fold increase in goblet cell count (30) and a greater increase in submucosal glandular area in the bronchial wall was observed in fatal asthma patients compared to those with non-fatal asthma ($p=0.003$) (31). In addition, mucus hypersecretion due to increased bronchial submucosal glandular areas has been demonstrated to be associated with asthma attacks (30). Thus, an increased mucous gland area and percentage of the relaxed lumen area occupied by mucus have been detected in asthma cases ($p < 0.05$) compared with healthy patients (32), with mucus hypersecretion being also partially related to goblet cell hyperplasia even in well-controlled asthma (33, 34). Moreover, mucus hypersecretion has been detected to be inversely correlated with FEV1 (Spearman's rho = -0.51, $p < 0.001$) and FEV1/FVC ratio (Spearman's rho = -0.54, $p < 0.001$) in a large cohort of asthmatic cases (35), data which are consistent with our observation.

Although an increased two- to four-fold compare to controls of the submucosal gland area in asthma has been detected, the glandular hypertrophy is not a constant morphologic feature in all asthmatic patients (36). This feature is in accordance with our results, showing a near-abnormal appearance of the submucosal glandular area in most cases. Moreover, the increased airway submucosal glandular area is due to hypertrophy of the mucous glands, demonstrated by the Ki-67 negative immunoexpression in all cases examined tissue fragments. Given their impact on lung function, preventing mucus plug formation driven by mucus hypersecretion represents a new therapeutic strategy to prevent airflow obstruction, particularly in patients with uncontrolled asthma.

CONCLUSIONS

Airway remodeling is characterized by long-term changes of the airway's architecture in asthmatic patients, associated with lung function decline. This process is characterized by the reorganization of the epithelial and subepithelial tissues, which also includes mucus hypersecretion and thickening of the bronchial smooth muscle layer. Increased areas of muscular and submucosal glands are mainly observed in patients with uncontrolled bronchial asthma, despite treatment with high doses of inhaled corticosteroids. Thus, the new therapeutic strategies should be targeted to prevent bronchial wall remodeling in asthmatic patients, by reducing the increase of the bronchial muscle area and limiting the mucus hypersecretion.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no funding regarding this research.

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GENDER DETERMINATION BY LINEAR MEASUREMENTS OF THE FRONTAL SINUS USING CBCT SCANS

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GENDER DETERMINATION BY LINEAR MEASUREMENTS OF THE FRONTAL SINUS USING CBCT SCANS (Abstract): This research aimed at identifying gender by performing linear measurements of the frontal sinus (FS) on images obtained from CBCT scans. **Material and methods:** We analyzed 30 CBCT scans from adult patients of Romanian origin aged between 18-65 years old using the Romexis 4.4.0. software (Planmeca, Helsinki, Finland). Several linear measurements were made: maximum height of the right frontal sinus, maximum height of the left frontal sinus, maximum length of the right frontal sinus, maximum length of the left frontal sinus, total length of the frontal sinus, maximum anterior-posterior diameter of the right frontal sinus, maximum anterior-posterior diameter of the left frontal sinus. All data was assessed on 0.200mm slice thickness images in different anatomical planes. **Results:** Based on both measurements of the maximum height of the right FS, 60% of females and 60% of males were correctly identified while the same measurements on the left FS achieved gender estimation accuracy up to 53.3% in females and 40% in males. The assessment of the maximum length of the right FS lead to a correct prediction of up to 53.3% in females and 53.3% in males, while for the maximum length of the left FS, female individuals were properly identified in 53.3% of cases and males in 46.7% of cases. Measuring the total length of the FS allowed us to identify up to 60% of females and 60% of males. Both measurements of the maximum anterior-posterior diameter of the left FS estimated correctly 66.7% females, 73.3% males. **Conclusions:** The best gender predictor variable of the FS is the maximum anterior-posterior diameter, with a correct estimation rate up to 80% in females and 73.3% in males for the right FS. Due to our small and homogenous study group, we recommend further research on a larger and ethnically diverse population. **Keywords:** CBCT, FRONTAL SINUS, GENDER DETERMINATION, LINIAR MEASUREMENTS.

The frontal sinus (FS) is an air-filled, pyramid-like cavity located within the frontal bone. As the other paranasal sinuses (maxillary, sphenoid and ethmoid), the FS is a paired space, lined with a mucous membrane. It serves several functions such as: reducing the weight of the skull, humidifying and regulating air temperature. Each FS develops independently and, oftentimes, one FS is more dominant than the other. Overall, the variability of the FS is well-acknowledged in the literature and its

unique pattern recommends it as an important anatomical area to be analyzed for forensic purposes, particularly for human identification and/or gender determination by using measurements performed on skulls (1), plain X-ray assessments, usually on lateral cephalometric projections (2, 3) and PA views (4, 5) and Caldwell views (6, 7, 8), conventional computed tomography (CT) (9, 10, 11) or cone-beam computed tomography (CBCT) examinations (6, 12, 13, 14).

Current literature did not reach a consensus regarding the age of maximum development of the FS and, because the bone development is completed around the age of 25 years, we chose to limit our study participants to adults only. However, some researchers consider that the FS is a precise indicator of somatic development maturity in children past puberty (15). Previous studies concluded that the FS reaches its maximum dimensions around the age of 20 years old (16) and that its expansion is in direct relation with the masticatory mechanical stress and hormonal levels, highlighting the idea that the FS continues to develop up to 40 years old (17).

Since current data highlights the superiority of the CBCT assessments over extraoral radiographs for forensic purposes (18), we included only 3D images in our study.

This research aimed at identifying gender by performing linear measurements of the FS on images obtained from CBCT scans. The study protocol was approved by the "Grigore T. Popa" UMPH Ethics Committee (reference number: 13084, July 1st, 2015).

The CBCT is a medical imaging device available on the market in Europe since 1996. It allows 3D scanning of the area of interest and uses a reduced radiation dosage and exposure time, rendering superior quality images compared with the conventional computed tomography (CT). Unlike the regular CT, the CBCT uses divergent X-rays, which form a cone. Its' indications include various branches of dental specialties, particularly oral surgery, implantology and orthodontics, being largely used both as a diagnostic and treatment planning exam in every day practice and in research. Due to its' capacity to offer detailed, accurate and reproducible images of the scanned structures, the CBCT has become quite popular in paranasal sinus lesions and forensics.

MATERIAL AND METHODS

We analyzed retrospectively 30 CBCT scans from adult patients of Romanian origin aged between 18-65 years-old. The images were retrieved from a large database of cranial CBCTs which were performed between September 2013 and December 2014 with a Planmeca ProMax 3D Mid (Planmeca, Helsinki, Finland) scanning device. Several linear measurements were made at the inner bony contour of the FSs: maximum height of the right frontal sinus, maximum height of the left frontal sinus, maximum length of the right frontal sinus, maximum length of the left frontal sinus, total length of the frontal sinus, maximum anterior-posterior diameter of the right frontal sinus, maximum anterior-posterior diameter of the left frontal sinus. Due to the software's ability to differentiate between various densities of the structures within the FS area, the sinus mucosa did not interfere with our measurements. All data was assessed on 0.200mm slice thickness images in different anatomical planes. The study included only CBCTs with FSs that did not present any pathology, previous treatments affecting the FS,

artifacts, uni- or bilateral agenesis. The images were exported in DICOM (.dcm) format, introduced into a laptop (Lenovo G50, Lenovo Group Ltd., Beijing, China) and read using the Romexis 4.4.0. software (Planmeca, Helsinki, Finland) by a dentoalveolar surgeon trained in CBCT and an experimented senior radiologist (A.G.B., D.H.), both of which made the measurements (Fig. 1). In the case of interpretation discrepancies, the final analysis was performed by consensus between the researchers. The assessment was repeated following one month from the first measurement.

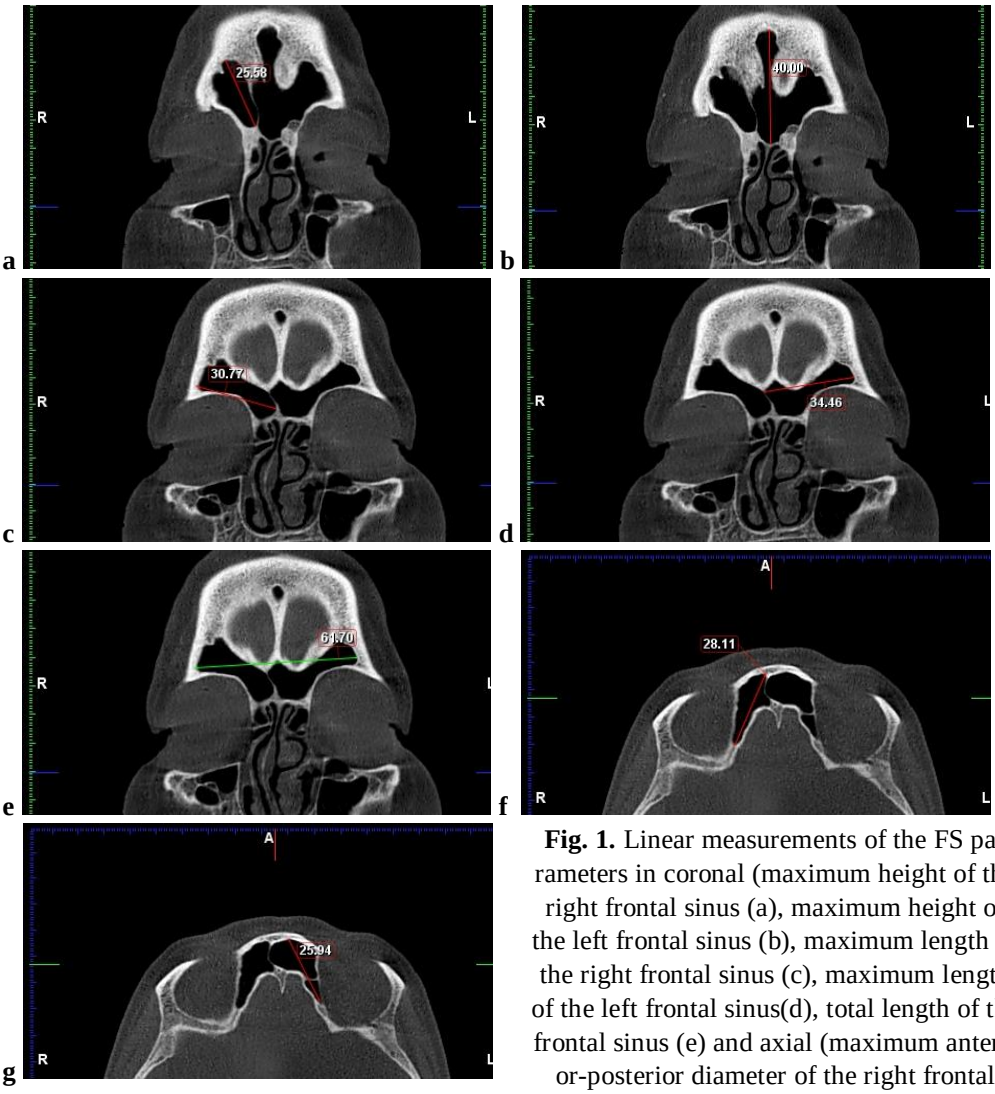

Fig. 1. Linear measurements of the FS parameters in coronal (maximum height of the right frontal sinus (a), maximum height of the left frontal sinus (b), maximum length of the right frontal sinus (c), maximum length of the left frontal sinus(d), total length of the frontal sinus (e) and axial (maximum anterior-posterior diameter of the right frontal sinus (f), maximum anterior-posterior diameter of the left frontal sinus(g) view in a 54 years old female patient.

Statistical Analysis

The statistical analysis was performed using *SPSS 22.0* (SPSS Inc., Chicago, IL, USA). For statistical purposes, the reference value of the signification level was p=0.05, which corresponds to a confidence interval (CI) of 95%. For mean values comparison of the linear characteristics, the Kruskal-Wallis test was performed. The prediction power estimate of the linear characteristics of the FS regarding patients' gender was evaluated using logistic regression. For the accuracy assessment of various parameters used for gender estimation, the kappa coefficient (k) was used. The Landis and Koch reference scale was used for analysis, which allowed us to evaluate the agreement quality as being "poor" (k<0), "fair" (k=0-0.20), "acceptable" (k=0.21-0.40), "moderate" (k=0.41-0.60), "very good" (k=0.61-0.80), and "almost perfect" (k=0.81-1.00).

RESULTS

The first measurement (M1) of the maximum height of the right FS presented significantly higher values ($H_{Kruskal-Wallis}$=5.05, p=0.0245, 95%CI) in male patients (males: $M_{MaxH(M1)}$ - 33.36±8.98SD vs. females: $M_{MaxH(M1)}$ - 27.33±7.6SD) (tab. I).

The calculated value of the area under the ROC curve (AUC=0.6689, p=0.0246, 95%CI: AUC→0.529-0.8081) demonstrates a good prediction rate of the patients' gender based on the first measurement (M1) of the maximum height of the right frontal sinus. Also, the results indicated a 32.8 cut-off value of the first measurement (M1) of the maximum height of the right FS; this threshold offers a good estimation of the patient's gender. The method of gender prediction based on the first measurement (M1) of the maximum height of the right FS presents an 80% sensibility and a 53% specificity (tab. II). Thus, values lower than 32.8 of the first measurement (M1) of the maximum height of the right FS indicate with a high probability that the patient is female. The concordance of the results based on the first measurement (M1) of the maximum height of the right FS was insignificant, the kappa coefficient (k=0.20, p=0.273, 95%CI) indicated a superficial agreement degree (tab. III).

The second measurement (M2) of the maximum height of the right FS presented significantly higher values ($H_{Kruskal-Wallis}$=4.79, p=0.028, 95%CI) in male patients (males: $M_{MaxH(M2)}$ - 33.36±9.05SD vs. females: $M_{MaxH(M2)}$ - 27.48±7.68SD) (tab. I).

The calculated value of the area under the ROC curve for the second measurement (M2) of the maximum height of the right FS being higher than 0.5 (AUC=0.664, p=0.02866, 95%CI: AUC→0.5242-0.8047) confirms a good prediction rate of patients' gender based on the second measurement (M2) of the maximum height of the right FS. The study results indicated a 32.63 cut-off value of the second measurement (M2) of the maximum height of the right FS, which is a reference value for the estimation of patients' gender. An 80% sensibility and a 53% specificity corresponds to this value. Thus, values lower than 32.63 of the second measurement (M2) of the maximum height of the right FS indicate with a high probability the fact that the patient is female (tab. II). Based on the second measurement of the maximum height of the right FS, 60% of female patients and 60% of male patients were correctly predicted. However, the concordance of the results was insignificant, the kappa coefficient (k), (k=0.20, p=0.273, 95%CI) indicated a su-

perficial agreement degree (tab. III).

The first measurement (M1) of the anterior-posterior maximum diameter of the right FS presents significantly higher values ($H_{Kruskal-Wallis}$=9.28, p=0.0023, 95%CI) in male patients (males: $M_{AP\ Max\ Ø\ (M1)}$ - 25.12±5.19SD vs. females: $M_{AP\ Max\ Ø\ (M1)}$ - 21.2±5.45SD) (tab. I).

TABLE I.

Linear measurements characteristics of the FS at the study population

Frontal Sinus	Male n = 15 (†)	Female n= 15(†)	p-value (95%CI) (‡)
Age (years)	34.47 ± 13.01	41.27 ±15.05	H_{KW}=0.897, p=0.3436
Right FS Max H (M1)	33.36±8.98	27.33±7.60	H_{KW}=5.505, p=0.0245*
Right FS Max H (M2)	33.36±9.05	27.48±7.68	H_{KW}=4.792, p=0.0286*
Left FS Max H (M1)	32.54±9.79	31.09±7.88	H_{KW}=0.0079, p=0.9293
Left FS Max H (M2)	32.69±10.03	31.88±7.89	H_{KW}=0.0219, p=0.8824
Right FS Max L (M1)	28.68±8.26	28.91±6.85	H_{KW}=2.101, p = 0.1472
Right FS Max L (M2)	31.74±8.38	29.63±6.72	H_{KW}=1.1979, p=0.2737
Left FS Max L (M1)	33.00±9.39	30.70±6.74	H_{KW}=0.9529, p=0.329
Left FS Max L (M2)	33.32±9.61	30.75±6.87	H_{KW}=1.1979, p=0.2737
FS Total Max L (M1)	60.24±15.21	54.23±11.67	H_{KW}=11.3309, p=0.0248
FS Total Max L (M2)	60.26±15.14	50.15±11.76	H_{KW}=10.951, p=0.0132*
Right FS AP Max Ø (M1)	25.12±5.19	21.20±5.45	H_{KW}=9.2834, p=0.0023*
Right FS AP Max Ø (M2)	25.19±5.37	21.16±5.35	H_{KW}=7.89, p=0.005*
Left FS AP Max Ø (M1)	25.76±3.73	21.82±5.03	H_{KW}=10.208, p=0.0014*
Left FS AP Max Ø (M2)	25.85±3.96	21.78±4.99	H_{KW}=10.211, p=0.0014

† Values were expressed as number (%) mean ± SD or percent at%; (*) Marked effects are significant at p < .05000
‡ Kruskal-Wallis; Chi-square test or Fisher's exact test.

The insignificant calculated value of the area under the ROC curve (AUC=0.728, p=0.0023, 95%CI: AUC→0.597-0.860) demonstrates a good power of prediction of the values for the first measurement (M1) of the anterior-posterior maximum diameter of the right FS regarding patients' gender. Also, the minimum AUC value calculated in this case (0.597) is higher than the reference value (0.5), which confirms the power of the prediction method. The study results indicated a 23.36 cut-off value of the first measurement (M1) of the maximum diameter of the right FS regarding gender estimation, which corresponds to a 80% sensibility and 66.7% specificity. These results confirm the discriminatory power of the first measurement (M1) of the values for the maximum diameter of the right FS regarding patients' gender. Values higher than 23.4 accurately estimate that the patients are males (tab. II). Based on the first measurement (M1) of the maximum diameter of the FS, 80% females and 66.7% males were correctly predicted. The concordance of the results was significant, the kappa coefficient (k=0.467, p=0.01, 95%CI) indicated a moderate degree of agreement (tab. III).

TABLE II.

Sensitivity, Specificity, cut-off value for the linear characteristics of the FS regarding patients' gender prediction

Frontal sinus. Anatomical particularities	Sensitivity	Specificity	Cut off value	p-value (95%CI)
Right FS Max H (M1)	0.8000	0.5333	32.77	.02463*
Right FS Max H (M2)	0.8000	0.5333	32.63	.02866*
Left FS Max H (M1)	0.8667	0.3333	36.84	0.9293
Left FS Max H (M2)	0.8667	0.3333	36.94	0.8825
Right FS Max L (M1)	0.8667	0.4667	35.94	0.1474
Right FS Max L (M2)	0.8667	0.4667	35.82	0.2739
Left FS Max L (M1)	0.8667	0.4000	36.63	0.3292
Left FS Max L (M2)	0.8667	0.4000	36.84	0.2739
FS Total Max L (M1)	0.5330	0.6667	54.130	0.2488
FS Total Max L (M2)	0.400	0.800	49.96	0.3292
Right FS AP Max Ø (M1)	0.8000	0.6667	23.36	0.00232*
Right FS AP Max Ø (M2)	0.8000	0.6667	23.15	0.004969*
Left FS AP Max Ø (M1)	0.667	0.800	23.22	0.0014*
Left FS AP Max Ø (M2)	0.6667	0.9333	19.55	.001406*
Statistical test - 95%CI (Confidence Interval) - (*) Marked effects are significant at p < .05000				

TABLE III.

The predictability degree of the linear characteristics of the FS regarding gender determination

Frontal sinus characteristics	Correct Predicted Percentage		Kappa Accord (k)	p-value‡ (95%CI)
	Masculine	Feminine		
Right FS Max H (M1)	60%	60%	0.200	0.273
Right FS Max H (M2)	60%	60%	0.200	0.273
Left FS Max H (M1)	40%	46.7%	-0.133	0.464
Left FS Max H (M2)	40%	53.3%	-0.067	0.713
Right FS Max L (M1)	53.3%	46.7%	0.000	1.000
Right FS Max L (M2)	53.3%	53.3%	0.067	0.715
Left FS Max L (M1)	46.7%	53.3%	0.000	1.000
Left FS Max L (M2)	46.7%	53.3%	0.000	1.000
FS Total Max L (M1)	60.0%	53.3%	0.133	0.464
FS Total Max L (M2)	60%	60%	0.179	0.273
Right FS AP Max Ø (M1)	66.7%	80%	**0.467**	0.010*
Right FS AP Max Ø (M2)	66.7%	80%	**0.467**	0.010*
Left FS AP Max Ø (M1)	73.3%	66.7%	**0.410**	0.028*
Left FS AP Max Ø (M2)	73.3%	66.7%	**0.450**	0.028*
Statistical test - 95%CI (Confidence Interval) - (*) Marked effects are significant at p < .05000				

The second measurement (M2) of the maximum anterior-posterior diameter of the right FS presented significantly higher values ($H_{Kruskal-Wallis}$=7.89, p=0.005, 95%CI) in males (males: $M_{AP\ Max\ Ø\ (M2)}$ - 25.19±5.37SD vs. females: $M_{AP\ Max\ Ø\ (M2)}$ - 21.16±5.35SD) (tab. I). The insignificant calculated value of the area under the ROC curve (AUC=0.711, p=0.0049, 95%CI: AUC→0.576-0.845) demonstrates a good prediction rate of the values of the second measurement (M2) of the of the maximum anterior-posterior diameter of the right FS regarding patient's gender. Also, the minimum value of the calculated AUC in this case is significant (0.576); this confirms our conclusion.

The results of the study indicated a cut-off value of the second measurement (M2) of the maximum diameter of the right FS of 23.15 for gender prediction, which has a corresponding sensibility of 80% and specificity of 66.7%. The prediction power of this parameter regarding the patient's gender is high (tab. II). Based on the second measurement (M2) of the maximum diameter of the right FS, 80% of females and 66.7% of males were correctly predicted. The concordance of the results was significant, the kappa coefficient (k=0.467, p=0.01, 95%CI) indicated a moderate agreement degree (tab. III).

DISCUSSION

Due to the ability of CBCT devices to generate 3D reconstructions of the scanned structures, most research in this field focuses on volumetric measurements. However, linear measurements performed on CBCT images with the aim of gender estimation have been successfully completed in the past. In a previous study performed on CBCT scans from 77 Romanian patients, up to 92% females were correctly identified by using the frontal sinus index (6).

A study performed on 200 Persian patients (100 females, 100 males) using conventional computed tomography (CT) scans that analyzed various linear measurements of the FSs and other characteristics including pneumatizing and septa concluded that best gender predictor was the maximum height of the left FS (61.3%) (9). However, these researchers consider that the FS parameters should be used for gender determination as part of a comprehensive evaluation that should include other factors. These results are in accordance with other publications (19, 20, 21). Our study found a low prediction capacity of the maximum height of the left FS (40% in males and a maximum of 53.3% in females).

In a multi-detector, computed tomography (MDCT) study conducted on 100 patients (50 females and 50 males), the anterior-posterior length of the FS was the best predictor for gender with an accuracy of 67% (70% for females and 64% for males (22). Our research shows that the best gender estimator was also the maximum anterior-posterior diameter of the FS in all CBCT view. Based on the first measurement (M1) of the maximum diameter of the FS, 80% of female patients and 66.7% of male patients were correctly identified. The concordance of the results was significant; the kappa coefficient (k=0.467, p=0.01, 95% CI) indicated a moderate agreement degree. Based on the second measurement (M2) of the maximum diameter of the FS, 80% of female individuals and 60 % of male individuals were correctly predicted. The concordance of the predicted results based on the actual measurements was significant, the kappa coefficient (k=0.46, p=0.01, 95%CI) indicated a moderate degree of agreement. Using the first measurement (M1) of the

maximum diameter of the left FS, 66.7% of females and 73.3% of males were correctly identified. The concordance of the predicted results based on the actual measurements was significant, the kappa coefficient (k=0.41, p=0.028, 95% CI) indicated a moderate agreement degree. Considering the maximum diameter of the left frontal sinus (M2), 66.7% of females and 73.3% of male individuals were correctly assessed. The concordance of the results was significant, the kappa coefficient (k=0.45, p=0.028, 95% CI) indicated a moderate agreement degree.

CONCLUSIONS

The best gender predictor variable of the FS is the maximum anterior-posterior diameter in the all CBCT view, with a correct estimation rate up to 80% in females and 73.3% in males for the right FS.

Due to our small and homogenous CBCT study group, we recommend further research on a larger and ethnically diverse population, including children past puberty. We also recommend the quantification of unilateral and bilateral agenesis of the frontal sinus in the Romanian population.

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NEWS

THE ANTI-CARCINOGENIC ROLE OF NICOTINAMIDE

Nicotinamide is an amide form of vitamin B3 that has the potential to treat dermatological conditions such as rosacea, acne, or atopic dermatitis. According to Nazarali *et al.*, nicotinamide has anti-carcinogenic properties and can be used in topical or oral forms as a chemo preventive agent against skin cancer. This can be explained by its ability to enhance DNA repair and prevent the immunosuppression induced by UV light. It is also a precursor of key coenzymes such as nicotinamide adenine dinucleotide, used in the synthesis of adenosine triphosphate (ATP). The author concludes that nicotinamide plays an important role in energy-dependent cellular processes such as DNA repair. (Nazarali S, Kuzel P. Vitamin B Derivative (Nicotinamide) Appears to Reduce Skin Cancer Risk. *Skin Therapy Lett*. 2017 Sep; 22(5): 1-4.)

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INDICATORS OF PROGNOSIS IN COMMUNITY-ACQUIRED BACTERIAL MENINGITIS

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INDICATORS OF PROGNOSIS IN COMMUNITY-ACQUIRED BACTERIAL MENINGITIS (Abstract) Bacterial meningitis is a medical, neurological and sometimes neurosurgical emergency, being an important cause of morbidity and mortality worldwide. Aim: Analysis of some epidemiological parameters with possible role in prognosis prediction, and analysis of therapeutic conduct in relation with clinical evolution. **Material and methods**: Retrospective study of the medical records of 70 patients with a discharge diagnosis of bacterial meningitis. The patients were admitted to the Iasi Infectious Diseases Hospital in 2014 and 2015. **Results**: A larger number of patients under 50 years of age exhibited the symptomatic associations (fever, headache, stiff neck) compared to those aged over 50 years ($p < 0.05$). Discordance between diagnoses, found in 65.71% of patients correlates with adverse events and death ($p = 0.037$). A larger proportion of patients on modified therapy (46.67%) died as compared to those in whom treatment was not changed (22.50%) ($p = 0.033$). We found that a CSF glucose level under 0.4 g/L is more frequently associated with coma ($p = 0.002$). Statistically significant associations were found between CSF glucose level <0.7 g/L and the following clinical signs and symptoms: fever, headache and neck stiffness ($p = 0.043$). Patients with pathological changes found at CT examination were more likely to have an unfavorable outcome ($p = 0.014$). **Conclusions**: The high rates of comorbidities, the presence of coma and abnormal CT findings on admission, as well as the discordant admission and discharge diagnoses may be considered factors of negative prognosis. **Keywords**: BACTERIAL MENINGITIS, PROGNOSIS FACTORS, DIAGNOSIS.

Bacterial meningitis is a medical, neurological, and sometimes neurosurgical emergency being an important cause of morbidity and mortality worldwide. It is estimated that the mortality rate of bacterial meningitis ranges from 7% to 30% in the developed countries, and reaches even 50% in the developing world. Neurological sequels such as hearing loss and psychomotor disorders are reported in almost half of the survivors. The importance of the problem is evidenced not only by the large number of days of medical care for survivors, but also for rehabilitation (1, 2).

Statistical data underline the importance of the correct therapeutic management of

the patient suspected of or diagnosed with bacterial meningitis. In these circumstances, the initial assessment of the patient and the evaluation of prognostic factors are essential. Given the high risk of sequels and even death, identifying the elements with prognostic role as immediately after the diagnosis was made may decrease the risk of adverse events.

The objectives of our study are: analysis of some epidemiological parameters (age, gender, personal medical history) with possible role in prognosis prediction, be it favorable or unfavorable, in a group of patients with bacterial meningitis confirmed by clinical and laboratory findings, and analysis of therapeutic management (appropriate/inappropriate, early/late) in the patients in which a complete cure was obtained, compared to those who developed complications/sequelae or died.

MATERIAL AND METHODS

Our study retrospectively reviewed the medical records of 70 patients consecutively admitted and treated for bacterial meningitis to the Iasi "Sfânta Parascheva" Infectious Diseases Hospital during the period 2014-2015. Cases of posttraumatic meningitis, postoperative bacterial meningitis, or ventriculo-peritoneal shunt (VPS)-associated meningitis were excluded from the study. The diagnosis of meningitis was based on clinical criteria (fever, headache and neck stiffness, disorders of consciousness) and cerebrospinal fluid (CSF) criteria (leukocytosis >10/mm^3, ± increased and decreased CSG glucose levels, positive cultures or direct exams). No written consent was given by the participants, as data were collected and analyzed anonymously. The data were statistically analyzed using Epi Info and the used parametric test was the two-tailed test.

RESULTS

For comparative analysis by age group, we decided to divide the 70 patients into two groups: patients aged less than or equal to 50 years (36 cases, 51.42%), and patients aged over 50 years (34 cases, 48.57%). As one can see in Table I, a larger number of patients under 50 years of age exhibited the described symptomatic associations as compared to those aged over 50 years.

TABLE I

Age-correlated clinical manifestations

Signs and symptoms	>50 years of age No (%)=34	<50 years of age No (%)=36	*p*
Fever, headache, stiff neck	10 (29%)	23 (63.8%)	0.003
Headache, stiff neck	13 (38.24)	26 (72.22)	0.004
Fever, headache	19 (55.88)	31 (86.11)	0.005
Fever, stiff neck	13 (38.24)	25 (69.44)	0.008
Fever, headache, vomiting	8 (23.53)	18 (50.00)	0.021

Although there are significant percentage differences between the two groups in the following reported parameters: hospital stay over 14 days (p = 0.816), evolution towards death (p = 0.149), coma (p = 0.136), other neurological disorders (p = 0.871), CSF albumin levels over 3 g/L (p = 0.879) or CSF glucose levels under 0.7 g/L (p = 0.065), no statistically significant correlations have been found. Decreased CSF glucose levels were more frequently found in patients < 50 years of age (tab. II).

Discordance between initial and final diagnosis, found in 65.71% of patients (46 patients), correlated with adverse events and death (p = 0.037), correlation that may be probably due to the inappropriate management. A number of 19 deaths (41.30%) were recorded in the group in which diagnoses did not coincide, as compared to 4 deaths (16.67%) in the group with concordance between diagnoses (tab. III).

TABLE II

Biological parameters and case evolution according to age category

Parameters	> 50 years of age No (%)=34	<50 years of age No (%)=36	*p*
CSF albumin > 3 g/L	10 (29.41)	10 (27.78)	0.879
CSF glucose <0.7 g/L	23 (38.65)	31 (86.11)	0.065
Coma	12 (35.29)	7 (19.44)	0.136
Other neurological disorders	23 (67.65)	25 (69.44)	0.871
Hospitalization for >14 days	17 (50.00)	19 (52.78)	0.816
Evolution towards death	14 (41.18)	9 (25.00)	0.149

TABLE III

Clinical course and therapy reshuffling reported to the concordance between diagnosis upon admission and diagnosis at discharge

Parameters	Discordance No (%)=46	Concordance No (%)=24	*p*
Deaths	19 (41.30%)	4 (16.67%)	0.037
Cured	27 (58.70%)	20 (83.33%)	
Therapy reshuffling	23 (50.00)	7 (29.17)	0.094

No statistically significant correlations could be established between diagnostic concordance and treatment reshuffling (p = 0.094), although there were percentage differences between the two groups in terms of the above-mentioned parameters (tab. III). A statistically significant association was that between the less-than-2-day-period to admission (and treatment) and a CSF albumin level of over 3 g/L (p = 0.033) (tab. IV).

Of the 20 patients in which CSF albumin level was greater than 3 g/L, 35.71% were hospitalized and received treatment in less than two days (tab. IV). This could be explained by the existence of a rapid and more pronounced inflammatory reaction at the CSF level.

TABLE IV

Mortality rate was similar in the two groups differentiated through the onset-to-hospitalization time

	Treatment at <48 hours No (%)=25	Treatment at >48hours No (%)= 45	*p*
CSF albumin >3g/L	11 (44.00)	9 (20.00%)	0.033
Death	8 (32%)	15 (33.33%)	0.909

Therapy reshuffling also influenced the clinical course: a larger proportion of patients receiving modified therapy (46.67%) died as compared to those in which treatment was not changed (22.50%) (p = 0.033; OR = 3.013) (tab. V).

TABLE V

Associations between treatment reshuffling and evolving features

Parameters	Reshuffling No (%)=30	Without reshuffling No (%)=40	*p*
Hospital stay >14 days	20 (66.67%)	16 (40%)	0.027
Death	14 (46.67%)	9 (22.50%	0.033
Coma present	9 (30%)	10 (25%)	0.641
CSF albumin >3g/L	7 (23.33%)	13 (32.50%)	0.400
CSF glucose <0.7 g/L	21 (70%)	32 (80%)	0.334
Neurological disorders	22 (73.33%)	26 (65%)	0.457

One can notice that in most cases therapy reshuffling was required when symptoms on admission persisted or worsened. In descending order of frequency, the reasons were: the results of antibiotic sensitivity tests and the persistence or worsening of symptoms present on admission or overlap of disorders of consciousness occurring during hospital stay. Of the 70 patients, 21 (30%) have received antibiotic therapy prior to their admission to the Infectious Disease Clinic (tab. VI).

TABLE VI

Clinical manifestations associated with prior antibiotic therapy

Parameters	No Prior AB treatment No (%)=49	Prior AB treatment No (%)=21	*p*
Fever, headache and disorders of consciousness	7 (14.29%)	3 (14.29%)	1
Headache and disorders of consciousness	9 (18.37%)	5 (23.81%)	0.601
CSF elements >1,000/mm³ (Nr=21)	13 (26.53%)	8 (38.10%)	0.333

In addition, all patients in the group with elevated white blood cell counts were more prone to exhibit disorders of consciousness (coma) (p = 0.0008; OR = 2.162). In 89.47% of coma cases, WBC count was over 11,000/mm^2. Thus, it is the systemic inflammatory response that has been statistically correlated with an unfavorable course (tab. VII).

We have found that CSF glucose level below 0.7 g/L was more frequently associated with coma than a normal or an increased level (p = 0.002; OR = 6.98), 52.63 % of the patients with CSF glucose below 0.7 g/L presenting disorder of consciousness. Neck stiffness was also correlated with CSF glucose below 0.7 g/L (p = 0.006). Forty of the 54 patients with CSG glucose below 0.7 g/L (74.07%) and only 6 of the 16 patients with CSF glucose over 0.7 g/L (37.50 %) have experienced this symptom (tab. VIII).

Statistically significant associations were established between CSF glucose

level <0.7 g/L and the following clinical signs and associations of signs and symptoms: neck stiffness (p = 0.006), fever and neck stiffness (p = 0.035), fever, headache and neck stiffness (p = 0.043), headache and neck stiffness (p = 0.024).

TABLE VII

Clinical and biological manifestations related to serum white blood cell count

Parameters	WBC > 11,000/mm³ No (%)=40	WBC <11,000/mm³ No (%)=30	*p*
Fever	38 (95%)	21 (70%)	0.004
Fever, headache	29 (72.50%)	21 (70.00%)	0.818
Coma (n=19)	17 (42.50 %)	2 (6.67%)	0.0008
Death (n=23)	20 (50%)	3 (10%)	0.0004
CSF elements> 1,000/mm³ (21)	16 (35%)	5 (13.33%)	0.040
ESR > 50 mm/h	17 (42.5%)	9 (30%)	0.284

TABLE VIII

Clinical manifestations and death rate related to the initial CSF glucose level

	CSF glucose <0.7 g/L No (%)=54	CSG glucose > 0.7 g/L No (%)=16	*p*
Coma	10 (18.52)	9 (56.25)	0.002
Neck stiffness	40 (74.07)	6 (37.50)	0.006
Fever, neck stiffness	33 (61.11)	5 (31.25)	0.035
Fever, headache, neck stiffness	29 (53.79)	4 (25.00)	0.043
Headache, neck stiffness	34 (62.96)	5 (31.25)	0.024
Other neurological disorders	37 (68.52)	11 (68.75)	0.986
Death	15 (27.78)	8 (50.00)	0.096

No statistically significant associations have been found between CSF glucose level and progression to death (p = 0.096) or neurological disorders, other than those of consciousness (p = 0.986) (tab. VIII). In the patients who underwent blood cultures, 39 were negative and only 10 were positive *for S. aureus* (8 cases), *Salmonella enterica* (1 case), Streptococcus Group D (1 case).

Of the 70 patients, 23 (30%) had positive CSF cultures for: *S. aureus* (8 cases), *N. meningitidis* (4 cases), *Listeria monocytogenes* (3 cases), *Streptococcus pneumoniae* (3 cases), *Streptococcus Group D* (2 cases), *Salmonella enterica* (1 case), coagulase-negative Staphylococcus (1 case), and alpha hemolytic Streptococcus (1 case). All 8 cases of *S. aureus* meningitis had positive blood cultures which proved the hematogenous spread of the pathogen from a primary site. Positive results were obtained in 23.40% of survivors, while the microbial agent was isolated in 52.17% of the deceased patients.

A greater number of patients with negative CSF cultures presented low CSF glucose and elevated CSF albumin level over 3 g/L, but the differences were not statistically significant (tab. IX).

Patients with pathological changes detected at CT examination or associated comorbidities were more likely to have an unfavorable course by contrast with those with normal CT scans (p = 0.014) or no associated comorbidities, respectively (tab. X, tab. XI).

TABLE IX

Frequency of CSF culture positivation related to clinical manifestations and biological parameters

Parameters	Positive CSF cultures No (%)=23	Negative CSF cultures No (%)=47	*p*
CSF albumin > 3 g/L	7 (30.43%)	13 (27.66%)	0.809
CSF glucose < 0.7 g/L	16 (69.57 %)	38 (80.85%)	0.401
Previous treatment	7 (30.43%)	20 (42.55%)	0.327
Coma	9 (39.13%)	10 (21.28%)	0.114
Other neurological disorders	14 (60.87%)	34 (72.43%)	0.331

TABLE X

CT appearance related to CSF albumin level and correlated with unfavorable outcome

Parameters	Pathologic CT exam No (%)=28	Normal CT exam No (%)=20	*p*
Death	17 (60.71%)	5 (25.00%)	0.014
CSF albumin >3 g/L	3 (10.71%)	10 (50.00%)	
CSF albumin <3 g/L	25 (89.29%)	10 (50.00%)	0.002

TABLE XI

Associations between comorbidities and clinical course and imaging manifestations

Parameters	Comorbidities and APP No (%)=54	No comorbidities and APP No (%)=16	*p*
Fever, headache, neck stiffness	22 (40.74%)	11 (68.75%)	0.048
Oher neurological disorders	37 (68.52%)	11 (68.75%)	0.986
Death	20 (37.04%)	3 (18.75%)	0.171
Pathological CT exam	25 (46.30%)	3 (18.79%)	0.048
Coma	14 (31.82%)	5 (31.25%)	0.966

DISCUSSION

Data from the literature underline that clinical manifestations of bacterial meningitis are rather blurred or unusual in patients over 50 years of age. This has also been observed in our patient group which showed significant differences regarding the presence of age-group specific symptoms of meningitis. Similar results have been reported by van de Beek (3), 44% of the cases presenting the fever-neck stiffness-impaired consciousness triad, which could not be associated with a higher mortality rate.

Lucena et al. (4) reported that fever, neck stiffness and vomiting were present in most patients, and their absence was associated with adverse prognosis. Domingo et al. reported that 50% of the elderly patients presented the meningeal triad compared with 8% of the younger patients (5).

Another negative prognostic factor is hospitalization in the context of disorders of consciousness (6, 7, 8). Mendizabala et al. in their study (9) showed that the absence of headache and vomiting was directly associated with a greater risk of death, as the lack of suggestive signs may delay

diagnosis and treatment initiation. In our study an altered level of consciousness was more frequently found in patients aged over 50 years, and the data obtained by us are like those reported by other authors which report disorders of consciousness in 68% of the patients over 65 years of age(5, 10, 11).

The current study does not report significant differences in the prognosis of patients in whom treatment was initiated within more than 48 hours compared to less than 48 hours of symptom onset, but this could be accounted for by the heterogeneity of the compared groups in terms of symptom severity, age group and etiology, requiring a more detailed statistical analysis on a larger number of cases. Similarly, Mendizabala et al. (9) found no statistical association between the progression to death and the time elapsed between symptom onset and hospital admission. Contrary, Aronin et al. (12) argue that the delay in administering treatment worsens prognosis. The results of the study may be explained by the initiation of appropriate antimicrobial therapy without delay and low incidence of resistant bacterial strains.

We observed changes in the spectrum of acute bacterial meningitis compared with a study performed in the same hospital between 2000 and 2009 (13). *Staphylococcus aureus* was the predominant cause in this 1-year study while *S. pneumoniae* was the most common pathogenic agent of adult meningitis over the 10-year period. We also found a lower rate of positive CSF cultures (30% *vs.* 51%), although 30% of the patients enrolled in both Romanian studies had benefited from pre-admission antibiotic therapy (13).

The present study, as other references in the literature, demonstrates that the isolation of the causative agent from biological fluids (blood or CSF) is associated with an adverse prognosis. Mendizabala et al. (9) showed that positivation of blood cultures increases the risk of death, with 49.1% of cases presenting positive cultures compared to 19.5% in the control group. Also, McMillan et al. (14) showed that death is more likely to occur in patients with positive blood cultures and lower CSF cellularity (<1,000 elements). Moreover, van de Beek (3) asserts that the risk of death increases 6 times with the isolation of the causative agent.

Analysis of biochemical data from CSF suggested that a CSF glucose level below 0.7 g/L accompanied in a greater proportion a more severe clinical presentation, even severe, marked by coma. This low level could be correlated with the progression to death. The same finding is reported by McMillan et al. (14) in their study. However, Mendizabala et al. (9) claim that CSF glucose does not influence the prognosis of the patients.

Among the limitations of the study we mention the small number of patients investigated during only one year of study and the absence of the comparison between clinical and biological parameters based on etiology, due to the small number of patients with positive CSF cultures.

CONCLUSIONS

Our study shows that the high rates of comorbidities, the presence of coma and pathologic CT exam on admission, as well as the discordant admission and discharge diagnoses may be considered negative prognostic factors. At the same time, we underline the importance of symptomatology which depends on patient's age. The present study demonstrates that the isolation of the causative agent from biological

fluids (blood or CSF) is associated with a negative prognosis. Another important result of our study was that CSF glucose below 0.7 g/L accompanied by a severe clinical presentation could be correlated with progression to death. It is very important to recognize different aspects of meningitis for good clinical practice and prognosis. We believe that our study is a good tool for achieving this goal.

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SEVERITY OF DUODENAL HISTOLOGY AND TISSUE TRANSGLUTAMINASE ANTIBODY LEVELS CORRELATE WELL IN ADULT CELIAC DISEASE IRRESPECTIVE OF CLINICAL FEATURES

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SEVERITY OF DUODENAL HISTOLOGY AND TISSUE TRANSGLUTAMINASE ANTIBODY LEVELS CORRELATE WELL IN ADULT CELIAC DISEASE IRRESPECTIVE OF CLINICAL FEATURES (Abstract): Celiac disease (CD) is a chronic immune-mediated enteropathy that occurs in genetically predisposed individuals. The clinical phenotypes range from classical gastrointestinal manifestations to only atypical signs, thus being a challenge to diagnose. **Aim:** To investigate the relationship between duodenal histology, specific antibody levels and clinical presentation in adult Romanian CD patients. **Material and methods**: Study design: retrospective retrieval of information prospectively entered into a structured database including 81 adult patients diagnosed with CD admitted with symptoms of abdominal disturbances (diarrhea, heartburn, nausea, vomiting, regurgitation, abdominal pain) to the Iasi Institute of Gastroenterology and Hepatology, "Sf. Spiridon" Emergency Clinical County Hospital between January 2012 and December 2016. The demographic, clinical, serological, and histological characteristics of individuals with CD were reviewed. **Results:** The female: male ratio was 3:1, 60 (71.1%) female patients, mean age 40.02±12.14 years. A total of 48.1% patients presented with gastrointestinal (GI) complaints and 51.9% presented mostly with non-GI manifestations, with a slightly more advanced age at symptom onset in the latter (38 yrs. *vs.* 47 yrs.). Marsh-Oberhuber classification was used to assess mucosal injury and Marsh 3c lesions were found in 25 (30.9%) cases. When assessing the serological parameters, IgA anti-tissue transglutaminase (IgA-tTG) antibody (61.45±76.458 u/mL *vs.*162.02±106.179 u/mL, p=0.001) and IgA anti-gliadin antibody (IgA-AGA) levels (61.83±69.41u/mL *vs.* 77.15±71.02 u/mL, p=0.001) correlated with intestinal villous atrophy (Marsh 3a and 3c) in CD patients by Spearman rank correlation. Of the symptoms, abdominal distention and diarrhea were associated with abnormal histology. Hemoglobin levels were determined and anemia was diagnosed in 61.7% of the patients with elevated IgA-tTG levels (r= -0.316; p=0.004), IgA-AGA (r= -0.301; p=0.006) and Marsh 3b-3c lesions (p=0.0048). Of the biological markers included in the statistical analysis, low iron levels (cut off 30 mg/dl), hypocholesterolemia and low protein levels were associated with Marsh 3b lesions (p=0.006) and elevated tTG-IgA levels (r= -0.384; p=0.001). **Conclusions**: IgA-tTG and AGA levels correlate with duodenal villous atrophy in adult CD patients. An IgA-tTG level >160 was nearly always associated with severe CD histopathology. GI and non-GI symptoms are not reliable predictors of CD. **Keywords:** CELIAC DISEASE, MARSH CLASSIFICATION, CLINICAL MANIFSTATIONS, ANTI-TISSUE TRANSGLUTAMINASE.

Celiac disease (CD) is a chronic immune-mediated enteropathy that occurs in genetically predisposed individuals (1, 2) and the most frequent food intolerance with an increasing prevalence over the last three decades (3). This has been partially prompted by globalization, which led to an increased consumption of gluten-containing food worldwide. The development of new sensitive and specific serological tests and their extended availability, as well as the best knowledge of the disease including a wide clinical spectrum have greatly facilitated CD diagnosis, being indirectly responsible for the rising prevalence (4). In Europe, a recently published large international, multicenter study investigating a wide population sample revealed an overall prevalence of 1% (5).

Unfortunately, no one test for CD has a perfect sensitivity or specificity. The clinical phenotypes range from classical gastrointestinal manifestations to only atypical signs, thus making the clinical diagnosis a challenge (6-8). As a rule, CD diagnosis can be made by serological tests, searching for ant tissue transglutaminase (tTG) and anti-endomysium (EMA) auto-antibodies, but confirmation of intestinal damage relies on small bowel biopsy and histological analysis, mainly in Europe (9). Nonetheless, the prevalence of seronegative CD is 6-22% of all diagnosed cases (10), the histological abnormalities associated with CD can be patchy (11,12), and a normal endoscopic appearance of the duodenum may occur in the presence of villous atrophy (13-16). The aim of the study was to investigate the relationship between duodenal histology, specific antibody levels and clinical presentation in adult CD Romanian patients.

MATERIAL AND METHODS

The study design included a retrospective retrieval of information prospectively entered into a structured database carried out at the Iasi Institute of Gastroenterology and Hepatology, which serves as a tertiary care referral center. The study was conducted between January 2012 and December 2016 and included 81 adult CD patients. In all CD cases, the diagnosis was established by a combination of positive IgA-tTG, IgA-AGA and biopsy results. Abdominal pain, diarrhea, constipation, bloating, nausea and vomiting were considered GI manifestations, and enclosed fatigue, joint pain, irritability, anorexia, anemia, aphthous stomatitis, short stature non-GI manifestations.

After obtaining a written informed consent, all subjects underwent upper endoscopy during which duodenal biopsies were obtained. The final Marsh score for each patient was graded according to the most affected site (highest Marsh score). The biopsies were evaluated by a single experienced pathologist blinded to all clinical data. The mucosal specimens were graded independently according to the Marsh-Oberhuber classification as follows: Marsh 0 denotes normal villi and crypt depth with no excess of intraepithelial lymphocytes (IEL), Marsh 1-2 denotes normal villi but intraepithelial lymphocytosis without (1) or with (2) hyperplastic crypts, and Marsh 3a-c denotes varying degrees of villous atrophy with hyperplastic crypts (17). Multiple biopsies (minimum of three) were taken during upper endoscopy from the bulb and distal duodenum. Immunohistochemical studies were performed using anti-CD3 antibody and an IELs count was noted (lymphocytosis >25/100 enterocytes).

Quantative analysis was performed us-

ing ELISA technique. IgA and IgG-tTG were reported according to the manufacturer's cut off as UI/mL and classified as negative <8 IU/mL and positive ≥8 IU/mL. Serum samples containing more than 12 U/mL were considered positive for tTG and for AGA, respectively, as recommended by the manufacturer. Total IgA serum levels were measured. A serum IgA concentration below 0.07g/dl was graded as IgA deficiency by an immunoturbidimetric assay. Detailed information regarding demographics, symptoms at presentation, duration, type of investigations performed, type of treatment including gluten-free diet were recorded.

Statistical analysis was performed using *SPSS 18.0* (*SPSS Chicago Inc., IL, USA*). Continuous data were expressed as mean ± SD and categorical data as percentage. A P-value less than 0.05 was considered statistically significant. The study was approved by the ethical committee and all participants were informed about the study according to the study protocol and gave their written informed consent.

RESULTS

A total of 81 patients met the inclusion criteria of the study with a female to male ratio of 3:1, with a majority of 60 (71.1%) female patients, mean age 40.02±12.14 years (fig.1). The baseline characteristics of the participants are shown in Table I. Family history of CD was identified in 12 (14.8%) cases. The proportion of newly diagnosed CD patients was high: 68 (84%), 48 female patients (70.6%), mean age 40.09±12.67. The prevalence of associated diseases was low, 7 (8.6%), 3 (3.7%) cases being diagnosed with thyroid disease and 4 (4.9%) cases with chronic idiopathic hypertransaminasemia.

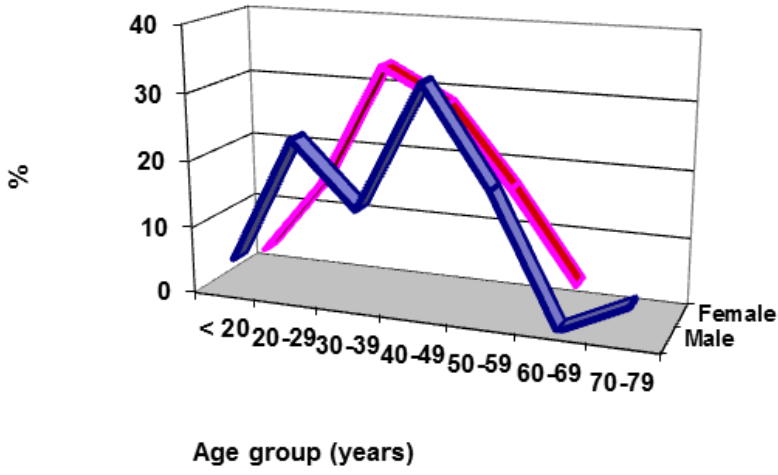

Fig. 1. Prevalence of CD by age and gender

A total of 39 (48.1%) patients presented with GI complaints and 42 (51.9%) of patients presented mostly with non-GI manifestations, with a slightly more advanced age at symptom onset in in the latter (38 yrs. *vs.* 47 yrs.). Interestingly, GI symptoms were more common in patients with lower IgA-tTG <100U/mL

levels, 21 (26.9%), compared to patients with levels >100 U/mL, p=0.007. Marsh-Oberhuber classification was used to assess mucosal injury and varying degrees of villous atrophy were identified in 59 (72.8%) cases as follows: complete atrophy lesions (Marsh 3c) in 25 (30.9%) cases, Marsh 3a in 24 (29.6%), Marsh 3b in 10 (12.3%), and Marsh 1,2 in 22 (27.1%) cases. The severity of duodenal lesions was not influenced by patients' age (F=1.691; df=3; p=0.176).

TABLE 1
Characteristics of celiac disease patients

Patient characteristics	n (%)
Age, median (range), yrs.	38(18-72)
Females, n, (%)	60 (71.1%)
Gastrointestinal symptoms, n, (%)	39(48.1%)
Extra intestinal symptoms, n, (%)	42(51.9%)
Family history of CD, n, (%)	12 (14. 8%)
Newly diagnosed patients, n, (%)	68(84%)
Patients on a gluten free diet, n, (%)	10(12.3%)
CD associated comorbidity*, n, (%)	7(8.6%)

*thyroid disease, dermatitis herpetiformis, ataxia, type 1 diabetes, autoimmune liver disease, unexplained hypertransaminasemia, selective IgA deficiency.

When assessing the serological parameters, IgA-tTG antibody levels (61.45± 76.458 u/mL *vs.* 16.02±106.179 u/mL, p=0.001) and IgA-AGA antibody levels (61.83±69.41u/mL *vs.* 77.15±71.02 u/mL, p=0.001) correlated with intestinal villous atrophy (Marsh 3a and 3c) in CD patients by Spearman rank correlation (fig.2). Mild enteropathy (Marsh 1, 2) was identified in 22 (27.1%) cases with mean tTG levels of 32.44 ± 37.994 U/mL. Strongly positive antibody levels (IgA-tTG>160 units) were highly specific (>97%) for Marsh 3c lesions. Of the GI symptoms anemia, nausea and diarrhea were associated with abnormal histology (Marsh 3a-c lesions) when compared to mild duodenal lesions (Marsh 1, 2). The prevalence of non-GI manifestations was similar and was not influenced by the severity of mucosal lesions. Nevertheless, patients with villous atrophy presented more often with anemia compared with patients with mild enteropathy (p=0.048).

Hemoglobin, ferritin, iron, vitamin B12, albumin, folate, calcium and total protein levels were measured. When assessing the hematological parameters, hemoglobin levels were determined and anemia was diagnosed in 61.7% patients with Marsh 3b and 3c histological lesions (p=0.0048), elevated IgA-tTG levels (r=-0,316; p=0.004) and IgA-AGA (r=-0,301; p=0.006). Of the biological markers included in the statistical analysis, low iron levels (cut off 30 mg/dL), hypocholesterolemia and low protein levels were associated with Marsh 3b lesions (p=0.006) and elevated IgA-tTG levels (r=-0,384; p=0.001). The lowest mean iron levels were found among patients with subtotal and total villous atrophy, p=0,004.

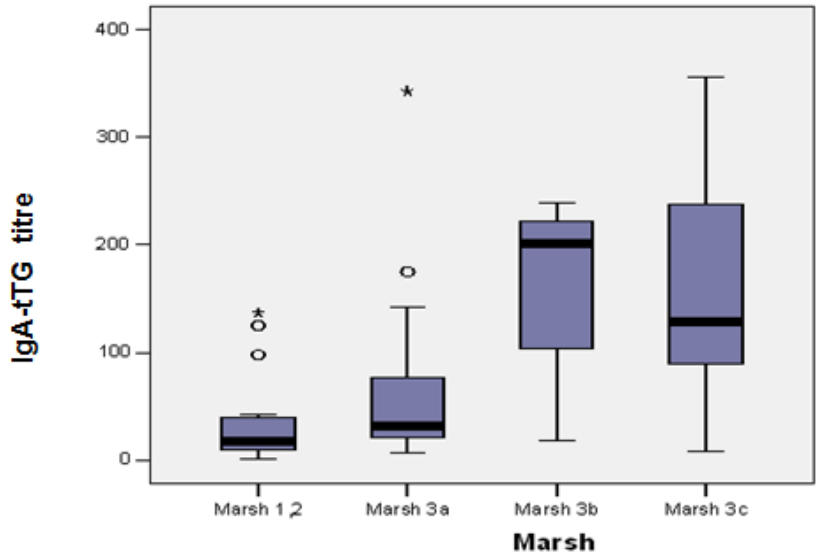

Fig. 2. Correlation between IgA-tTG level and severity of mucosal damage according to Marsh-Oberhuber classification.

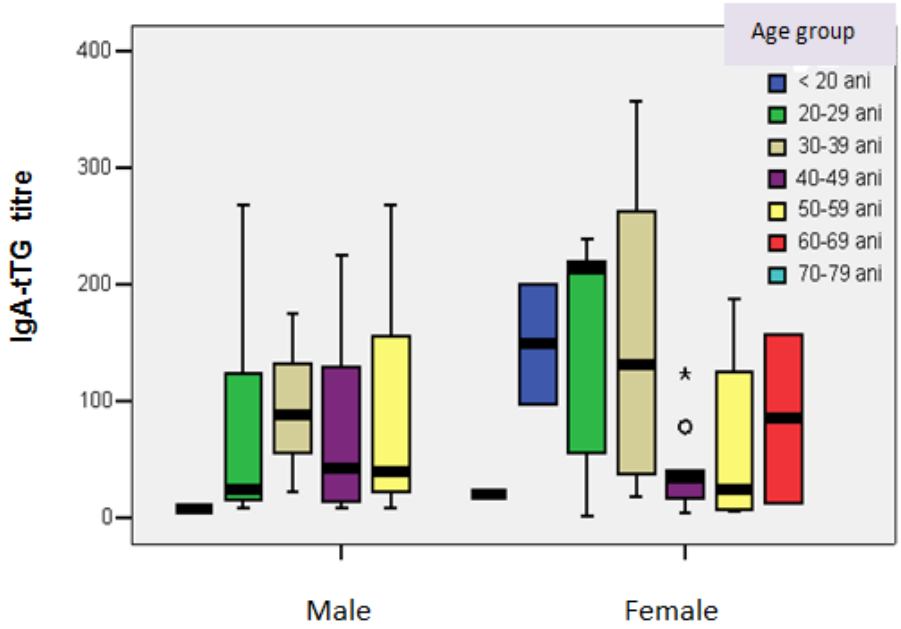

Fig. 3. Gender and age-group distribution of IgA-tTG level

DISCUSSION

Gastrointestinal symptoms alone cannot accurately differentiate CD from other common gastrointestinal disorders (20-50% of patients with CD met the Rome criteria for irritable bowel syndrome) (18,19). Whilst current literature suggests a discriminatory role for a high-risk group (anemia, weight loss, diarrhea, dermatitis herpetiformis) our data suggest that no single clinical feature is a reliable predictor or risk stratifier for CD and the disease occurs irrespective of clinical presentation (20,21). According to our results, CD is more frequent among young female patients, with a shift in clinical pattern occurrence towards atypical presentation in older patients, which is associated with misdiagnosis and delayed diagnosis.

The results showed significant correlations between degrees of small-bowel mucosal morphologic damage in CD. Patients with IgA-tTG levels>160U/mL virtually always have CD, whereas the disease can be histologically absent in a significant number of patients with low serum tTG level. In our study, strongly positive antibody levels (IgA-tTG>160 units) were highly specific (>97%) for Marsh 3c lesions. We also noted that GI symptoms were more common in patients with lower IgA-tTG compared to patients with elevated levels. Alterations of laboratory parameters were particularly evident for the parameters expressing intestinal malabsorption, reasonably reflecting a more severe impairment of intestinal absorptive function in patients with villous atrophy.

The relationship between specific antibody level, degree of mucosal damage and clinical presentation in CD remains controversial. In the article by Taavela *et al*, the ratio of small-bowel villous height to crypt depth and results from serology tests correlate with the reported symptoms and quality of life of patients with CD. Patient-reported outcomes are therefore of value, in addition to histology findings, in assessing patients with CD (22). Mubarak *et al*. aimed to investigate whether CD patients with tTG levels ≥100 U/mL are different from patients with lower tTG levels. Patients with high tTG levels had lower average body weight-for-height standard deviation scores than did patients with lower tTG levels. In the low tTG group, GI symptoms were more common; specifically, abdominal pain and nausea were more frequent among patients with low tTG. In contrast, patients with solely extra intestinal manifestations were only present in the high tTG group (23). Dahlbom *et al*. demonstrated that high levels of IgA-tTG antibodies were associated with the grade of mucosal villous atrophy and a more severe clinical presentation. The combined measurement of IgA-tTG enables a noninvasive prediction of small intestinal villous atrophy with high accuracy, and may reduce the need for biopsy in patients with suspected CD (24).

Our study has some limitations. Our investigation is uncontrolled and retrospective but reflects current clinical routine practice. The inclusion of all adult CD patients over a 5 year- study period, with the availability of a small number of cases forms nonetheless the basis for future prospective studies to create a national CD patient database to access reliable information responding to the specific needs of the Romanian society and provide knowledge and even prevention tools (25).

CONCLUSIONS

Our study confirms the chameleonic na-

ture of CD. Adult Celiac disease is currently much more common than previously estimated and there is a need for well-designed epidemiological studies to increase detection rate. Celiac disease is confirmed to be more prevalent in female than in male patients (F:M = 3:1). IgA tTG levels correlate with duodenal villous atrophy in adult CD patients. An IgA-tGA level >160 was nearly always associated with CD histopathology. GI and non-GI symptoms are not reliable predictors of CD. No single clinical feature on its own is a reliable predictor or risk stratifier for CD.

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NEWS

GIANT CELL MYOCARDITIS: A BRIEF REVIEW

Giant cell myocarditis (GCM) was first described in 1905 and is characterized by a mixed myocardial infiltrate with multinucleated giant cells and cardiomyocyte necrosis. In larger autopsy series, the incidence of GCM has been reported to range from 0.007% to 0.051%. Given that autopsies are not routinely performed on an unselected population, and patients dying of GCM may not undergo autopsy, it is likely these figures underestimate the true incidence. In the United States in the 1990s, a group of major heart failure referral centers reportedly diagnosed GCM (via autopsy, endomyocardial biopsy, apical wedge resection, or cardiac explantation) an average of once every 21 months. Giant cell myocarditis is a rare, progressive inflammatory myocardial disease with high mortality, affecting a relatively young population. Although much progress has been made during the past decades in understanding the pathophysiology, there are still many deficiencies in our knowledge. Rapid diagnosis is critical, as the management differs from that of other myocardial processes containing giant cells, and early institution of combined cyclosporine-based immunosuppression can dramatically impact the disease course. Promising future directions include the development of serum biomarkers and utilization of gene expression profiling to potentially more rapidly diagnose and monitor GCM progress (Xu J and Brooks EG. Giant Cell Myocarditis: A Brief Review. Archives of Pathology & Laboratory Medicine 2016, 140 (12): 1429-1434).

Doina Butcovan

CLINICAL SIGNIFICANCE OF VARIABILITY OF ATHEROGENIC INDEX OF PLASMA IN PATIENTS WITH LOWER LIMB PERIPHERAL ARTERIAL DISEASE ON LIPID-LOWERING THERAPY

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CLINICAL SIGNIFICANCE OF VARIABILITY OF ATHEROGENIC INDEX OF PLASMA IN PATIENTS WITH lower limb peripheral arterial disease ON lipid-lowering therapy (abstract): Atherosclerosis, as the pathophysiological substrate of lower limb peripheral arterial disease (LLPAD), is a major health problem given the multitude of associated diseases and its severe course. Atherogenic index of plasma (AIP), defined as the logarithmically transformed ratio of concentrations of triglycerides (TG) to HDL-cholesterol is one of the main evaluation parameters of the atherosclerotic process, its variability being an important predictive factor of treatment response. **Aim**: The evaluation of clinical significance of AIP in patients with LLPAD on lipid-lowering therapy with statins and fibrate association. **Material and methods**: Retrospective study of a group of 160 patients aged 40 to 89 years diagnosed with LLPAD and on lipid-lowering treatment with statins and fibrate, hospitalized at the Cardiovascular Rehabilitation Clinic of the Iaşi Rehabilitation Hospital. During the 2 study years (January 1st, 2012-December 31st, 2013), the subjects received daily a constant maximum therapeutic dose of statin and fibrate. Demographic and anthropometric variables were considered and serological parameters were evaluated (total cholesterol-TC, TG, HDL-cholesterol, LDL-cholesterol and AIP) both before and after starting the treatment. In addition, the following symptoms were evaluated on a scale of 0 to 10 at the beginning and at the end of the study: intermittent claudication, feeling of cold feet, paresthesia. **Results and discussion**: There were significant reductions in TC, TG and implicitly in AIP levels. Analysis of clinical response showed that patients who achieved an AIP under 5 (117 cases-73%) experienced a significant reduction of symptoms, associated with a change in disease staging classification; in patients with an AIP over 5 (43 cases-27%) no variations of symptoms were recorded, the previous staging classification remaining unchanged. **Conclusions**: A 2-year constant dose of statin and fibrate combination in patients with LLPAD is associated with a decrease in AIP levels and correlates with symptoms reduction and improvement in the disease staging classification. Thus, an adequate control of lipid profile has beneficial effects on the clinical manifestations of LLPAD. **Keywords**: ATHEROGENIC INDEX OF PLASMA, LIPID-LOWERING THERAPY, LOWER LIMB PERIPHERAL ARTERIAL DISEASE, ATHEROSCLEROSIS.

Peripheral artery disease (PAD) comprises a multitude of different diseases in terms of etiology and pathogenesis that have in common damage to lower and up-

per extremity arteries (1). This group of diseases includes the chronic peripheral artery disease of lower limbs and its acute form (acute peripheral arterial occlusion), thromboangiitis obliterans (Buerger's disease) and the functional vascular disorders: Raynaud's disease, acrocyanosis, livedo reticularis. *Lower Limb Peripheral Arterial Disease* (LLPAD) is an obstructive disease that causes progressive narrowing of the lumen and decreased blood flow to the limbs, atherosclerosis being the most common cause (2). The prevalence of arterial occlusive diseases is dependent on age and sex, men over 70 years with multiple cardiovascular risk factors being most commonly affected. The prevalence of PAD in Romania varies directly with age, from 2.5% in the population aged 40-59 years to 18.8% in the population aged 70-79 years (2, 3).

The clinical picture of LLPAD includes a multitude of symptoms, the most important being intermittent claudication, paresthesia and cold feet. Of these, intermittent claudication is the key element in Leriche-Fontaine classification, as follows: stage I-asymptomatic; stage IIA -intermittent claudication after more than 200 meters of walking; stage IIB-intermittent claudication after less than 200 meters of walking; stage III-rest pain; stage IV-ischemic lesions: ulcers, gangrene, necrosis (4, 5, 6).

Atherogenic index of plasma, defined as the logarithm of the ratio between the concentration of triglycerides (TG) and HDL-cholesterol is one of the main benchmarks of the atherosclerotic process and hence of LLPAD, the variability of which is an important factor of predictability of treatment response (7, 8).

MATERIAL AND METHODS

A single-center, observational, descriptive and retrospective study was conducted on a sample of 160 subjects aged 40 to 89 years, hospitalized at the Cardiovascular Rehabilitation Clinic of Iasi Rehabilitation Hospital between January 1st, 2012 and December 31st, 2013. Inclusion criteria for the study were: LLPAD stage II (A or B) or III, a baseline TG level of more than 150 mg/dL, TC level over 220 mg/dL and HDL cholesterol under 35 mg/dL, absence of lipid-lowering therapy at study initiation. Exclusion criteria were: LLPAD stage I or IV, normal TG level, HDL-cholesterol level over 35 mg/dL and TC under 220 mg/dL, lipid-lowering medication. During the 2 years of study, the subjects received constant daily maximum therapeutic dose of statin and fibrate. Demographic variables, anthropometric and serological parameters (TC, TG, HDL-cholesterol, LDL-cholesterol, AIP) were assessed both before and after starting treatment. The following symptoms have also been evaluated on a scale of 0 to 10: intermittent claudication, cold feet, paresthesia, both at the beginning and end of the study.

The obtained data have been processed using *SPSS 21.0* (SPSS, Inc., Chicago, IL, USA).

RESULTS

After clinical examination, the patients included in the study were stratified according to Leriche-Fontaine classification as follows: stage IIA-20 (12.5%) patients, stage IIB-67 (60.6%) patients, stage III-73 (26.8%) patients (fig. 1).

At the end of the two years of study, after a constant daily maximum dose of statin and fibrate, the distribution of patients according to disease stage was: stage IIA-107 (66.8%) patients, stage IIB-40 (25 %) patients, and stage III-13 (21.6%) patients (fig. 2).

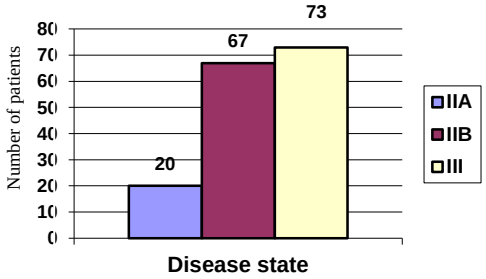

Fig. 1. Distribution of patients according to disease stage at baseline

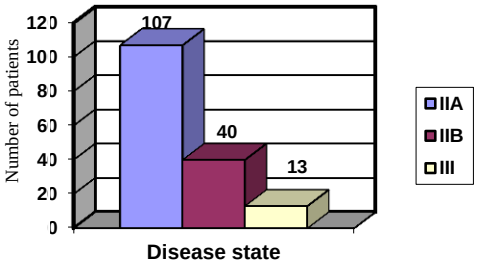

Fig. 2. Distribution of patients according to disease stage at study endpoint

The data obtained and illustrated in the above figures show that, as a result of the treatment administered during the two-year study, 30 patients (18.7%), previously staged as stage III, were reassigned as stage II, while 87 patients (54.3%), previously staged as stage IIB, were reassigned as stage IIA.

As mentioned above, every symptom present on admission (paresthesia, intermittent claudication and cold feet) were evaluated on a scale of 0 to 10, with 0 meaning the absence of symptom and 10 the highest intensity. During the study, revaluations were performed every 6 months, significant improvements in the grades of the patient being recorded (fig. 3).

Thus, a total of 110 patients (68.7%) showed an improvement by least two points on the pain scale (fig. 4).

Regarding AIP, at the end of the two-year study a significant number of patients (117/73%) had a decrease in value below 5, while in a smaller percentage (43/27%) the value remained above 5 (fig. 5).

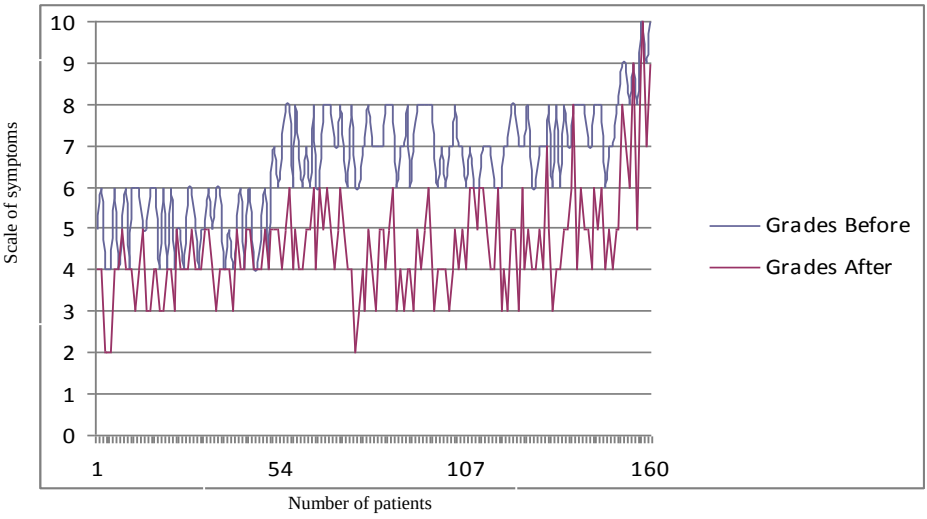

Fig. 3. Change in disease staging

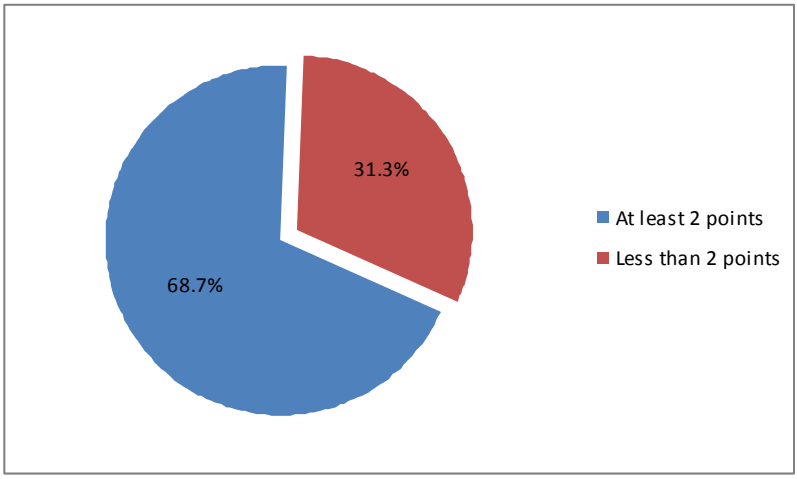

Fig. 4. Improvement on the pain scale

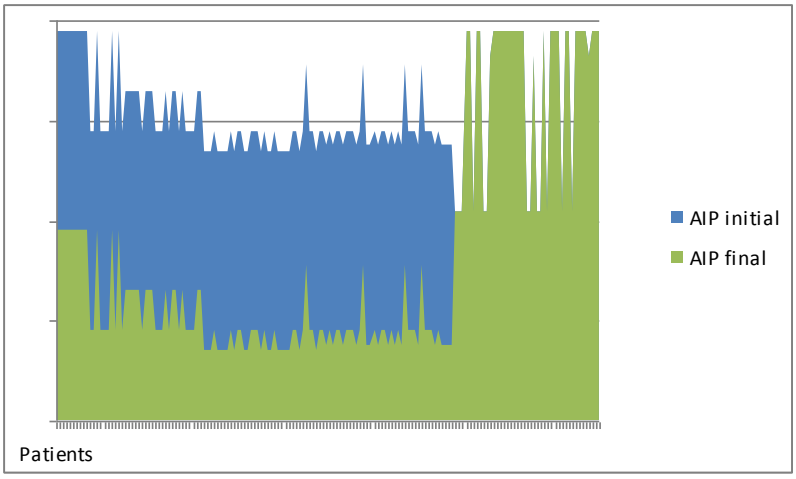

Fig. 5. AIP variability during the study

DISCUSSION

The process of atherosclerotic lesion formation is slow and remains asymptomatic until lumen compromise or vascular occlusion (9, 10). The pathophysiological processes involved are enhanced as well by classical risk factors for developing PAD: cigarette smoking, hypertension, diabetes and hyperlipidemia (11). Lipid abnormalities are known to have a critical role in the development of atherosclerosis, so that the administration of lipid-lowering medication has beneficial role both in preventing the occurrence of atherosclerotic lesions and in slowing the pathophysiological processes, with default improving of symptoms (12, 13).

Although the main goal of lipid-lowering therapy is to reduce the LDL-cholesterol levels, it is well known that high levels of TG and TG-rich lipoproteins (TRLs) are a cardiovascular risk factor

(14). On the other hand, hypertriglyceridemia is associated with low HDL-cholesterol levels; thus, although the lipid-lowering therapy is not targeted at correcting the HDL level, it is used to reduce hypertriglyceridemia (15). In the literature, there are case reports of patients with normal TG levels associated with low HDL-cholesterol (16, 17, 18). Due to the fact that HDL-cholesterol level is a marker of cardiovascular risk, uncorrelated with the atherogenic process, its isolated correction is not associated with a decreased risk (19). Recent studies have shown that aggressive treatment with lipid-lowering agents (maximum therapeutic dose) is associated with better prevention of cardiovascular events (20).

CONCLUSIONS

The present study demonstrates the efficacy of lipid-lowering therapy with statin and fibrate in constant maximum therapeutic doses administered for 2 years in patients with stage II and III LLPAD. Thus, there was a significant reduction of symptoms associated with improvement in disease staging, through the improvement of the lipid profile and therefore a decrease in AIP.

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NEWS

NEW PROTECTIVE ROLE OF IL-22 IN *MYCOBACTERIUM TUBERCULOSIS* INFECTIONS

In a study by Treerat *et al*., the functional role of IL-22 pathway in immunity against *Mycobacterium tuberculosis* strains was assessed. A member of the IL-10 cytokine family, IL-22 has both protective and pathological functions on mucosal surfaces. The study used as a prototype *M. tuberculosis* HN878, a member of the W-Beijing lineage and showed that the microorganism stimulates IL-22 production, using a TLR2 dependent mechanism. The study showed that IL-22 mediates protective immunity in chronic stages of infection in mice and that IL-22-dependent pathway in macrophages and epithelial cells mediate protective mechanisms for infection control (Treerat P, Prince O, Cruz-Lagunas A, Muñoz-Torrico M, Salazar-Lezama MA, Selman M, *et al.* Novel role for IL-22 in protection during chronic *Mycobacterium tuberculosis* HN878 infection. Mucosal Immunol. 2017 Mar 1. doi: 10.1038/mi.2017.15.).

Teodora Vremera

POLYCYSTIC OVARY SYNDROME, A COMPLEX ENTITY INVOLVING REPRODUCTIVE AND METABOLIC IMPAIRMENTS: A SYSTEMATIC REVIEW

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POLYCYSTIC OVARY SYNDROME, A COMPLEX ENTITY INVOLVING REPRODUCTIVE AND METABOLIC IMPAIRMENTS (Abstract): A SYSTEMATIC REVIEW (Abstract): We attempt to synthesize main data in what concerns pathogenesis, signs and symptoms, diagnosis and management of polycystic ovary syndrome PCOS. The issue was the systematization of main data regarding different features of PCOS and stressing its peculiar association with characteristics of metabolic syndrome, main diagnostic criteria and most important therapeutic options, finally contouring an updated general picture of PCOS. We pointed also on associated morbidities, such as endometrial cancer, insulin resistance and need for individualized therapy, including changing in lifestyle, especially weight loss.
Keywords: POLYCYSTIC OVARY SYNDROME (PCOS), INSULIN RESISTANCE, ANOVULATION, HYPERANDROGENEMIA, GLUCOSE TOLERANCE TEST (GTT).

Polycystic ovary syndrome (PCOS), also known as Stein-Leventhal syndrome or hyperandrogenic anovulation is characterized by irregular menstrual periods or amenorrhea, hirsutism, acne, infertility and *acanthosis nigricans* (1).

The syndrome was first described in 1935 by American gynecologists Irving F. Stein and Michael L. Leventhal, giving the initial name of Stein-Leventhal syndrome. The earliest description of the disease dates back from 1721 in Italy (2).

In 2010, World Health Organization has estimated the syndrome being present in 116 million women all over the world, 3.4 % of the female population.

Ultrasonography has found polycystic ovaries in 8-25 % of normal women (3, 4).

ETIOLOGY AND PATHOGENESIS

Genetic factors are strongly involved in the genesis of the disease. Evidences include: hereditary features of PCOS, familial clustering and greater concordance in monozygotic than in dizygotic twins (5).

The disease is transmitted on an autosomal dominant way; in women with the allele, the phenotype is revealed by high androgen levels originating in the techa cells of the ovarian follicles. The precise affected genes have not yet been identified, but accumulated data suggest that the syndrome is a complex multigenic disorder (6).

The severity of the PCOS symptoms seems to be related to obesity and features of metabolic syndrome, since symptoms of PCOS are partially reversible, thus supporting the overlapping with main traits of metabolic syndrome (3).

The cysts in the ovaries are a symptom of the disease and not its primary cause; recent data is featuring the syndrome as a multisystem disorder with primary hypothalamic hormonal dysregulation. The term of disease is used to describe the situation in which ovarian cysts are evident in ultrasonography, while the term of syndrome is rather describing a complex condition with a wide spectrum of symptoms and presenting ovarian cysts in only 15 % of the cases (7).

Main pathogenic issue is represented by the overproduction of androgens, especially testosterone generated by the high levels of luteinizing hormone (LH) and hyperinsulinemia, in women whose ovaries are peculiarly sensitive to insulin (6, 8).

The increase of hypothalamic GnRH pulses results in an elevated LH/FSH ratio. Most PCOS cases show insulin resistance and/or obesity. Elevated insulin levels are triggering features of PCOS since are increasing the frequency of GnRH pulses. Increased LH/FSH ratio is inducing ovarian androgen overproduction, decreased follicular maturation and low SHBG binding, leading to the development of PCOS. Aromatase present in the adipose tissue is converting androstenedione into estrone and testosterone into estradiol; obesity is responsible for both overproduction of androgens (causing hirsutism and virilization) and estrogens (inhibiting FSH secretion) (9).

Chronic inflammatory mediators are believed to be involved in the development of different symptoms of PCOS, such as anovulation (10).

DIAGNOSIS

Diagnosis of polycystic ovary syndrome is based on two of the three criteria: anovulation, high androgen levels and ovarian cysts in ultrasound examination (Rotterdam criteria established in 2003).

Common signs and symptoms of PCOS are represented by: menstrual disorders, more often oligomenorrhea or amenorrhea, but other menstrual disorder may also be present; infertility caused by chronic anovulation; high levels of androgens resulting in acne and hirsutism, sometimes determining hypermenorrhea, alopecia or other symptoms; metabolic syndrome with central obesity and signs of insulin resistance (3, 11).

Diagnosis may be established exclusively on clinical signs, such as the history of menstrual pattern, obesity, hirsutism and acne (12).

Ultrasonography should demonstrate presence of small ovarian cysts resulted from the so called follicular arrest, in which several follicles reach a size of 5-7 mm, but they do not develop furthermore. No follicle reaches the pre-ovulatory size of at least 16 mm.

Recent studies support the opinion that at least 25 such follicles should be present in an ovary, in women aged 18-35 years, to confirm the ovarian morphology of PCOS. The cysts must appear in periphery of the ovary, showing the aspect of a string of pearls (13).

An ovarian volume of at least 10 ml is also considered a criterion for the PCOS morphology, besides the presence of cysts. Laparoscopy may put in evidence a thickened, pearl-white surface of the ovary.

Plasma elevated androgen levels (androstenedione and testosterone) is also a common finding in the polycystic ovary

syndrome. 60% of PCOS patients show high free testosterone levels.

Free androgen index: ratio of testosterone to sex hormone-binding globulin (SHBG) is correlating to the degree of obesity. Among obese PCOS cases, low levels of SHBG are common. Anti-Müllerian hormone shows high levels in PCOS; this may become a diagnostic criterion (14).

Insulin resistance demonstrated by abnormal two-Hour oral glucose tolerance test (GTT) is present in 15-33 % of women with PCOS; insulin resistance is appearing in overweight, as well as in normal weight subjects. Diabetes is associated in approximately 65% of women with the disease.

Elevated insulin levels are a common finding; insulin resistance in such cases is revealed at GTT (GTT insulin levels) by higher insulin levels and lower blood glucose, than in fasting condition.

Glucose tolerance test is a better diagnostic tool than fasting glucose in PCOS, since while fasting glucose may be normal, oral glucose tolerance is impaired in 38 % of the asymptomatic cases (15).

Differential diagnosis should consider different other causes of amenorrhea and virilization: hypothyroidism, congenital adrenal hyperplasia (21-hydroxylase deficiency), Cushing's syndrome, androgen secreting tumors or hyperprolactinemia.

MANAGEMENT AND PROGNOSIS

Therapy includes changing in lifestyle, medications and surgery.

Main aims of the therapy are consisting in reducing insulin resistance, recovery of fertility, therapy of hirsutism and acne; obtaining regular menstruation and prevention of endometrial hyperplasia and endometrial cancer.

Significant controversies are ruled out in what concerns optimal therapy, especially because of the lack of therapies comparing large-scale clinical trials. Weight loss is the most effective method in restoring normal ovarian function (ovulation, menstruation). Unfortunately, many women found it difficult to achieve. There are studies which are pointing on low glucids diets, which are bringing greater benefit in what concerns regular menstruation (16).

Medications used in the treatment of PCOS are especially oral contraceptives and metformin (oral hypoglycemiants).

Oral contraceptives are increasing SHBG levels and thus the binding of free testosterone. In this way are improved symptoms of hirsutism and normal menstruations are appearing. In many cases besides treating insulin resistance, metformin is effective in restoring ovarian function and normal ovulation. Spironolactone is also used for its antiandrogenic effects (17).

For anovulation (cause of infertility in PCOS) besides recommended weight loss, ovulation inducting medications are used: clomiphene-citrate and FSH (19). Metformin was also used in the treatment of anovulation but was found to be less effective than clomiphene; an important aspect in what concerns the use of metformin is that is not increasing the risk of birth defects (18).

In vitro fertilization may be used in case of non-responsiveness to clomiphene therapy.

Laparoscopic procedure consisting in puncturing 4-10 small cysts using electrocautery or laser, known as ovarian drilling is used for its result in restoring normal ovulation, yet there are controversies in what concerns the long-term effect of this

surgical procedure (19).

Anti-androgen drugs, such as flutamide and spironolactone are used to treat hirsutism. At the same time, metformin may reduce hirsutism possibly by diminishing insulin resistance, for this being used in the treatment of associated conditions such as diabetes and obesity (17).

The risk of endometrial hyperplasia and endometrial cancer through hyper stimulation of the uterine lining by estrogens, might be induced also by associated conditions such as obesity, hyperandrogenemia (20).

Insulin resistance and type II diabetes are commonly associated with PCOS, even in the cases with normal body mass index (7, 21).

Other associated conditions in PCOS are including: dyslipidemia non-related to insulin resistance; depression and anxiety; cardiovascular disease; hepatic steatosis; *acanthosis nigricans* and autoimmune thyroiditis (22, 23).

CONCLUSIONS

In our evaluation, besides its direct related pathology, PCOS is drastically affecting life quality; the need for changes in lifestyle (diet, physical exercise) does not need any more to be stressed; however, the difficulty in adopting different living habits makes from PCOS a subject of continuous challenge for patients, as well as for physicians.

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NEW DEVELOPMENTS IN THE APPROACH AND DIAGNOSIS OF SARCOPENIA

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NEW DEVELOPMENTS IN THE APPROACH AND DIAGNOSIS OF SARCOPENIA (Abstract): Sarcopenia, the key point of frailty syndrome, leads to decreased physical activity, with important consequences upon the quality of life in elderly. The prevalence of sarcopenia is still uncertain because of the lack of homogeneity of the studied populations, and also because of the variety of techniques. The development of biological markers that can be used in a cost effective manner to guide diagnosis and facilitate monitoring patients with sarcopenia, would mark an important step in managing the care of geriatric patients. Nutrition combined with physical activity is the key component of the management of sarcopenia, with a synergistic effect that helps combat malnutrition and improve the quality of life. **Keywords**: SARCOPENIA, BIOMARKER, NUTRITION, VITAMIN D.

Sarcopenia is defined as the loss in muscle mass and strength that occurs with age, generating a decrease in physical activity. It causes a progressive decrease in muscle mass of about 8% per decade by age 40-70 years, and of 15% after the age of 80. This condition affects 30% of patients over 60 years of age and about 50% of those over 80 years old (1).

The factors that contribute to the development of sarcopenia are multiple: physical inactivity, vitamin D deficiency, chronic inflammatory status, hormonal changes (low testosterone levels) and neuromuscular changes (decrease in the number of muscle fibres and motor neurons). The consequences will be severe, as the decreased muscle mass and strength will lead to a decline in physical activity, increased body instability and risk for falls and ultimately depression, poor quality of life and disability.

The decline in physical activity due to sarcopenia leads to a decrease in physical strength and force and will eventually lead to sedentary life and dependency with direct implications on quality of life and an increased risk of disability (2).

Sarcopenia decisively influences the risk of falls and fractures in older people: nearly half of accidental deaths in people over 65 are related to falls and their consequences (prolonged immobilization, respiratory tract infection) (3).

Unlike cachexia, that occurs secondary to a chronic disease, usually neoplasia,

sarcopenia refers to a decrease in muscle mass, not necessarily associated with weight loss (4). Thus, the concept "sarcopenic obesity" was introduced, where muscle tissue is infiltrated by fat tissue, in addition to excess subcutaneous fat, which translates to lower quality muscle fibre and muscle strength. Sarcopenia is a reliable marker of global presence of fragility syndrome (5). However, sarcopenia can lead to frailty, but not all sarcopenic patients are frail.

DIAGNOSIS

Decreased muscle mass makes the diagnosis of sarcopenia. The discrepancy between muscle mass and strength makes the assessment of muscle mass alone insufficient (6). Longitudinal studies have shown that the decline in muscle strength far exceeds changes in muscle mass, particularly in patients with constant body weight. In addition, preservation of muscle mass does not prevent the sensation of muscle weakness in the elderly (7).

The prevalence of sarcopenia is still uncertain because of the lack of homogeneity of the studied populations, but also because of the variety of techniques used to assess muscle mass. Full body scan by Dual X-Ray Absorptiometry (DXA) is currently considered the golden standard for assessing muscle mass (8). Other methods are bioelectrical impedance analysis, computed tomography (CT), magnetic resonance imaging (MRI), each of them providing objective and reliable information on muscle mass and fat (9). Unfortunately, such imaging equipment is not always available. In addition, MRI and CT examinations are quite expensive. Also, each of these techniques provides different estimates of body composition profile in terms of anatomical regions, making it difficult to compare the results (10). These and other drawbacks limit their use in routine practice. Muscle mass is only one dimension of sarcopenia. The functional decline arising from muscle coordination and balance disorders are clear signs of aging, which significantly affect the quality of life (11).

Muscle strength can be easily measured through performance tests: Short Physical Performance Battery (SPPB), 4-meter walk test, handgrip strength test. These tests can be significantly influenced by comorbidities that are often present in older people, mainly through inflammatory or degenerative osteoarticular diseases (12). While assessing the combined muscle mass and strength is a prerequisite for identifying sarcopenia, one of the biggest current problems is to define thresholds to differentiate between "physiological" and "pathological" aging (13). This limits the applicability of imaging tests and biomarkers in clinical practice and research.

The development of biological markers that can be measured in body fluids, used in a cost effective manner to guide diagnosis and facilitate monitoring patients with sarcopenia, would mark an important step in managing the care of geriatric patients.

Recent studies show that the combination of biochemical assays with imaging methods increase the accuracy of sarcopenia detection (12). However, the list of biomarkers proposed is very long and includes markers of inflammation (CRP, IL-6 and tumor necrosis factor TNF-alpha), urinary creatinine, hormone levels (testosterone, insulin-like growth factor 1, vitamin D), markers of oxidative stress. It was recently proposed a new set of serum markers directly associated with changes in skeletal muscle mass and function.

N-terminal peptide of type III procollagen (P3NP) (10) seems to be correlated with muscle remodeling after lifestyle change, exercise done consistently (14) or after hormone therapy with testosterone or growth factor (15). Framingham Offspring study conducted on 687 people showed that P3NP levels were related to total muscle mass in postmenopausal women but not in elderly men, therefore this biomarker is highlighted as a gender restrictive one (16).

C-terminal Agrin Fragment (CAF) (17) seems to be a reliable marker in skeletal muscle mass assessment. Excessive cleavage of the native motor neuron-derived agrin by neurotrypsin into a C-terminal Agrin Fragment (CAF) leads to functional disintegration at the neuromuscular junction and may consecutively cause sarcopenia. Elevated plasma levels of CAF were correlated with impaired neuromuscular junction, which in turn is involved in fiber denervation, muscle atrophy and dysfunction (18).

Similar to CAF, plasma concentrations of extracellular heat shock protein 72 (eHsp72) were inversely related to muscle mass and function (19). Production of eHsp72 has been correlated with inflammation (20) and the phenomenon of apoptosis. However, its implication in the development of sarcopenia is currently unclear.

Listed parameters and their partial association with pathophysiological processes involved in the development of sarcopenia underline the idea that we cannot consider the existence of a single biomarker. Comorbidities (cardiovascular disease, chronic renal disease, diabetes, lung disease, cancers) can also influence the biomarkers.

In conclusion, a complex approach can provide useful information about the pathophysiological mechanisms that lead to frailty and sarcopenia, with further implications in prevention or therapeutic strategies.

PREVENTION AND TREATMENT OF SARCOPENIA

Screening elderly people in order to detect those at risk of malnutrition is the key point and involving geriatricians, nutritionists and physiotherapists is essential.

In 2008 the Society for Sarcopenia, Cachexia and Wasting convened an expert panel to develop a guide of nutritional recommendations for prevention and management of sarcopenia. It concluded that protein and calorie intake is key measures associated with appropriate resistance training and aerobic exercises. However, nutritional management for sarcopenia remains unknown, recommendation ranging from one study to another.

1. PROTEIN INTAKE

In 2015, Rizzoli reported their recommendations for optimal protein intake from 1.0 to 1.2 g / kg, with an equal distribution for each meal (21).

Paddon-Jones et al. have proposed a diet plan that includes 25 to 30 grams of high quality protein per meal. In addition, adding to the diet 15-20 grams of whey proteins has increased the effects of exercise on muscle (22).

The intake of proteins and amino acids remains the most important anabolic stimulus of lean muscle tissue. Previous studies demonstrated anabolic effect of essential amino acids, particularly the branched chain (leucine). The β-Hydroxy β-Methylbutyrate (HMB), is a metabolite of the amino acid Leucine that, along with KIC (α keto-isocaproate) and isovaleryl-CoA, mediate the effects of leucine. Approximately 5% of dietary leucine is oxi-

dized into HMB, and HMB appears to be the main metabolite of leucine that more effectively prevents the breakdown of muscle protein. HMB is proposed as a potential food supplement for patients confined to bed for a long period of time (23), but further studies are needed.

In addition, diets supplemented with amino acids and carbohydrates consumed one hour after physical exercise increase muscle protein synthesis.

2. PHYSICAL ACTIVITY

Exercise is another important aspect in the prevention and management of sarcopenia. There are four types of recommended exercise, depending on the particularities of each elderly. Endurance exercises have a positive effect on the cardiovascular system. Strength training increases muscle mass and combined with nutritional supplements, improve muscle strength. Complex or simple balance exercises standing on one leg with eyes closed and hand support may decrease the risk of falls. Flexibility exercises, such as yoga or stretching can help prevent or recover from falls (24). Regardless of the type of exercise, it is recommended practicing them at least three times a week.

Nutritional intake combined with exercise has a synergistic effect that helps combat malnutrition and improves the quality of life. Physical activity should be recommended but in different intensity and complexity depending on age and possibilities of travel. Patients should be evaluated by a physiotherapist in order to assess range of motion, strength and endurance and to determine the need of using assistive devices such as canes, grab bars, shower seats.

3. VITAMIN D

Vitamin D deficiency is also frequent in older people, regardless of race or ethnicity. The level of 25-hydroxy vitamin D (25) (OH) decreases with age and is associated with muscle weakness. Dosing of vitamin D should be made to all person with sarcopenia, followed by adequate treatment (doses sufficient to increase the level of over 100 nmol/L) (25). The effects were observed in postmenopausal women. Doses up to 50,000 IU of vitamin D2 or D3 per week are allowed. But recent studies show that increased doses do not provide additional benefits compared to usual doses (26).

CONCLUSIONS

The analysis of recent literature makes us conclude that sarcopenia is a fundamental element in the diagnosis of frailty syndrome. According to our results, the geriatric evaluation must include the evaluation of sarcopenia, in order to assess the response to treatment and the evolution of frailty. From our experience several nutritional supplements, such as whey protein, amino acids (leucine, glutamine), vitamin D appear to be beneficial in promoting healthy muscle mass.

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NEWS

ORGANOPHOSPHATE PESTICIDES DURING PREGNANCY AND NEUROLOGICAL DEVELOPMENT DURING EARLY CHILDHOOD

The authors studied prenatal exposure to organophosphate pesticides, which are ubiquitous in the environment, and child's neurological development. The study was conducted between 2003-2006 on a sample of 327 pairs mother / infant in Cincinnati, Ohio, to determine if there are associations between prenatal exposure to organophosphate pesticides and neurodevelopment in children. In pregnant women, urinary concentrations of metabolites of organophosphate pesticides and non-specific concentrations diethylphosphates, dimethylphosphates and dialkylphosphate were measured twice. In children, Bayley Scales of Infant Development, Second Edition-Mental and Psychomotor Developmental indices at the age of 1, 2, 3 years, the Clinical Evaluation of Language Fundamentals-Preschool, Second Edition, at age 4, and the Wechsler Preschool and Primary Scale of Intelligence, Third Edition, at age 5. Mothers with higher urinary total dialkylphosphate concentrations reported higher levels of socioeconomic status and an increased fresh fruit and vegetable intake. The authors found no association between prenatal exposure to organophosphate pesticides and cognition in children aged 1-5 years. It is possible that higher socioeconomic status and a healthy diet protect the fetus from potential side effects of exposure of pregnant women to organophosphate pesticides or that dietary exposure to metabolites is harmless or not an ideal measure of exposure to the parent compound. (Donauer S, Altaye M, Xu Y, Sucharew H, Succop P, Calafat AM, Lanphear B, Yolton K. An observational study to evaluate associations between low-level gestational exposure to organophosphate pesticides and cognition during early childhood. *Amer J Epidemiol* 2016; 184, 5, 410-418)

Alina Manole

NEW CLASS OF DRUGS: THERAPEUTIC RNAi INHIBITION OF PCSK9 AS A SPECIFIC LDL-C LOWERING THERAPY

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NEW CLASS OF DRUGS: THERAPEUTIC RNAi INHIBITION OF PCSK9 AS A SPECIFIC LDL LOWERING THERAPY (Abstract): Hyperlipidemia is a well-known risk factor for coronary heart disease, the leading cause of death for both men and women. Current lipid-lowering treatment is not always efficient, therefore new pharmacological interventions that reduce LDL cholesterol (LDL-C) have been developed. This paper presents new class of specific LDL lipid-lowering drugs under investigation in phase II or III clinical trials. The inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9), a key enzyme in cholesterol homeostasis, improve the liver's ability to clear LDL from the plasma, reducing LDL-C levels. Currently, three monoclonal antibodies PCSK9 inhibitors (alirocumab, evolocumab and bococizumab) are evaluated in clinical outcome trials. ALN-PCSsc, the new first-in-class therapeutic RNA interference (RNAi) inhibitor of proprotein convertase subtilisin/kexin type 9 (PCSK9) is also the first-in-class investigational medicine that acts by turning off PCSK9 synthesis in the liver. The development leadership of ALN-PCSsc has now transferred from Alnylam Pharmaceuticals® to The Medicines Company, who has initiated the ORION-1 Phase II study at the beginning of 2016. ALN-PCSsc has significant potential given its highly competitive profile as compared with monoclonal antibodies anti-PCSK9 MAbs, a recently approved class of LDL-C lowering drugs. **Keywords**: RNA INTERFERENCE (RNAi), PCSK9 PROTEIN, ALN-PCSsc.

Hyperlipidemia is a well-known risk factor for coronary heart disease, the leading cause of death for both men and women. Current lipid-lowering treatment is not always efficient, therefore new pharmacological interventions that reduce LDL-C have been developed.

The inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9), an enzyme discovered almost a decade ago (1) as a key player in cholesterol homeostasis (2,3,4), is now considered an exciting approach (5,6,7) in the reduction of the incidence of cardiovascular events (8,9,10). The PCSK9 protein is a plasma protein synthesized by several organs in the body (liver, intestine, etc.) and it can bind to LDL receptors. This binding prevents the LDL receptors from continuously clearing LDL from the plasma, resulting in elevated LDL-C levels. The new class of drugs, PCSK9 inhibitor drugs, serves to improve the liver's ability to clear LDL from the plasma, reducing LDL-C levels and reducing the risk of cardiovascular diseases.

When added to a maximized statin regimen, PCSK9 inhibitors work synergistically with statins to provide additional LDL-lowering via a different mechanism of action (11). The progress from PCSK9 discovery to the development of targeted treatment (12, 13) has been unprecedented in terms of scale and speed. The first suggestion of a link between PCSK9 and hypercholesterolemia was published in 2003 (14); a decade later, two meta-analyses of clinical trials comparing anti-PCSK9 treatment to placebo or ezetimibe, including >10,000 hypercholesterolemic individuals, were published (15). This paper presents new groups of lipid-lowering drugs under investigation in phase II or III clinical trials.

PCSK9 INHIBITION WITH MONOCLONAL ANTIBODIES

Currently, three PCSK9 inhibitors (all anti-PCSK9 MAbs: alirocumab, evolocumab and bococizumab) are being evaluated in clinical outcome trials and the results will determine the future of these lipid-lowering therapies by establishing their clinical efficacy in terms of cardiovascular event reduction, safety, and the consequences of prolonged exposure to very low levels of LDL-cholesterol. Irrespective of their outcomes, the exceptionally rapid development of these drugs exemplifies how novel technologies, genetic validation, and rapid clinical progression provide the tools to expedite the development of new drugs.

Hypercholesterolemia is a condition characterized by very high levels of cholesterol in the blood which is known to increase the risk of coronary artery disease, the leading cause of death in the world. However, a large proportion of patients with hypercholesterolemia are not achieving adequate LDL-C levels with currently available therapies such as statins, including genetic familial hypercholesterolemia patients, acute coronary syndrome patients, high-risk patient populations (e.g., patients with coronary artery disease, diabetes, symptomatic carotid artery disease, etc.) and other patients that are statin intolerant. There is a significant need for novel therapeutics to treat patients with hypercholesterolemia whose disease is inadequately managed by existing therapies. One potential drawback of monoclonal antibodies and other PCSK9 inhibitors in development is the need for repeated injections of the drugs.

PCSK9 INHIBITION WITH RNA INTERFERENCE (RNAi) INHIBITORS

In efforts to reduce the number of injections required for effective treatment, Alnylam Pharmaceuticals® and The Medicines Company recently reported positive results from their phase I clinical trial with ALN-PCSsc, an investigational RNAi therapeutic targeting PCSK9 which would likely be dosed quarterly. RNAi (RNA interference) is a revolution in biology, representing a breakthrough in understanding how genes are turned on and off in cells, and a completely new approach to drug discovery and development. Its discovery has been heralded as "a major scientific breakthrough that happens once every decade or so," and represents one of the most promising and rapidly advancing frontiers in biology and drug discovery today which was awarded the 2006 Nobel Prize for Physiology or Medicine. RNAi is a natural process of gene silencing that occurs in organisms ranging from plants to mammals. By harnessing the natural biological process of RNAi occurring in our cells, the creation of a major new class of medicines, known as RNAi therapeutics, is on the horizon (16).

Small interfering RNA (siRNA), known as short interfering RNA or silencing RNA, the molecules that mediate RNAi and comprise Alnylam's RNAi therapeutic platform, target the cause of diseases by potently silencing specific mRNAs, thereby preventing disease-causing proteins from being made (fig.1). RNAi therapeutics have the potential to treat disease and help patients in a fundamentally new way (17, 18, 19).

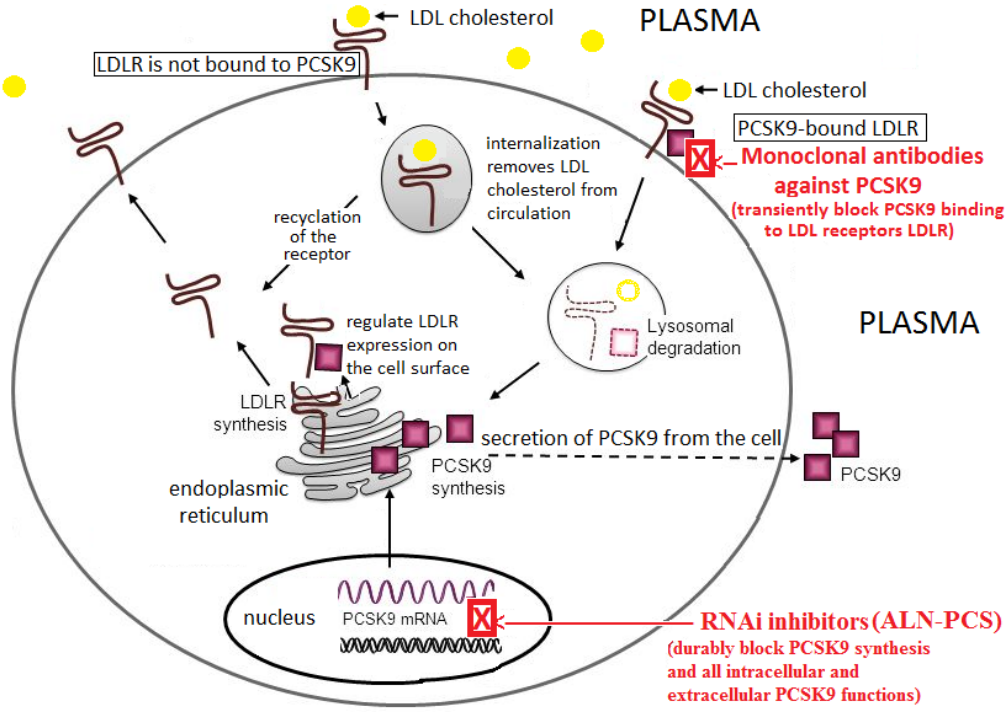

Fig. 1. PCSK9 Therapeutic Hypothesis [Adapted from (17)]

New delivery platforms were introduced for ALN-PCSsc. GalNAc-siRNA conjugates are a proprietary Alnylam delivery platform and are designed to achieve targeted delivery of RNAi therapeutics to hepatocytes through uptake by the asialoglycoprotein receptor. Alnylam's Enhanced Stabilization Chemistry (ESC)-GalNAc-conjugate technology enables subcutaneous dosing with increased potency and durability, and a wide therapeutic index. This ESC-GalNAc-conjugate delivery platform is being employed in nearly all of Alnylam's pipeline programs, including ALN-PCSsc and several other programs in clinical development (20).

PHARMACOLOGYCAL EFFECTS

The effects of ALN-PCSsc were found to be highly durable, with clinically significant and clamped reductions in LDL-C, supportive of a potential bi-annual subcutaneous dose regimen. Specifically, an up to 53 percent maximal and 47 percent least squares mean reduction in LDL-C was achieved at day 180 after just a single, low volume injection (tab I). In addition, ALN-PCSsc was shown to reduce a number of

atherogenic lipids, including lipoprotein (a) - or "Lp(a)" - and total cholesterol, which are associated with increased risk of cardiovascular disease. ALN-PCSsc was generally well tolerated with no clinically significant drug-related adverse events, but it is not known the long-term clinical benefit (21).

TABLE I
Comparative efficacy of MAbs and RNAi inhibitors of PCSK9

Study name	PCSK9 inhibitor	Dosing	LDL-C reduction vs. placebo
ODYSSEY (7)	Alirocumab	300 mg once-monthly	56%
RUTHERFORD (7)	Evolocumab	420 mg once-monthly	55%
Ballantyne et al.(7)	Bococizumab	300 mg once-monthly	27–53%
ORION-1 (18)	ALN-PCSsc	500 mg on days 0 and 90	59%

CONCLUSIONS

These anti-PCSK9 agents, now in phase II or III clinical trials, are still years away from approval. These new drugs appear to be promising in the reduction of the incidence of cardiovascular events because they reduce LDL-C, but there are basic facts not understood. Although the current recommendation for PCSK9 inhibitors is to be used in conjunction with statins and they have a steep price compared to statins, there are still not enough data about their long-term safety and efficacy and about the place of PCSK9s in the treatment of hypercholesterolemia.

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ETHICAL AND GENETIC ASPECTS REGARDING PRESYMPTOMATIC TESTING FOR NEURODEGENERATIVE DISEASES

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ETHICAL AND GENETIC ASPECTS REGARDING PRESYMPTOMATIC TESTING FOR NEURODEGENERATIVE DISEASES (Abstract) Neurodegenerative diseases, such as Alzheimer's dementia, Huntington's chorea, Parkinson's disease or spinocerebellar ataxia, manifests into adulthood with an insidious onset, slowly of progressive symptoms. All of these diseases are characterized by presimptomatic stages that preceded with many years of clinical debut. In Parkinson's disease, more than half of the dopaminergic neurons of the black substance are lost before the advent of motor characteristic manifestations. In Huntington's chorea, the progressive neurodegenerative disease could be diagnose prenatal and presymptomatic by analyse of the number of CAG repeats in exon 1 of the huntingtin gene. A similar mechanism represented by expansion of trinucleotide repeats during hereditary transmission from parents to children was identified in fragile X syndrome, spinocerebellar ataxia, spinal muscular and bulbar atrophy, or myotonic dystrophy. Presymptomatic diagnosis in all these progressive diseases raise many ethical issues, due to the psychological impact that can cause the prediction of a disease for which there is currently no curative treatment. Therefore, a positive result can produce serious psychological trauma and major changes in the lifestyle of the individual, instead, a negative result can bring joy and tranquillity. But the problem arises if presymptomatic testing in these neurodegenerative diseases brings greater benefits compared to the possible psychological damage, which can add the risk of stigmatization or discrimination. **Keywords**: NEURODEGENERATION, DYNAMIC MUTATION, PRESYMPTOMATIC TESTING, ETHICS ASPECTS.

Neurodegenerative diseases (ND) are hereditary or sporadic conditions characterized by progressive nervous system dysfunction, often associated with atrophy of central or peripheral nervous system structures. They are a heterogeneous group of age-associated, chronic illnesses of varying aetiologies, the main challenges for healthcare systems, including informal care and long-term care facilities, in the coming decades (1).

ND had a bad prognosis, after a period of 7–12 years of progressive deterioration of multiple areas of brain, including memory, reasoning, communication and the skills needed to carry out daily activities (2).

The recent discoveries of disease-causing genes in neurodegenerative diseases have generated considerable interest and debate for both physicians and patients regarding routine genetic testing of some ND in the clinic. Molecular genetics provide a powerful tool in the diagnosis of many neurodegenerative diseases and can help better define and classify many of the heterogeneous inherited neurodegenerative disorders. Genetic testing could allow early confirmation of diagnosis and appropriate institution of genetic counselling, but also may provide a genotype-phenotype correlation, select specific patients for clinical drug trials, and offer a better understanding of pathogenesis of the disease (3). Pre-symptomatic testing is usually performed at risk-population and investigate a person with a clear-cut family history, who has no symptoms of ND at the time of testing (4) and looking for genetic mutations that have a high penetrance, such as homozygous or compound heterozygous mutations in recessively inherited genes (e.g. *PINK1* and *DJ1* genes, in Parkinson's disease) or dominantly inherited mutations *(*like *SNCA* or *LRRK2* genes, in Parkinson's disease*)* (5).

Various positions have been advanced regarding the issue of predictive genetic testing for unpreventable or untreatable adult-onset disorders (6). On the basis of a medical benefit argumentation, some have argued that the absence of measures to prevent the disease or its complications or to treat the disease is a reason not to perform this test (7). Although still acknowledging the importance of medical benefit as a justification for predictive genetic testing, it has been recognized that in exceptional circumstances to apply the predictive test could be better than wait that child will arrive in adulthood (8).

Often, the communication of genetic information is a difficult issue and may have a powerful psychosocial impact on the child and its parents. Thus, in the case of a positive test, children could present anxiety or depression, and the parents could present a feeling of guilty. This process could be complicated by several factors. Usually, genetic testing is made only one time during the life and could have important consequences. Thus, the individual needs to think very carefully before deciding to take such a test. In majority of diseases is still not an ideal biomarker able to improve differential diagnosis, track disease progression and measure treatment efficacy and the method could have not conclusive results or be genetically uninformative (7, 8).

However, the feasibility of routine genetic testing of ND in the clinic is still fraught with many unanswered questions. “Which gene must be analysed in a particular case?” “Which clinical features are more adequate to select patients for genetic testing?” “Is the sensitivity and specificity of the genetic test high enough?” “Which are the consequences of a positive or ambiguous result?” “Which will be the patient's and physician's attitudes in case we will discover a genetic abnormality?” (6).

Genetics of neurodegenerative diseases

Alzheimer's disease

Alzheimer's disease (AD) is the most prevalent ND, accounting 50-70 % of all dementias. In a report of Commission of the European Communities, from 2009 is estimated that 7,300,000 peoples from Europe present AD and probably in 2020 the number will be double (9). The pathogenic mechanisms imply structural and chemical changes in the brain, leading to the death of neurones. For late-onset AD

cases the mechanism implies interaction of multiple genetic and environmental factors. Early-onset (before age 65) AD tends to cluster within families, but represents only 10% of AD (10). Among these, one half is caused by a heritable mutation in gene encoding one of 3 proteins: presenilin-1 (PS1), presenilin -2 (PS2), or amyloid precursor protein (APP). All these mutations are dominant, and the carriers develop AD in nearly 100% of cases. For multifactorial forms the most important gene seems to be the gene of apolipoprotein E (APOE). The gene is located on chromosome 19 and presents three variants: APOE ε2, APOE ε3 and APOE ε4. Only APOE ε4 has been validated, by genetic linkages, as an AD risk factor. The heterozygous for APOE ε4 have a 3-5 time higher risk, while homozygous for APOE ε4 have a 8-18 time higher risk for late-onset AD. However, a genetic test for APOE ε4 has little predictive value (11, 12).

Other genes which are linked to increased risk of AD are *CLU, PICALM, CR1, BIN1, ABCA7, MS4A, CD33, EPHA1* and *CD2AP*. Variants of these genes are linked to significant differences in risk of Alzheimer's, but their effects are much smaller than for APOE (10-13).

Despite the progresses concerning genetic, behavioural, cardiovascular, and nutritional risk factors, long-term prevention studies have yet to be conducted to see if the incidence of AD can be reduced.

Parkinson disease

Parkinson disease (PD) is characterized by a loss of dopaminergic cells in the *substantia nigra pars compacta* and the presence of Lewy bodies (14). The recent new discoveries of a number of disease-causing genes (such as α-synuclein, parkin, *UCHL1, PINK1, DJ-1, LRRK2*) in PD have generated considerable interest for presymptomatic genetic testing of PD in the clinic. Early-onset autosomal-recessive PD forms were associated with mutation in *PINK1* and *DJ-1 (PARK7)* genes (15, 16). *LRRK2 (PARK8)* gene is implied in autosomal-dominant PD with similar phenotype to sporadic PD, but the brain changes generated by LRRK2 mutations are extremely variable. The most common mutation of *LRRK2* gene is G2019S substitution present in different populations worldwide. Other possible target genes are *PRSS25, UCHL1*, synphilin and *NURR1* (17). However, the promises and limitations of genetic testing in PD must be confirmed by detailed scientific studies, which will allow also guidelines and recommendations for such genetic testing.

Huntington disease

Huntington disease (HD) is a neurodegenerative, autosomal dominant disorder with late-onset, caused by the expansion of a CAG repeat in the first exon of the HD gene, located on chromosome 4p16.3. Usually symptoms begin in 30s or 40s decades by involuntary choreic movements, loss of voluntary control of movement, followed by cognitive impairment and personality disorder tending to depression, anger and temper outbursts (18).

The number of CAG repeats is correlated with clinic manifestation of the disease. Normal individuals have 6 – 26 CAGs (class 1) and the allele is stable. In the presence of 27–35 CAGs (class 2) phenotype is normal, but the allele is expandable during spermatogenesis. The individuals with 36–39 CAGs (class 3) have a phenotype with reduced penetrance. The presence of more than 40 CAGs (class 4) is associated with fully penetrated forms (19, 20).

The test for the HD gene is fast, rela-

tively inexpensive, and highly sensitive, with positive and negative predictive values approaching 99 percent. Genetic testing can be performed at any stage of development, even before the onset of symptoms. Many ethical aspects could be identified in the predictive testing of HD, like autonomy, beneficence, non-maleficence, inequality, paternalism or even medical errors. An example of ethical issue could be the case of monozygotic twins, when one brother would to apply the predictive test and other brother do not accept this. In this case is an ethical conflict between autonomy and coercion, but also is necessary to quantify which action is more beneficial: to know if mutation is present or to not know is the mutation is present (21).

Spinocerebellar ataxia diseases

Spinocerebellar ataxias (SCAs) constitute a large group of heterogeneous disorders characterized by a degeneration of the spinal cord and the cerebellum, that cause imbalance, poor coordination, and speech difficulties. Some types of ataxia are inherited, including dominantly inherited diseases and about 30 different gene mutations have been found. Among the more common inherited ataxias are *Friedreich's ataxia* and *Machado-Joseph disease.* Ataxia can also be acquired after stroke, multiple sclerosis, tumours, alcoholism, peripheral neuropathy, metabolic disorders, and vitamin deficiencies. One of the common genetic defects is an expansion of a CAG triplet repeat in genes that regulate the production of different proteins, but the number of CAG repeats varies among the different types of SCAs. Normal individuals with premutation have an increased risk of developing symptoms late in life and also to have abnormal children (22, 23). The correlation between the mutations and the disorders remains unclear, but one explanation may be that the encoded abnormal protein has a toxic effect on neurones, which are successively destroyed. Molecular genetic testing for CAG repeat length is a highly specific and sensitive diagnostic test (24). If the mutation is known in the family, prenatal diagnosis and pre-implantation diagnosis (PGD) are possible. Genetic testing is available for spinocerebellar ataxia types 1, 2, 3, 5, 6, 7, 8, 10, 12, 13, 14, 17, 28; dentate-rubro-pallidoluysian atrophy (DRPLA); ataxia-telangiectasia (A-T); Friedreich's ataxia (FRDA); Ataxia with oculomotor apraxia types 1 and 2; Marinesco-Sjogren Syndrome; Ataxia with Vitamin E deficiency; Fragile X associated tremor/ataxia syndrome (FXTAS); mitochondrial recessive ataxia syndrome (MIRAS); autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS); and Episodic ataxia types 1 and 2 (20, 22-26).

Particularities of genetic testing for a neurodegenerative disease?

In hereditary forms of ND genetic testing can be applied before or after the setup of symptoms. However, the test does not predict the age of setup and the evolution of disease. The major reason for undergoing testing is to find out for the sake of one´s children, or for family planning. However, the use of this test for the minor persons is denied, and the only exception is the presence of symptoms before the age of 18 (27).

Most clinical genetic units follow recommended guidelines or programmes, as the waiting process and dealing with the result are very stressful situations, both for the person tested and for the family. The guidelines indicate the need of several meetings with a multidisciplinary team

(that include a geneticist, a neurologist, a psychiatrist, an ethical counsellor) started by a preliminary meeting when the data from familial history is stored. After this meeting, follow a pretesting meeting (when are discussed the beneficial and the disadvantages of testing) and finally a meeting when are presented the results and are discussed the consequences (positive or negative) of genetic analyse. In the present, the diagnoses of ND are frequently made on clinical grounds, sometimes supported by radiological methods. The application of genetic testing for ND should be reserved to patients who have a family history of ND or those with very young age at onset (28-30).

Ethical aspects of genetic testing for adult-onset disorders and ethical aspects

Testing of young people for adult-onset disorders by presymptomatic and predictive genetic testing is the most controversial predictive testing because we have not any treatment that could prevent the onset and progression of the disease (30).

A big problem concerning presymptomatic genetic testing in high risk individuals for ND is related to answer to question - *Do people really want to know the truth?*. Other difficulties are related to collect DNA samples from relatives, who need neurological examination and genetic counselling, problems that rais complex psychological, ethical, legal and moral conundrums (31).

Presymptomatic genetic testing for neurodegenerative diseases generates some ethical conflicts. The authors identified four types of issues. First, in some ND exists juvenile form (for example HD) and this could complicate the decision to make or not the test. Second: in some diseases the preventive measures or the therapies are uncertain. Third: the best interest is interpretable; in some situation the best interest for child is not identical with best interest for family and in these situations the physicians must protect the rights of child in relation with family. And finally, the guidelines must be concordant with local legal or cultural context (32).

Most people have these tests in hopes of negative results, but inevitably, such hopes are often dashed by tests that come up positive. Many other subjects (in particular, those in families with a history of disease) choose to be tested so that, in the event of a positive test, they can make lifestyle changes or pursue clinical interventions that may alter the course of the disease to which they are predisposed. Unfortunately, a positive genetic testing for ND do not allow a preventive or curative treatment like in other diseases (i.e. breast cancer, colorectal cancer) and this could generate psychological trauma. Thus, a positive DNA test for HD or (early-onset) AD means that, if the person lives to middle or late middle age, the disease *will* manifest, and will be unstoppable once it does. This explains why in generally only about 15-25 percent of people at risk get tested (33).

For some ND, the pathogenic mechanism is linked to a variable amplification of DNA repeats sequences during vertical transmission and generates the phenomenon of anticipation. Also, the severity of the symptoms is correlated with the size of the amplification, but it is difficult to predict severity in a future generation based on mutation size in a parent (34).

Other problem that complicates the genetic testing in ND is the varied penetrance. For example, the penetrance of G2019S mutation associated with Parkin-

son's disease is highly age dependent, increasing from 17% at age 50 years to 85% at age 70 years (35).

So, in the absence of a possible clinical response, a test confirming or even suggesting future development of a fatal neurodegenerative condition can have a devastating psychological impact on the patient. These risks may include changes in the individual's perception of self, stresses in relationships with friends or family, discrimination in the workplace or community, difficulties obtaining or keeping insurance, unfavourable adjustment of disability benefits, and other concerns related to privacy and confidentiality. A positive result could be beneficial, because sometime is preferable to known that you have a certain type of mutation in opposition with the anxiety generated by the absence of a clear answer. Also, the result of genetic testing could profoundly influence patient decision making with respect to finances (i.e., providing for inevitable hospice care), family planning (i.e., marriage or reproduction), and fostering personal relationships. Finally, the weighing of risks and benefits of genetic tests is a decidedly personal endeavour (36).

Although, the guidelines for presymptomatic genetic testing in ND vary among countries, some key elements include the following steps: neurologic neuropsychologic and psychiatric evaluation, genetic counselling, sign informed consent document, psychological assessment, review of the potential impact of the test, disclosure of results in person and arrange post-result follow-up. For the testing will exclude patients with significant psychiatric disorders or those undergoing stressful life circumstances causing emotional upheaval. During the pre-test counselling should be provided the explanations concerning implications of positive and negative results. All DNA determinations must be carried out independently at least twice and the results will be analysed after a genetic linkage computer analysis of haplotypes (the data from relatives must be analyzed anonymously to prevent the transmission of inappropriate information). Usually, the process is long and need many counselling sessions as the patient needs a psychological support. If the patient agrees the idea to inform other members of family about its genetic status, the patient could be accompanied by a relative during a minimum one session and also for the disclosure session when the results will be communicated. The diagnostic information must be given in a face- to-face session, because patient needs to discuss what this information may mean, even if the outcome is genetically uninformative. Long-term follow-up is essential, particularly for those who test positive for the gene. The testing are applied only to persons aged 18 years or older, must be totally voluntary and the results remain totally confidential (9,30-36).

Conclusions

The neurodegenerative diseases represent a group of diseases with late onset, invariable evolution to neurological and cognitive degradation, have not yet any pathogenic therapy and have a genetic component more or less important. In some such conditions the predictive genetic testing before onset of symptoms is possible, but is not available for general population. Such testing may be useful where a family member has already been diagnosed to carry a known mutation.

The genetic testing must be preceded by a genetic counselling respectful of the reli-

gious and cultural perspectives and traditions of individuals. Genetic counselling is complicated by a multitude of aspects, such as the apparition of a de novo mutation in a negative history family or familial factors, such early parental deaths, adoption, and illegitimacy.

Although the medical advances will be inherent and hopefully beneficial to the persons suffering from ND, the psychological and social support by sensitive, timely, and accurate counselling will remain an essential aspect in presymptomatic genetic testing.

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THE NEED FOR HBV GENOTYPING: A COST-EFFICIENT APPROACH

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THE NEED FOR HBV GENOTYPING: A COST-EFFICIENT APPROACH (Abstract): The outcome of chronic HBV infection is variable; approximately one half of individuals transition to an inactive carrier state, 30% progress to cirrhosis, and the remainder to chronic hepatitis. Ten different HBV genotypes and many subtypes have been identified with distinct geographical distributions. Over the years, a lot of studies presented the efficiency of different genotyping methods; for this reason we aimed to present a cost efficient genotyping diagnosis algorithm of CHB infected patients, especially useful to identify those at risk of disease progression and determine optimal anti-viral therapy as useful instrument for physicians. **Keyword**s: HBV, CHRONIC HEPATITIS, GENOTYPE, ANTIVIRAL THERAPY.

More than one third of the world's population has been exposed to HBV, and approximately 350 million persons are chronically infected (1). Persons with chronic infection are at risk for the development of complications such as cirrhosis, hepatic failure, and hepatocellular carcinoma. In the US, 3050 cases of acute HBV infection were reported in 2013 (2); in the same year, 19 930 cases of HBV infection were reported in 28 EU/EEA Member State. 13 644 (68.5%) of these reported cases were chronic (ECDC); the true figure is estimated to be considerably higher (3).

Since 1984, universal newborn immunization and mass population screening and immunization programs have reduced the rate of chronic HBV infection and liver cancer in endemic regions (1). Persons at high risk of HBV infection should be tested for HBV (Management Guidelines) (4). Screening and diagnosis involve serology assays (detection of HBsAg and Anti-HBs, Anti-HBc (total antibodies and IgM type), HBeAg and Anti-HBe) and HBV DNA assays. These methods, together with the liver histology are used to establish the most suitable diagnostic algorithm.

In clinical practice, HBV DNA measurement is used to assess the state of HBV infection, stratify risk of disease progression to cirrhosis and hepatocellular carcinoma, aid in the decision to begin therapy, and monitor treatment efficacy and failure.

A serum HBV DNA level > 2000 IU/mL has been proposed as a cutoff level to differentiate patients with HBeAg-negative chronic hepatitis from those in an inactive carrier state (ie, hepatitis B e antigen – HBe Ag - negative, persistently normal alanine aminotransferase) (5).

10 different genotypes (and many subtypes) of HBV have been recognized and are designated by letters A-J HBV genotypes. These genotypes are recognized having different geographic distribution and they influences natural history, clinical outcomes, and response to therapy (6).

METHODOLOGY

This review has two objectives: 1. to present comparatively the HBV genotyping techniques in order to indicate the most sensitive and specific method and 2. to assess the clinical utility of HBV genotyping assays. We have searched the PubMed library using next key words: *hepatitis B virus, genotype, genotyping methods, antiviral therapy, chronic B hepatitis* and we have found 668 articles, from which 112 clinical trials. Our clinical question included several components: the Population, Intervention, Comparison, Outcome and Method (PICOM). The components of the clinical questions were: **P**atients – individual with chronic HBV hepatitis; **I**ntervention or exposure – HBV genotyping; **C**omparison – antiviral therapy efficiency according with the HBV genotype; **O**utcomes – decrease HBV DNA level, absence of HBsAg; **M**ethod of study design for this narrative review – controlled trials.

1. The HBV genotyping methods

The search of Pub Med data base using the key words *hepatitis B virus*, and *genotyping methods* revealed 301 articles, from which we selected 2 for analysing, the most representative of them (7, 8). The most common assays used for HBV genotyping were: Genomic sequencing and filogenetic analysis, INNO-LiPA, RFLP (*Restriction fragment length polymorphism*), Multiplex PCR (reacții PCR multiple), Serologic subtyping, *Oligonucleotide Microarray chips*, FT-RDB (*Flow-through reverse dot blot*), RFMP (*Restriction fragment mass polymorphism*), PCR *invader assay*, Real Time-PCR (table I). In terms of identifying mixed genotypes of the same sample, INNO-LiPA achieve excellent results (100%) in each study, contrast with genomic sequencing that can not detected the co-infection. However, this latter technique the "gold standard", is characterized by the ability of detection of mutations in the viral genome (100% results), for which INNO-LiPA results were obtained for 93.75% (6). Finally, the best consistent results (99.56%) was also obtained by these two techniques, INNO-LiPA and sequencing genome, which apparently are distinguished by their benefits and results. In terms of cost-benefit analysis, technique INNO-LiPA seems to be the most advantageous, this standing out for the price of low cost, high sensitivity, specificity 100%, use easy method, but the most important feature recommending this technique is the detection capability of mixed genotypes. The disadvantages of the technique can be translated as: cost price higher than PCR-based methods; low sensitivity to the presence of mutations in the sequences to be analyzed; it can reduce the effectiveness of the exogenous DNA contamination; inability to detect in a single reaction both the genotype and characteristic mutation, thus the time to result being increased two-times (7, 8).

Regarding subtypes of HBV genotypes, sequencing and phylogenetic analysis, proved their efficacy and accuracy, although sequencing not yet achieved the level of "gold standard" and from this point of view.

TABLE I

Comparison of HBV genotyping methods (7, 8)

Genotyping method	Se	Sp	Limit of detection	Advantage	Disadvantage
Genomic sequencing	100%	100%	4 x 10^2 UI/ml	Gold standard; detect the genotypes recombination	Expensive; can not be used at large scale
INNO-LiPA	100%	100%	1 x 10^3 UI/ml	Cheaper than sequencing; high specificity	Expensive in comparison with serological assays; time consuming
RFLP	87%	100%	2 x 10^3 UI/ml	Simple technique; cost efficient; can be used in large populational studies	The polimorfism of nucleotides for the prime affect sensitivity
Multiplex PCR	99%	100%	-	Simple technique, cost effcient; can be used in large populational studies	The polimorfism of nucleotides for the prime affect sensitivity
Serologic subtyping	-	-	1 x 10^2 UI/ml	No needed PCR, simple technique, cost efficient	The polimorfism of nucleotides for the prime affect sensitivity
Real Time-PCR	99%	100%	500 IU/ml	Low risk for contamination of samples; high sensitivity; can be analyzed a high number of samples.	Low capacity to distinguish between genotypes with closed temperatures of denaturation

*Se – sensibility, Sp - specificity

2. HBV genotype influence of viral therapy in CHB patients

Therapy for HBV infection currently includes interferon and nucleos(t)ide analogues (NA) that inhibit HBV polymerase. Five nucleoside / nucleotide analogues are currently approved for HBV treatment: lamivudine, telbivudine, adefovir, entecavir, and tenofovir. Targeting other steps in the viral life cycle (eg, encapsidation and viral entry), stimulating the innate immune response, and therapeutic vaccination, as well as RNA interference–based strategies are currently under investigation.

Numerous studies have reported differences in the natural history, clinical outcomes, and responses to therapy based on hepatitis B virus (HBV) genotype (9, 10). Data show that patients infected with genotype B achieve better clinical outcomes than patients infected with genotype C, including lower disease activity, a younger age at hepatitis B e antigen (HBeAg) seroconversion, a higher chance of sustained remission after HBeAg seroconversion, and a reduced risk of hepatocellular carcinoma (11). In addition, patients infected with HBV genotypes A and B have been shown

to have higher rates of HBeAg seroconversion when treated with peginterferon vs patients with HBV genotypes C and D (9). Genotype D has been correlated with the precore mutation leading to HBeAg-negative chronic hepatitis B (11).

TABLE II

The antiviral therapy in CHB patients according with HBV type (12 – 23)

Clinical trial author, year, no of cases	Given therapy	Genotype effect on antiviral therapy efficiency
Marcellin P, 2014, 266 CHB	tenofovir disoproxil fumarate (TDF)	The strongest independent predictors of HBsAg loss were Caucasian race with genotype A/D
Ito K, 2014, 212 CHB	nucleotide analogs (NAs)	The time to disappearance of HBsAg was significantly longer for genotype A patients than non-A genotypes
Sonneveld MJ, 2013, 803 CHB	peginterferon (PEG-IFN)	The response rates were low in patients with genotypes A or D and in comparison with genotypes B or C
Lampertico P, 2013, 128 CHB	peginterferon α-2a (PegIFN)	PegIFN treatment was well tolerated and the post-treatment virological response improved significantly (120 genotype D)
Sonneveld MJ, 2012, 221 CHB	PEG-IFN +/- lamivudine	On-treatment HBsAg decline significantly varied according to HBV genotype (A and B more than C and D).
Liaw YF, 2011, 544 CHB	peginterferon alfa-2a (PEG-IFNα-2a)	PEG-IFNα-2a treatment regimen was the most efficacious and beneficial for HBeAg-positive patients predominantly infected with hepatitis B virus genotypes B or C.
Gish RG, 2010, 354 CHB	entecavir or lamivudine	Caucasian patients, and those infected with HBV genotype A or D, were significantly more likely to lose HBsAg.
Lu HY, 2008, 71 CHB	lamivudine-IFN alpha-2b	HBV genotypes C and B had no obvious influence on intrahepatic HBV DNA load or cccDNA load.
Zou HB, 2007, 90 CHB	lamivudine and alpha-interferon	NO influence on the lamivudine treatment effects for the HBV genotype B and C CHB patients; alpha-interferon treatment for HBV genotype B CHB patients is more effective than that for the genotype C ones.
Lu HY, 2007, 71 CHB	lamivudine, IFN-alpha 2b	Intrahepatic HBV DNA level (before and after treatment) was not significantly affected by the patients' HBV genotype.
Luo K, 2007, 224 CHB	interferon-alpha (IFN-alpha)	NO significant differences in clinical characteristics and response to IFN-alpha between genotypes B and C were found
Yuen MF, 2006, 80 CHB	lamivudine	NO difference in treatment response between patients with genotype B and C.
Zhao H, 2007, 230 CHB	pegylated IFN-alpha -2b	HBV genotype B and patient age (< or =25 years) were 2 independent factors associated with sustained combined response.
Bonino F, 2007, 518 CHB	peginterferon alpha-2a +/-lamivudine / only lamivudine	In the peginterferon alpha-2a and lamivudine monotherapy arms, patients with genotypes B or C had a higher chance of response than genotype D infected patients, the latter responding better to the combination than to peginterferon alpha-2a monotherapy.
Flink HJ, 2006, 266 CHB	Peg-interferon alpha-2b.	Treatment with Peg-interferon alpha-2b is the best therapy to achieve HBsAg clearance in patients with genotype A.

From the 112 clinical trials only 15 full filed our search criteria. The given therapy (interferon / nucleotide analogues), the effect according with the HBV genotype can be observed in Table II. Although new therapies for hepatitis B introduced in the past 10 years have improved the efficacy of viral suppression and have lowered the risk of antiviral resistance, in certain patient populations, there remain complex issues surrounding hepatitis B prevention and treatment. For the second aim of this study we formulate the review question (PICOM) and we have included only clinical trials, like a proof of study quality. We need reviews in order to increase ***power*** to detect intervention effects, to prevent delay in the introduction of effective therapies, to better select and individualise therapy. The extracted data from these revealed that 5/15 clinical trials concluded that antiviral therapy efficiency was not influenced by the HBV genotype. The results of the remained 10 studies are not homogenous, but we still can appreciate that therapy with NA is more efficient for genotype A and D of HBV, and interferon therapy is having better outcome for B and C genotypes. Future studies will be necessary to support a final clear conclusion.

CONCLUSION

Although the WHO 2015 Report and US guidelines do not involve the HBV genotyping in evaluation of antiviral therapy of CBH patients (1, 24) we consider that according with consistent results of presented studies, the genotyping methods are useful in identify those patients at risk of disease progression and determine optimal anti-viral therapy, useful tool for practitioners. It is accepted that HBV genotype influences the prevalence of pre-core mutations, but the functional role of this mutation in liver disease is unclear. In the future, well designed studies will be necessary to evaluate the efficiency of interferon treatment *versus* NA therapy according with HBV genotype.

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ARTERIAL HYPERTENSION IN THE ELDERLY – THE HEART OF THE MATTER

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ARTERIAL HYPERTENSION IN THE ELDERLY – THE HEART OF THE MATTER (Abstract): Arterial hypertension is a public health issue that has not been completely solved from an epidemiological point of view in terms of its assessment and particular management. Poor data from trials allow only general recommendations limiting the decisions in clinical practice. Elderly hypertensives are a special group characterized by a particular blood pressure profile, most often an isolated systolic hypertension, and prone to orthostatic hypotension, pseudohypertension and blood pressure variability that impose strict rules and various methods of blood pressure measurement for making appropriate therapeutic decisions. The approach of the elderly hypertensive patient is also complex as it requires rigor and flexibility, well-balanced choice of therapy, careful, regular monitoring, polypharmacy avoidance, and a close relationship between the physician and the patient in order to increase compliance. **Keywords**: HYPERTENSION, ELDERLY, BLOOD PRESSURE MEASUREMENT, TARGET BLOOD PRESSURE.

Arterial hypertension (AH) in the elderly remains a topic of broad interest as disease prevalence is markedly increasing and it is the most important modifiable risk factor and predictor for cardiovascular morbidity and mortality. In this group of patients, AH management is closely related to the cardiovascular physiology and pathophysiology of aging. The most important issue is lack of objective data from trials. Frequently, age is an exclusion criterion and studies that include elderly have no specific results for this group. Clinical practice lacks a specific guideline and is currently based on recommendations from general guidelines for arterial hypertension. Actually, the only document addressing this problem is ACCF/AHA 2011 Expert Consensus Document on Hypertension in the Elderly (1).

ARTERIAL HYPERTENSION EPIDEMIOLOGY IN THE ELDERLY

AH prevalence has been increasing sharply in the elderly, a population that has undergone evident growth, especially in Europe and Japan. Data from a study that cumulates results from national registers of 6 European countries, US and Canada, shows that the AH prevalence in the elderly aged between 65-74 years, ranges between 53% for Canada and the US and 78% for Europe (2). SAGE study (Study on Global

Ageing and Adult Health) conducted by the WHO concludes the same trend in countries in Asia, Africa, South America and the Russian Federation (3). NHANES report indicates that rates of AH control in the elderly are low compared to the middle aged hypertensive adults, especially in patients older than 80 years (4). Framingham Heart Study data are consistent, as rates of controlled blood pressure (BP) are decreasing both in males and females with advancing age (38% for ages younger than 60 compared to 23 to 28% in patients aged over 80) (5). It seems that older women have a much higher prevalence of AH after 65 years, a situation worsened by poor rates of controlled blood pressure and unfavorable prognosis (6). Regarding the influence of race, NHANES data highlights the highest prevalence in black population compared with white population (40% versus 27%), highest stroke mortality, coronary artery disease and heart failure, as well as a lower rate of control (20.7% *vs.* 30%) (1). On the other hand, for the Asiatic elderly patients, it revealed a lower prevalence compared to the white race (16.1% *vs.* 28.5%), with a genetically influenced response to treatment among different subpopulations (1). Arterial hypertension epidemiology data in the elderly are non-homogeneous before and after 2000s due to disease definition. Changing the concept regarding the importance of systolic BP (SBP) for cardiovascular risk demonstrates the increase of isolated systolic hypertension (ISH) prevalence. BP behavior with age is not uniform as ISH appears as a separate clinic entity, but the BP profile can be also systolic-diastolic or isolated diastolic. Diastolic AH is a much more rare entity, occurring in less than 10% of hypertensives aged over 70 (1).

ARTERIAL HYPERTENSION AND CARDIOVASCULAR RISK

In the elderly, SBP irrespective of the increase in diastolic BP (DBP) maintains its role as a predictor of morbidity and mortality. Therefore, the risk of all-causes mortality increases by 1% for every 1 mmHg increase in BP (1). The risk is continuous for coronary heart disease, while SBP lowering doubles the absolute decrease in clinical events (7). The level of SBP is a better predictor of cardiovascular risk than DBP. For instance, MRFIF study shows that the incidence of cardiovascular events and mortality in grade 1 ISH is doubled compared to the optimal level of SBP (8), and the risk of stroke increases by 1.42 times in the Physician's Health Study trial. (9). Similarly, SBP Cardiovascular Health Study considers SBP as the best predictor of cardiovascular events and uncontrolled AH could explain 25% of coronary events (10). Cardiovascular risk in the elderly is influenced by almost constant association with other cardiovascular risk factors. Dyslipidemia is obviously more frequent and is a common therapeutic target with proven benefits, as ALLHAT-LLT trial demonstrates (1, 11). The prevalence of diabetes increases exponentially with age and remains an important predictor for progression of AH to heart failure at the age over 65 (12). Obesity and overweight increase the risk of AH especially in women (13), although the obesity paradox is discussed also in the elderly (INVEST study) (14). It discusses the role of hyperuricemia induced frequently by thiazide diuretics and complex causal relationship with osteoarticular diseases, accompanied by an excess of cardiovascular morbidity and mortality caused by AH (52% to 73%) (1).

PATHOPHYSIOLOGICAL FEATURES

In the elderly, the arterial system shows changes similar to the hemodynamic effect of AH at younger ages. In hypertensive patients, atherosclerosis being initially an adaptive process is accompanied by inflammation and endothelial dysfunction, the substrate of vulnerable plaques and acute atherothrombotic complications (15). Direct functional consequences are decreased flow-mediated vasodilation and baroreceptor sensitivity correlated to the level of SBP. The process of arteriosclerosis, age-specific and accelerated by AH, is responsible for arterial stiffness, characterized by thinning and fragmentation of elastin, collagen deposit growth, Ca^{2+} ions fixing (16). For small arteries, atherosclerosis is the consequence of vascular remodeling with increased pulse pressure and peripheral vascular resistance. Changes are especially evident in coronary arteries and blood vessels in the circle of Willis. Arterial stiffness is the tisular biomarker of early vascular aging syndrome (EVA) and reflects the influence of age-associated cardiovascular risk factors on the arterial wall (17).

Arterial stiffness is frequently measured by pulse wave velocity, with pathological values > 13 m/s in the elderly. Arterial stiffness causes the increase of central BP, changes of the cerebral and renal local hemodynamics, cardiac structural and functional impairment. Basically, there appears an early reflection of pulse wave from proto-diastole to tele-systole with aortic SBP amplification and direct effect on heart-vessel coupling (left ventricular hypertrophy - LVH). At the same time it causes a reduction of DBP, pulse pressure amplification, and therefore, damage of coronary and myocardial perfusion. In the kidney, decrease of distensibility of juxtaglomerular apparatus, reduction of the ability to produce renin at any level of Na^+ input, damage of the ATP-dependent Na^+-K^+ pump occur. Specific to the elderly is the inability of rapid Na^+ excretion after an increased intake, increased sensitivity to salt and growth of BP. Increased sensitivity to salt is caused by the generation of natriuretic substances, such as dopamine and prostaglandin E2, as well as insufficiency of an inhibitor of Na + pump called marinobufagenin (18,19). Alpha-ducin cytoskeletal protein modulates the sodium balance, the BP level, the elastic properties of the arteries, and explains the complicated evolution of hypertension by coronary heart disease or stroke (20). It has been shown that the diurnal variation in glomerular filtration is absent in the elderly, affecting the chrono-pharmacological profile of renal excreted drugs. The reduction of renal mass and a decrease in aldosterone production it is also associated with impaired potassium excretion (1, 21). Another feature in the elderly is the hyperactivity of catecholamines, reduced sensitivity of target-organs to noradrenaline, increased activity of alpha-receptors. It results in a poor vasodilation, to which also contributes low parasympathetic activity. These changes are less correlated with the BP per se, but rather with its variability. Overall, the elderly hypertensive patient has a particular hemodynamic profile, characterized by normal cardiac function at rest but with a tendency to decreased function during exercise, slight tendency towards bradycardia, predominant increase of SBP, hypervolemia and decreased activity of plasma rennin, accentuated variability of BP.

CLINICAL AND EVOLUTIVE FEATURES

Accurate office BP measurement is extremely important and is required by par-

ticular aspects as orthostatic hypotension (OH), pseudohypertension, BP variability (22, 23, 24). BP difference between arms is common (about 20% of cases) and SHEP study finds the increased frequency of OH (17.3% to 26%), having an impact on mortality, risk of stroke and dementia (25). Postprandial OH is another specific and frequent feature, caused by the inadequate response to vasodilator effect of gastrointestinal peptides and insulin, and reported in 70% of the elderly aged over 70 (26). Detection of pseudohypertension in the elderly with false resistant AH is also important. This phenomenon found in the elderly is defined as an artificial increase of BP due to stiffened arteries that cannot be adequately compressed during the cuff inflation. Electronic gauges or "finger" type devices are useful technical alternatives (Finapres) (1). AH and white coat-effect (15-25%), masked AH (21-45%), BP variability ("non-dipper" or "extreme-dipper" profiles) are also more frequent, and in this case ambulatory BP monitoring acquires a special importance (27). The prognostic value of the method is supported by multiple studies. Each increase by 10 mmHg of daytime and night-time SBP increases the risk of cardiovascular death by 10% and 18% respectively. Awakening and bed-time increase of SBP are related with long-term cardiovascular complications. A parameter with prognostic significance for mortality is also the ambulatory arterial stiffness index (28,29). Home BP measurement, although having a particular importance, cannot be used properly because of frequent physical and mental disabilities or associated arrhythmias, so measurement errors are common in the elderly (30). Overcoming these common problems in the elderly would be possible by using memory devices, telemonitoring or smart phone applications (1).

Recommendations for the diagnosis of secondary forms of AH are generally similar to other age groups, although the onset in the elderly is rare. The onset of disease in elderly patients, sudden worsening of previously well-controlled AH, resistant AH are the main clinical scenarios for a secondary form. Inadequate life style may increase SBP and induce pseudoresistance. Smoking has the most aggressive impact and frequent use of NSAIDs remains significant by determining 1.82-times higher risk of AH (1).

In terms of clinical evolution, geriatric AH often is characterized by unpredictable outcomes, 3 to 4 times higher frequency of myocardial infarction or ischemic stroke (1), simultaneous impairment of cognitive function up to vascular dementia (35% *vs.* 13% in non-dippers *vs.* dippers) (31). Meta-analysis of 31 randomized trials in adults and elderly does not show the superiority of one therapeutic class (32), but the early initiation of treatment clearly influences clinical outcomes (PROGRESS, SHEP, Syst-Eur cit. by 1). Heart failure is closely related to AH, LVH being a determinant mechanism. The studies reported up to 82% incidence in elderly over 65, with a high frequency of preserved systolic function form of heart failure (22.6%) mainly determined by myocardial ischemia and diabetes (Lakki E cit. by 1). The association of peripheral arterial disease accelerates the progression of the disease, so the attention should be focused on screening strategy (PREVALENT study) (33). SBP is an independent predictor for renal function decline in the elderly with ISH. In turn, renal dysfunction correlates independently with risk of heart failure and is more common in diabetic patients (1).

THERAPEUTIC APPROACH

The two recently published guidelines for the management of AH (23,24) provide the same recommendations on initiation of antihypertensive drug therapy in the elderly (tab. I).

TABLE I

Recommendations for initiating antihypertensive treatment in the elderly

(adapted after 23, 24)

2013 ESH/ESC Guidelines	2014 JNC8th
Initiation	***Initiation***
- BP ≥ 160 mmHg (I A) - BP 140-159 mmHg (< 80 years old) if therapy is well-tolerated (IIb C)	- SBP ≥ 150 mmHg or DBP ≥ 90 mmHg
BP target	***BP target***
- < 80 years old: SBP 140-150 mmHg (I A); SBP < 140 mmHg may be considered in some patients, but generally depending on individual tolerabiity (IIb C) - > 80 years old – SBP 140-150 mmHg (I B)	- < 150/90 mmHg (grade A) - if SBP < 140 mmHg is well-tolerated, the adjustment of therapy is not necessary (grade E – expert opinion)

Both guidelines underline lack of additional benefit of lowering SBP less than 140 mmHg for clinical outcomes, starting from the observation that in the trials this level of BP has not been reached (34). The same statement applies for "the very elderly" according to data of HYVET trial (35). Lacking the consistent data on the benefits/disadvantages of lowering SBP less than 140 mmHg, a prospective trial is currently ongoing. The estimated completion date is set for 2018, and the trial will enroll 10,000 patients aged over 55 followed-up 4 to 8 years. It aims to assess the impact on stroke, cardiac complications, progression of chronic kidney disease and age-related cognitive dysfunction. SPRINT Senior sub-study will enroll 1,750 participants over 75 years to assess whether benefits are similar (36).

In terms of clinical practice, it is important to lower BP slowly and gradually, with lower drug doses, carefully used to avoid tissue hypoperfusion, especially at the cerebral level. Intervention by non-pharmacological measures is mandatory as in any hypertensive patient. TONE trial offers reference information in this respect (37). It started in 1995 and it aims to assess the role of decreased sodium intake (daily less than 80 mEq), reduction of body weight (≥ 10 lb) or of both strategies in controlling BP level in treated hypertensive elderly patients (37). Weight loss of 3.5 kg in hypertensive patients aged 60-80 reduces BP on average by 4/1.1 mmHg. Salt restriction has an additive effect on lowering BP (average of 4.3/2 mmHg after 3 months) in combination with exercise and without medication. Two trials included in a large meta-analysis studying the role of potassium supplementation show that the BP reduction was consistent in the elderly (4.3 to 10 mmHg/1.7 to 6 mmHg) (1), although serum potassium levels should be closely monitored. The well-known DASH trial reveals the benefits of diet rich in fruits and vegetables, low in saturated fat at any age and presents the importance of sodium restriction. SBP decreased on average by 8.1 mmHg in hypertensive patients between 55-76 years compared with 4.3 mmHg in adults aged 23-41 (38).

General rules of pharmacotherapy in elderly patients are: start with small doses,

reduced by approximately 50% of the adult dose, with gradual and slow growth; doses are adjusted according to orthostatic BP, drugs that can cause OH are avoided as first-line choice (i.e. central sympatholytics); pseudoresistance should be considered but also the drugs that may enhance the antihypertensive effect (i.e. cimetidine, antifungals, grapefruit juice etc.); convenient therapeutic regimens are used, preferably with a single administration as to increase compliance, polypharmacy is avoided; attention is given to adverse effects as frequent comorbidities are associated. ESH/ESC guidelines states that all five classes of antihypertensive drugs may be recommended for initiation, but diuretics and calcium channel blockers (BC) remain the first option, while the JNC8th do not favor beta blockers. The initial selection of drugs in the elderly should especially take into account associated diseases (2/3 of hypertensive patients). An important aspect is the principle of combination therapy. In addition to the general benefits related to pharmacokinetics medication, the elderly is included by the cardiovascular risk scores systems into additional high and very high risk group. Therefore, the therapeutic scheme should include drugs with cardiovascular protective and antiatherosclerotic effects, such as ACE inhibitors and sartans drugs. There is no consistent data in new trials on the elderly patients. HYVET shows the favorable effect of the combination of ACE inhibitors-diuretics and ACCOMPLSH reports the superiority of the association of ACE inhibitors-BC (39). Fixed-dose combinations are a viable alternative both in uncomplicated and complicated AH for secondary prevention. A special mention should be made for octogenarians, in which effective treatment substantially reduces cardiovascular risk and mortality, similar to other age groups. In this case, it is preferred to start with one antihypertensive drug, and depending on response and individual tolerability, a second drug will be added. Low-dose thiazide diuretics may be used, BC and where appropriate, ACE inhibitors/sartans drugs are recommended with careful and regular monitoring. 2011 American Consensus recommended that SBP/DBPs levels are not lowered under 130/60 mmHg (1).

Compliance to treatment, a general problem in AH, becomes even more important in the elderly. Age is one of the major predictive factors of non-adherence, it is explainable because of an asymptomatic disease until cardiovascular complications appear, where polypharmacy, complicated therapeutic schemes, adverse effects, high-costs in socially disadvantaged patients with multiple comorbidities are common (40,41,42). A common reason of non-compliance is polypharmacy. Schall et al. show that the average number of drugs prescribed to American elderly patient is about 6, and antihypertensive drugs but also medication directly affecting BP is frequently included (40). A method of treatment improvement for clinicians has been the elaboration of an information system to correct prescriptions in the elderly (STOPP) and to develop individualized prescription (START) in order to fight polipharmacy (43). A second cause is deficiency in reaching blood pressure targets. Therefore, only 1 out of 3 patients continued treatment for hypertension after 1 year (44).

CONCLUSIONS

After consulting the dedicated literature, we can conclude that AH in the elderly is a complex issue for which evidence-based information is incomplete. We have at our disposal only recommendations provided by general guidelines for AH, based

primarily on observational data and the opinions of experts. AH prevention in the elderly is not standardized and optimal therapy remains a sensitive issue that requires clinical experience and individualization. In addition, the elderly should be correctly defined and consequently the therapy optimized.

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NEW PERSPECTIVES IN THE DIAGNOSIS AND THE TREATMENT OF CHILDHOOD FEBRILE EXANTHEMAS

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NEW PERSPECTIVES IN THE DIAGNOSIS AND TREATMENT OF CHILDHOOD FEBRILE EXANTHEMAS (Abstract): A child with a febrile exanthema is a complex medical problem involving diagnostic challenges, epidemiological threats and a great concern for the parents and any physician, should be prepared to deal with it. Many of the classical ones (measles, rubella, chickenpox) have now a decreased incidence due to a high vaccine coverage, which makes even harder for the physician to establish an early diagnosis. To the untrained eye most of them are difficult to differentiate. Their prompt recognition is necessary in order to manage them adequately and to prevent spreading of the disease.
Keywords: RASH, BLISTERS, ANTIBODIES.

The majority of the febrile exanthemas of the childhood have viral origin and have autolimitant spontaneous evolution. Even that, through its frequency, epidemiological implications and potential viral, bacterial or immunologic complications, these represents an important and delicate medical and epidemiologic problem. Due to high vaccination coverage, some of them are now rarely seen.

MEASLES

Measles, also known as morbilli, is an infection of the respiratory system, immune system and skin caused by a virus, specifically a paramyxovirus of the genus *Morbillivirus*. Symptoms usually develop 7–14 days (average 10–12) after exposure to an infected person and the initial symptoms usually include a high fever (often > 40 °C), Koplik's spots (spots in the mouth, these usually appear 1–2 days prior to the rash and last 3–5 days), malaise, loss of appetite, hacking cough (although this may be the last symptom to appear), runny nose and red eyes (1). After this a spot-like rash comes, covering much of the body. The course of measles, provided there are no complications, such as bacterial infections, usually lasts about 7–10 days. Measles is an airborne disease that is spread through respiration (contact with fluids from an infected person's nose and mouth, either directly or through aerosol transmission via coughing or sneezing), and is highly contagious-90% of people without immunity

sharing living space with an infected person will catch it. An asymptomatic incubation period occurs nine to twelve days from initial exposure. The period of infectivity has not been definitively established, some saying it lasts from two to four days prior, until two to five days following the onset of the rash (i.e., four to nine days infectivity in total), and whereas others say it lasts from two to four days prior until the complete disappearance of the rash. The rash usually appears between two and three days after the onset of illness.

The classic signs and symptoms of measles include four-day fevers and the three Cs-cough, coryza (head cold), and conjunctivitis along with fever and rashes (2). The fever may reach up to 40 °C. Koplik's spots seen inside the mouth are pathognomonic for measles, but are not often seen, even in confirmed cases of measles, because they are transient and may disappear within a day of arising. Their recognition, before the affected person reaches maximum infectivity, can be used to reduce spread of epidemics.

The characteristic measles rash is classically, described as a generalized, maculopapular, erythematous rash that begins several days after the fever starts. It starts on the back of the ears and, after a few hours, spreads to the head and neck before spreading to cover most of the body, often causing itching. The measles rash appears two to four days after the initial symptoms and lasts for up to eight days. The rash is said to "stain", changing color from red to dark brown, before disappearing. Complications with measles are relatively common, ranging from mild and less serious complications such as diarrhea to more serious ones such as pneumonia (either direct viral pneumonia or secondary bacterial pneumonia), otitis media, acute encephalitis and corneal ulceration (leading to corneal scarring). Complications are usually more severe in adults who catch the virus. In underdeveloped nations with high rates of malnutrition and poor healthcare, fatality rates have been as high as 28%. In immuno compromised persons the fatality rate is approximately 30%. Clinical diagnosis of measles requires a history of fever of at least three days, with at least one of the three C's (cough, coryza, conjunctivitis). Observation of Koplik's spots is also diagnostic of measles. Alternatively, laboratory diagnosis of measles can be done with confirmation of positive measles Ig M antibodies or isolation of measles virus RNA from respiratory specimens. In patients where phlebotomy is not possible, saliva can be collected for salivary measles-specific Attesting Positive contact with other patients known to have measles adds strong epidemiological evidence to the diagnosis. The contact with any infected person in any way, including semen through sex, saliva, or mucus, can cause infection. There is no specific treatment for measles. Most patients with uncomplicated measles will recover with rest and supportive treatment. It is, however, important to seek medical advice if the patient becomes more unwell, as they may be developing complications. Some patients will develop pneumonia as a sequel to the measles. Other complications include ear infections, bronchitis (either viral bronchitis or secondary bacterial bronchitis), and encephalitis. Acute measles encephalitis has a mortality rate of 15%. While there is no specific treatment for measles encephalitis, antibiotics are required for bacterial pneumonia, sinusitis, and bronchitis that can follow measles.

RUBELLA

Rubella, also known as *German measles or three-day measles*, is a disease caused by the rubella virus. This disease is often mild and attacks often pass unnoticed. The disease can last one to three days. Children recover more quickly. Infection of the mother by rubella virus during pregnancy can be serious; if the mother is infected within the first 20 weeks of pregnancy, the child may be born with congenital rubella syndrome (CRS), which entails a range of serious incurable illnesses. Miscarriage occurs in up to 20% of cases (3). The disease is caused by Rubella virus, a togavirus that is enveloped and has a single-stranded RNA genome. The virus is transmitted by the respiratory route and replicates in the nasopharynx and lymph nodes. The virus is found in the blood 5 to 7 days after infection and spreads throughout the body (4).

Rubella is a common childhood infection that is seldom fatal usually with minimal systemic upset although transient arthropathy may occur in adults. Serious complications such as deterioration of the skin are very rare. Apart from the effects of transplacental infection on the developing fetus, rubella is a relatively trivial infection. Rubella has symptoms that are similar to those of flu. However, the primary symptom of rubella virus infection is the appearance of a rash on the face which spreads to the trunk and limbs and usually fades after three days. The facial rash usually clears as it spreads to other parts of the body. Other symptoms include low grade fever, swollen glands (sub occipital and posterior cervical lymphadenopathy), joint pains, and headache and conjunctivitis lesions. During this incubation period, the patient is contagious typically for about one week before he/she develops a rash and for about one week thereafter. In children Rubella normally causes symptoms which last two days and include the following: rash beginning on the face which spreads to the rest of the body, low fever, posterior cervical lymphadenopathy (5). The swollen glands or lymph nodes can persist for up to a week and the fever rarely rises above 38°C the rash of German measles is typically pink or light red. The rash causes itching and often lasts for about three days. The rash disappears after a few days with no staining or peeling of the skin. When the rash clears up, the skin might shed in very small flakes where the rash covered it. Forchheimer's sign occurs in 20% of cases, and is characterized by small, red papules on the area of the soft palate. Other symptoms are swollen glands, coryza (cold like symptoms), and aching joints (especially in young women). Diagnosis is done through determination of Rubella virus specific IgM antibodies present in people recently infected by Rubella virus, but these antibodies can persist for over a year and a positive test result needs to be interpreted with caution. The presence of these antibodies along with, or a short time after, the characteristic rash confirms the diagnosis (5, 6). There is no specific treatment for Rubella.

CHICKENPOX

Chickenpox is an airborne disease which spreads easily through coughing or sneezing by ill individuals or through direct contact with secretions from the rash. Chickenpox is a highly contagious disease caused by a primary infection with varicella zoster virus (VZV). It usually starts with a vesicular skin rash mainly on

the body and head rather than on the limbs. The rash develops into itchy, raw pockmarks, which mostly heal without scarring (5, 7). On examination, the observer typically finds skin lesions at various stages of healing, and ulcers in the oral cavity and tonsil areas. The disease is most commonly observed in children. Children with chickenpox are infectious one to two days before the rash appears. They remain contagious until all lesions have crusted over (this takes approximately six days). Immunocompromised patients are contagious during the entire period as new lesions keep appearing. Crusted lesions are not contagious. Chickenpox is a highly contagious disease caused by primary infection with varicella zoster virus (VZV). It usually starts with a vesicular skin rash mainly on the body and head rather than on the limbs. The rash develops into itchy, raw pockmarks, which mostly heal without scarring. On examination, the observer typically finds skin lesions at various stages of healing, and ulcers in the oral cavity and tonsil areas. This is followed by the characteristic rash or oral sores, malaise, and a low-grade fever that signal the presence of the disease. Oral manifestations of the disease (enanthem) not uncommonly may precede the external rash (exanthema). In children the illness is not usually preceded by prodromal symptoms, and the first sign is the rash or the spots in the oral cavity. The rash begins as small red dots on the face, scalp, torso, upper arms and legs; progressing over 10–12 hours to small bumps, blisters and pustules; followed by umbilication and the formation of scabs (8).

At the blister stage, intense itching is usually present. Blisters may also occur on the palms, soles, and genital area. Commonly, visible evidence of the disease develops in the oral cavity and tonsil areas in the form of small ulcers which can be painful or itchy or both; this enanthem) can precede the exanthem by 1 to 3 days or can be concurrent. These symptoms of chickenpox appear 10 to 21 days after exposure to a contagious person. Because watery nasal discharge containing live virus usually precedes both exanthem (external rash) and enanthem (oral ulcers) by 1 to 2 days, the infected person actually becomes contagious one to two days prior to recognition of the disease. Contagiousness persists until all vesicular lesions have become dry crusts which usually entail four or five days, by which time nasal shedding of live virus also ceases. Chickenpox is rarely fatal, although it is generally more severe in adult men than in women or children. Non-immune pregnant women and those with a suppressed immune system are at highest risk of serious complications. The diagnosis of varicella is primarily clinical, with typical early "prodromal" symptoms, and then the characteristic rash and oral-cavity sores. Confirmation of the diagnosis can be sought through either examination of the fluid within the vesicles of the rash, or by testing blood for evidence of an acute immunologic response. Vesicular fluid can be examined with a Tzanck smear, or better by testing for direct fluorescent antibody. The fluid can also be "cultured", whereby attempts are made to grow the virus from a fluid sample.

SCARLET FEVER

Scarlet fever is an infectious disease of children. The disease itself is caused by secretion of pyrogenic exotoxins by the

infecting *Streptococcus*. Exotoxin A (*speA*) is probably the best studied of these toxins (9). Symptoms include sore throat, fever and a characteristic red rash. Scarlet fever is usually spread by inhalation. Scarlet fever is usually spread by the aerosol route (inhalation) but may also be spread by skin contact or by fomites. Asymptomatic carriage may occur in 15–20% of school-age children. The incubation period is 1–4 days. Scarlet fever is characterized by: fever, sore throat, bright red tongue with a "strawberry" appearance, Forchheimer spots (fleeting small, red spots on the soft palate). A characteristic rash, which is fine, red, and rough-textured, blanches upon pressure appears 12–72 hours after the fever starts generally begins on the chest and armpits and behind the ears. It may also appear in the groin on the face, often shows as red cheeks with a characteristic pale area around the mouth (circumoral pallor) is worse in the skin folds (so-called Pastia lines), where the rash runs together in the armpits and groin, appear and can persist after the rash is gone) may spread to cover the uvula begins to fade three to four days after onset and desquamation (peeling) begins. This phase begins with flakes peeling from the face. Peeling from the palms and around the fingers occurs about a week later. Peeling also occurs in the axilla, the groin, and the tips of fingers and toes. The rash is the most striking sign of scarlet fever. It usually appears first on the neck and face (often leaving a clear, unaffected area around the mouth). It looks like bad sunburn with tiny bumps, and it may itch. It then spreads to the chest and back and finally to the rest of the body. In the body creases, especially around the axillae (underarms) and elbows, the rash forms the classic red streaks known as Pastia lines. On very dark skin, the streaks may appear darker than the rest of the skin. Areas of rash usually turn white (or paler brown, with dark completed skin) when pressed on. By the sixth day of the infection, the rash usually fades, but the affected skin may begin to peel (5, 10).

Usually there are other symptoms that help to confirm a diagnosis of scarlet fever, including a reddened and sore throat, fever and swollen glands in the neck. Scarlet fever can also occur with a low fever. The tonsils and back of the throat may have a whitish coating, or appear red, swollen, and dotted with whitish or yellowish specks of pus. Early in the infection, the tongue may have a whitish or yellowish coating. Also, an infected person may have chills, body aches, nausea, vomiting, and loss of appetite.

When scarlet fever occurs because of a throat infection, the fever typically subsides within 3 to 5 days, and the sore throat passes soon afterward. The scarlet-fever rash usually fades on the sixth day after sore-throat symptoms started, and begins to peel (as described above). The infection itself is usually cured with a 10-day course of antibiotics, but it may take a few weeks for tonsils and swollen glands to return to normal. Scarlet fever can be diagnosed by clinical signs and symptoms. Complete blood count findings characteristic of Scarlet fever would show marked leukocytosis with neutrophil a and conserved or increased eosinophils, high erythrocyte sedimentation rate and C-reactive protein and elevation of antistreptolysin O titer. The treatment and course of scarlet fever are no

different from those of any strep throat-penicillin or macrolides.

Other diseases with febrile exanthema, but with a lower frequency, are epidemic megalerythema hand-foot-mouth disease, roseola infantum or the consequence of infection with different subtypes of enteroviruses or adenoviruses (11).

CONCLUSIONS

Any childhood febrile exanthema is a complex medical problem involving diagnostic challenges, epidemiological threats and a great concern for the parents and any physician, especially the infectious disease specialist, pediatrician or dermatologist should be prepared to face them.

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ASPECTS OF PHYSICIAN – PATIENT COMMUNICATION IN THE PROGRAM OF SMOKING CESSATION

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ASPECTS OF PHYSICIAN – PATIENT COMMUNICATION IN THE PROGRAM OF SMOKING CESSATION (Abstract): The doctor-smoker patient communication is essential for smokers to realize the harmful effects of tobacco on health and the benefits of smoking cessation. Nicotine found in cigarettes is a powerful drug and a direct dependency generator, which makes smoking cessation difficult, the withdrawal syndrome being hard to overcome for many smokers. The doctor - smoker patient communication is a complex process of data, information and knowledge transmission, subjected to some semiotic rules. In the Counseling Center for Smoking Cessation (CCSC) from the Rehabilitation Clinical Hospital of Iasi the medical and psychological counseling and the pharmacologic therapy for smoking cessation is ensured by the qualified personnel. CCSC was founded in 2005, when the hospital was included in European Program: „European Network Smoke-Free Hospital", and experienced an important development in 2007 with the initiation "Stop Smoking" National Program of Ministry of Health. The doctor - smoker patient communication in the CCSC was conducted during the smoker's recruitment, therapeutic and post therapeutic period, a special place being occupied by the doctor - medical staff communication, including smoker medical students. The number of people who became nonsmokers after being counseled at our center was the evidence of the effectiveness of this communication. The obtained results determined us to join the global fight against smoking and to propose the introduction of the smoking cessation program in the curriculum of the medical education institutions. **Keywords:** DOCTOR - PATIENT COMMUNICATION, SMOKING, CESSATION.

ISRA Center Marketing Research conducted a study in 2007 for the Pfizer Romania Pharmaceutical Company which aimed to evaluate the incidence of tobacco use and the assessment of smoking behavior in Romania. This study showed that among the 3326 people aged over 18, 1550 were smokers, the incidence being higher for males. Of

these, 43.9% said that they thought to quit smoking. Geographically, in Muntenia the percentage of smokers was 51%, 49.7% in Moldova, 40.1% in Bucharest and 42.4% in Banat. Being asked: "Is it possible to have health problems due to smoking?", the majority answered: "Yes!" (1).

Because the incidence and prevalence of smoking increases from year to year, a worrying phenomenon also in Romania, as shown in the above study, in the same year - 2007, the Ministry of Health initiated The National Program "Stop Smoking", which provides medical and psychological counseling and therapy for smoking cessation.

Smoking refers to the consumption of tobacco in form of cigarettes and it is a habit voluntarily acquired and which causes addiction. During the "Stop Smoking" program, there were established counseling and therapy centers for smoking cessation across the country, which were coordinated by pulmonologists with special training regarding the counseling of smokers, one of these being in Rehabilitation Clinical Hospital from Iasi (2).

Of the over 4,800 toxic substances existing in cigarette smoke, nicotine is the one that causes addiction, which is classified by The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) (3) and Fifth Edition (DSM-5) (4) as a disease. According to this authority, the criteria for this diagnosis include at least three of these phenomena during the last year to a smoker:

- Smoking more intense than normal;
- Strong desire to smoke, despite all efforts to reduce the number of cigarettes smoked per day;
- Tolerance to nicotine, manifested by the reduction of its effect and the need of increasing the number of cigarettes to achieve the same effect;
- Withdrawal symptoms after discontinuing smoking;
- Increase time spent smoking and acquiring cigarette, giving up the participation to different social or educational activities;
- Continue smoking despite the danger of ill health awareness.

The nicotinic withdrawal is also classified as a disease induced by nicotine consumption, according to DSM-IV-TR and DSM-5. It occurs in some people with major symptoms in the early days: very strong desire to smoke, difficulty concentrating, headache, nervousness, insomnia, irritability and even depression, increased appetite, reduced heart rate. These symptoms may disappear within a month.

Smoking is responsible worldwide for more than 85% of all deaths due to lung cancer, being the major cause of emphysema, bronchitis, Chronic Obstructive Pulmonary Disease (COPD) and cardiovascular diseases. Tobacco may also determine neoplasms located in the oral cavity, esophagus, pancreas, stomach, larynx, it can complicate surgery recovery, delay healing of wounds and fractures, decrease bone density, causing osteoporosis and it can affect the excretory and genital systems, skin and mucous membranes etc. (5, 6, 7, 8).

Counseling for smoking cessation has major and immediate health benefits, regardless the smoker’s age. This medical counseling requires meetings and discussions where the physician explains to the patient the harmful effects of smoking on health and its negative economic consequences. Some smokers want to quit smoking but can not do it alone. In the "Stop Smoking" National Program of the Minis-

try of Health, after the counseling session conducted by the specialist doctor, patients who wanted to be included in the program have returned and attended medical and psychological counseling provided by the physician and psychologist and pharmacological therapy.

Drug treatment was made with Bupropion and Varenicline for 2-3 months. This treatment helps the smoker to overcome the withdrawal syndrome by the action of drugs on the central nervous system, inhibiting the wish to smoke. After the initial consultation, follows a period of 2 weeks of treatment then the patient went to the consultation to assess efficacy, followed by another period of treatment, until 2 or 3 months, depending on the drug used. After completing this treatment, the patient is monitored for another 6 months. The patient-medical specialist meetings involve direct communication.

The medical specialist- patient communication

In the entire world, the interpersonal communication is located to the top of communication hierarchy. It is a complex process of transmission of information, data and knowledge from one source to another, connecting individuals, communities, groups, companies, ensuring cooperation and collaboration between them.

The doctor-patient communication represents a social interaction which implies the existence of the two partners, one being the emitter (source message), and the other being the receiver (target message). Both partners have a common set of signs and they follow a common set of semiotic rules.

These semiotic rules are classified into three categories:

1. Syntactic - defines the formal properties of signs and symbols;

2. Pragmatic - concerns the relations between signs, expressions and their uses;

3. Semantic - reflects interactions between symbols and signs and their meanings (9).

Interpersonal communication is examined and analyzed according to several possible classifications. After the channel used, communication can be: auditory, olfactory, visual, tactile. Another classification is: verbal (words are vehicles for information) and non-verbal (physical methods are vehicles for information: signs and body language, paralanguage, eye contact, touching etc.).

Communication can be written (letters, recommendations, reports, statements etc.) or oral (discussion, exposure).

After the means used in the transmission of information, communication can be realised through: auditory media (radio, teleconferences), print media (newspapers), audiovisual media (television), and electronic media (e-mail, audio and video files).

Considering communication conditions, it can be: face to face (when all the four senses are involved) or remote communication (involving hearing, sight, or both) (10).

General aspects of the physician-patient relationship

The chronic respiratory pathology that dominates the daily hospital activity has brought us face to face with the consequences of smoking, due to the patients suffering from COPD, chronic bronchitis and pulmonary emphysema. The communication with these smoking patients is present in the daily activity, but even more within the anti-smoking program at the Rehabilitation Clinical Hospital from Iasi.

The communicational component is a process that needs abilities in the interpersonal relationships: listening, asking, speaking, analysis and evaluation. These partially native skills need to be shaped by a specific education and also on the basis of the personal experiences, as the ability of advising is acquired in time.

"Stop Smoking" the National Program cannot take place without the addressability of people, which has risen up from zero to a high number of smokers who have asked for help in giving up smoking. The first measure of the plan was to inform people in Iasi about the existence of this program, to present them the facilities of it and also to tell them about this Counseling Center for Smoking Cessation within the Rehabilitation Clinical Hospital. This Center was founded in 2005, when the hospital was included in European Program: „European Network Smoke-Free Hospital", and experienced an important development in 2007 with the initiation "Stop Smoking" National Program of Ministry of Health. We used therefore all the available means of communication, ranging from inpatients, hospital staff and students, to written press, radio and television. The second main element was the message itself which was delivered to people in order to encourage them to contact the counseling center. Giving up smoking is possible, encouraging smokers and their entourage to take action, supplying all the details on how to apply for the services of the counseling center. In all of this, a balance was made between the positive and negative features of the message.

We tried to change the vision of smokers and non-smokers, convincing smokers to be more responsible towards their own health and of the people around them and the nonsmokers to convince the smokers to give up this habit. The main point of the message was that immediate action is urged because it is possible to give up smoking, being possible for the health status to be recovered. All the details were previously debated with the media representatives, all the aspects concerning the communication being paid attention, even to the apparently minor ones. When the message was delivered audio-visually, the aspects regarding the posture, voice, audio-visual amplification were carefully analyzed. During the radio and television broadcasts, the persons showing interest could interact with us live and that were advised to address to our center. Each time possible, the interaction with the audience was ensured, avoiding as much as possible the speeches. The message was insistently repeated using as much as possible present tense and imperative verbs.

The written press was used to underline the consequences on morbidity and mortality of the diseases caused by the active and passive smoking. These consequences equally affect both the men and women and depend on the number of cigarettes smoked per day, age and moment of start. The message was spread presenting advantages of giving up smoking on short and long periods, urging immediate action in giving up. Messages that underlined the negative aspects of smoking and the positive ones of giving up, confessions of the ex-smokers were used for the inpatients and the smokers in the hospital's staff, emphasizing the possibility of direct addressing to the center. The inpatients with chronic addiction had two different attitudes after discussing with the physician. Some of them requested help in giving up smoking, some others had no reaction. For the smoking in patients who did not request out help, we initiated

the minimal advice - three minutes intervention, which is the most efficacious prevention action. The chances to succeed are bigger if the patient attends the counseling center compared to trying to give up by them. We avoided mentioning percents and we used assertions such as: 'A smoker dies each 5 minutes!' or 'Give up smoking gives years to the life and life to the years'. If the medical staff would give the *three minutes intervention minimal advice* to each smoker, 2200 years of life every 1 million people would be saved each year by preventing the occurrence of diseases caused by smoking (11). Psychologically, the nonsmoking staff is more credible than the smoking one. Mass-media gave us the chance to implement educational programs which advised the nonsmokers not to take up smoking and the smokers to give up by applying to the anti-smoking counseling center within the Rehabilitation Clinical Hospital from Iasi. It is important to mention that the smokers usually tend to ignore the danger in which they put people around them, who are passive smokers- having the same risks. The process of counseling is possible, useful and needs to take place gradually.

The physician-smoker patient communication during the therapeutic period

The therapeutic period starts at the moment when the patient comes to the counseling center for giving up smoking, admitting that he wants to give up, this representing the first step in the therapeutic success. At this moment, the communication between the physician and the patient has a special meaning, within the process of giving up smoking. The first step when communication is initiated is to explain to the patient the path that needs to be followed, were the nicotine withdrawal syndrome is very important. This syndrome is defined by the totality of symptoms which occur when cutting off the intake of nicotine after a prolonged period of use. It is given a special attention to it in order to realize that giving up is difficult and in order to recognize it and in this way to avoid panic. Therefore, the appointments in the first period are frequent, asking the smokers to contact us each time the fear occurs. At this moment, the communication with the smokers and their relatives takes place in all the four situations earlier mentioned:

- one emitter and one receptor: face to face communication with the patient during the session;
- one emitter and more receptors: the communication with the smoker and his relatives or a group of smokers, during the first session;
- more emitters and one receptor: medical counseling followed by the psychologically one, both given by a specialized person;
- more emitters and more receptors: the public presentations with supportive and educational role (8, 10, 11).

The style of communication is influenced by the personal sensitivity and the persuasion skills of the person who gives the message, each physician having a particular style of interaction with patients, shaped up by instruction, exercise and experience. The persuasion capacity of the physician is revealed by his ability to ask questions and make affirmations in the moment of interaction with the patient, while the personal sensitivity represents the degree of the control over expressing his emotions. Four personal communication styles are known to influence the quality of

physician-patient relationship: analytical, respectful, expressive, and directive. The difficult relationships may occur between the persons who belong to the analytical-expressive and respectful-directive styles. The verbal and nonverbal communication between the physician and the smoker has different percents, depending on the emitter and the receptor, the verbal component - 7-10%, the vocal one (clarity, inflexion, speaking speed and firmness) - 35-38% and the visual one (mimics and acts, visual contact) - 55%, being the most representative (10, 11).

Communication between transmitter and receiver can be disrupted by psychological, social, structural or environmental barriers that are technically called "noise". This can create a situation in which the transmitter and receiver do not see the same meaning of the message. To check the reception of the message by the patient, the medical provider uses different opened questions that let the liberty of several response options (example: What you did not understand?).

In the process of communication with the smokers I assured a persuasive communication, so that the smoker listens to the message, accepts it and implements it. Once the smoker knows how dangerous smoking is and tries to abandon tobacco, usually problems occur due to psychological dependence because smoking cessation causes numerous demotivating issues such as weight gain, withdrawal symptoms, sudden desire to smoke, depression, insomnia.

Therefore addiction requires medical treatment that consists of two essential components: the behavior intervention (education, information, advice) and the pharmacological support (treatment of the withdrawal syndrome), medical intervention increasing the success rate.

Stages of smoking cessation were formalized by British researchers in the 5 A:

- ASK (ask the subject if he is a smoker to force him to admit their status)
- ADVISE (advising to quit smoking)
- ASSESS (evaluation of the desire to give up smoking)
- ASSIST (help him to make an attempt to quit smoking)
- ARRANGE (maintaining constant contact for support)

For the smokers with compliance issues we can apply to the 5 R in persuasive form:

- RELEVANCE (personalized information)
- RISKS (both for the smokers and their entourage)
- REWARDS (smoking cessation benefits)
- ROADBLOCKS (obstacles that may arise)
- REPETITION (repeating information)

Non-pharmacological therapy is based on social support during and after treatment. Educational practice includes: educational models (social interaction, the processing of information, personal preferences, and behavioral system), educational strategies (direct, indirect and interactive training, personal documentation), educational methods and educational materials (texts, pictures, posters, multimedia interactions) (10, 11).

All problems encountered were analyzed and continuously improved over these 7 years of activity in this field.

Specifics of doctor-patient communication in the post-therapeutic period

In post-treatment period we should keep in touch and to ensure free addressability when the patient feels the need to discuss

with us. Doctor-patient relationship is a key component of a good clinical practice in any situation, including the anti-tobacco treatment throughout its duration, but also in post-treatment period in order to consolidate the success and avoid relapse. The pre- and post-therapy counseling depends entirely on the doctor-patient relationship, the patient's level of participation and the patient's satisfaction.

Practical experience has put us in front of all situations regarding possible types of patient-physician relationship. There are 3 well-known views of the physician-patient relationship: as a consensual event – Parson model (1951) (12), Szasz and Hollender model (1956) (13), Stewart and Roter model (1989) (14), as a conflict event - model Freidson (1971), or as a process of negotiation.

Because the patient goes to the counseling center on his own initiative or on the entourage's initiative, we try to take control using the types of relationships based on the degree of control identified by Stewart and Roter (1989):

• Paternalistic (doctor-centered relationship; use of closed questions; model focused on defining illness and diagnosis).

• Consumerist (patient-centered relationship; he knows exactly what he wants).

• Absent (in this case focusing on patient fails; the doctor abandons the control of the patient, he is not willing to accept the doctor; the final result is a stalemate).

• Reciprocity (using open questions, encouraging the patient to talk about his suffering) (14).

These theoretical models concern the physician-patient relationship in terms of consensus, being static models, but in practice the relationship has a dynamic character due to problems occurring during the treatment and post-treatment. The situation is maintained even after exiting the program as any event can cause a relapse and long-term failure, if abandoning tobacco was not consolidated as a habit.

The dynamic nature of the doctor-patient relationship considered in the conflict and negotiation models, and the many reasons that can damage the communication, put us in the position to decide whether or not we continue the treatment, so that we can take a decision regarding the patient, approaching problems directly and identifying under what circumstances the partnership can continue.

Particularities regarding the communication doctor-medical staff, including medical students

Practical activity in the anti-smoking counseling center has made us confront with different issues regarding the medical staff of the hospital, various medical units and students from "Grigore T. Popa" University of Medicine and Pharmacy, Iasi (15).

The habit of smoking on healthcare professionals has an influence on their professional credibility and effectiveness in tobacco control activities. Studies shown that non-smoking male doctors advise patients more frequently to quit smoking, than the male doctors that smoke. The negative attitude of the latter affect the success of their therapeutic practices. For women doctors, differences are less significant between smokers and nonsmokers. Smoker healthcare professionals, as well as medical students, are a special target group because they can provide an assessment of the effectiveness of anti-smoking counseling program (16).

Based on the initiatives taken by the National Institute of Cancer of including

the anti-smoking education programs in the American medical education institutions (17), we considered healthcare professionals a very important target group and we proposed, along with other colleagues involved in"Stop Smoking" National Program, to also include in the Romanian medical education institutions, training classes about the harmful effects of smoking and smoking cessation treatment modalities.

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NEUROENDOCRINE TUMORS: CHOOSING APPROPRIATE IMAGING METHODS

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NEUROENDOCRINE TUMORS: CHOOSING APPROPRIATE IMAGING METHODS (Abstract): With an overall increased incidence in general population neuroendocrine tumors (NETs) are often late diagnosed. Symptoms are nonspecific and almost 50% of all patients have regional or distant metastases at the time of diagnosis. This article provides an overview of the current state of the imaging modalities used for primary tumor visualization, staging and follow-up. Detection of NETs and patient monitoring relies mainly on anatomical imaging such as computed tomography (CT), magnetic resonance imaging (MRI), and ultrasonography (US) under certain conditions. Molecular imaging techniques in combination with CT or MRI (hybrid imaging) greatly benefit patient management, including better localization of occult tumors and better staging. Octreoscan or somatostatin receptors (SSTRs) scintigraphic imaging in combination with CT represents the standard diagnostic of NETs in most countries. However, it is rapidly surpassed by SSTRs PET/CT with 68Ga-labelled somatostatin analogues with a superior spatial resolution and faster imaging (one-stop shop principle). Other more specific tracers are 18F-L-DOPA, 11C-L-DOPA and 11C-5-hydroxytryptophan, which have demonstrated excellent results in previously published studies. Diagnosis of patients with NETs is a complex process and, it is unlikely that any single diagnostic modality to be effective. Thus, NET diagnosis is a process utilizing a variety of methods including blood, urine and tumor tissue samples in combination with anatomical or hybrid imaging for localization, delineation and staging of the disease. Diagnostic approach to patients with NETs should focus on including hybrid imaging methods, which might play an important role in the future. **Keywords:** NEUROENDOCRINE TUMORS, ANATOMIC IMAGING, FUNCTIONAL IMAGING.

Neuroendocrine tumors (NETs) include a variety of malignancies that can arise from neuroendocrine cells throughout the body. They can store and secrete different peptides and neuroamines that cause characteristic hormonal syndromes, often late diagnosed (1, 2). Nonspecific symptoms are characteristic to the disease, and almost 50% of all patients have regional or distant metastases at the time of diagnosis (3). NETs can arise in many different areas of the body and are most often localized in the gastrointestinal tract (70%) and the bronchopulmonary system (25%), with substantial variations in both clinical presentation and tumor biology. NETs are mainly spo-

radic but may occur in a familial context of autosomal dominant inherited syndromes such as MEN1, MEN2, Von Hippel-Lindau disease, neurofibromatosis type 1 and multiple tuberous sclerosis. The annual incidence of clinically significant neuroendocrine tumors is approximately 2.5-5 per 100,000 (4, 5).

Anatomic imaging. *Ultrasonography* (US) is frequently used in the evaluation of gastroenteropancreatic neuroendocrine tumors (GEP-NETs), with a low success rate, between 13–27%. It is valuable for the localization of pancreatic and liver metastases and particularly in the preoperative assessment of patients (fig. 1).

Endoscopic US (EUS) could be an excellent imaging modality to detect pancreatic NETs, with pooled sensitivity and specificity of 87 and 98%, respectively. US performed intraoperatively combined with intraoperative palpation could raise the sensitivity up to 91% (6).

Computed tomography (CT) is the most commonly used technique for primary tumor localization, staging, therapeutic monitoring, and follow-up of patients with NETs. Triple-phase intravenous contrast-enhanced CT (early arterial, portal-venous, and equilibrium) is usually performed (fig. 2). In primary NETs, the average sensitivity of a CT scan is 73%. A better sensitivity of 80% in detecting NET metastases is reached for liver metastases and 75% sensitivity for extrahepatic metastases (7).

Magnetic resonance imaging (MRI) findings provide a broad spectrum of information for both primary and metastatic tumors. On non-contrast MRI they can be hypointense or isointense on T1-weighted images. Metastases to the liver are typically hyperintense on T2-weighted images. A primary tumor is best identified on dynamic gadolinium contrast images (fig. 3).MRI may be superior to CT regarding liver lesion characterization but may not be as useful in the identification and follow-up of extrahepatic disease (13). A study comparing MRI, CT and standard somatostatin receptor scintigraphy (SSR) reported 95.2% sensitivity for MRI, 78.5% sensitivity for CT and 49.3% sensitivity for the OctreoScan in detecting hepatic metastases (8, 9).

Functional imaging. These techniques depict the somatostatin receptor status, metabolic activity, or specific amine or peptide regulatory profile. The human somatostatin receptor family consists of 5 subtypes each differentially distributed throughout the body (SST1-SST5).

The radiolabeled somatostatin analogue (SSA) 111 In-pentetreotide is the most commonly used agent in somatostatin receptor scintigraphy. The tracer is commercially available as 111In-DTPA-D-Phe1-octreotide (Octreoscan, Mallinckrodt Medical, St. Louis, MO, USA) and used for scintigraphic localization of primary and metastatic neuroendocrine tumors that express somatostatin receptors subtype 2 and 5 (fig. 4) (10).

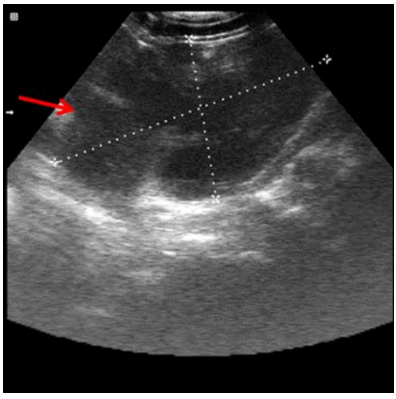

Fig. 1. Nonfunctional pancreatic neuroendocrine tumor

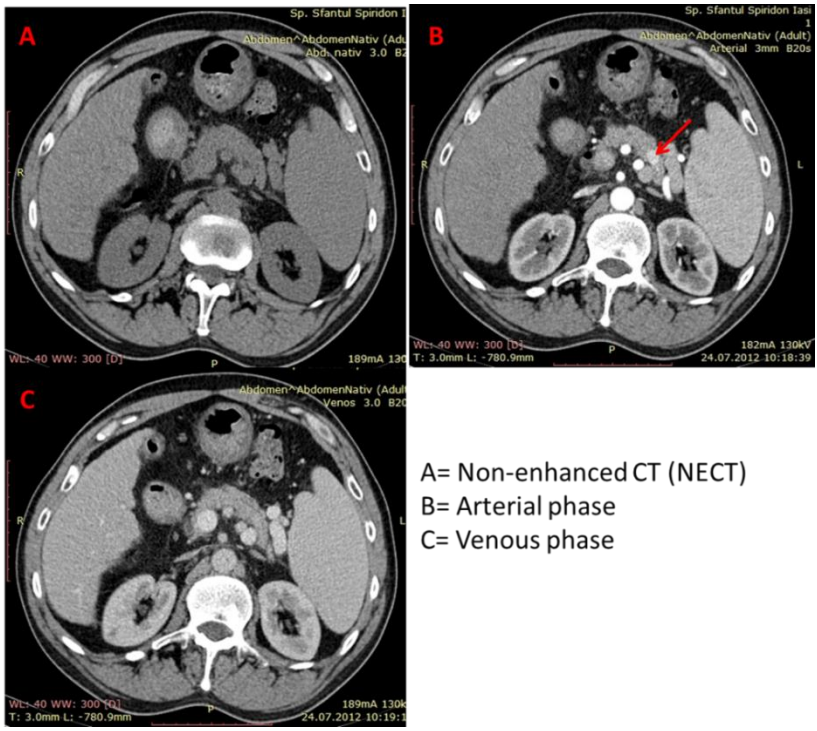

Fig. 2. Computed tomography of insulinoma of the pancreas. Typically, insulinomas are hyper vascular (arrow) and demonstrate more enhancement than normal pancreatic parenchyma during the arterial phase.

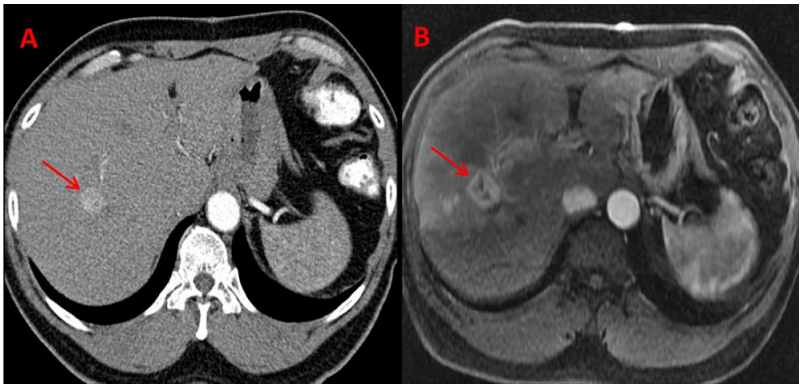

Fig. 3. Liver metastases from a neuroendocrine tumor (arrows) CT (A) versus MRI (B). The hyper vascular liver metastases are more clearly observed on the T1w gadolinium enhanced MRI (B) compared to the arterial phase CT (A).

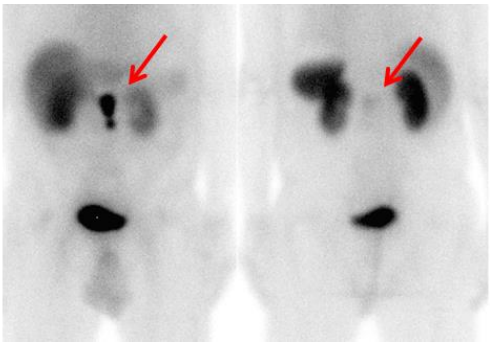

Fig. 4. Octreotide (111 In-pentetreotide) anterior and posterior images obtained at 24 hours. Increased activity is depicted in the pancreatic region corresponding to a neuroendocrine carcinoma. No distant metastases were visualized.

Alternatively, iodine metaiodobenzylguanidine (^{131}I /^{123}I MIBG) scintigraphy is an imaging test that confirms the presence of and monitors primary or metastatic pheochromocytoma (PCC) and paragangliomas (PGLs), and certain other NETs (neuroblastoma) (fig. 5).

False-negative results could occur with extra-adrenal tumors. Several drugs, such as opioids, tricyclic antidepressants, and antihypertensive drugs like labetalol can also affect MIBG uptake, leading to less intense or false-negative scans. MIBG scintigraphy performs poorly for head and neck PGLs and may also miss metastatic disease. ^{131}I MIBG imaging is performed after 24 hours, 48 hours and, if necessary, at 72 hours; only planar views are obtained. ^{123}I MIBG scintigraphy is performed after 24 hours and, if necessary, at 48 hours; single-photon emission computed tomography (SPECT) can be performed (11).

Hybrid imaging. *F-18 FDG PET/CT.* Positron emission tomography (PET) together with CT, using ^{18}F-fluorodeoxyglucose (FDG PET/CT) has limited usefulness in NETs due to their low metabolic activity. 18F-FDG PET/CT may instead be considered for imaging of high-grade NETs (poorly-differentiated), where the relationship between standardized uptake value (SUV) and overall survival can provide predictive information regarding overall survival and progression (fig. 6) (12).

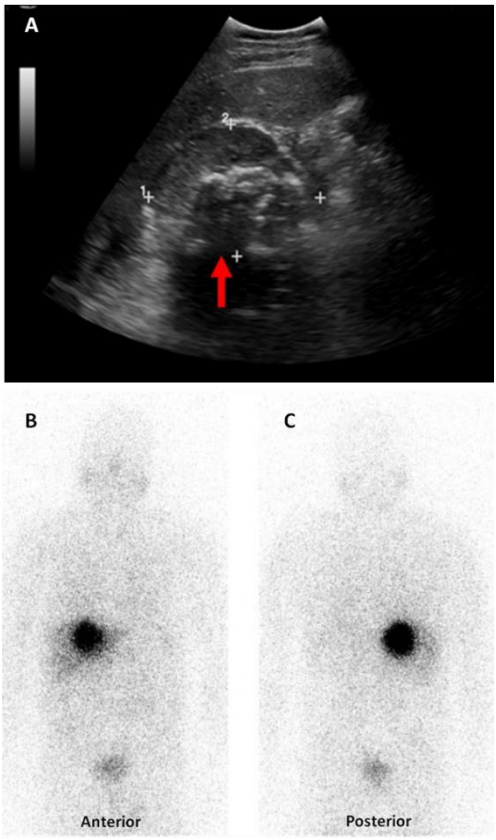

Fig. 5. Pheochromocytoma: US reveals a large echogenic lesion with calcifications on the right adrenal gland (arrow) (A). 131 I-MIBG scan shows a dark sphere (B, C).

18F-DOPA PET/CT. 18F-DOPA (L-3, 4-dihydroxy-6-18F-fluorophenylalanine), fluorodopa (FDOPA) is a tracer which depicts catecholamine metabolism and

exhibits an intense concentration in NETs, independent of somatostatin receptor. The main indication for FDOPA may be for the patients with insulinoma or paraganglioma. The reported sensitivity and specificity rates of [18F] DOPA PET or PET/CT for the diagnosis of thoracic and gastroenteropancreatic NETs were 77% and 95%, respectively (13). In patients with pheochromocytoma or paraganglioma the rates were 92% (sensitivity) and 92% (specificity), respectively.

PET/CT Imaging with Somatostatin Analogs. The introduction of somatostatin receptor PET/CT, somatostatin analogs (SSA) labeled with gallium-68 (Ga-68-DOTA-peptides) has allowed an accurate imaging of SSTRs by PET. DOTA peptide PET/CT has a significant role in the staging of well-differentiated gastroenteropancreatic tumors. It is recommended for clinical use in the initial diagnosis and for the evaluation of tumor response. The overall sensitivity of 68Ga-SSA-PET/CT for NETs is >90%, while the specificity ranges from 92 to 98%.The clinical impact of differences in SSTR binding affinity has not been proven, and therefore no preferential use of one compound over the others can be advised (14).

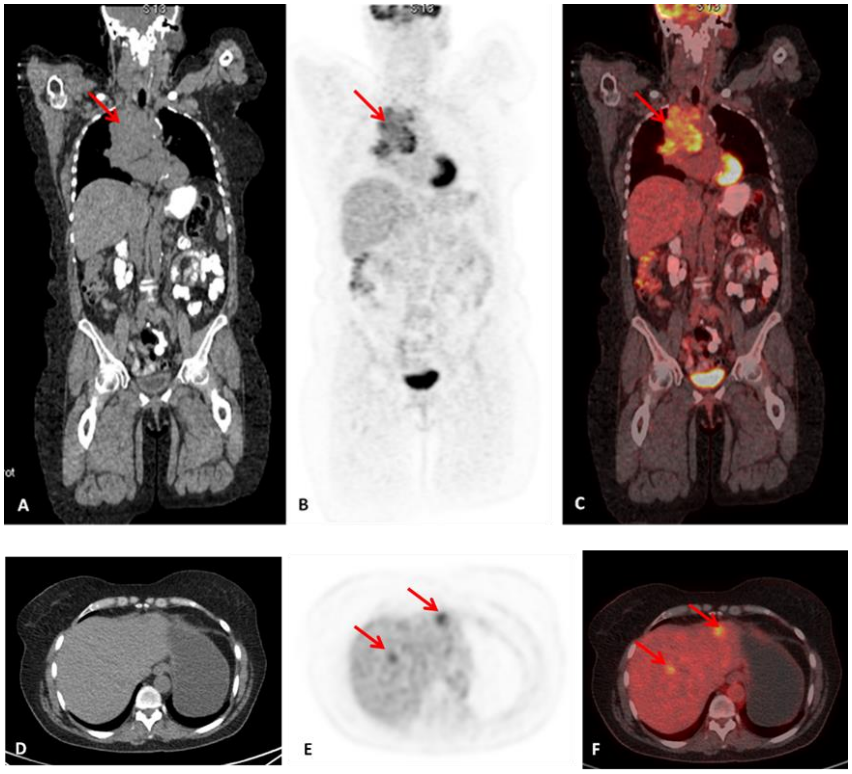

Fig. 6. Upper row: Patient with bronchopulmonary carcinoid, Ki-67 30-40%. Positive FDG PET uptake at the tumor level (B, arrow).
Bottom row: Liver metastases from a neuroendocrine tumor of midgut origin, Ki 67 10%. Faint FDG uptake (maximum SUV, 2.5 and 2.9 g/ml, E, arrows).

Future techniques. Other investigational tracers include ^{18}F-labeled amine, ^{18}F-fluorothymidine (FLT) and ^{11}C-labeled precursors, such as levodopa and serotonin, but the clinical experience is still limited (27).

There is great interest in the emergence of the so-called pan-receptor somatostatin analogs. Pasireotide (SOM230) is a novel multi receptor-targeted somatostatin analog with high binding affinity for SST receptor subtypes 1, 2, 3, and 5.

CONCLUSIONS

Our overview suggests that a single diagnostic test will not be effective to assess the NETs. The diagnosis will use a variety of methods including blood and urine tests and tumor biopsy in combination with anatomical and molecular imaging for localization, delineation, and staging of the disease, as the basis for optimal selection of therapy.

Future advances in NET diagnosis need to consider two realities. Firstly, the proper approach focused on validation of the present techniques. Secondly, a long-term strategy to develop a multilevel integration scheme of biologic and genomic diagnosis to provide added value to the imaging techniques. Thus, a fusion product of molecular and genomic information with tumor imaging is likely to be the essence of future NET diagnosis.

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NEWS

CARDIOTOXICITY OF LEVOFLOXACIN AND CIPROFLOXACIN

Fluoroquinolones are a class of antibiotics widely used in the treatment of common bacterial infections and are unusual because they are synthesized, not isolated from living organisms. One potentially serious adverse effect is prolongation of QT interval that may lead to *torsades de pointes* or ventricular arrhythmias. A group of researchers did a study on animals using Wistar albino rats .The study was divided into two experiments to determin the effects of levofloxacin and ciprofloxacin on rats with acute myocardial dysfunction (AMI) induced by isoproterenol (sixty rats into five groups of twelve rats each) and rats without AMI (twenty five rats into five groups of five rats each). They analised the changes in ECG pattern, the serum level of cardiac enzymes and the histopathologic features in the myocardium. In rats without AMI, the ECG pattern and the heart beats were normal but the rats with MI presented an abnormal pattern with specific change in ST segment and abnormal Q waves. The level of lactate dehydrogenase was increased in both experiments. The creatine kinase-MB activity was increased compared to the saline controls, however in the MI experiment treatment with any dose of levofloxacin or large dose of ciprofloxacin (300 mg/kg) the CK-MB activity was significantly increased. The histopathologic findings in cardiac sections, stained with hematoxylin and eosin, revealed different degrees of inflammatory cell infiltration, cardiomyocyte degeneration, intermuscular edema, loss of the cardiac striations and hemorrhage as an effect of levofloxacin and ciprofloxacin. The conclusion of the study was that it is important to monitor patients with a history of cardiac disease requiring treatment with these antibiotics (Ahmed M. Abdelradya, Sawsan A. Zaitoneb, Noha E. Faragd, et al. Cardiotoxic effect of levofloxacin and ciprofloxacin in rats with/without acute myocardial infarction: Impact on cardiac rhythm and cardiac expression of Kv4.3, Kv1.2 and Nav1.5 channels. *Biomedicine & Pharmacotherapy* 2017; 92: 196-206).

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ALCOHOLIC LIVER DISEASE - EPIDEMIOLOGY AND RISK FACTORS

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ALCOHOLIC LIVER DISEASE - EPIDEMIOLOGY AND RISK FACTORS (Abstract): Alcoholic liver disease (ALD) accounts for the majority of chronic liver diseases in Occidental countries and remains a major cause of liver-related mortality in worldwide. The spectrum of ALD includes steatosis in patients which consume over 80g of alcohol per day, alcoholic steatohepatitis and liver cirrhosis in approximately 15% of patients. Once cirrhosis is established, the annual risk for hepatocellular carcinoma is about 1–2%. Environmental factors such as drinking patterns, coexisting liver disease, obesity, diet and co-medication may affect the natural course of ALD. Abstinence is the hallmark of therapy for ALD, and nutritional therapy is the first line in therapeutical intervention. **Keywords:** ALCOHOLIC LIVER DISEASE, ALCOHOL CONSUMPTION, ENVIRONMENTAL FACTORS, ABSTINENCE

DEFINITION

Alcoholic liver disease (ALD) covers a large spectrum of structural and functional hepatic modifications, from benign and frequent forms (asymptomatic hepatomegaly) to the most severe ones (acute liver failure), modifications that share the same etiologic factor: systematic and long term consumption of alcoholic beverages (1-3). The spectrum of clinical manifestations and hepatic morfopathological modifications varies widely: from liver steatosis (alcoholic fatty liver) to inflammatory and necrotic lesions (alcoholic hepatitis), progressive fibrosis (hepatic cirrhosis) (4, 5, 6).

EPIDEMIOLOGY

ALD is probably one of the oldest forms of manifestation in chronic liver disease, considering that fermented alcoholic beverages have been consumed for hundreds of years, ever since the Neolithic Period (7, 8).

After 1970, many countries registered a fall in mortality by ALD; unfortunately, during the last decade, we have witnessed an increase in the incidence of this condition, and, implicitly in mortality, reaching 14.3/100,000 in France and 7.9/100,000 in the United States (9).

A lot of information concerning incidence, risk factors and ALD evolution are outdated, as while analyzing existing literature, we can easily see that most studies were carried out 15 or 20 years ago.

The real incidence and prevalence of ALD are difficult to evaluate, as many cases are asymptomatic, remaining thus

undiagnosed and unreported. On a group of 1,604 patients with chronic alcohol consumption which underwent liver biopsy, in 20% of them morfopathological modifications in alcoholic hepatitis have been registered (10). Other studies estimate alcoholic hepatitis incidence in 10-35% of chronic alcohol consumers.

Although alcohol consumption is increasing, numbers related to mortality by ALD remained constant in the last 24 years, the United States even witnessing a reduction from 6.9/100,000 inhabitants in 1980, to 4.4%/100,000 in 2003 (11, 12).

Mortality by ALD mainly depends on the severity of hepatic affectation, varying from 20% in light forms, to 30-60% in cases of severe alcoholic hepatitis (13). Acute hepatitis has the highest mortality rate, over 15% within 30 days and up to 39% within 1-year follow up (14,15). Great Britain is one of the countries where mortality by chronic liver disease has raised dramatically in the course of a few decades (1979-2005), the main cause of death by hepatic affectation being ALD. In this area, mortality by ALD has jumped from 1.7 to 10.4/100,000 in men, and from 0.9 to 5.0/100,000 in women with alcoholic hepatitis, the increasing trend in alcohol consumption and mortality among young people aged between 25 and 34 years causing concerns (16).

On the long term, survival in patients with uncomplicated alcoholic hepatitis reaches 58%, and only 35% in patients with alcoholic hepatitis developed on cirrhotic background (17).

ALD patients who continue to consume alcohol have a reserved prognosis on the short term as well as on the long term: 5-year survival in compensated cirrhosis is almost 90% in abstinent and drops below 70% in those who continue to drink, while the occurrence of decompensation indicates a chance of survival under 30% (18).

A study carried performed on a group of patients with alcoholic hepatitis fallowed up during 5 years through annually evaluations by liver biopsy has shown the occurrence of cirrhotic morfopathological modifications in almost 40% of them (19), as well as the possibility that resolution of histological modifications be complete in 10% of abstinent (20). Regrettably, in many abstinent it can be noticed a certain progression towards liver cirrhosis, while women, even in conditions of total abstinence, seem to be more exposed to an aggravation of lesions.

In an earlier study, Bird specifies that the risk of developing hepatic cirrhosis in patients with alcoholic hepatitis is 10-20% every year, and in the end, over 70% of them, in time, develop cirrhosis with all its complications (21).

ALD occurrence is influenced by many factors, mainly alcohol consumption, thus geographic distribution and prevalence of the disease correlate with distribution of alcohol consumption.

ALCOHOL CONSUMPTION – A SOCIAL PROBLEM

Excessive alcohol consumption implies high economic and social costs. Alcohol accounts for 1.8 million deaths worldwide, while in Europe, alcohol consumption is responsible for more than 55,000 deaths amongst those aged between 15 and 29. In the United States, alcoholism prevalence is 20% in hospitalized adults (22). In Great Britain, 1 in 3 patients assisted through primary care services has an alcohol addiction.

ALD incidence is strongly correlated with geographic distribution of alcohol consumption. According to a recent WHO

report (*Global Status Report on Alcohol and Health*), published in 2011, alcohol consumption is a major global health issue (23). The WHO report estimates that 4% of deaths worldwide are caused by excessive alcohol drinking, totalizing more deaths than AIDS, tuberculosis or violence (24). Alcohol consumption is considered to be the most important risk factor leading to death in men aged between 15 and 59.

The lowest mortality rate determined by alcohol-induced pathology is registered in regions with Islamic population, where alcohol consumption is reduced; in Western Europe also mortality is relatively low (in comparison with the East), although numbers referring to alcohol consumption are significant.

Globally, pathology determined by alcohol consumption accounts for 6.2% of registered deaths in males, beside 1.1% in females. Damaging alcohol drinking is fatal especially in youngsters.

Annually, 320,000 young people (15-29 years of age) worldwide die from alcohol consumption related causes, accounting for 9% of the total number of deaths at this age.

Quantity of alcohol consumed

The largest quantity of alcohol is consumed in developed countries. Although Western European countries report an important consumption of alcohol, alcohol induced mortality is significantly lower (while morbidity rate is considerable), as opposed to Eastern countries with the highest levels of alcohol consumption, and, probably, it is the type of beverages and consumption that determine much higher morbidity and mortality rates (9).

Alcohol drinking is largely spread, numbers are in some areas alarming, but a considerable percentage of the population is totally abstinent: almost half of men and over 2/3 of women have not drunk alcohol at all in the last years. From the point of view of the geographic distribution, populations in North African and South Asian regions are the most abstinent - areas with Muslim inhabitants, where alcohol is forbidden by religion.

In 2005, worldwide, the level of consumption of pure alcohol per inhabitant (persons over 15 years of age) was 6.13 liters. This largely varies geographically: developed countries, especially from the Northern hemisphere, report the highest values, but so do Argentina, Australia and New Zeeland. South African, South and North American countries register a moderate level of consumption, while the North African and Sub Saharian, East Mediterranean and South-East Asia regions show reduced levels of alcohol drinking (23).

It must be underlined that an important percentage (28.6%) of this consumption, the equivalent of 1.76 liters, is represented by unregistered alcohol (homemade, produced in illegal conditions or sold without taxes (23). Consumption of illegal beverages is much more dangerous (to the hepatic toxic effect of alcohol that of noxious substances is added as well as the impurities contained by these inappropriately produced beverages).

Alcohol drinking in Eastern European countries exceeds the European average level, in Russia and the Community of Independent States (CIS) every fifth death is caused by excessive drinking. According to the WHO report, Romania is among the countries with the highest alcohol drinking levels in Europe: the national average is 15.3 liters/inhabitant compared to 12.2 liters, which is the EU average; we exceed by far the European average, while the ex-Soviet states are ahead of us.

Type of alcohol

Worldwide, spirits are the most consumed beverages: over 45% of the total quantity of alcohol consumed (23). However, as there are geographical differences in quantities consumed, so are in types of drinks: beer, wine, spirits etc.

Traditionally, in Europe there were marked differences concerning preferences: it was well known that Nordic countries preferred beer, while the South-East consumed mostly wine; at present, these practices tend to change (35). Spirits are consumed mostly in Asia and Eastern Europe, whereas wine tops the preferences list of South Americans, as well as of some European states. Beer is one of the most favorite alcoholic drinks, being consumed by most Westerns countries, Northern Europe, Australia and most Africans states.

Alcohol consumption may be: close to risk level, damaging consumption and alcohol addiction. In the United States, it is estimated that there are 4.65% persons that meet the criteria for damaging consumption and 3.81% alcohol addicts (24).

ALCOHOLIC LIVER DISEASE – RISK FACTORS

The occurrence of hepatic lesions in alcohol consumers is influenced by a series of risk factors pertaining to: *alcohol consumption* (quantity drunk, type of alcohol, duration, and frequency), *constitutional factors (*sex, ethnicity), *risk factors for hepatic simultaneous affectation* (viral chronic hepatitis, iron overload syndrome, obesity, genetic factors etc.).

Alcohol consumption

Although there isn't a linear progression type of relationship between ALD incidence and alcohol consumption, numerous data confirm the strong connection between the quantity of alcohol consumed and the debut of alcoholic liver cirrhosis (25, 40).

It is difficult to establish which of the chronic alcohol consumers will develop a severe liver disease. Though it is considered that the occurrence risk for liver cirrhosis is high in consumers with over 10 years of alcohol drinking, 60-80 grams/day in men and 20 grams/day in women, only 6-41% of them develop cirrhosis (8). Some studies show that after large quantities of alcohol consumed (approximately 120 g per day), only 13.5% of consumers are affected by cirrhosis (7). At the same time, an epidemiological study on a large scale proves that every liter of alcohol consumed in excess (irrespective of type) leads to an increase in cirrhosis incidence with 14% in males and 8% in females.

It is relatively difficult to say who will develop or not ALD according to the quantity of alcohol consumed. Nevertheless, risk thresholds as defined by WHO are 21 glasses per week in men (approximately 3 glasses per day, not more than 5 glasses on occasions) and 14 glasses per week in women (about two glasses per day). These thresholds are not risk-free, as there are differences according to nutrition, sex, physical and mental health, and consumption time.

While hepatic steatosis occurs in almost all persons with sustained alcohol consumption, only 10-35% of them develop alcoholic hepatitis, and less, 8-20%, develops liver cirrhosis (6). If the risk for cirrhosis occurrence is no more than 1% in those who consume 30-60 grams of alcohol per day, this risk increases to over 5.7% in persons who drink daily 120 grams of alcohol (23). There is clear and sufficient evidence to confirm that, irrespective of other risk factors associated, the quantity of alcohol consumed is the key element causing ALD.

Beverage type and consumption pattern

All alcoholic drinks are toxic to the liver, but it seems that the risk for ALD is greater in individuals that prefer beer and spirits, and more reduced in wine consumers (18). Also, the risk for liver damage is 2.7 higher in those who usually drink outside meals (9). Daily (or very frequent) alcohol consumption represents a more significant risk for the occurrence of severe forms of ALD than excessive periodical or occasional drinking; the earlier the start, the higher the risk to develop ALD (24).

Sex

The fact that the percentage of females with ALD is smaller than that of men does not mean that women are less likely to develop ALD, but it is a consequence of the fact that men drink alcohol more frequently. Female alcohol consumers are more exposed ALD than men, the main factors which favor the disease being: reduced level of gastric alcohol dehydrogenase, high fat mass, and hormone status (6). While in men the risk for hepatic disease is significant at 14-27 drinks per week, in women, the risk increases at a smaller number of drinks, 7-13 per week (11). Women are twice more sensitive to liver alcohol toxicity and usually develop more aggressive forms of ALD, though they drink less and for a shorter period of time as compared to men (51).

A number of studies showed that at the same quantity of alcohol consumed, blood alcohol content is significantly higher in women than in men (9). It is estimated that women without any other liver condition can consume 14 units of drink per week (one unit equals 8 grams of alcohol) without risk to develop ALD (in the case of men, the "*risk-free*" dose is 21 drinks per week) (23, 30); quantities mentioned are relative, as they do not give any warranty. Other studies lower the risk-free limit to 7 units per week.

Race and ethnicity

Some studies mention that African and Hispanic populations in America register higher rates in mortality and incidence of alcoholic liver cirrhosis as opposed Caucasian population (23). As these differences do not seem to correlate with differences in alcohol quantity consumed, we are inclined to think of the role played by race or some genetic factors in favoring alcohol induced hepatic lesions.

Obesity

Obesity is an independent risk factor for hepatic disease, and non-alcoholic hepatic steatosis incidence, frequently associated to overweight, has been increasing in the past years. The association of the toxic effect of alcohol with non-alcoholic steatosis lesions raises the risk for ALD, with evolution towards cirrhosis; obesity intensifies ALD severity irrespective of the stage: steatosis, alcoholic hepatitis or liver cirrhosis. Taking that into account, for some years now, obesity has become an important health care problem for developed countries, with alarming rising incidence, and it is expected that in the following years we should face a significant increase in ALD cases, in number as well as severity (18).

Nutritional deficiencies

Many alcoholic patients are confronting with different nutritional deficiencies, and in those with alcoholic hepatitis it has been noticed that death risk is strongly correlated with the degree of nutrition (9, 12).

In these cases, mortality increases proportionately with malnutrition severity, reaching 80% in patients with severe nutritional deficiency, as compared to less than 50% in those with normal nutritional status. Malnutrition is a result of poor diet, anorexia, neuropsychological disorders, while

nutritional deficits of vitamin A and E are the most frequently met.

Hepatitis C virus infection

Almost 30% of ALD patients are infected with hepatitis C virus and more than 70% of those with viral chronic hepatitis C have previously consumed significant quantities of alcohol. The relationship between hepatitis C and ALD is proved, the infection with hepatitis C virus being directly involved in the occurrence of severe and advanced forms of ALD (27). The presence of lesions caused by hepatitis C virus is a major risk factor for hepatocellular carcinoma in patients with alcoholic cirrhosis, with a 10-year absolute cumulative incidence of 81% compared to 19% in those without VHC antibodies (28).

Alcohol drinking aggravates hepatic lesions in patients with viral chronic hepatitis C and accelerates the progression towards fibrosis and liver cirrhosis. It has also been noticed that in chronic alcohol consumers, ARN VHC level is significantly higher than in abstinents; last but not least, response to antiviral therapy in alcoholics is much weaker.

In viral hepatitis B, there is no certain data to confirm aggravation of hepatic lesions in patients which associate alcohol drinking (study results are even contradictory), but the effects of alcohol on an already damaged liver are very likely to be more significant than on undamaged liver.

Iron overload syndromes

There is irrefutable proof that hepatic iron overload is a frequent disorder in alcohol consumers; ALD patients have increased hepatic iron load, deposited at hepatocytes level and Kupffer cells. Hepatic iron overload in patients with ALD is considered a predictive factor for mortality (1).

Sinergical hepatotoxic effect of iron and alcohol is proved most convincingly by alcohol drinking association in patients with hereditary hemocromatosis: patients that drink alcohol have a significantly greater risk for severe hepatocytary lesions and cirrhosis occurrence at younger ages in comparison with abstinents (1, 9).

Genetic factors

There are numerous genetic factors that indicate a predisposition to alcoholism as well as to ALD; monozygotic twins are twice likely to become alcoholic and develop cirrhosis than are dizygotic twins (1, 11). It has been noticed that, generally, adopted children from alcoholic parents had a significantly higher alcohol addiction rate (18%) than adopted children with non-alcoholic parents (5%) (6, 11).

Recent studies have shown that variations of *patatin-like phospholipasedomain-containing protein 3* (*PNPLA3*) influence occurrence of alcoholic liver cirrhosis in Caucasian patients.

ALD ethiopathogenesis is not yet fully clear and has several unknown elements; still, one thing is certain: whatever may be the associated risk factors or genetic predisposition, the main triggering element in the genesis of the disease is alcohol consumption.

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NEWS

EVALUATION OF ADJUNCTIVE SYSTEMIC DOXYCYCLINE WITH NON-SURGICAL PERIODONTAL THERAPY WITHIN TYPE 2 DIABETIC PATIENTS

The aim of a study realized by a group of researchers from King Abdulaziz University, King Saud University, Riyadh, Kingdom of Saudi Arabia was to evaluate the effects of systemic doxycycline on clinical and microbiological parameters of diabetic subjects with chronic periodontitis. The 9-month multi-center, randomized, parallel, single-blinded study was conducted from different hospitals in Riyadh, Saudi Arabia between april 2010 - december 2010. A total of 76 diabetic subjects with chronic periodontitis were randomized into 2 groups: control group (CG) received only scaling and root planing (SRP), and the treatment group (TG) receiving systemic doxycycline during the reevaluation visit 45 days after the completion of SRP. Probing pocket depth, clinical attachment level, gingival index, plaque index, and bleeding on probing were collected at baseline, 45 days after SRP, and one, 3, and 6 months after the use of systemic doxycycline. Microbiological analysis comprised the detection of *Tannerella forsythia* (Tf), *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), and *Prevotella intermedia* (Pi) by polymerase chain reaction method. Sixty-eight (33 CG and 35 TG) subjects completed the study. Greater reduction in the population of Tf, Pg, and Pi were observed in TG compared with CG in the first month after the administration of systemic doxycycline. The TG showed a significant improvement in gingival index scores compared with the CG by the end of the first and 6 months after the administration of doxycycline. The conclusion of the study was that the adjunct systemic doxycycline can be associated with a reduction of Tf, Pg, and Pi in the first month after the administration of doxycycline with an improvement in the GI. (Al-Nowaiser AM, Al-Zoman H, Baskaradoss JK, Robert AA, Al-Zoman KH, Al-Sohail AM, Al-Suwyed AS, Ciancio SG, Al-Mubarak SA. Evaluation of adjunctive systemic doxycycline with non-surgical periodontal therapy within type 2 diabetic patients. *Saudi Med J*, 2014 ; 35 (10) :1203-1209)

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TRACHEOMALACIA IN CHILDREN AND ADULTS - NOT SO RARE AS EXPECTED

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TRACHEOMALACIA IN CHILDREN AND ADULTS - NOT SO RARE AS EXPECTED (Abstract): Tracheomalacia remains a special entity present also in children and adults. Tracheomalacia refers to a weakness of the trachea. Bronchoscopy is the "golden standard" for diagnosis. Differential diagnosis includes foreign body aspiration, difficult controlled asthma and other diseases. This disease may be congenital or it may be acquired. Acquired tracheomalacia can be treated. The main symptoms in tracheomalacia are: dyspnea, sputum production, hemoptysis and cough in adults and expiratory stridor and cough in children. Tracheomalacia could be progressive in some patients. We want to bring to your attention the tools for diagnosis and different methods of treatment. Tracheomalacia is not a rare disease and therefore we need to consider it. **Keywords**: TRACHEOMALACIA, STRIDOR, BRONCHOSCOPY

Tracheomalacia (TM) is still an underdiagnosed disease in both pediatric and adult patients but is not a rare disease. The term malacia is derived from the Greek world "malakia" which means soft.

In TM we can find a reduction and / or an atrophy of the longitudinal elastic fibers of the pars membranacea or impaired cartilage integrity. This cause is the reason of the dynamic narrowing of the transverse or sagittal diameters of the tracheobronchial lumen.

CLASSIFICATION

The most common classification for TM is primary (congenital disease) and secondary (acquired disease). TM is the most common congenital disease of the trachea in premature infants. Acquired TM is more frequently than congenital TM.

The respiratory distress syndrome in premature infants is the most common cause for acquired TM. Other etiology for acquired TM could be: prolonged intubation, tracheostomy, double aortic arch, left atrial hypertrophy, posttraumatic. Compression (vascular, tumors, infections, posttraumatic) could affect the tracheal wall with an increase in the intrathoracic pressure (1, 2).

DIAGNOSIS

Bronchoscopy remains the 'golden standard' for the diagnosis of TM (1). The

pulmonary function test (spirometry) is important because it describes the flow limitation on the expiratory part of flow volume loop with a decreased FEV1, low peak expiratory flow, reduced mid expiratory / mid inspiratory ratio and preservation of the shape of the inspiratory part (2).

It is believed that a reduction by 50-70% of the tracheal lumen is a diagnostic tool for TM. Endoscopic evaluations have resulted in the identification of 3 major models of tracheal lumen narrowing: narrowing of the lateral wall of the trachea ("scabbard type"), antero-posterior narrowing of the trachea ("floppy membrane type') and circumferential narrowing that combines the first two variants (3).

Over the years a lot of diagnosis tools were used: tracheograms, radiographs with comparative inspiratory and expiratory views, CT (computed tomography) scan, MRI (magnetic resonance imaging) scan. CT scan is noninvasive and it reveals anatomic details and the data can be reconstructed into two-dimensional and three-dimensional images (virtual bronchoscopy) (4). Dynamic CT scan images reveal the range intrathoracic tracheal diameters during forced maneuvers. MRI is important for the evaluation of the extrinsic airway abnormalities, for example vascular compression.

Differential diagnoses in children include: foreign body aspiration, difficult asthma and recurrent croup.

PATHOPHYSIOLOGY

Tracheal diameter is influenced during ventilatory movements (exhalation, inhalation), by variations of the intrathoracic pressure. During inspiration the intrathoracic pressure decreases, size of the trachea diameters get larger, and during exhalation, as intrathoracic pressure increase the caliber of the trachea will decrease. These physiological changes enhance in tracheomalacia and become obviously clear in conditions that accompany increased intrathoracic pressure: forced exhalation, coughing, Valsalva maneuver (1, 2).

CLINICAL SIGNS AND SYMPTOMS

The signs and symptoms of TM may appear during the first weeks to the first months of life (5). The most frequently symptoms are expiratory stridor, wheezing and cough. Symptomatology can vary from low-grade intermittent to continuous and severe. Children with TM have an increased relative risk of lower tract illness and a delayed recovery from intercurrent illness. We can find also: cyanosis, respiratory distress, sternal and intercostal retractions, spontaneous hyperextension of the neck, prolonged expiratory phase (6). Some children may have feeding difficulties like regurgitation and dysphagia (7, 8). In severe TM cases can appear increase sputum retention, reflex apnea and stridor during tidal breathing. Sometimes trachea is compressed by the esophagus which produces arterial desaturation, respiratory obstruction and weight loss.

TREATMENT

Intervention is not necessary in some children with TM, because when the child grows the tracheal cartilage strengthens, especially in children with mild and moderate TM, by 18-24 months (9).

Surgical intervention resolves the underlying cause (10).

Asymptomatic patients do not need therapy.

Initially, even in symptomatic patients the treatment is started with conservative

measures (chest physical therapy, control of infection, control of secretions, slow feedings, air humidifier) (11).

When conservative measures failed, surgical therapy is necessary. Tracheostomy, prosthetic stenting, tracheoplasty or tracheal resection with *end-to-end* anastomosis is some options regarding treatment for proximal or diffuse tracheomalacia (8, 9, 10).

Intraoperative bronchoscopy with concomitant aortopexy is the option in case of distal tracheomalacia with tracheo-esophageal fistula or vascular anomalies (12, 13, 14).

In adult patients we can find tracheomalacia in patients who have chronic obstructive pulmonary disease (COPD). It is necessary to treat first the COPD (8, 9, 11).

Stents (metal, silicone) can be placed for tracheomalacia and some authors reported long-term pulmonary function improvement (15, 16, 17). Metal stents are expandable, but could provide secondary reaction: granulation tissue, fracture of the stent (18, 19, 20). Silicone stents could be placed and repositioned by rigid bronchoscopy using general anesthesia but can migrate from inset (21). There are also newer hybrid biodegradable stents, whose efficiency will be evaluated. Some patients may benefit from stenting in the long-term.

CPAP (Continuous Positive Airway Pressure) is very important as treatment for severe TM. Long-term use of CPAP could affect speech and oral feeding (22, 23).

Administration of inhaled bronchodilators in patients with TM is not helpful (24).

CONCLUSIONS

Tracheomalacia is a pathologic condition which causes dynamic narrowing of tracheal lumen. With new tools for diagnosis, TM is identified increasingly often in children and adults too. Early diagnosis of TM and individually management of disease could improve respiratory symptoms and lung function parameters.

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ACUTE CARDIOGENIC PULMONARY EDEMA - ETIOLOGICAL SPECTRUM AND PRECIPITATING FACTORS

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ACUTE CARDIOGENIC PULMONARY EDEMA (APE) - ETIOLOGICAL SPECTRUM AND PRECIPITATING FACTORS (Abstract): **Aim:** the analysis of the predisposing and precipitating factors encountered in the anamnesis of the patients hospitalized with acute pulmonary oedema, in order to establish some correlations with the evolution and prognosis. **Material and methods:** The study included 50 patients, 32 males and 18 females, admitted to the Cardiology I Clinic between 2009 and 2013, diagnosed with acute pulmonary oedema upon admission. The following aspects were investigated: cardiovascular antecedents, prodromal elements of the current episode of acute pulmonary oedema (APE), risk factors and associated co morbidities, previous treatments followed at home as well as the triggering factors of the acute episode. **Results:** The main pathology on which the acute pulmonary oedema (APE) episode occurred was represented by: arterial high blood pressure (HBP), dilated cardiomyopathy, ischemic heart disease, valvular heart disease, pre-existing atrial rhythm disorders. The analysis of the factors that precipitated the acute pulmonary oedema episode revealed the following possible triggering conditions: inadequate physical effort on the background of treatment discontinuation, high sodium diet, a prolonged ischemic episode due to inadequate physical effort, rhythm disorder with rapid ventricular response (atrial fibrillation, atrial flutter). In some cases, the risk factors were cumulated. **Conclusions:** Acute pulmonary oedema represents a major emergency that requires immediate admission to hospital and rapid treatment in the emergency department concurrent with the identification of the triggering and precipitating factors. **Keywords:** PULMONARY EDEMA, RISK FACTORS.

Cardiogenic pulmonary oedema represents an important pathology with 10% incidence in patients known with cardiovascular diseases and with a morbidity rate of 50% (1, 2). It is caused by the excessive accumulation of blood plasma at the level of alveoli and pulmonary interstitial space secondary to the increase of the filling pressure in the left heart, which leads to the increase of the hydrostatic pressure in the pulmonary veins and capillaries (1, 2, 3). The main cause is represented by the left heart dysfunction, but there are also some other cardio circulatory conditions that lead to the increase of the pressure in the pulmonary capillaries with the appearance of

the transudate: left heart valvular disorders, cardiac tumours, rhythm disorders with rapid ventricular response (atrial fibrillation, atrial flutter), pulmonary embolism, high blood pressure (2, 3, 4, 5). It represents a major cardiovascular emergency, with life threatening risk, reasons for which the therapeutic intervention has to be fast, prompt, addressing both the involved physiopathological mechanisms and the etiology (5).

Aim of the study: the analysis of the predisposing and precipitating factors encountered in the anamnesis of the patients hospitalized with acute pulmonary oedema, in order to establish some correlations with the evolution and prognosis.

MATERIAL AND METHODS

The study included 50 patients, 32 (64%) males and 18 (36%) females, admitted to the Cardiology I Clinic between 2009 and 2013, diagnosed with acute pulmonary oedema upon admission. All patients were admitted to the emergency unit of the hospital and they were brought by ambulance in most of the cases. Patients were aged between 52 and 85. Being a major emergency, the first therapeutic measures for all patients were initiated in the emergency care unit concomitant with a cardiological examination.

The following steps were undertaken:

- The anamnesis of the patient, with the mention that in the situations in which the condition of the patient did not allow the direct anamnesis, the data was obtained from his relatives or from the previous hospital release documents (patient's previous medical folder respectively).

The following aspects were investigated during the anamnesis:

- Patient's cardiovascular antecedents: high values of blood pressure (HBP), chronic ischemic disease, acute myocardial infarction (IMA), valvular heart diseases, dilated cardiomyopathy (CMD), previously known rhythm and/or conduction disorders, potential surgical interventions at the level of the cardiovascular system;
- Identifying the number of previous episodes of left ventricular insufficiency (cardiac asthma, acute pulmonary oedema) if these occurred;
- Identifying some prodromal elements of the current episode of acute pulmonary oedema (paroxysmal nocturnal dyspnoea);
- The associated risk factors having an impact on the cardiovascular system (smoking, alcohol intake, diabetes);
- Associated comorbidities (thyroid gland disorders such as hypo or hyperthyroidism, chronic obstructive bronchopneumopathy (BPCO), stroke);
- Treatments followed at home up to the moment of coming to the emergency care unit;
- The emphasis was targeted on identifying the precipitating and aggravating factors of the acute pulmonary oedema episode: inadequate physical effort, alcohol intake, paroxysmal rhythm disorder, conduction disorder, prolonged anginous episode, intercurrent respiratory infection, fever, treatment discontinuation, excessive salt intake.

The complete examination of the patients involved: assessing the general state, consciousness, pulmonary auscultation, heart and peripheral arteries (carotid) auscultation, checking of blood pressure (BP).

The paraclinical data were collected concomitantly with the application of the first aid measures in the emergency care unit and therapy:

- Measuring the levels of SaO2, PO2, PCO2, Astrup;

- 12-lead electrocardiogram;
- Continuous EKG monitoring;
- Continuous monitoring of blood pressure (BP) and SaO2;
- Blood sampling for the absolutely necessary usual blood tests: hemoleucogram, renal function, electrolytes, glycaemia, myocardial cytolysis enzymes (aspartate aminotransferase (AST), creatine kinase (CK), creatine kinase MB (CKMB), lactate dehydrogenase (LDH)), coagulation tests;
- Echocardiography in the Emergency Room: 2D, M mode and Doppler in order to assess left ventricular systolic and diastolic function and to confirm the etiology of heart failure.

RESULTS AND DISCUSSION

In our study 50 patients, 32 males and 18 females, aged between 52 and 85, diagnosed with acute pulmonary oedema were admitted to the Cardiology I Clinic, between 2009 and 2013 (fig.1).

Age distribution of the patients from the group was as follows (fig.2).

Fig. 1. Gender distribution of patients

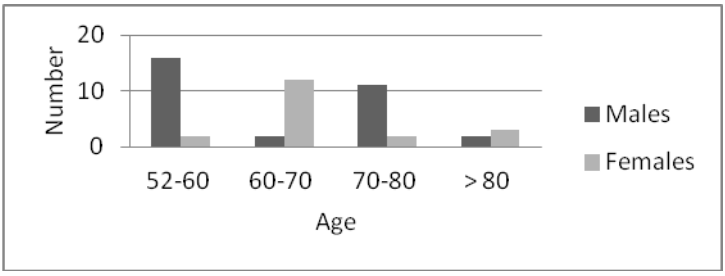

Fig. 2. Age distribution for the patients with pulmonary oedema

The anamnesis of the patients in the group revealed the following data:

- high blood pressure present in 20 patients, 11 males and 9 females;
- dilated cardiomyopathy present in 15 patients, 13 males and 2 females;
- 3 patients presented significant hemodynamic valvular heart disorders: one male presented aortic stenosis and 2 females presented mitral stenosis and aortic stenosis respectively;
- the ischemic cardiac disease was present in 12 patients from the group with high blood pressure, 8 males and 4 females respectively, of which: 2 males had acute myocardial infarction (AMI) before, 6 males presented different forms of angina pectoris; one female had acute myocardial infarction (AMI) before and 3 females, different forms of angina pector-

is;

- rhythm disorders in 16 patients as follows: 12 with atrial fibrillation (7 males and 5 females), 4 with atrial extrasystolic arrhythmia and ventricular extrasystolic arrhythmia (2 males and 2 females).

Of the total number of patients, 33 (66%) were at their first acute pulmonary oedema episode, and 17 (34%) at their second (fig. 3).

The analysis of the associated risk factors in the patients from the group revealed the following: of the total number of patients, 21 were smokers, 23 reported chronic alcohol consumption, dyslipidemia was present in 18 patients, 6 of them being under treatment with statin. Diabetes was present in 6 males and 3 females, 5 of them being under treatment with insulin; 3 patients had associated thyroid pathology; 4 of them had antecedents of stroke; 5 had chronic obstructive bronchopneumopathy (BPCO) and presented chronic renal disease (fig.4).

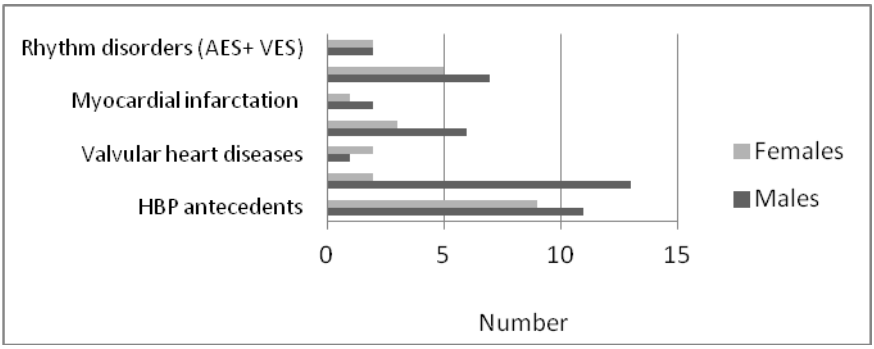

Fig. 3. Pathological antecedents present in patients in the study

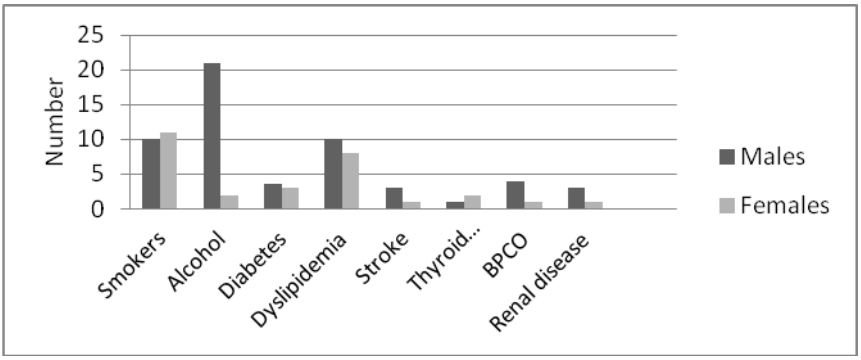

Fig. 4. Risk factors present in the patients diagnosed with acute pulmonary oedema.

Treatments followed by the patients in the group before the acute pulmonary oedema (APE) episode were different. Patients with high blood pressure were under different therapeutic schemes with calcium blocker, beta blocker, conversion enzyme inhibitor (ACE) and diuretic. The oral anticoagulant was used in 22 patients, 14 of them receiving platelet antiaggregants.

Three patients from the group of those with thyroidian pathology were under treatment with antithyroid drugs or Euthyrox.

Patients known with chronic obstructive bronchopneumopathy had Miofilin in their scheme of treatment (used as a bronchodilator) and sympathomimetic medication in some cases associated with corticotherapy in minimal doses (fig.5).

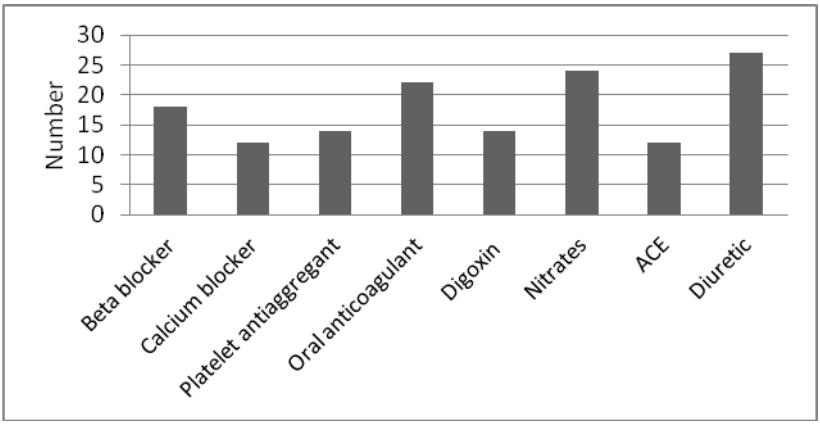

Fig. 5. The medication administered before the APE episode.

The analysis of the factors that precipitated the acute pulmonary oedema (APE) episode revealed the following possible triggering conditions:

- Inadequate physical effort in the context of treatment discontinuation in 27 patients (20 males and 7 females);

- High sodium diet associated with inadequate physical effort and treatment discontinuation in 12 patients (8 males and 4 females);

- Prolonged ischemic episode in the context of inadequate physical effort in 5 patients (3 males and 2 females). All these patients were known with ischemic cardiac disease;

- Rhythm disorder with rapid ventricular response (atrial fibrillation, atrial flutter) in 15 patients (13 males and 2 females);

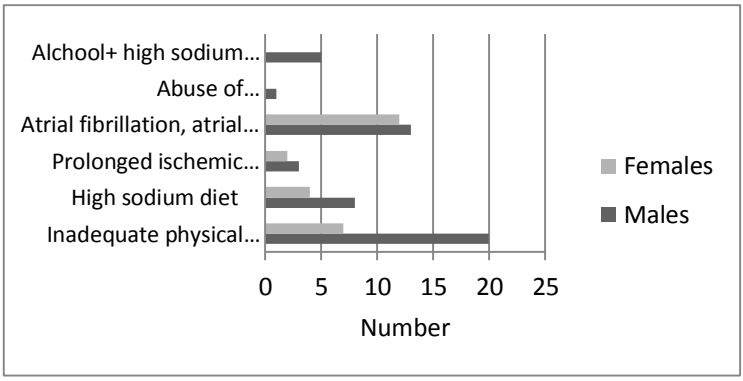

Fig. 6. Factors that precipitated the onset of acute pulmonary edema

- Sympathomimetic drugs abuse reported by one patient;

- For 5 patients (all male) the precipitating factors were cumulated: inadequate physical effort associated with high sodium diet, together with alcohol intake, on the background of treatment discontinuation (fig. 6).

CONCLUSIONS

The acute pulmonary oedema episode was solved faster in the case of the patients for whom the triggering factor was the high blood pressure (HBP) and of those who were at their first episode.

In patients having dilated cardiomyopathy and mitral or aortic valve disorders as an etiologic substrate, the evolution was more severe, requiring special therapeutic measures. The severity was amplified by the coexistence of the low cardiac output syndrome in the respective cases.

Patients who were at the second episode of acute pulmonary oedema had a more severe evolution.

Treatment discontinuation at home in the case of some patients associated with chronic alcoholism was a clear aggravating factor for the evolution.

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PRURITUS IN THE ELDERLY. PATHOPHYSIOLOGICAL, CLINICAL, LABORATORY AND THERAPEUTIC APPROACH

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PRURITUS IN THE ELDERLY. PATHOPHYSIOLOGICAL, CLINICAL, PARA-CLINICAL AND THERAPEUTIC APPROACH (Abstract): Generalized pruritus is a common symptom in elderly patients, with severe impact on the quality of life. The diagnosis of senile idiopathic pruritus is made after the exclusion of a systemic disease such as chronic renal disease, hepatobilliary disease with cholestasis, thyroid dysfunctions, drug-induced hypersensitivity reactions, visceral or hematological neoplasia, and primitive dermatological distinct conditions. The pathophysiological mechanisms are still unclear. A critical role is considered to be played by changes related to skin aging, cutaneous nerve supply and other nervous system components. The clinical approach requires a thorough assessment of general health status. In primary skin conditions, a biopsy and direct immunofluorescence (DIF) are required, while in pruritus associated with a systemic disorder, the assessment of hematological, biochemical and immunological parameters and imaging are necessary. The treatment of a patient with chronic pruritus is often palliative and individualized, with emollients, sedating and non-sedating antihistamines, tricyclic antidepressants, gabapentinum, and narrow-band UVB phototherapy. Pruritus associated with systemic disease may be alleviated by etiologic treatment. **Keywords**: PRURITUS, PATHOPHYSIOLOGY, ELDERLY, SYSTEMIC DISEASE, TREATMENT

Generalized pruritus is a common symptom in elderly patients, with severe impact on their quality of life. It is an annoying symptom, considered an “alarm system” or a self-defense mechanism, the same as other cutaneous sensations such as pain, tactile sensation, vibrations, cold or hot.

Pruritus is a symptom occurring in about 20% of the adult population.

An underlying systemic disease is reported in 10 - 50% of the patients who see a doctor for pruritus. Thus, it occurs in 22 - 66% of the hemodialysis patients, 30% of the patients with chronic renal failure not undergoing dialysis, 60% of the patients with primary biliary cirrhosis, 48-70% of the patients with polycythemia vera, 4 - 11% of the patients with untreated Graves disease, 35% of the patients with Hodgkin’s disease, 10% of the patients with non – Hodgkin lymphoma.

The malignancy rate in patients with generalized pruritus is thought to range between 1 and 8%. Pruritus occurs in very few cases of diabetes, hypothyroidism and leukemia (1, 2).

PATHOPHYSIOLOGY

Various mechanisms involve a complex network of cutaneous cells (keratinocytes, mast cells, inflammatory infiltrate cells: lymphocytes, eosinophils), which interact with unmyelinated slow-conducting polymodal C fibers and A-delta nociceptive neurons, with free terminations, located in the vicinity of the dermoepidermal junction or in the epidermis (2, 3, 4, 5).

The following mediators were found to play an important role: histamine, serotonin, bradykinin, protease (for instance mast cell tryptase), endothelin, NGF, brain-derived neurotrophic factor, neurokinins, neuropeptides (substance P), gastrin-releasing peptide, cytokines such as IL31, autotaxin, histamine H_4 receptors (6).

Senile pruritus or Willan's itch is induced by age-related tegument changes and cutaneous nervous fibers distribution alteration. Also, any increase in the expression of the vanilloid transient receptor 1 (TRPV1), which is a member of the non-selective cation channel family whose activation determines an influx of bivalent and monovalent cations (for instance, Ca2+, Na+, Mg2+) in aged human skin, would be suggestive of the involvement of this receptor in different senile cutaneous symptoms such as pruritus and neurogenic inflammation (2, 6, 7).

In renal pruritus (chronic renal failure, especially in hemodialysis patients), pruritus is rather due to the high level of bivalent ions (calcium, magnesium, phosphate), also detected in patients with cutaneous pruritus of different etiology, than to the high level of circulating histamine associated with a large number of mast cells in various organs. Other factors that may be involved in renal pruritus are: lower transepidermal release rates of pruritogenic substances, xeroderma, higher bile acid levels in the serum, higher vitamin A levels in the epidermis, serum serotonin increase. Also, in chronic renal failure, pruritus may be a possible symptom of peripheral neuropathy. Enolaso-positive non-specific nerves were found to proliferate in the epidermis of uremia patients. Also, opioid substance accumulation and μ-opioid receptor over-expression and activation could account for renal pruritus. Another hypothesis supports the role of systemic inflammatory reactions in these patients, which trigger the over-expression of activated Th1 lymphocytes, which secrete the IL2 responsible for pruritus; hence, the efficiency of the UVB phototherapy, and thalidomide and tacrolimus treatment, which target inflammation mediators. Pruritus severity in uremia patients is correlated with high ferritin and low transferrin and serum albumin levels (2, 6).

In cholestatic pruritus, the accumulation of specific pruritogenic intermediaries in bile salt synthesis is thought to determine hepatic lesions and pruritogenic substance release. Rifampin and ursodeoxycholic acid diminish the intrahepatic concentration of bile salts and ameliorate hepatic pruritus. This would also involve the accumulation of endogenous opioids, which play a certain role in pruritus modulation and cerebral opioidergic tonus increase; therefore, cholestatic pruritus is partially ameliorated by opioid antagonist delivery (2, 6).

In hematologic pruritus, iron deficit may lead to pruritus occurrence through a variety of metabolic pathways. In *polycythemia vera*, pruritus is influenced by the large number of circulating basophils and skin mast cells, and it is typically enhanced when the contact with hot water ceases. In addition to iron, other major hematologic

pruritus mediators are mast cell prostaglandins and platelet degranulation products, which induce serotonin and prostanoid release (2, 6).

In endocrine thyropathy pruritus, the thyroid hormone hypersecretion specific to hyperthyroidism may activate the kinins resulting from high tissue metabolism or it may reduce pruritus threshold as a result of heating and vasodilation. In hypothyroidism, pruritus follows xerosis. In diabetic patients, pruritus is correlated with metabolic anomalies, anhidrosis, diabetic neuropathy and autonomic dysfunction (2, 6).

Pruritus associated with malignant processes may be accounted for by the release of tumor toxins. Pruritus mediators in Hodgkin's disease are leukopeptidase and bradykinin released as a self-immune response directed against malignant lymphoproliferative cells. In carcinoid syndrome, pruritus is triggered by serotonin (2, 6).

CLINICAL APPROACH

The detailed history and thorough general physical and tegument examination play a critical role in determining the cause of pruritus.

The detailed history should include the onset, duration, severity, location and temporal relation with triggering factors such as drugs, bath, emotional stress, and data on possible alcohol abuse, which may indicate a chronic hepatic disease. The history should also provide information on patient's mental health in order to exclude a possible psychiatric cause of their pruritus.

The clinical examination may reveal xeroderma as a possible cause or just as a coincidental symptom, as well as signs that allow differentiating systemic causes from primary dermatological conditions. Thus, the skin may look normal or it may exhibit secondary lesions: excoriation, prurigo nodularis, lichen simplex chronicus, signs of secondary suprainfection, "butterfly sign" (hypo-pigmented or normal skin area on the postero-median thorax and post-inflammatory pigmentation areas which are easy to reach by patient's hands).

Renal pruritus may be accompanied by: diffuse xerosis, "half and half nails" associated with signs of peripheral neuropathy and uremia.

Signs of hepatic condition present in cholestatic pruritus are: jaundice, spider angiomas, white nails, xanthelasma, Dupuytren's contracture, gynecomastia in males, ascites, splenomegaly.

Hematological pruritus determines: paleness (anemia, hyposideremia), glossitis and angular cheilitis, "red face" (perioral, cheek, nose and ear erythema), whereas in polycythemia vera it also associates hypertension and splenomegaly.

Well-defined hyperpigmentation, ichthyosis, painless lymphadenopathy and splenomegaly are characteristic of paraneoplasic pruritus in Hodgkin's disease.

Diagnostic keys: generalized pruritus due to a systemic disease has an insidious onset; generalized pruritus in an old man with iron deficit but without anemia raises the suspicion of a neoplasm.

The causes of pruritus occurrence in systemic diseases are partially elucidated. Bile salts, histamine, opioids and unidentified pruritogenic substances in injured hepatocytes are thought to be involved in cholestatic pruritus. Pruritus may be correlated with primary biliary cirrhosis cholestasis, sclerosing cholangitis, chronic virus C hepatitis, choledocholithiasis, obstructive pancreas or bile duct carcinomas, pregnancy, and terminal hepatic illnesses regardless of etiology. Also, pruritus may be

associated with drug-induced cholestasis: chlor-propamide, salbutamol, phenothiazine, anabolic steroids, oral birth control pills, and erythromycin.

Hematologic prurits may either be induced by iron deficit, or occur in polycythemia rubra vera, hypereosinophilic syndrome, primary thrombocythemia, myelodysplastic syndromes.

Endocrine pruritus accompanies hypo-/hyperthyroidism, diabetes mellitus, hyperparathyroidism, hypoparathyroidism.

Pruritus associated with neoplastic conditions occurs in Hodgkin's disease, non-Hodgkin lymphomas, leukemia, paraproteinemia, carcinoid syndrome, SIPPLE syndrome (multiple endocrine neoplasia), solid tumors (gastro-intestinal tumors, SNC, pulmonary tumors).

Other conditions that materialize in pruritus are drug-induced rash-free pruritus (prurit sine materia), mastocytosis, HIV infection, AIDS.

LABORATORY APPROACH

Whenever primary cutaneous lesions are present (dermatitis herpetiformis, bullous pemphigoid, mastocytosis), cutaneous biopsy and possibly direct immunofluorescence and special stains are required.

Suspicion of a systemic cause requires: hematologic, biochemical and immunological tests (complete blood count, leukocyte differential count, ESR, electrolyte dosage, total protein test and protein electrophoresis, functional renal tests, glycemia, hepatic enzymes, uric acid, serum iron, alkaline phosphatase, bilirubin (high in cholestasis), hepatitis B and C serology, thyrotropin and thyrosine indicative of thyroid dysfunctions, IgE, PSA, urine exam for hydroxyl-indol-acetic acid (5-HIAA) and mast cell metabolites, folic acid dosage, zinc, vitamin B_{12}, porphyrin, antimitochondrial antibodies (positive and with 98% specificity in primary biliary cirrhosis), parathormone level, coproparasitologic exam, hidden hemorrhage); diagnosis confirmation procedures: retrograde cholangio-pancreatography, colonoscopy; necessary imaging investigations: chest X-ray, abdominal ultrasonography, tumor detecting CT/ IMR, ganglion ultrasonography.

THERAPEUTIC APPROACH OF PRURITUS

Possible treatment stages include: when the cause in not identified: avoid triggering factors, emollients, symptomatic therapy (non-sedating H_1 antihistamines), topical corticotherapy; when the cause has been detected: etiologic treatment; when the cause is unclear and the patient has not responded to previous therapies, and when secondary lesions are present: administer capsaicin, calcineurin inhibitors, cannabinoid agonists, naltrexone, gabapentinum, UV therapy, immunosuppressants (cyclosporine), tricyclic antidepressants (doxepin) and tetracyclic antidepressants (mirtazapine).

Elderly patients in whom pruritus is suspected to have a systemic cause are given: gastroenterological exams (to detect hepatobiliary conditions), hematologic/ oncologic exams (to detect malignant hematologic conditions, organic neoplasm), nephrological exams (to detect chronic renal conditions), endocrine exams (to detect thyroid conditions), surgical procedures (for renal/ hepatic transplant or obstruction removing stent in primary sclerosing cholangitis).

ETIOLOGIC TREATMENT

Therapeutic options available in renal pruritus are represented by physical, topical

and systemic treatment. Physical treatment - UVB phototherapy - reduces cutaneous phosphorus, decreases the number of dermal mast cells, and reduces vitamin A level in the epidermis. Topical treatment is used in localized pruritus and includes 0.025% capsaicin cream, 0.03% tacrolimus ointment (calcineurin inhibitor able to decrease Th1 lymphocytes differentiation and reduce IL2 production), 3-6% gabapentinum cream (ameliorates localized neurogenic pruritus), gamma-linolenic acid. Systemic treatment: the most common therapy of renal pruritus in dialysis patients is narrow-band UVB phototherapy associated with activated carbon administered orally. Another option in patients not responding to conventional therapy is thalidomide which decreases Th lymphocytes and inhibits TNF alpha production, or nicergoline and free fatty acids as the ones found in common evening primrose, gabapentinum or opioid antagonists, butorphanol and nalfurafine (2, 8, 9).

Cholestyramine is the first line therapy in cholestatic pruritus. It is followed by rifampine (rifadin, an antibiotic inhibiting RNA synthesis in bacteria and also hepatic biliary acid re-uptake, by detoxifying them), opioid antagonists (i.v. naloxone or oral naltrexone or oral butorphanol), ursodeoxycholic acid and s-adenosyl-methionine (efficient in pregnancy cholestasis and generally in patients with intrahepatic cholestasis) (2, 8).

Iron therapy in hematologic pruritus normalizes ferritin levels. The first therapeutic option in polycythemia vera is aspirin, which decreases platelet degranulation and serotonin and prostaglandin release. Other therapeutic options are cimetidine, danazol, UVB phototherapy, cholestyramine. In Hodgkin's disease, pruritus may be ameliorated by thalidomide (2, 8).

Pruritus due to hypothyroidism is ameliorated by topical therapy with emollients, whereas systemic pruritus responds well to substitution thyroid hormones.

Pruritus associated with chronic dermatosis responds to basic disorder therapy or to non-specific treatments such as topical cannabinoid agonists.

Uncontrollable pruritus may be reduced by UVB phototherapy, cholestyramine, naloxone and activated carbon.

Limited data suggest that gabapentinum, focal/zonal cutaneous stimulation, serotonin antagonists and UVB phototherapy may ameliorate senile idiopathic pruritus (6, 7).

PATIENT EDUCATION AND PROGNOSIS

Common cutaneous xerosis in the elderly enhances pruritus. Consequently, recommendations include emollients, avoidance of excessive bathing, dry environments, alkaline soaps and irritating fabrics.

Prognosis depends on complications, which are: debilitating insomnia, suicidal ideation, prurigo nodularis, and lichen simplex chronicus.

Poor prognosis is correlated with symptom severity. Thus, generalized pruritus is an example of long-term mortality marker of approximately 3 years in hemodialysis patients and it has poor significance in Hodgkin's disease patients. The recurrent nature of pruritus after visceral neoplasm therapy is a marker of cancer recurrence.

CONCLUSIONS

Pruritus is a common symptom in the elderly, yet age, just as sex, does not correlate with pruritus occurrence in patients with systemic conditions.

Idiopathic senile pruritus is an exclusion diagnosis, which requires investigation of a basic systemic disease (thyroid, hematologic, cholestatic or chronic renal condition, or more rarely as paraneoplastic phenomenon or cerebral infarction aftermath). Initially negative paraclinical tests do not exclude a systemic disease and the screening must be repeated every 3-6 months if the clinical suspicion remains.

Pruritus in old age is a difficult symptom to treat. The elucidation of the pathological mechanisms involved may have therapeutic advantages both in idiopathic senile pruritus and in pruritus triggered by systemic diseases.

A symptomatic patient-specific therapy is preferred in the absence of an etiological diagnosis, especially considering that the mere use of emollients may be impossible because the physical disabilities proper to seniors and the adverse effects of therapy are more common in seniors, since the associated diseases may alter drug metabolite pharmacokinetics and polypragmasia increases the risk of adverse reactions correlated with drug interactions, and failing cognitive functions induce low therapy compliance in the elderly.

The therapeutic options in severe therapy-resistant pruritus of systemic origin are: IL31 antagonists, neurokinin 1 receptor anagonists or anti H4 which are still under clinical study.

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THE QUALITY OF MEDICAL CARE DURING AN ACUTE EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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THE QUALITY OF MEDICAL CARE DURING AN ACUTE EXACERBATIONS OF COPD (Abstract): Chronic obstructive pulmonary disease (COPD) is a significant cause of global morbidity and mortality, with a substantial economic impact. Acute exacerbations of COPD (AECOPD) represent a dramatic event in the course of the disease; is an important cause of morbidity and the fourth cause of mortality worldwide. During the hospitalization for AECOPD mortality is 10%. AECOPD are also associated with a significant reduction of functional capacity and health-related quality of life. Despite these alarming evidence-based data the response of the healthcare system globally is not adequate to the gravity of the situation. A recently published study done in a Canadian hospital reveals that the treatment of the AECOPD is sub-optimal. The management of the COPD exacerbations prior, during and after the hospitalization showed inadequate adherence of the physicians (respirologists, internists and hospitalists) to the current guidelines. This review outlines the worrisome findings of this study and the proposed measures suggested by the authors in order to optimize the management of AECOPD. **Keywords:** COPD, ACUTE EXACERBATION OF COPD, LUNG ATTAK, TRIPLE THERAPY FOR COPD, HOSPITALIZATION, ADHERENCE TO GUIDELINES

Sandhu et al. published in the May 2013's issue of the Canadian Respiratory Journal a study ("Variations in the management of acute exacerbations of chronic obstructive pulmonary disease") that highlights worrisome observations regarding the management of acute exacerbations of Chronic Obstructive Pulmonary Disease (COPD). The lack of optimal care for these patients is disappointing in the light of documented poor prognosis associated with acute exacerbations of COPD (AECOPD). Our intention is to discuss the findings of this study and its proposed solutions.

COPD is defined by Global Initiative for Chronic Obstructive Lung Disease (GOLD) as "a common, preventable and treatable disease characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and in the lung to noxious particles or gases. Exacerbations and co morbidities contribute to the overall severity in individual patients" (1). Burden of Obstructive Lung Disease (BOLD) study estimate the prevalence of moderate and severe COPD, confirmed by spirometry, at 10% respectively 11.8% in

men and 8.5% in women (2).

COPD exacerbation represents an acute event characterized by the worsening of the respiratory symptoms beyond daily variations and which necessitate the change in the medication. The acute exacerbations and comorbidities contribute to the severity of COPD which is an important cause of morbidity and mortality globally (1).

COPD exacerbation, known also as a "lung attack" represents the fourth cause of mortality in the world and is a dramatic event; mortality in the hospital with an acute exacerbation is 10% and the mortality at 1 year following a hospitalization for an acute exacerbation is 25% (one in four patients will die in the following year after a hospitalized acute exacerbation) (3).

The average COPD patient with moderate or severe disease experiences 2-3 acute exacerbation/year (4). There is a documented decline in the lung function following an acute exacerbation (5, 6). Acute exacerbations of COPD are associated with significant reduction in functional exercise capacity and health-related quality of life measurements even in patients that underwent pulmonary rehabilitation prior to the "lung attack" (7). Early and adequate therapy improves the quality of life of these patients and can lower the hospitalization risk (8). This evidence – based data is worrisome but the response of healthcare systems around the world is disappointing (9).

A group from Vancouver (Sandhu et al.) did a retrospective study of the charts of 293 patients, admitted in a tertiary hospital for COPD exacerbation in 2009 (10). They examined the management of the COPD exacerbations prior, during and after the hospitalization to assess the adherence of the physicians to the current guidelines.

Inclusion criteria refer to self-reported previous diagnosis of COPD and primary discharge diagnosis of AECOPD (additional hospital visits under 28 days from the primary discharge were considered relapses).

Exclusion criteria were: the patient didn't have a pre-existing diagnosis of COPD; severe pre-existing mental issue and the AECOPD was the secondary diagnostic criteria.

The demographic characteristics of these patients are: mean age: 74.2+/- 11.4; sex: 52.9 % male and 47.1% female; previous family doctor: 95.5%; current smokers: 34.9% and co morbidities: 0 – 25.6%; 1 – 33.1%; 2 – 28.0%; 3 – 13.3% or co morbidities: 21% - heart failure; 9.5% - lung cancer; 23.6% - diabetes mellitus; 10% - depression.

Patient analysis shows that no information on lung function was collected (FEV1). The patients were admitted to respirologists, internists and hospitalists. Arterial blood gases (ABG) were performed in patients admitted to the respirology service: 68.4% compared with 48.4% for internists and 21.0% for hospitalists. Chest-X-rays were universally done (96-98%).

Patients'COPD maintenance medications on arrival were: 58% salbutamol, 40% ipratropium, 47.1% on a combination of ICS (inhaled corticosteroids) and LABA (long acting bronchodilator agonist), 17.7% tiotropium, 13.7% prednisone and 18.6% antibiotics. In total 82.6% of the patients were treated with > 1 maintenance medication.

This Canadian study reveals some worrisome findings. Current guidelines (1, 11, 12) highlight the "triple therapy" for the maintenance of moderate and severe COPD: ICS + LABA +/- LAMA (long acting anticholinergic muscarinic agonists). Before admission to the hospital less than half of the COPD patients were treated

with a combination of LABA + ICS or with LAMA. Only 82.6% of the patients were treated before the admission in the hospital with one or more COPD medication and only 17.7% of them were treated with an inhaled long acting anticholinergic (ipratropium). A small percentage of these patients were seen by a respirologist as outpatients (15.4%). Only 18.6% of the patients received antibiotics prior to their admission and only 13.7% received systemic cortisol.

The current guidelines for AECOPD (1, 14) recommend the prescription of a short acting bronchodilator with or without a short acting anticholinergic. Oxygen should be given to maintain the saturation between 88-92% and systemic corticosteroid (30-40 mg Prednisolon/day, 10-14 days) are recommended.

Antibiotics are recommended only in the presence of three cardinal symptoms: worsening of dyspnea + increased sputum production + purulent sputum or antibiotics for the patients that necessitate mechanical ventilation. During the hospitalization only half of the COPD patients of this study received the "acute triple therapy" (inhaled bronchodilator + systemic corticoids + antibiotics). 16% of the admitted patients did not receive antibiotics.

GOLD guidelines recommend to test the arterial gases only if the patient is hypoxemic (SO_2 < 92%). The spirometry is not recommended during an AECOPD. During the hospitalization of the COPD patients of this study very few arterial blood tests were performed.

The patients were admitted at: 44.9% internal medicine, 27.5% at respirology and 5.1% in Intensive Care Unit (ICU). Suboptimal treatment was offered by all three categories of physicians but the data shows that the internist and the hospitalists managed less optimal than the respirologists. An explanation might be the fact that the patients admitted under the internal medicine and hospitalists were older than those admitted under the respirologists (14). The elderly have more comorbidities compared with the younger patients admitted by respirologists and the prescription of systemic corticosteroids may have been limited by possible drug side effects. The data shows that the patients admitted under respirology were sicker: respiratory rate was higher (tachypnea), SO_2 lower, $PaCO_2$ higher (CO_2 retention), more acidotics.

The respirologists performed more arterial blood gases (64.4% versus 48.4% internists and 21% hospitalists). 78.9% of the patients admitted under the respirologists received corticosteroids compared with only 53.2 % of the patients admitted under the internists.

Despite the fact that the management of the patients with acute exacerbation admitted under the internists and hospitalists was less optimal compared with the management offered by respirologists, it is interesting to noticed that the deficiency was not translated into a significant increased of relapses or by an increase of mortality. 52.6% of the total number of the patients had a new exacerbation at up to 28 days after discharge.

Clinical studies showed that the maintenance "triple therapy" (LABA + ICS + LAMA) reduces the number of the acute exacerbations with risk of hospitalization (severe exacerbations) but does not reduce the general risk of exacerbation, for the patients with an FEV1 < 65% (15). At discharge, only 25.5% of the COPD patients received a prescription for the maintenance "triple therapy" (LABA + ICS + LAMA).

There is no mention of recommending Pulmonary Rehabilitation even with the recent evidences that the use of pulmonary rehabilitation immediately after an acute exacerbation of COPD is beneficial (16).

CONCLUSIONS

The management of the AECOPD is deficient especially when the patients are treated by the non-respirologists. This study enrolled only 293 patients thus it might be possible that it does not have enough statistical power. A suggestion made by the authors of this study is the introduction of the pre-printed prescriptions (orders); a list format. These prescriptions should be available for the physicians who admit these patients to the hospital (emergency room).

The prescription should contain: a bronchodilator, an inhaled corticosteroid and an antibiotic if the patient has purulent sputum or requires mechanical ventilation. The admitting physician is asked to check the appropriate items on the form and to sign the prescription thus the nurse can start the administration of the treatment right away.

Consequently, will not be necessary that the internist or the hospitalist to know the current recommendation for the treatment of stable or acute COPD nor to know when to prescribe the maintenance "triple therapy" at the discharge.

This Canadian study highlights the importance of continue medical education and it is not surprising that the respirologists managed better the acute exacerbations of COPD compared with the internists and hospitalists.

The respirologists adhere more to the current guidelines and this study invites them to reflect about the modalities to improve the management of these very sick patients. It is the respirologists duty to do a better job regarding the implementation of the current guidelines in regards with the acute and maintenance therapy for COPD in internal medicine and family practice, because when you can't breathe, nothing else matters !

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NEWS

ADHESION AND BIOFILM FORMATION BY PERIODONTOPATHOGENIC BACTERIA ON DIFFERENT BRACKETS

The aim of the study realized by a group of italian researchers from the Department of Public Health and Infectious Diseases- Sapienza University of Rome was to compare early bacterial adhesion and biofilm formation of common and uncommon periodontal pathogens on a variety of commercial brackets in vitro. In vitro adhesion and biofilm formation of 4 bacterial strains on 15 different commercial brackets, in standard culture mediums with and without addition of either serum or human saliva was evaluated by quantitative real time PCR after extraction of bacterial DNA. The results showed that the materials significantly influenced bacterial adhesiveness in a species-specific way. Titanium and gold brackets constantly yielded the lowest values with all tested bacteria and in all tested conditions. Bracket materials and medium of growth significantly influenced biofilm formation. The conclusions of the authors were that the materials and environmental conditions significantly influence biofilm formation by periodontal pathogens at the surface of brackets. Whenever possible brackets should be kept far from the gingival margin and if this is not possible, brackets made of gold, titanium, and ceramic should be preferentially used. (Passariello C, Gigola P. Adhesion and biofilm formation by periodontopathogenic bacteria on different commercial brackets. *Eur J Paediatr Dent*. 2013;14 (3) :199-203).

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HEART RATE PARTICULARITIES IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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HEART RATE PARTICULARITIES IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (Abstract): Autonomic nervous system dysfunction proved in chronic obstructive pulmonary disease (COPD) patients might determine an elevated cardiovascular risk by heart rate alteration. **Aim:** To assess the particularities of heart rate as a possible cardiovascular risk factor in COPD patients. **Materials and Methods:** This prospective, case-control study comparatively analyzed the pulse rate continuously recorded with a polygraph in 32 COPD patients and 29 healthy subjects during rest (supine and sitting position) and during submaximal exercise (6-minute walk test). The relation between pulse rate and respiratory, functional or clinical alterations was analyzed in COPD patients. **Results:** The mean pulse rate was significantly higher during rest and exercise in COPD patients compared with the controls. However, the chronotropic response determined by exercise was similar in COPD and control groups: 55.19 beats/minute and 57.21 beats/minute, respectively ($p=0.686$). The mean pulse rate during exercise correlated with hypoxemia ($r=-0.354$, $p=0.47$) and with resting pulse rate ($r=0.871$, $p<0.001$ for supine position). **Conclusions:** COPD associates elevated pulse rates during both rest and exercise. Hypoxemia and resting pulse rate are determinatives of chronotropic response during submaximal exercise in COPD patients. **Keywords**: COPD, RESTING PULSE RATE, CHRONOTROPIC RESPONSE

One of the specific characteristics in COPD patients is represented by the presence of complex chronic comorbidities (1). Cardiovascular diseases represent important comorbidities, resulting in 26% of cause-specific mortality in COPD patients: 16% sudden death, 4% stroke, 3% myocardial infarction, 3% congestive heart failure (2). COPD associates an increased risk of developing acute cardiovascular events such as cardiac arrhythmia, deep vein thrombosis, pulmonary embolism, myocardial infarction, and stroke (3). However, the complex pathogenic relation between COPD and cardiovascular diseases is poorly understood, although there is evidence for the involvement of some common risk factors, such as smoking.

Patients with COPD have a lower heart rate recovery after exercise than healthy people. This alteration was proved as a strong predictor of mortality in COPD patients (4). Some researchers suggest that autonomic nervous system dysfunction

demonstrated in COPD patients may alter the cardiac autonomic modulation and subsequently the heart rate (5). These alterations might partially explain the increased cardiovascular risk in COPD patients. Given the pathogenic and prognostic implications of heart rate, our study aimed to assess the particularities of heart rate as a possible cardiovascular risk factor in COPD patients.

MATERIAL AND METHODS

To achieve our goal we performed a prospective, case-control study in which we compared heart rate in COPD patients and subjects without lung diseases.

The study included patients admitted to the Iasi Clinic of Pulmonary Diseases in the interval April 2011 - September 2012 diagnosed with COPD according to the criteria in Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines: chronic dyspnea, cough, or sputum production, and persistent airflow limitation, confirmed by spirometry (a ratio of forced expiratory volume in 1 second (FEV1) to forced vital capacity (FVC) of <0.7 after bronchodilator administration (6). The control group included subjects without history of lung diseases and without clinical symptoms compatible with COPD diagnosis or respiratory dysfunction, according to the results of pulmonary function tests. COPD patients and controls were matched for sex and age. Male gender and age between 45 and 85 years were inclusion criteria for both COPD and control group. History of heart rhythm disorders represented an exclusion criterion for both COPD and control group.

The study protocol included clinical and anthropometric assessment of the subjects: body height and weight were measured and body mass index (BMI) was calculated. The degree of dyspnea was estimated for COPD group using the Modified Medical Respiratory Council (MMRC) dyspnea scale (7). Health impairment in COPD patients was measured by St George's Respiratory Questionnaire (SGRQ). This provides three component scores: symptoms, activity and impacts scores, in addition to SGRQ total score (8). Smoking history was expressed by the number of pack-years (py). Pulmonary function was assessed by spirometry, including postbronchodilator FEV1 measuring, to confirm the diagnosis in COPD group. All subjects underwent heart rate evaluation at rest and during exercise (6-minute walk test, 6MWT). Using a polygraph (SOMNOcheck® Effort, Weinmann®, Germany), pulse rate and oxygen saturation (SpO2) were continuously recorded as follows: 6 minutes with the patient in supine position, 6 minutes in sitting position and 6 minutes during exercise (6MWT). The 6-minute walk test was performed according to the standards of American Thoracic Society (9).

The study was approved by the Ethics Committee of Iasi Clinic of Pulmonary Diseases, and the patients were enrolled only after they provided appropriate informed consent.

Data analysis was performed using Statistical Package for the Social Sciences (SPSS v.16.0.). The data were presented as mean ± standard deviation (SD). The equality of means for quantitative variables of the control and COPD groups were assessed for significance using the unpaired Student's t-test. In case the assumption of normality was not met, a nonparametric test was applied (Mann–Whitney U-test). Analysis of variance (ANOVA) was used to test the means equality in case of more than two samples. The Pearson correlation coefficient or the Spearman rank correlation coefficient was calculated to assess the

relationships between various clinical and functional parameters in COPD patients. All statistical tests were 2-tailed, and p values < 0.05 were considered significant for all analyses.

RESULTS

We enrolled a total of 32 patients in the COPD group and 29 subjects in the control group. The COPD group had a higher smoking index and a lower body mass index than the control group. The 6-minute walking distance was significantly higher for the control group (tab. I).

Regarding pulse rate, both case and control groups shared a similar trend, the mean values significantly increasing from supine to sitting position and from sitting position to exercise during 6MWT ($p<0.001$ for both COPD and control groups). The results demonstrate significantly higher pulse rate mean values in COPD subjects than in controls, during rest as well as during exercise: supine position, sitting position and 6-minute walk test, respectively (tab. II, fig. 1). However, no significant differences were found between study groups in pulse rate increase (mean pulse rate differences) from supine to sitting position ($p=0.479$) and from sitting position to exercise during 6MWT ($p=0.065$).

TABLE I

Anthropometric, clinical and respiratory functional characteristics in the study groups

	COPD			**Control**			
Subjects n	n=32			n=29			
	Mean ± SD	**Min**	**Max**	**Mean ± SD**	**Min**	**Max**	**p value**
Age yrs	65.75 ± 8.79	51.0	85.0	61.72 ± 8.51	48.0	80.0	0.068
Weight kg	68.09 ± 17.48	45.0	110.0	83.37 ± 15.93	58.6	125.0	<0.001
Height cm	168.59 ± 7.27	154.0	192.0	171.17 ± 5.75	159.0	180.0	0.133
BMI $kg \cdot m^{-2}$	23.81 ± 5.18	17.6	38.1	28.37 ± 4.79	20.5	43.3	<0.001
Smoking py	38.64 ± 19.43	10.0	100.0	17.96 ± 20.13	0.0	80.0	<0.001
MMRC	2.28 ± 0.95	1	4	-	-	-	
FEV1	38.9 ± 13.5			102.5 ± 14.8			<0.001
FEV1/FVC	52.6 ± 11.1			80.7 ± 7.3			<0.001
6MWT m	281.4 ± 107.5			426 ± 77.4			<0.001

TABLE II

Mean pulse rates - comparison between COPD and control groups

	COPD			**Control**			
Subjects n	n=32			n=29			
Pulse rate $beats \cdot min^{-1}$	Mean ± SD	Min	Max	Mean ± SD	Min	Max	p value
Supine	78.16 ± 16.61	52.2	111.3	66.42 ± 8.57	51.2	87.9	0.001
Sitting	82.87 ± 17.03	52.7	115.4	71.49 ± 8.88	53.8	92.4	0.002
6MWT	97.07 ± 2.36	90.1	103.3	90.6 ± 2.39	80.0	96.9	<0.001

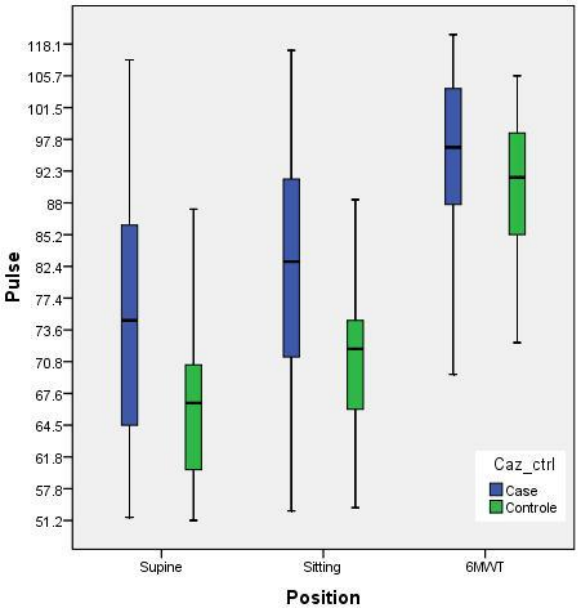

Fig. 1. The trend of pulse rate in the study groups

Even if pulse rate increase seems to be higher in controls than in COPD group as a result of the transition from rest to exercise (6MWT), the difference was on the borderline of statistical significance. The resting pulse rate (mean pulse rate for supine position) and peak pulse rate (maximum value during 6MWT) were assessed, the calculated difference between peak rate and resting rate representing the chronotropic response to exercise. The chronotropic peak moment was determined as the time of the peak in pulse rate during the 6 minute period of walk test. The chronotropic response and the chronotropic peak moment did not show significant differences between COPD and control groups (tab. III).

TABLE III

Chronotropic response – comparison between COPD and control groups

	COPD	**Control**	
Subjects n	n=32	n=29	
Pulse rate variation beats·min^{-1}	**Mean ± SD**	**Mean ± SD**	**p value**
Supine to sitting	4.71 ± 8.49	5.07 ± 4.6	0.479
Sitting to 6MWT	14.25 ± 8.58	19.1 ± 11.47	0.065
Chronotropic response (beats·min^{-1})			
	55.19 ± 30.93	57.21 ± 26.39	0.686
Chronotropic peak moment (sec)			
	173.9 ± 114.7	181.9 ± 111.3	0.783

We searched for potential determinatives of pulse rate alterations in COPD patients, assessing the relation of this parameter with other clinical or functional parameters (tab. IV). The mean pulse rate during 6 minute walk test inversely correlated with mean SpO2. In COPD patients, SpO2 demonstrated an opposite trend than pulse rate in relation with exercise, significantly decreasing during walk test as compared with rest in supine position (p=0.019). However, we did not find other correlations between pulse rate alteration, either during rest or exercise, and any other functional or clinical parameter in COPD patients, including MMRC dyspnea score, FEV1 and SGRQ scores. Resting pulse rate seems to determine the pulse rate increase during exercise, 6MWT mean pulse rate strongly correlating with mean pulse rates for both supine and sitting position (p<0.001).

TABLE IV

Potential determinatives for the pulse rate alteration in COPD group

		Correlation coefficient r	p value
Supine pulse rate	Sitting pulse rate	0.871	<0.001
Supine pulse rate	6MWT pulse rate	0.701	<0.001
Sitting pulse rate	6MWT pulse rate	0.819	<0.001
6MWT pulse rate	6MWT SpO2	-0.354	0.047

DISCUSSION

COPD associates an important risk of cardiovascular disease, the factors responsible for this association remaining largely unknown. Elevated resting heart rate was proved to be a strong independent risk factor for cardiovascular diseases in healthy subjects (10).

Our results have proved a significantly higher resting heart rate in COPD patients compared with controls without lung disease, for either supine or sitting position. Jensen et al. showed that resting heart rate was associated with cardiovascular mortality in a study including 16,696 COPD patients. They also found a strong association of the resting heart rate with COPD severity assessed as airway obstruction (11). We did not find any association between resting heart rate and FEV1 percent of predicted value (p=0.54), probably because of the small size of our study group. Furthermore, Van Gestel et al. found a significant correlation between resting heart rate and exercise capacity assessed by 6MWT distance (12). Our study did not prove any association between resting heart rate and health impairment measured by SGRQ in COPD patients in either symptoms, activity, impacts, or total scores of SGRQ. Dyspnea score (MMRC) did not correlate with resting heart rate, too. The higher resting heart rate might be explain by autonomic nervous system dysfunction in COPD patients, resulting in alterations of cardiac autonomic modulation and enhanced sympathetic tone at rest (5). Hypoxemia seems to be one of the determinant factors of this dysfunction, given the effect of oxygen supplementation which significantly and favorably alters autonomic modulation in COPD (13). There are also studies which obtained divergent results. Chen et al. found that resting autonomic nervous function of COPD patients is not different from that of normal controls.

The airway obstruction was not related

to the cardiac autonomic nervous function, but a worse oxygenation status was associated with increased cardiac vagal and decreased cardiac sympathetic activities in COPD patients (14).

Most of the researches regarding resting heart rate in COPD have assessed the patients in supine position. However, the transition from supine to sitting position is proved to improve hemodynamics, to decrease the respiratory functional residual capacity and airflow limitation, and to allow the accessory muscle use in breathing (15). These functional mechanisms which explain the orthopnea in COPD as well as in heart failure may also result in autonomic modulation and consequently in heart rate modulation. We found a significant increase in heart rate determined by transition from supine to sitting position in COPD patients. The heart rate increase in COPD patients was similar with the heart rate increase in healthy subjects (4.71 beats/minute and 5.07 beats/minute, respectively). However the mean heart rate for sitting position was significantly higher in COPD patients than in the control group subjects, maintaining the same trend as in supine position. The exercise represented by 6MWT determined a similar chronotropic response in COPD group compared with control group (55.19 beats/minute and 57.21 beats/minute, respectively). However, the mean heart rate during exercise was significantly higher in COPD patients than in controls, maintaining the same trend as at rest. The mean time for chronotropic peak reaching, within the 6 minute period was also similar in COPD and control groups (173.9 seconds and 181.9 seconds, respectively). Our results on heart rate assessment during exercise are different from the results obtained by Inal Ince et al. They found a peak heart rate and chronotropic index significantly lower in COPD patients than in healthy subjects ($p<0.05$). Other interesting finding in their study was the relation between chronotropic response, BMI, and dyspnea score (MMRC), respectively. Weight and body mass index were significantly higher than in COPD patients with normal chronotropic response ($p<0.05$). MMRC dyspnea score explained 44% of the variance in chronotropic index (16). We did not find any correlations between chronotropic response and functional or clinical parameters in COPD patients, including MMRC dyspnea score, FEV1 and SGRQ scores. However, we determined the chronotropic response during a submaximal exercise test (6MWT), unlike the study by Inal Ince et al. who used incremental exercise testing, although the COPD group in their study included only 25 patients.

The results obtained by Bartels et al. suggest that cardiac autonomic modulation is altered in patients with COPD during maximal exercise. Autonomic changes were not significantly correlated with age, gender, body mass index, spirometry, lung volumes, resting gas exchange, or oxygen saturation during exercise (17). In our study, oxygen saturation and resting pulse rate seem to be the only possible determinatives of pulse rate increase during submaximal exercise, strongly correlating with the mean pulse rate during 6MWT.

CONCLUSIONS

COPD associates elevated pulse rates during both rest and exercise. Pulse rate increasing in transition from supine to sitting position and chronotropic response during exercise are similar in COPD and healthy subjects. Hypoxemia and resting pulse rate are determinatives of chrono-

tropic response during exercise in COPD patients. Pulse rate alterations do not correlate with airway obstruction, dyspnea, or health impairment in chronic obstructive lung disease. Although more data are needed, elevated heart rate associated during both rest and exercise might result in increased cardiovascular risk in COPD.

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THE ROLE OF RIFAXIMINE IN THE PREVENTION OF THE SPONTANEOUS BACTERIAL PERITONITIS

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THE ROLE OF RIFAXIMINE IN THE PREVENTION OF THE SPONTANEOUS BACTERIAN PERITONITIS (Abstract): Cirrhosis is characterized by an increased susceptibility to infection. Spontaneous bacterial peritonitis (SBP) is the most common infectious complication, the main causes being the bowel bacterial overpopulation, the increased intestinal permeability and bacterial translocation. Antibiotic prophylaxis with Norfloxacin increases the rate of PBS with G+ multiresistant bacteria. **Purpose**: to demonstrate the advantage of using rifaximin, nonresorbable broad spectrum antibiotic, in SBP prophylaxy. **Material and methods**: The study is a prospective case-control, which included 46 patients diagnosed with Child class C cirrhosis and refractory ascites, based on clinical, biological, ultrasound and endoscopic findings, followed over a period of 6 months. Protein level in ascitic fluid (AF) was higher 14g/dl. SBP was defined as the presence of > 250 PMN/mm^3. Patients were divided into 2 groups, group 1 comprised 22 patients who received rifaximin treatment during the study for a previous episode of hepatic encephalopathy (HE), and group 2 consisted of 24 patients who did not receive antibiotic treatment during follow-up. **Results**: Rifaximin significantly decreases the polymorpho-nucleares (PMN) in ascitic fluid from patients, one single case of neutroascitic SBP with negative cultures were recorded in this group, with a net improvement of the general condition. In group 2 who did not follow any antibiotic treatment, SBP was recorded in 4 patients, an increase of PMN in ascitic fluid at 14 patients, an approximately constant value in 4 patients, a decrease in 2 subjects. **Conclusions**: The study suggests that rifaximin causes a significant decrease in PMN in AF, producing a decrease in SBP frequency and improvement of life in cirrhotic patients with refractory ascites. In this study, the effects of rifaximin on intestinal bacterial overpopulation and bacterial translocation, decisive factors in SBP, are consistent with literature data. Use of rifaximin as an alternative method to prevent SBP deserves more attention. **Keywords**: SPONTANEOUS BACTERIAL PERITONITIS, BACTERIAL TRANSLOCATION, PROPHYLAXY TREATEMENT, RIFAXIMIN.

Cirrhosis is characterized by an increased susceptibility to infection. In this way, spontaneous bacterial peritonitis (SBP) is a major complication of liver cirrhosis, the incidence in hospitalized patients with ascitic cirrhosis ranging between 7% and 23%, but increased when ascites associated low protein concentration (below 1g/100 ml)

and severe hepatic impairment (1).

In most cases, SBP is a monomicrobial infection, the culture of the ascitic fluid reveals a single microorganism, and the neutrophils being above 250/mm^3. The essential pathogenic elements are the intestinal bacterial overpopulation, bacterial translocation, increased intestinal permeability and immune deficiency.

Translocation of bacteria is defined as the passage of viable bacteria from the gastrointestinal tract to extra-intestinal sites, such as mesenteric lymph nodes (MLN), liver, spleen, kidneys, and blood flow. Overgrowth of the intestinal gram-negative bacilli will be followed by translocation in mesenteric lymph nodes, in the conditions of disruption of the intestinal barrier (2). Unlike the aerobic bacteria, which translocate through an intact mucosa, the anaerobic bacteria translocate through an intestinal mucosa defect (3). In fact, by eliminating the anaerobic bacteria, the intestinal bacterial overgrowth is facilitated, which one of the main factors is promoting bacterial translocation (4).

The long term prognosis of patients with SBP is unfavorable, the recurrence rate being 43% at 6 months. Despite the use of empirical antibiotic therapy, particularly cefotaxime, as well as antibiotic prophylaxis, currently mortality ranges from 10-30% (5).

A recent study shows that 30.8% of patients with Child class C cirrhosis, who did not have antibiotic treatment, have enteric bacteria in the mesenteric lymph nodes, while only 4.5% of those who were treated for decontamination have an enteric bacterial population in the mesenteric lymph nodes (6). Induced antisecretory hypochlorhydria favors the intestinal bacterial overgrowth. In patients with liver cirrhosis there is an intestinal motility disorder, which promotes intestinal bacterial overpopulation.

An important problem which has been recently discussed is the prophylactic treatment of spontaneous bacterial peritonitis with Norfloxacin (400 mg orally every 12 hrs.) considering the possibility to select quinolone-resistant bacteria species. However, special attention and a high degree of suspicion should be maintained in patients with spontaneous bacterial peritonitis that followed the prophylactic treatment. If the standard treatment fails, a 48h control paracentesis should be associated with vancomycin and imipenem (7).

Since 1994, investigators have explored the possibility of resistant bacteria species in patients receiving norfloxacin 400 mg daily to prevent SBP (8). By observing quantitative stool cultures of 31 patients, no resistant organisms were isolated from 15 patients, however, fluoroquinolone-resistant organisms were isolated from 16 patients between days 14 and 43, inclusive *Staphylococcus spp aureus* coagulase-negative, *Citrobacter freundii, Enterobacter cloacae, Klebsiella oxytoca*, and *Proteus rettgeri*.

In this way, considering that antibiotic prophylaxy of SBP with Norfloxacin increases the G+ multiresistant bacteria, the aim of the present work was to proove the advantage of using rifaximin, non-resorbable broad-spectrum antibiotic in SBP prophylaxis.

MATERIAL AND METHODS

The study is a prospective case-control, which included 46 patients diagnosed with Child class C cirrhosis and refractory ascites, based on clinical, biological, ultrasound and endoscopic findings, followed

over a period of 6 months. Patients were divided into 2 groups, group 1 comprised 22 patients who received rifaximin treatment during the study for a previous episode of hepatic encephalopathy, and group 2 consisted of 24 patients who did not receive antibiotic treatment during follow-up. Protein level in Af was higher than 1.4 g/dl. SBP was defined as the presence of > 250 PMN/mm^3. Diagnostic paracentesis was done with white blood cells (WBC) and PMN counting, and biochemical determinations (proteins, glucose). Cultures of the AF have also been done.

Our data were statistically analyzed by using one-way analysis of variance (ANOVA). All results are expressed as mean ± SEM. F values for which $p<0.05$ were regarded as statistically significant.

RESULTS

Regarding the distribution of the PMN values, as a result rifaximin administration for 6 months, we could observe that a high percentage of the treated patients manifested a decrease in their levels of PMN (20 patients out of 22 ~ 90 %). Also, only one cases each, for stable PMN and SBP (5 %) is reported for this group (fig. 1)

When analyzing the changes observed after 6 months in the untreated group, we observed that most of the patients (58 %) experienced an increase in their levels of PMN, with only 8 % of them having decreased number of PMN and 17 % each for stable values of their PMN or SBP (fig. 2).

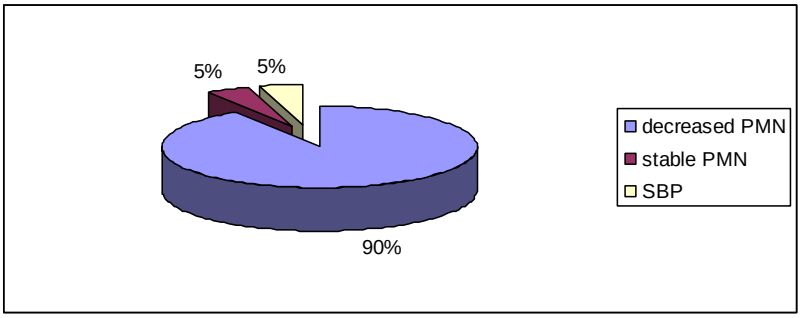

Fig. 1. The percentage distribution of the PMN values, after 6 months of rifaximin administration

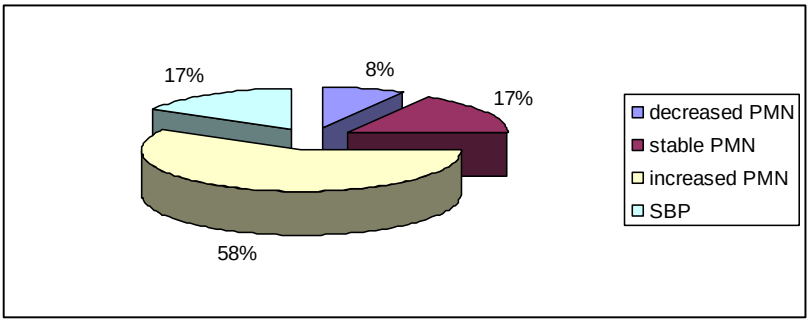

Fig. 2. The percentage distribution of the PMN values, after 6 months, in the untreated patients

Table I presents the detailed average values for the PMN levels at the baseline point, as well as after 6 months, in both our study groups (treated vs. untreated) (tab. I). We could observe a significant difference (F(1.42)=6, p=0.022) between the baseline point and after 6 months of treatment in our group of patients treated with rifaximin. Conversely, there were no significant differences (F(1.46)=1, p= 0.066) between baseline levels and after 6 months levels in the group of untreated patients, as shown by ANOVAs One Way (tab. I).

TABLE I

The PMN values, at the baseline point and after 6 months, in treated and untreated patients

	Treated			Untreated		
	Baseline	**6 months**	**p**	**Baseline**	**6 months**	**p**
PMN	101.09 ± 8.67	62.5 ± 13.82	0.022	54.41 ± 7.41	104.33 ± 20.41	0.066

Moreover, our final analysis focused on the possible differences between groups of patients after 6 months (treated vs. untreated). As can be seen in the Figure 3, we observed an increase of the PMN values in the untreated group, when compared with patients treated with rifaximin (F (1.44) =3, p=0.1) (fig. 3).

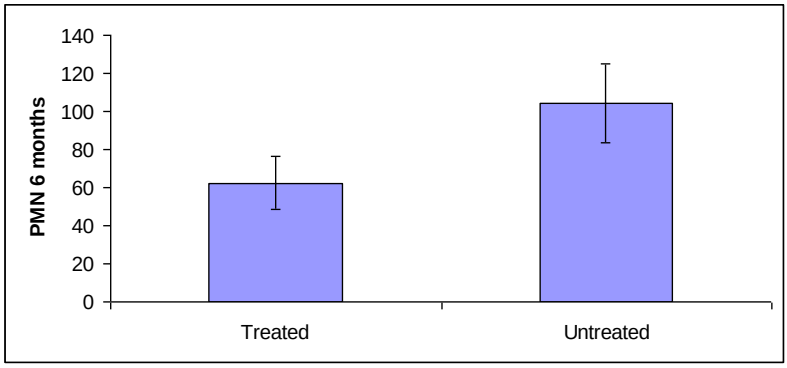

Fig. 3. PMN values after 6 months, in patients treated with rifaximin vs. untreated group

DISCUSSION

The present study demonstrated that rifaximin is associated with a significant decrease of PMN in the ascitic fluid, which could suggest that it could be implicated in the decrease of SBP frequency, and also in improving the quality of life in cirrhotic patients with refractory ascites.

These aspects could be very important considering that rifaximin, nonresorbable broad-spectrum antibiotic, has less adverse effects and does not increases the G+ multiresistant bacteria.

Cirrhotic patients have frequent episodes of infections due to immunodeficiency and malnutrition, which justifies prophylactic antibiotic treatment. Establishing the optimal therapeutic dose should

avoid the selection of multiresistant bacteria (9).

Among hospitalized patients, it was found that 16% had a multidrug-resistant bacteria, (most often Staphylococcus), mostly those who received prior antibiotic treatment. Germs are often 10 times more aggressive in 33% of cases, resulting a longer hospitalization period and also increased hospital mortality (10).

A direct relationship between micro biota composition and bacterial translocation was demonstrated in mice. Bacterial overgrowth that occurs in cirrhosis due also to the intestinal hipomotility was correlated with bacterial translocation, endotoxemia and the development of spontaneous bacterial peritonitis (11).

Authors warned against antibiotiotic profylaxis in patients with cirrhosis. Subsequent studies have shown changes in the epidemiology etiology associated with PBS antibiotic prophylaxis (11).

In consideration of two time periods (1991-1995 and 1996 2000), Singh et al. have shown that the incidence of SBP with multidrug-resistant organisms in patients who were admitted in a liver transplant unit increased from 8.3% to 38.5%, the most resistant pathogens (71%) were either ESBL -producing organisms and methicillin-resistant *S. aureus* . The researchers noted that asymptomatic colonization of the gastrointestinal tract with ESBL-producing organisms normally precedes the symptoms. With the occurrence of gram-positive methicillin-resistant (MRSA) and gram-negative ESBL-producing germs, it seems prudent to evaluate patients with resistant pathogens SBP. (12). Thus, if patients who have received prior fluoroquinolones as SBP prophylaxy do not respond to empiric treatment after 48 hours, vancomycin (Vancocin, Viro Pharma) should be added to the treatment. Local epidemiological results also support the transition to antibiotics such as ertapenem (Invanz) or tigecycline (Tigacil). (12, 13).

In a recent study, it was demonstrated that in the cirrhotic patients which were not treated with fluoraoquinolone , the dominant spectrum in the ascitic fluid is represented by gram-negative bacteria (67%), whereas in those treated the dominate germs were gram positive bacteria. (14)

Given the increased cost and risk of developing resistant organisms, antibiotic prophylaxis is indicated in the following three groups: the history of SBP, those hospitalized for upper gastrointestinal bleeding and those with low-protein-concentration in AF.

Selective decontamination by prophylactic antibiotic aims to reduce the bacteria from the gastrointestinal tract in order to reduce the risk of developing spontaneous bacterial peritonitis.

Also, a recent study by Kalambokis et al. (15) demonstrated that administration of a therapy for four weeks with rifaximin 1.200 mg / day significantly reduced the number of neutrophils in the ascites fluid of cirrhotic patients with sterile ascites, in accordance with a significant decrease in plasma endotoxin. The observations are consistent with recent findings that show a significantly reduced rate in developing SBP after 5 years in cirrhotic patients receiving rifaximin.

CONCLUSIONS

The present study suggests that rifaximin cause a significant decrease in PMN in AF, causing a decrease in SBP frequency and improvement of life in cirrhotic patients with refractory ascites. In this study,

the effects of rifaximin on intestinal bacterial overpopulation and bacterial translocation as decisive factors in SBP, are consistent with literature data. Therefore the use of rifaximin as an alternative method to prevent SBP deserves more attention.

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BODY COMPOSITION ALTERATIONS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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BODY COMPOSITION ALTERATIONS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (Abstract): Metabolic disorders are involved in the pathogenesis of some co morbid conditions such as atherosclerosis and cardiovascular diseases, which frequently occur in COPD patient. These disorders may also associate alterations in body composition. **Aim:** To determine the particularities of body composition, assessing its relationship with respiratory function and clinical status in COPD patients. **Material and methods:** A prospective case-control study including 31 patients with COPD admitted to the Clinic of Pulmonary Diseases Iasi from April 2011 to September 2012, and 29 subjects without lung diseases which served as controls. Body composition (body fat, lean body mass, body water) and basal metabolic rate were measured using bioelectrical impedance analysis. Other assessments included anthropometric parameters, spirometry, 6-minute walk test. In COPD patients, dyspnea scale and health impairment assessment by St George's Respiratory Questionnaire (SGRQ) were used. **Results:** In COPD group, 67% of the patients were normal weight, 10% underweight, 10% overweight and 13% obese. The results showed that lean body mass ($p<0.001$), dry lean mass ($p<0.001$), total body water ($p<0.001$) and basal metabolic rate ($p<0.001$) were lower in the COPD patients as compared to the controls. These findings significantly correlate with age, dyspnea, severity of airway obstruction and exercise capacity in patients with COPD. No significant differences between study groups were observed in body fat mass. **Conclusions:** Lean body mass, dry lean mass, and total body water are altered in COPD patients and significantly correlate with age, clinical and functional severity of COPD. **Keywords:** COPD, BODY COMPOSITION, FAT MASS, LEAN MASS.

Chronic obstructive pulmonary disease (COPD) is associated with an increasing prevalence and a high mortality in the coming decades, being a challenge for the clinicians and a leading public health problem worldwide (1). One of its specific features associated with increased morbidity and mortality is the presence of chronic co morbidities. The complex pathogenic link between COPD and co morbidities seems to involve systemic inflammation which represents another important feature of COPD (2).

The metabolic syndrome is frequently diagnosed in overweight COPD patients, its association with increased systemic in-

flammation independent of lung function impairment being thus proved (3). However, data on metabolic alteration in COPD are still poor and inconsistent. The "obesity paradox", defined as an inverse association of good health, survival and obesity in COPD patients was described. A higher mortality was found among underweight COPD patients, whereas overweight patients had a lower overall mortality (4). Other researchers have found a low fat-free mass frequently associated in COPD patients, even when body mass index (BMI) was normal. Furthermore, low fat-free mass is considered an independent predictor of mortality in COPD (5).

Plasma adiponectin, an adipose tissue-derived specific protein with anti-inflammatory and anti-atherosclerotic effects, correlates with body weight loss and systemic inflammation in COPD, some researches concluding that adiponectin may reduce cardiovascular events in underweight patients (6).

However, cardiovascular diseases, many of these involving atherosclerosis and metabolic disorders, represent an important comorbidity accounting for 26% of the cause-specific mortality in COPD patients (7).

COPD patients have excessive visceral fat even in advanced stages of the disease despite the absence of obesity (8). Physical inactivity, a commonly observed feature in COPD, might be a determinant of visceral fat accumulation (9).

Given the pathogenic and prognostic implications of fat mass alteration, our study aimed to determine the particularities of body composition, including fat body mass, lean body mass, body water and basal metabolism, in view of assessing their relationship with respiratory function and clinical status in COPD patients.

MATERIAL AND METHODS

We conducted a prospective case-control study in which we determined and compared body composition in patients with COPD and subjects without lung disease. The study included patients admitted to the Clinic of Pulmonary Diseases Iasi in the interval April 2011 - September 2012, diagnosed with COPD according to the criteria of Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines: chronic dyspnea, cough, or sputum production, and persistent airflow limitation, confirmed by spirometry (a ratio of forced expiratory volume in 1 second (FEV1) to forced vital capacity (FVC) of <0.7 after bronchodilator administration) (1).

The control group included subjects with no history of lung diseases, clinical symptoms suggestive of COPD or respiratory dysfunction, based on the results of pulmonary function tests. COPD patients and controls were matched for sex and age. Male gender and age between 45 and 85 years were inclusion criteria for both COPD patients and controls. Cachexia was the exclusion criterion for the control group.

The study was approved by the Ethics Committee of the Clinic of Pulmonary Diseases Iasi and the patients were enrolled only after they provided appropriate informed consent.

The study protocol included the clinical and anthropometric assessment of the subjects: body height and weight were measured and BMI was calculated as weight/height^2 (kg/m^2). We used the World Health Organization classification of BMI which categorizes BMI into the following categories: underweight (BMI$\leq$18.5 kg/m^2), normal weight (BMI: 18.5-24.99 kg/m^2), overweight (BMI: 25-29.9 kg/m^2), and

obesity (BMI>30 kg/m^2) (10). Smoking history was expressed as the number of pack-years (py). For the COPD group the degree of dyspnea was estimated using the Modified Medical Respiratory Council (MMRC) dyspnea scale (11).

Pulmonary function was assessed by spirometry, including post bronchodilator FEV1 measuring, to confirm the diagnosis of COPD in the corresponding group. FEV1 was expressed as percentage of predicted value. Exercise capacity was assessed in all subjects by the 6-minute walk test (6MWT). This was performed according to the standards of the American Thoracic Society (12). Health impairment in COPD patients was measured by St George's Respiratory Questionnaire (SGRQ). This provides three component scores: symptoms, activity, and impacts, in addition to SGRQ total score (13). Body composition was measured using bioelectrical impedance analysis with BodyStat 1500 analyzer (Bodystat Ltd, Douglas, Isle of Man). The device measures/estimates the following parameters: body fat percentage and fat weight (kilograms), lean mass percentage and lean mass (kilograms), body water percentage and total body water (liters), basal metabolic rate (kilocalories), and body impedance.

For analysis data were expressed as mean ± standard deviation (SD). Quantitative variables were compared using the unpaired Student's t-test. When the assumption of normality was not met, a nonparametric test was applied (Mann–Whitney U-test). Pearson correlation coefficient or Spearman rank correlation coefficient were calculated to assess the relationships between various clinical and functional parameters in COPD patients. All statistical tests were 2-tailed, and p value < 0.05 was considered significant for all analyses.

RESULTS

We enrolled a total of 60 male subjects, of which 31 were COPD patients and 29 controls. The most important clinical and anthropometric characteristics, respiratory function and body composition data are shown in table I.

TABLE I

Clinical characteristics, respiratory function and body composition

	Case	Control	p value
Age (years)	65.77 ± 8.86	61.41 ± 8.69	0.056
Smoking (py)	37.95 ± 18.83	19.34 ± 20.23	<0.001
Weight (kg)	66.80 ±16.27	81.51 ± 16.45	<0.001
Height (cm)	168 ± 6.11	171.34 ± 6.00	0.061
BMI (kg/m^2)	23.57 ± 5.09	27.74 ± 5.26	0.001
FEV1 (%)	37.59 ± 13.47	102.28 ± 15.02	<0.001
FEV1/FVC (%)	51.87 ± 10.90	80.95 ± 7.43	<0.001
6MWT distance (m)	276.09 ± 106.68	424.2 ± 77.9	<0.001
Fat (%)	17.89 ± 8.70	18.10 ± 7.24	0.842
Fat (kg)	12.83 ± 8.11	15.45 ± 9.24	0.185
Lean (%)	81.92 ± 8.56	81.89 ± 7.24	0.750
Lean (kg)	54.44 ± 11.21	66.22 ± 10.56	<0.001
Dry lean (kg)	8.35 ± 5.50	12.86 ± 4.33	0.001
Water (%)	70.60 ± 11.19	66.48 ± 8.2	0.111
Water (L)	46.09 ± 7.08	53.15 ± 6.86	<0.001
Basal metabolism (kcal)	1626.8 ± 290.9	1915.8 ± 262.9	<0.001
Impedance	410.8 ± 69.1	357.1 ± 57.2	0.002

A higher smoking index and lower weight were found in the COPD as compared to the control group. The 6-minute walking distance was significantly higher in the control group. BMI calculation in the patients in the COPD group showed: 67% were normal weight, 10% underweight, 10% overweight and 13% obese (fig. 1).

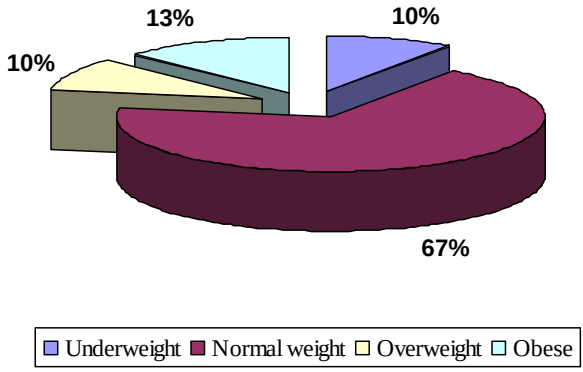

Fig. 1. Distribution of COPD patients according to BMI

The results of body composition assessment revealed certain particularities in COPD patients. The dry lean mass was significantly lower than in the healthy subjects. The absolute lean mass value, expressed in kilograms, was also lower in COPD patients, but no significant differences in percentage values between the two study groups were found.

Body water, in liters, was higher in the control compared with COPD group. However, body water percentage was higher in COPD patients than in controls, without reaching statistical significance. No difference in body fat mass, expressed either as percentage or absolute value, between COPD patients and healthy subjects was found. The different patterns of body composition determined a significant difference in body impedance between the study groups, which was significantly higher in COPD patients. Bioelectrical impedance analysis allowed the estimation of the basal metabolic rate. This was significantly higher in the control group than in COPD patients.

We also assessed the relationships between the results of body composition analysis and other clinical or functional parameters in COPD patients (tab. II).

In COPD patients, lean mass and dry lean mass, expressed in kilograms, were inversely correlated with age and dyspnea score (MMRC), and positively correlated with FEV1 and 6MWT distance (6MWTd). Body water percentage correlated with age and water volume (liters), inversely correlated with dyspnea score and positively correlated with FEV1 and 6MWTd. Basal metabolic rate, determined by bioelectrical impedance analysis, inversely correlated with age and MMRC and positively correlated with FEV1 and 6MWTd. No significant correlations between body composition components and health impairment in COPD patients, estimated by the three SGRQ component scores including symptoms, activity and impacts were found.

TABLE II

Correlations between body composition, clinical and functional parameters in COPD patients

		Age	MMRC	FEV1%	FEV1/FVC	6MWTd
Lean (kg)	Correlation Coefficient	-0.395	-0.473	0.47	0.475	0.505
	p value	0.002	<0.001	<0.001	<0.001	<0.001
Dry lean (kg)	Correlation Coefficient	-0.523	-0.398	0.361	0.402	0.474
	p value	<0.001	0.002	0.005	0.001	<0.001
Water (%)	Correlation Coefficient	0.329				
	p value	0.01				
Water (L)	Correlation Coefficient		-0.482	0.491	0.465	0.415
	p value		<0.001	<0.001	<0.001	0.001
Basal Metabolic Rate (kcal)	Correlation Coefficient	-0.37	-0.459	0.464	0.465	0.493
	p value	0.004	<0.001	<0.001	<0.001	<0.001
Impedance	Correlation Coefficient		0.392	-0.42	-0.34	-0.318
	p value		0.002	0.001		0.013

DISCUSSION

Even though the majority (67%) of COPD patients had a BMI within normal limits, the subjects in the control group presented a significantly higher BMI, which is in line with the results reported by Engelen et al. in patients with pulmonary emphysema (14).

Unlike Ischaki et al. we have proved a significant correlation of BMI, not only with 6MWTd but also with FEV1 which is the main determinant of COPD stage (15). A major difference is that, unlike previous studies, we found no differences in body fat mass between COPD patients and healthy controls and no correlation between body fat mass and clinical or functional parameters (8).

Ischaki et al. have demonstrated correlations between free-fat mass index and MRC dyspnea scale, 6MWT distance and percentage predicted FEV1 (15). Our results support these findings, proving an inverse correlation between lean mass/dry lean mass, expressed in kilograms, and dyspnea score (MMRC) and a positive correlation with FEV1 and 6MWTd. We also found a significantly lower lean mass in the COPD than in the control group.

Body water was also significantly lower in COPD patients than in healthy subjects. As lean body mass, body water inversely correlated with dyspnea score and positively correlated with FEV1 and 6MWTd. Previous researches demonstrated that COPD patients associating fat-free mass depletion are characterized by increased extracellu-

lar/intracellular water ratio (16).

Body impedance analysis also provided an estimation of the basal metabolic rate. This was lower in COPD group, inversely correlated with age and dyspnea severity and positively correlated with FEV1 and exercise capacity. However, other researchers have used more accurate methods for assessing metabolic rate in COPD. Using indirect calorimetry adjusted for the influence of fat-free mass, Creutzberg et al. have proved that hypermetabolism commonly occurs in COPD but cannot be linked to aging and fat-free mass depletion (17).

CONCLUSIONS

Although more than a half of our COPD patients are normal weight, they may present important disturbances in body composition. Lean body mass, dry lean mass and body water are significantly lower in COPD patients than in healthy subjects. These significantly correlate with age, dyspnea, severity of airway obstruction, and exercise capacity in patients with chronic obstructive lung disease.

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NEWS

INCREASED RISK OF ACUTE PANCREATITIS BY ORAL CORTISONE TREATMENT

Acute pancreatitis is a common disease of the pancreas favored by the lithiasis, smoking, consumption of alcohol and medication, such as oral cortisone used to treat asthma and autoimmune diseases. In 15% of cases, the disease can threaten the patient's life. Into a recent study Dr Omid Sadr-Azodi and colleagues demonstrated the relationship between administration of cortisone and acute pancreatitis. The results show that treatment with oral cortisone determined a 70 percent higher risk of developing acute pancreatitis. The researchers found that the risk of acute pancreatitis was highest 4 to 14 days after drug dispensation and attenuated thereafter. Also, the people who used aerosol cortisone have not manifested an increased risk for acute pancreatitis. They concluded that the current oral glucocorticoid use is associated with an increased risk of acute pancreatitis (Sadr-Azodi O, Mattsson F, Sjöberg Bexelius T et al. Association of Oral Glucocorticoid Use With an Increased Risk of Acute Pancreatitis. A Population-Based Nested Case-Control Study. *JAMA Intern Med.* 2013;():1-6. doi:10.1001/jamainternmed.2013.2737).

Cătălina Luncă

IMPACT OF CLOPIDOGREL RESPONSE ON THE CLINICAL EVOLUTION IN PATIENTS WITH ACUTE CORONARY SYNDROMES

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IMPACT OF CLOPIDOGREL RESPONSE ON THE CLINICAL EVOLUTION IN PATIENTS WITH ACUTE CORONARY SYNDROMES (Abstract): **Aim:** the purpose of the present study was to determine the relationship between the Clopidogrel genetic response (based on laboratory specific tests) and the worse evolution of the patients with acute coronary syndromes. **Material and methods:** The study included 80 patients (11 females and 69 males), aged between 45 – 85 years, admitted in the 1st Department of Cardiology, between January – June 2012, diagnosed with acute coronary syndromes (especially unstable angina). All the patients received Clopidogrel as antiplatelet agent, in some cases associated with aspirin. The diagnosis of acute coronary syndrome was based on clinical, ECG, echocardiographic and enzymatic signs. All the patients accepted to be included in the present study and signed a consent formulary. The Clopidogrel resistance was determined by specific genetic laboratory tests. **Results and discussion:** Of 80 cases, 4 patients were identified as normal responders (5%), 3 patients were low responders (3.75%), 58 patients were high responders (72.5%) and 15 patients were unpredictable (18.75%). In the high responders' group a lot of hemorrhagic disorders in different territories were identified. Normal responders had a good evolution. In patients with low response and unpredictable status the recurrence of angina and left ventricular failure as complications were frequent. **Conclusions:** The present study allowed a positive correlation between the haemorrhagic events and the high responder status to Clopidogrel. Low responder patients or those with an unpredictable phenotype had a worse evolution caused by the recurrence of angina or left ventricular failure. **Keywords:** CLOPIDOGREL, GENETIC RESISTANCE, ACUTE CORONARY SYNDROMES.

The concept of resistance to Clopidogrel dates a few years ago and has two implications: a clinical one, related to the unfavorable evolution and prognosis of some of the patients with acute coronary syndromes (ACS) and a second one, with practical implications, that enforces introduction on the market of some new antiplatelet agents, without the resistance phenomenon mentioned above (1, 2, 4, 6, 14).

Recent studies have shown that approximately 31% of the patients with ACS present resistance to Clopidogrel, defined as a weak response concerning platelet clotting, this category of patients having an unfavorable clinical evolution and prognosis.

Patients with early intrastent thrombosis are included in this category (3, 8, 11).

Of the liver enzymes involved in this process, a key role is held by CyP2C19 cytochrome, which has many different isoforms responsible for the variation of its catalytic activity (5, 7, 9, and 10). These variations of the catalytic activity determine variations of the conversion process of Clopidogrel into active metabolites (12, 13). According to the Clopidogrel conversion speed, patients could be slow or rapid metabolizes. Thus, some patients could have hypo response concerning the blocking of platelet clotting (followed by increased risk of thrombosis), whereas others have hyper response (clinically expressed as high hemorrhage risk) (15, 16, 17).

More importantly, the so-called "genetic resistance" to Clopidogrel is followed by dramatic increasing of stent thrombosis incidence in patients that receive Clopidogrel (2, 3, 5).

The study aimed to evaluate the degree in which the genetically determined response to Clopidogrel (demonstrated by specific lab tests) influences the evolution of patients with acute coronary syndromes.

MATERIAL AND METHODS

The study included 80 patients (11 females and 69 males), aged between 45 – 85 years, admitted in the 1st Department of Cardiology, between January – June 2012, diagnosed with acute coronary syndromes (in most of the cases different forms of unstable angina) that have accomplished the study inclusion criteria.

The acute coronary syndrome diagnostic criteria needed for patients' inclusion were the following ones:

- Clinical – anterior chest pain with angina characteristics that followed the criteria for unstable angina or important angina crisis;
- Paraclinical – suggestive electrocardiographical changes – subendocardic or subepicardic ischemic lesions or recent branch block associated with changes of myocardic cytolysis enzymes.

Patients with uncertain acute coronary syndrome criteria, those without Clopidogrel therapy, patients with contraindication of platelet antiagregant therapy (e.g., antecedents of hemorrhagic accidents or known hemorrhagic diatheses, previously known liver disease) as well as patients with psychiatric disorders or noncompliant ones were excluded.

The paraclinic investigation aimed to:

- Investigate associated risk factors: blood sugar, uric acid, complete lipid profile;
- Complete functional evaluation of the key organs involved in hypertensive and/or diabetic patients (renal function, electrolytes);
- Electrocardiogram 12 derivations – both for positive diagnosis of acute coronary syndrome and for diagnosis of different complications (e.g. rhythm and/or conduction disturbances), as well as for follow-up;
- Echocardiogram – 2D, M and Doppler – the following parameters were checked: LV and LA size, wall thickness (to appreciate left ventricular hypertrophy), systolic left ventricular function (expressed as ejection fraction and LV shortening), LV diastolic function, regional kinetic changes of LV, valvular regurgitation and other complications (intracavitar thrombus).

The patients were initially evaluated (at

the inclusion), following the complete check-up mentioned above. The final decision regarding study inclusion was taken after having a precise diagnosis and therapeutic scheme.

All the patients included in the study received platelet antiagregant therapy with Clopidogrel associated sometimes with Aspirin. In some cases the patients already had Clopidogrel therapy when included in the study, whereas for others Clopidogrel was started at the inclusion moment.

All the patients were previously informed about the aim of the study, those who agreed to the inclusion in the study signed an informed consent and then a blood sample was taken for DNA extraction.

Two samples of 2 ml peripheral blood were taken for every patient.

The anti-clotting agent was EDTA. The DNA extraction from integral blood was done -on the same day. Two extractions were done simultaneously, one for every sample taken.

The DNA extraction was performed using Wizard Genomic DNA Purification – Promega kit.

The extraction process followed 5 steps:

1. Red blood cells lysis;
2. White blood cells and nuclei lysis;
3. Cellular proteins precipitation;
4. Genomic DNA precipitation;
5. DNA rehydratation.

After rehydratation the DNA was quantified, aliquot and labeled in order to make a genomic DNA collection in the Molecular Biology Lab.

A part of the DNA was stored at -20°C in order to follow PCR experiments. The other samples were frozen at –80°C (long time storing).

TaqMan assay genotyping method used for the aimed SNPs is based on allelic discrimination by Real-Time PCR in TaqMan system.

Our study used specific probes for mutant alleles *2A, *3, *17 of the CYP2C19 gene versus specific probes for the wild allele, as well as probes for mutant allele 3435C>T versus wild type allele of the ABCB1 gene.

Work protocol: Amplification was done using Real Time PCR MX3005P Stratagene / Agillent termocycler.

Alongside the optimization and the performing of testing using TaqMan Assay, we have tried to identify an alternative method, at lower costs, that could discriminate alleles with more than 90% confidence and we have decided to use High Resolution Melting (HRM).

HRM is a technique that consists in the postPCR amplification analysis of the melting curves of the fragments of interest (amplicons).

In our study we have achieved HRM genotyping for all ABCB1c polymorphisms C3435T (rs1045642), CYP2C19*2 (c.G681A; rs4244285), CYP2C19*3 (c.G636A; rs4986893), and CYP2C19*17 (c.C806T; rs12248560).

RESULTS AND DISCUSSION

Our study includes a group of 80 patients (11 females and 69 males), aged 45-85 years old, admitted in the 1st Department of Cardiology, during January – June 2012, diagnosed with acute coronary syndromes (most of the cases with different forms of unstable angina) that have accomplished the study inclusion criteria.

Our study has achieved genotyping by means of the TaqMan system for all the patients and by means of HRM for 36 samples.

In the case of TaqMan genotyping, allelic discrimination is presented in Table I.

Using HRM we have got very good results (regarding amplification and results provided) for polymorphisms CYP2C19*2 and CYP2C19*17. For these polymorphisms the primers have functioned and we have got specific amplicons (without non-specific links and dimerization) and the results correlated 100% with the TaqMan genotyping. Allelic discrimination confidence exceeded 95%.

TABLE I.
Genotype frequencies for the studied genes and mutant alleles

Polymorphism	Genotype	Number of samples	Frequency
ABCB1 c.C3435T	CC	12	15%
	CT	48	60%
	TT	20	25%
	Allele T	55%	
CYP2C19*2,c.G681A	GG	62	77.5%
	GA	15	18.75%
	AA	3	3.75%
	Allele A	15.3%	
CYP2C19*3,g.-806C>T	CC	80	100%
	CT	0	0%
	TT	0	0%
	Allele T	0%	
CYP2C19*17, c.C806T	CC	4	5%
	CT	27	33.7%
	TT	49	61.25%
	Allele T	78.1%	

For alleles CYP2C19*3 and ABCB1 primers were not good enough and new steps of optimization will be needed.

Starting from the literature data, according to the genotyping variant of the studied alleles for gene CYP2C19, the patients were divided in several prediction categories:

1. *Normal metabolizer (N):* wild-type CYP2C19*2*3*17;

2. *Intermediate metabolizer (MI):* heterozygote for *2 and/or *3 and wild-type *17;

3. *Slow metabolizer (MS):* *2/*2, *2/*3, *3/*3, indifferent of also being heterozygote or homozygote for *17;

4. *Very quick metabolizer (MFR):* homozygote or heterozygote for *17 and wild-type for *2 and *3 ;

5. *Unpredictable metabolizer (MN):* heterozygote or homozygote for *17 and heterozygote for *2 and/or *3.

Thus, in our group study, we have identified 4 patients with normal metabolizer phenotype (5%), 3 slow metabolizer patients (3.75%), 58 patients with very quick metabolizer phenotype (72.5%) and 15 patients with unpredictable phenotype (18.75%). We didn't identify any patient with intermediate phenotype.

From the point of view of the clinical evolution, the 3 patients with slow metabolizer phenotype presented repeated episodes of intensified angina pain that needed

frequent admissions and revising of therapy scheme (increasing of nitrate and beta-blocker dose). One of the patients developed ischemic dilative cardiomyopathy, with dilated LV and important decrease of the ejection fraction. Two of the patients were sent to interventional cardiology services for coronarography and trans-luminal angioplasty with stent.

Of the 15 patients with unpredictable phenotype, 10 registered favorable evolution during the admission to hospital under therapy, without repetition of the angina pain and no other complications. The check-up after 3 months showed optimal clinical and functional parameters. 5 of the patients with unpredictable phenotype presented readmissions during the follow-up period due to the new angina pain with characteristics of unstable angor, fact that led to therapy scheme changing. 4 of these patients presented LV failure (cardiac asthma – 3 cases and acute pulmonary edema – 1 case). One of the patients (83 years old) presented a peripheral arterial embolus (in conditions of sinusal rhythm), that needed hospitalization in a vascular surgery service and emergency embolectomy. Favorable evolution was recorded later.

For the patients with rapid metabolizer phenotype we have recorded the following events:

- Prominent macroscopic haematuria (2 males aged 75 and 82 years respectively) – both needed change of therapeutic scheme without Clopidogrel;
- Cutaneous ecchymosis – 7 cases (4 females, 3 males);
- Cutaneous purpura – 1 female, associated with thrombocytopenia - 75,000/mm^3;
- Gum hemorrhage, independent of other causes – 2 cases of females. Bleeding stopped after excluding Clopidogrel from the therapeutic scheme.
- Superior digestive hemorrhage – melena – 1 male case in whom Clopidogrel was associated with Aspirin. No hemodynamic degradation was recorded in evolution.
- Haemoptysis – 1 male case (75 years old).

For the rest of the patients in this subgroup we didn't record hemorrhagic events during the follow-up period, but 15 patients needed readmission due to the aggravation of the pectoral angina (6 patients) and for left ventricular failure phenomena (12 patients).

The 4 patients with normal metabolizer phenotype had normal evolution, without complications during the follow-up period.

CONCLUSIONS

Although done on a relatively small group of patients, the study allowed a clear positive correlation between Clopidogrel rapid metabolizer phenotype and frequency of nonfatal hemorrhagic events. Hemorrhagic events which appeared during the Clopidogrel therapy are not important, but can manifest differently, in different areas (skin, oral mucosa, digestive tract, urinary) and disappear when therapy is stopped. The rapid metabolizer phenotype doesn't offer total protection against repetition of ischemic episodes. Moreover, all the patients with slow metabolizer phenotype had recurrent angina pain and needed emergency interventional cardiology services. Patients with unpredictable phenotype may have unpredictable evolution, some without angina events, some with recurrence and aggravation of the angina, evolution that certainly involves other factors.

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INFECTIVE ENDOCARDITIS IN NON-HIV IMMUNOSUPPRESSED PATIENTS

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INFECTIVE ENDOCARDITIS IN NON-HIV IMMUNOSUPPRESSED PATIENTS (Abstract): Infective endocarditis is a septic illness with sudden or insidious onset that causes heart lesions or aggravates the preexisting ones; during its course it can affect other organs, mortality being high. **Aim:** To identify non-HIV immunosuppressed patients with infective endocarditis, namely those with associated diabetes, hepatitis, cancer, or old age with multiple comorbidities, and to assess the nosocomial risk by Carmeli score. **Material and Methods:** This is a retrospective study using epidemiological, clinical, laboratory and treatment data from 56 patients admitted to and diagnosed with infective endocarditis at the Iasi Infectious Diseases Hospital in the interval January 2008 - May 2012. To these patients we looked for non-HIV immunosuppressed character, having in their pathology association diabetes, chronic hepatitis, malignancy, or other comorbidities. **Results:** From the infective endocarditis patients, 92.8% were found non-HIV immunosuppressed, 25% of them presenting renal failure, and 21.1% having diabetes. The most common Carmeli score obtained was score 2 in 57.1% of the cases. In these patients, the most frequent etiologic agent of infective endocarditis was *Enterococcus faecalis* (16%), followed by *Staphylococcus aureus* (10.7%). **Conclusions:** It was noted that in addition to endocardial damage due to the multiple etiologic agents involved, non-HIV immunosuppression in these patients plays an important role. The association of Carmeli score in patients diagnosed with infective endocarditis is significant, especially in case of adequate therapy initiation for a right treatment of this pathology. **Key words:** INFECTIVE ENDOCARDITIS, DUKE CRITERIA, CARMELI SCORE, NON HIV IMMUNOSUPPRESSION

Sepsis, a life-threatening medical condition, is characterized by whole-body inflammatory state (the so-called systemic inflammatory response syndrome or SIRS) and the presence of a known or suspected source of infection (1, 2). Worldwide, sepsis affects 18 million people every year. In the USA, organ dysfunction occurs in 300/100,000 inhabitants, and mortality from severe sepsis reaches 28%, in other words more than 500 patients die daily from severe sepsis. Recent studies have shown that in Europe the incidence of sepsis in intensive care units is 25% of admitted patients to which other 13% cases of nosocomial infections add up despite the progress made lately

in critical patient care (3).

Cardiac involvement in sepsis is especially due to the circulatory changes induced by chemical mediators and myocardial depressant factors, but also directly by the location of infection at this level: endocarditis, myocarditis, pericarditis, pancarditis (3).

Infective endocarditis (IE) is a septic illness with sudden or insidious onset that causes heart lesions or aggravates the preexisting ones, and which during its course can affect other organs (4). This particular form of systemic infection with unlimited course, in which the primary focus of germs multiplication is the endocardium, still has a great impact in the adult population and not only (5).

In the past 50 years major changes in infective endocarditis have occurred. Thus, there is a significant decrease in mortality, the etiologic spectrum has changed, new antibiotics and new laboratory techniques are now available, echocardiography has improved, and the susceptible population has changed the proportion of persons that are immunocompromised, have prosthetic valves or pacemakers increasing to the detriment of those with rheumatic valvular disease.

The incidence of infective endocarditis remains high, 1.7-6.2/ 100,000 persons / year in the U.S. and Europe, and the yearly mortality is of about 40% (6).

There is a wide diversity of involved etiological agents, these varying depending on valve type (native or prosthetic) and infection type (nosocomial or community acquired). Most infective endocarditis (> 80%) are determined by: *Streptococcus viridans* (30-40%), *Enterococcus species* (5-18%), *Staphylococcus speciae* (*Staphylococcus aureus*, *Staphylococcus epidermidis*: 10-27%), *Escherichia coli* (1.5 to 13%), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and Gram-negative bacteria of the HACEK group, responsible for 1-4% of endocarditis (3).

In the studies carried out during the last 4 decades, the risk factors for infective endocarditis were mainly represented by valvular degenerative injuries and heart surgery resulting or not in valve prosthesis, but, over time, with the advent of intravenous drugs, intravascular prostheses, nosocomial exposure, hemodialysis, and age-related valve sclerosis, more common than cardiac rheumatic diseases especially in developed countries, these underwent some changes (7).

Given the recent major changes in infective endocarditis epidemiology and bacteriological profile with strains resistant to various antibiotics, the use of Carmeli score, especially in immunodepressed patients or in those with multiple severe comorbidities became a must (8, 9).

Carmeli score, used to choose the appropriate antibiotic therapy, introduces a new term – health care associated infection, in addition to the two classic types: community acquired and nosocomial. It includes three parameters: contact with the health system, previous antibiotic treatment, and patient characteristics, the highest numeric value representing the value of the score (10).

MATERIAL AND METHODS

We conducted a retrospective study using epidemiological, clinical, laboratory and treatment data from 56 patients admitted to and diagnosed with infective endocarditis at the Iasi Infectious Diseases Hospital in the interval January 1, 2008 - May 31, 2012. Given the association in these

patients of diabetes, malignancy, or other severe comorbidities, we investigated them for non-HIV immunosuppression.

The diagnosis of endocarditis was established based on clinical picture, laboratory data, and Durack/Duke diagnostic and classification criteria (presence of positive blood cultures and echocardiographic appearance of valvular involvement – vegetations as major criteria, and minor criteria).

The non- HIV immunosuppresssed patients, namely those who besides infective endocarditis presented diabetes, chronic hepatitis, neoplasm, and old age, were subsequently identified.

In all these patients, Carmeli score was used to assess the nosocomial risk, according to the following algorithm:

Nosocomial risk assessment (Carmeli score)

A. Degree of contact between patient and the health care system:

1. without contact

2. contact (admissions, nursing home resident) without invasive procedures

3. admissions / prolonged contact with invasive procedures

B. Antibiotic treatment:

1. without antibiotic treatment

2. recent antibiotic therapy

C. Patient characteristics:

1. young without comorbidities

2. elderly + / - multiple comorbidities

3. immunosuppressed AIDS, COPD, malignancies, other causes of immune-depression.

RESULTS AND DISCUSSION

In the interval January 1, 2008 - May 31, 2012, at the Iasi Infectious Diseases Hospital 56 patients with infective endocarditis diagnosed according to the above mentioned criteria were admitted and treated. The yearly distribution of infective endocarditis cases during the study interval was as follows: most cases were recorded in 2010 (19 cases), 11 cases each in 2008 and 2009, 10 cases in 2010, and by May 2012 5 confirmed cases.

The patients were adults aged between 22 and 82 years, mean age 50 years, with most cases belonging to the age group 40-66 years (39 cases). Patients were predominantly males (44 cases), and from urban areas (31 cases).

A positive diagnosis of infective endocarditis was made according to Durack-Duke criteria. The patients presented specific signs and symptoms for infective endocarditis, in line with the inflammatory syndrome detected by laboratory investigations: presence of neutrophilic leukocytosis in the blood count, ESR, fibrinogen, elevated C reactive protein in 58% of cases. Also, a febrile syndrome was present in almost all cases (93%).

Blood cultures were positive in 46.4% of cases. The identified etiologic agents were: *Enterococcus spp., Staphylococcus spp., Streptococcus spp., E. coli, Klebsiella spp. Achromobacter spp.* In 53.6% of cases, blood cultures remained negative (tab. I).

The second major criterion for establishing a diagnosis of certainty of infective endocarditis was transthoracic/transoesophageal echocardiography the target of which is the identification, characterization, and localization of valvular vegetations, the pathologic hallmark of endocarditis. Thus, vegetations of different size and appearance, located on different valves were detected in 96.4% of cases. In only 3.5% of the cases the appearance was normal (tab. II).

The next step was to identify non-HIV immunosuppression in the patients diag-

nosed with infective endocarditis and presenting multiple associated comorbities. Thus, of a total of 56 patients diagnosed with infective endocarditis, 52 patients (92.8%) were found non-HIV immune-depressed (tab. III).

TABLE I

Distribution of positive blood cultures with identified etiologic agents and negative blood cultures

Blood cultures	Cases	
	Number	Percentage
Positive blood cultures	**26**	**46,4**
Enterococcus faecalis	9	16
Staphylococcus aureus	6	10.7
Streptococcus spp.	5	8.9
Escherichia coli	3	5.3
Klebsiella ozaenae	2	3.5
Achromobacter specialis	1	1.7
Negative blood cultures	**30**	**53,6**

TABLE II

Echocardiographic distribution of vegetations

ECHOCARDIOGRAPHIC LOCATION OF VEGETATIONS	**Cases**	
	Number	**Percentage**
Valvular location	**54**	**96,4**
Mitral valve	20	35.7
Aortic valve	13	23.2
Aortic and mitral valve	11	19.6
Tricuspid valve	3	5.3
Pulmonary valve	3	5.3
Location of mitral valve prosthesis	2	3.5
Location on pacemaker electrodes	2	3.5
Normal appearance	**2**	**3.5**

TABLE III

Distribution of non-HIV immunosupression cases

NON-HIV IMMUNOSUPRESSION IN IE PATIENTS	**Cases**	
	Number	**Percentage**
Acute/chronic renal failure	13	25
Insulin-dependent diabetes mellitus	11	21.1
Active chronic hepatitis B +D, C	10	19.2
Chronic heart insufficiency NYHA classified	10	19.2
COPD	5	9.6
Neoplasms	3	5.7

The nosocomial risk was assessed by using Carmeli score. Following this scoring system we found 1 (1.7%) infective endocarditis score 1 (community acquired), 32 (57.1%) score 2 (associated to health care or community acquired but with increased prediction of multidrug resistance), and 23 (41%) score 3 (usually nosocomial), (tab. IV).

The number of hospital stay days in patients with Carmeli score 2 and 3 ranged between 14 and 44 days, they requiring a long-term and targeted antibiogram-guided therapy after culture positivation.

TABLE IV

Nosocomial risk assessment – Carmeli score – in patients with infective endocarditis and non-HIV immunosuppression

Carmeli score	Cases	
	Number	Percentage
SCORE 1	1	1.7
SCORE 2	32	57.1
SCORE 3	23	41

CONCLUSIONS

It was noted that in addition to endocardial damage due to the multiple etiologic agents involved, non-HIV immunosuppression in these patients is an important presence. The association of Carmeli score in patients diagnosed with infective endocarditis is significant with respect to antibiotic resistance; especially when adequate therapy aimed at correctly treating this disease is initiated. Clinical impairment and the need for surgical treatment in some cases require a good knowledge of infective endocarditis assessment, both in terms of associated comorbidities and nosocomial risk.

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TUMORI OVARIENE SEROASE ŞI MUCINOASE. STUDIU CLINICOPATOLOGIC A 116 CAZURI

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SEROUS AND MUCINOUS EPITHELIAL OVARIAN TUMORS – A CLINICOPATHOLOGIC STUDY OF 116 CASES (Abstract). Surface epithelial tumors are the most important group of neoplasm of the ovary. These tumors are classified according to the following parameters: cell type, pattern of growth, amount of fibrous stroma, atypia and invasiveness. **Aim**: This study aimed to identify ovarian tumours, especially *borderline* type, by applying last parameters of criteria for histologic diagnostic of ovarian neoplasms. **Material and methods:** The retrospective study included 116 patients with ovarian tumors diagnosed, investigated, surgically treated at the Obstetrical and Gynecological Hospital Botoşani in the past 9 years (2002 - 2010). In this interval were recorded 116 cases of epithelial ovarian tumors. In sample processing, we used formalin for fixation, paraffin for specimen embedding and hematoxilin-eosin for staining. The age of the patients ranged between 19-86 years. **Results:** From 116 cases, 57 (49,1%) were benign, 40 (34,5%) malignant and 19 (16,4%) *borderline*. In our study of benign neoplasms, we included 37 cases of serous tumours (64,9%) and 20 cases of mucinous types (35%). In the group of *borderline* tumours, the serous type was the most frequent (13 cases), followed by mucinous types (6), including 5 cases of intestinal type and 1 of endocervical pattern. The malignant tumors, according with decreasing order of frequency, were the following: 19 serous (47,5%), 8 mucinous (20%), 11 endometroid type (27,5%) and one of each cases of clear cell and small cell tumors (2,5%). **Conclusions:** Of great interest in our research was to identify ovarian *borderline* tumours, still representing a controversial group, in the same time corresponding with a scientiphic progress. Because the spectrum of epithelial tumors is very large, extensive sampling is essential to carry out all these important determinations, because benign, *borderline* and malignant patterns can be found in the same tumor. **Key words**: SEROUS TUMORS, MUCINOUS TUMORS, BORDERLINE PATTERNS

Tumorile epiteliale reprezintă 90% dintre neoplaziile ovariene. Majoritatea se dezvoltă din celulele epiteliului de înveliş, chisturile de incluzie şi, rareori, din focare de endometrioză. Tumorile epiteliale sunt clasificate în funcție de tipul celular, tabloul arhitectural, cantitatea de stromă, gradul de atipie şi caracterul invaziv. Cele mai frecvente sunt de tip seros (46%), urmate de cele de tip mucinos (36%), endometroi-

de (8%), cu celule clare (3%), tranziționale (2%), nediferențiate (2%), mixte (3%) (1,2). Din punct de vedere al caracterului invaziv, tumorile ovariene se clasifică în benigne, *borderline* şi maligne.

Tumorile *borderline* constituie o entitate controversată în patologia neoplazică ovariană. Histologic, acestea prezintă unele caractere de malignitate (atipie celulară moderată, stratificare epitelială, activitate mitotică redusă) dar nu prezintă invazie stromală. Evoluția clinică a acestor tumori este cel mai adesea benignă, excepție făcând cele care prezintă implanturi peritoneale invazive care pot evolua similar tumorilor maligne (2).

Scopul acestui studiu îl reprezintă aprecierea valorii criteriilor histopatologice pentru diagnosticul tumorilor ovariene în colorație uzuală hematoxilină-eozină şi evidențierea caracteristicilor histopatologice ale tumorilor *borderline* în tabloul neoplazic ovarian.

MATERIAL ŞI METODE

Studiul retrospectiv a fost efectuat pe un lot de 116 cazuri de tumori ovariene epiteliale investigate şi operate la Spitalul de Obstetrică şi Ginecologie Botoşani între anii 2003-2010. Vârsta pacientelor a fost cuprinsă între 19 şi 86 ani.

Am efectuat o prelevare extensivă a fragmentelor tumorale, în special din ariile adiacente epiteliului superficial, ariile solide, formațiunile papilare şi baza acestora. Numărul de blocuri prelevate din fiecare tumoră a variat în funcție de dimensiunea tumorii şi aspectul macroscopic, în total au fost 843 blocuri.

Pentru diagnosticul anatomo-patologic am folosit tehnica uzuală: fixarea în formaldehidă 10%, includerea în parafină, secționarea la 5 microni şi colorația hematoxilină-eozină.

REZULTATE

1. Distribuția pe categorii de tumori

Din cele 116 cazuri investigate, 57 au fost cu tumori benigne (49,1%), 40 maligne (34,5%) şi 19 tumori ovariene *borderline* (16,4%). În cazul tumorilor epiteliale benigne, 64,9% au fost seroase şi 35% mucinoase. 19 dintre tumorile maligne identificate de noi au fost seroase (47,5%), 8 mucinoase (20%), 11 endometroide (27,5%), una cu celule clare (2,5%) şi 1 caz de carcinom cu celule mici (2,5%).

2. Tumorile seroase ovariene (69 de cazuri) au avut următoarea reprezentare: 37 (53,7%) benigne, 13 (18,9%) *borderline*, şi 19 (27,4%) maligne. 18,1% dintre tumorile seroase au avut afectare bilaterală.

Tumorile seroase benigne au prezentat aspecte microscopice variate: chisturi delimitate de epiteliu de tip tubar, unistratificat (chistadenom), stromă fibroasă (chistadenofibroame), atipie nucleară minimă sau absentă, mitoze rare. Rar, epiteliul a prezentat papile neramificate (chistadenom papilar).

Tumorile seroase borderline, identificate de noi în 13 cazuri, au fost în 92,3% din cazuri unilaterale şi în 7,3% din cazuri cu localizare bilaterală. La aceste paciente nu s-au evidențiat implanturi peritoneale.

Criteriile de diagnostic aplicate de noi tumorilor seroase *borderline* au fost: hiperplazie epitelială sub formă de pluristratificare, micropapile, aspecte cribriforme sau grupuri de celule, atipie uşoară sau moderată, cuiburi de celule detaşate intrachistic, activitate mitotică variabilă, de obicei minimă, prezență de corpi psamomatoşi. Proliferarea celulară variază de la celule mici, uniforme, cu nuclei hipercromatici, la celule mari, cu citoplama eozinofilă.

În cazul tumorilor *borderline* investigate de noi am indentificat următoarele aspecte histopatologice: absența invaziei stromale la toate cazurile, hiperplazie epitelială cu proliferare papilară cu aspect ramificat (92,3% dintre cazuri), proliferare glandulară (46,1%), cuiburi sau celule izolate detaşate (92,3%) (fig.1), celule detaşate intrachistic cu citoplasma eozinofilă şi nucleul hipercromatic (61,5%). Constant, am găsit atipie celulară uşoară sau moderată (fig. 2) şi un număr mic de mitoze. Un singur caz (7,6%) a prezentat aspect de proliferare epitelială cu arhitectură cribriformă. Am identificat corpi psamomatoşi în 15,4% dintre tumorile *borderline*. În 23% dintre cazuri, aspectele *borderline* au apărut pe arii mici, coexistând cu aspecte benigne.

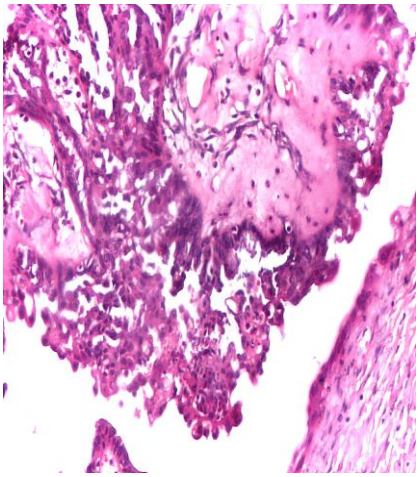

Fig. 1. Tumora seroasa *borderline* cuiburi celulare detaşate (HEx10)

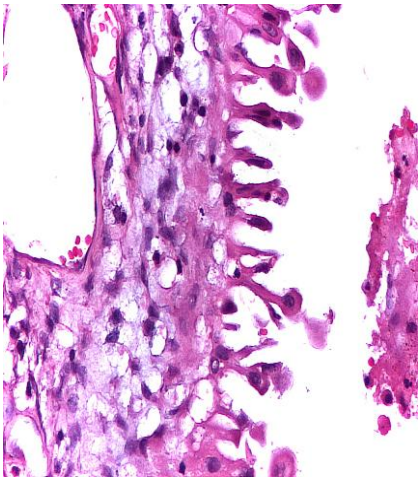

Fig. 2. Celule cu citoplasma eozinofilă, atipie nucleară moderată (HEx20)

Tumorile seroase maligne, în număr de 19, au fost reprezentate de chist-adenocarcinoame seroase slab diferențiate (63,2%) şi bine diferențiate (36,8%), distincție realizată în funcție de aspectul arhitectural, atipia citologică şi numărul de mitoze. Microscopic, modul de creştere al acestor tumori a fost sub formă de glande, papile cu arhitectură complexă, proliferare epitelială solidă cu prezență de spații sub formă de fantă. Au fost prezente atipiile celulare, şi caracterul invaziv. 26% dintre aceste tumori au prezentat corpi psamomatoşi.

3. Tumorile mucinoase identificate de noi au inclus cazuri benigne (58,8%), *borderline* (17,6%), maligne (23,6%).

Tumorile benigne au fost reprezentate de 18 chistadenoame mucinoase de tip intestinal, cu prezență de celule caliciforme, 2 cazuri de chistadenoame mucinoase de tip endocervical cu celule cilindrice, nuclei bazali şi o cantitate crescută de mucină intracitoplasmatică. Aceste tumori au prezentat o stratificare minimă sau absentă şi atipie celulară redusă sau absentă.

Cele 6 cazuri de *tumori borderline mucinoase* au fost diagnosticate ca fiind 5 de tip intestinal, unilateral, şi un caz de tip endocervical, unilateral. Tumorile *borderline* mucinoase de tip intestinal au prezentat papile filiforme intrachistice, unele cu aspect coalescent la nivelul extremității

distale, epiteliu de tip intestinal, cu prezență de celule caliciforme, stratificare celulară redusă la 2-3 straturi, atipie redusă, mitoze rare. Epiteliu cu aspect benign a coexistat la nivelul tumorilor polichistice. Tumora *borderline* mucinoasă de tip endocervical a prezentat proliferări papilare intrachistice acoperite de epiteliu mucosecretor de tip endocervical cu atipie moderată, celule detaşate intrachistice, infiltrat inflamator cu polimorfonucleare şi mitoze rare.

Tumorile maligne mucinoase au fost reprezentate de chistadeno-carcinoame mucinoase de tip intestinal. Microscopic, aceste tumori au prezentat proliferări glandulare cu arhitectură cribriformă sau cu aspect labirintic, glande cu contur neregulat cu caracter invaziv expansiv, proliferări celulare cu mai mult de 4 straturi, pierderea aspectului mucosecretor, atipie citonucleară. În 4 cazuri (50%) au fost identificate şi arii cu aspect *borderline*.

DISCUȚII

Tumorile epiteliale reprezintă cea mai mare parte dintre neoplaziile ovariene. Acestea se dezvoltă din epiteliul de înveliş al ovarului dar mai ales din epiteliul invaginat ce formează chisturile de incluzie (1,2). Într-un număr mai redus de cazuri, aceste tumori se pot dezvolta din focare de endometrioză: tumori endometroide (3) şi posibil cele mucinoase (2).

După OMS (1), tumorile epiteliale ovariene se clasifică în funcție de tipul celular (seros, mucinos, endometroid, celule clare, tranzitional), tabloul macroscopic (chistic, solid, mixt), proporția stromei fibroase, prezența atipiei, şi după caracterul invaziv.

Nucci et al. (2) recomandă, în cazul ovarului cu aspect macroscopic tumoral, prelevarea unui bloc pentru fiecare 1-2 cm din diametrul maxim al tumorii. Noi considerăm că pentru un diagnostic corect este necesară recoltarea multiplă, în special din ariile solide, ariile adiacente epiteliului de înveliş, formațiunile papilare şi baza acestora.

Tumorile seroase au prezentat în studiul nostru un procent de 59,4%, în timp ce în studiul efectuat de Nucci, acestea reprezintă aproximativ 50% din neoplaziile epiteliale.

Pe lotul analizat de noi, tumorile benigne au reprezentat 53,7% dintre neoplaziile seroase, procent apropiat de cel menționat de Rosai (50%) (3).

După clasificarea OMS, tumorile seroase *borderline* reprezintă aproximativ 15% din tumorile seroase. Acestea apar la vârste cu 10-15 ani mai mici decât în cazul adenocarcinoamelor, respectiv 45 ani versus 60 de ani (2).

În studiul nostru, proporția cazurilor *borderline* în cadrul neoplaziilor seroase a fost de 18,9%. Vârsta pacientelor a fost între 19-70 de ani, cu o medie de vârstă de 44,5 ani.

În literatură (4,5) au fost descrise alte trei variante rare (5-10%) de tumorii seroase *borderline*: varianta micropapilară, cea cu microinvazie, şi respectiv cea cu prezența carcinomului intraepitelial. În forma micropapilară au fost observate papile lungi şi subțiri, aspecte de tip cribriform pe o lungime de 5 mm (1,2), micropapilele fiind acoperite de celule cubice sau rotunde, cu raport nucleu/citoplasmă mărit (6,7). Criteriile microscopice pentru microinvazie au fost: celule citoplasma eozinofilă izolate sau în grupuri mici, neregulate, prezente în stroma fibroasă (8,9), de obicei înconjurate de mici spații libere. Proliferarea epitelială nu trebuie să depăşească 10 mm^2. Am identificat în studiul nostru un singur caz (7,6%) de microinvazie dezvoltat pe o tu-

moră *borderline* seroasă, ceea ce confirmă raritatea acestor leziuni.

Criteriile prognostice cunoscute în cazul tumorilor *borderline* sunt reprezentate de prezența implantelor peritoneale invazive, stadiul clinic şi aspectul histopatologic. Evoluția acestor tumori rămâne neschimbată chiar dacă există focare de microinvazie. Conform aprecierilor lui Rosai (3), prognosticul acestor tumori este mai favorabil (asemănător celor benigne) atunci când aspectele *borderline* apar doar focal într-o tumoră cu aspect benign (chistadenoame cu arii focale proliferative).

În lotul analizat, proporția carcinoamelor seroase (27,4%) a fost apropiată de valorile de referință (35%) (3) din cadrul tumorilor seroase. Ele au prezentat însă un procentaj mai mic (47,5%) comparativ cu alte studii (2, 5), în care ele au reprezentat 75% din cadrul neoplaziilor epiteliale maligne. Acest fapt se datorează faptului că noi am identificat în studiul nostru un număr crescut de tumori endometroide maligne. Aplicând criteriile de malignitate recomandate de OMS (1) şi Silverberg (4) la cazurile studiate de noi, a rezultat că 65% din tumorile maligne seroase au fost de grad înalt, iar 35% au prezentat un grad redus de malignitate.

Tumorile mucinoase, conform recomandărilor OMS (1), se împart din punct de vedere histologic în 2 categorii: tip intestinal şi tip endocervical.

În studiile efectuate de Rosai, tumorile benigne au fost cele mai frecvente neoplazii mucinoase ale ovarului (80%), dar pe lotul examinat de noi, frecvența tumorilor mucinoase benigne a fost mai redusă, în favoarea cazurilor maligne şi *borderline* (58,8%). Cele mai numeroase tumori benigne au fost cele de tip intestinal (90%), în timp ce doar 10% au fost de tip endocervical.

Noi am identificat tumorile *borderline* în procent de 17,6%, similar altor studii (15%) (3, 7). Cele mai frecvente au fost cele de tip intestinal (83%) urmate de tipul endocervical (17%). Toate cazurile de tumori *borderline* intestinale au prezentat şi arii cu aspect benign.

Tumorile mucinoase maligne au reprezentat în studiul nostru 23,5% din cazuri, comparativ cu Nucci et al. (2) unde procentul acestora a fost semnificativ mai mic (5%). Toate carcinoamele mucinoase au fost de tip intestinal iar în 50% din cazuri au fost prezente şi aspecte de carcinom *in situ* sau *borderline*. Carcinomul intraepitelial a prezentat aspecte focale de stratificare celulară intraepitelială cu arhitectură papilară, cribriformă sau solidă, atipie severă şi mitoze frecvente.

CONCLUZII

Tumorile ovariene epiteliale reprezintă o temă de actualitate prin incidența crescută a cazurilor raportate. Studiul nostru a identificat un spectru larg de tumori ovariene epiteliale, atât din punct de vedere al epiteliului proliferat, cât şi al gradului de proliferare celulară şi de atipie citonucleară.

Cele mai frecvente neoplazii epiteliale au fost de origine seroasă. Tumorile *borderline* au reprezentat 16,4% din cazuri şi au fost identificate la paciente cu o medie de vârsta de 44,5 ani.

Cercetările noastre au evidențiat faptul că pentru diagnosticul corect al tumorilor ovariene este necesară prelevarea de numeroase fragmente în cazul formațiunilor intrachistice şi în cazul tumorilor polichistice, pentru că pot coexista, în aceeaşi tumoră, aspecte benigne, *borderline*, microinvazie şi aspecte maligne.

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NOUTĂȚI

CHIRURGIA BARIATRICĂ VERSUS TRATAMENTUL MEDICAMENTOS INTENSIV LA PACIENȚII OBEZI CU DIABET ZAHARAT

Cercetarea continuă studiile observaționale care evidențiaseră ameliorarea controlului diabetului după realizarea chirurgiei bariatrice. Acest studiu randomizat a comparat eficacitatea terapiei medicamentoase față de terapia medicamentoasă în asociere cu procedurile de chirurgie bariatrică, înrolând 150 pacienți obezi, cu diabet tip 2 necontrolat. Vârsta medie a pacienților a fost de 49±8 ani, 66% fiind femei. La intrarea în studiu hemoglobina glicozilată avea valoarea medie de 9,2±1,5%, principalul obiectiv propus fiind scăderea acesteia la valoarea de 6% după 12 luni de tratament. 93% dintre pacienți au fost urmăriți până la finalizarea studiului. Obiectivul primar a fost realizat la 12% dintre pacienții aflați sub tratament medicamentos și la peste 35% dintre pacienții cu terapie de asociere chirurgicală și medicamentoasă (p=0,002). Controlul glicemic s-a ameliorat până la 7,5±1,8% în grupul cu terapie medicamentoasă, 6,4±0,9% în grupul cu by-pass gastric și 6,6±1,1% în grupul cu gastrectomie. Scăderea în greutate a fost mult mai semnificativă la pacienții care au beneficiat de chirurgie (p<0,001). Consumul de medicamente hipoglicemiante și hipolipemiante a scăzut semnificativ la pacienții care au beneficiat de intervenții chirurgicale și au crescut în celălalt lot. Indexul de insulinorezistență HOMA-IR s-a îmbunătățit semnificativ după chirurgie. Aceste rezultate sunt în favoarea tratamentului chirurgical dar trebuie confirmate pe termen lung. (Schauer PR, Kashyap SR et al. Bariatric surgery versus intensive medical therapy in obese patients with diabetes, *N Engl J Med* 2012; 366: 1567-1576)

Corina Dima-Cozma

STUDIU CLINIC DESCRIPTIV PE UN LOT DE PACIENȚI CU GONARTROZĂ, INTERNAȚI ÎN SPITALUL CLINIC DE RECUPERARE IAŞI

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DESCRIPTIVE CLINICAL STUDY ON A SAMPLE OF PATIENTS WITH GONARTHROSIS, ASSISTED IN THE HOSPITAL OF REHABILITATION IASI (Abstract)**: The aim** of our descriptive study was to assess a sample of patients with knee osteoarthritis. **Material and methods**: A sample of 295 patients was assessed regarding demographical features, co morbidities, some laboratory investigations and therapeutically interventions. **Results**: The predominance of females (81.4%), urban area (51.8%), age group over 50 (90.2%), primary bilateral disease (90.1%), associated with hip osteoarthritis. coxarthrosis (13.9%), essential arterial hypertension (10.9%), osteoporosis (9.5%) or obesity (8.1%) were the most frequent features of a patient admitted for knee osteoarthritis. Among therapeutically methods, patients received ultrasounds in 61.1% of cases, and kinetotherapy in 16.6%. **Conclusions**: Our study presented news aspects regarding knee osteoarthritis, in a geographical area of north-east of Romania. **Key words**: GONARTHROSIS, PREVALENCE, PHYSICAL THERAPY.

Gonartroza este o formă frecventă a reumatismului degenerativ cu localizare la nivelul articulației genunchiului, afecțiunea fiind supărătoare şi frecvent invalidantă. Boala este o problemă majoră de sănătate publică, la nivel mondial, precum şi în România, prin frecvența mare la populația de peste 40 de ani, afectând 25-30% din persoanele în vârstă de 45-64 de ani şi peste 60% dintre cele peste 65 de ani (1, 2). Etiologic, gonartroza poate fi primitivă şi secundară. Gonartroza primitivă este mai frecventă la femeie; apare la vârsta de 40-70 de ani. Gonartroza secundară poate fi urmarea unor tulburări ale biomecanicii articulare de origine traumatică, de origine statică sau a unor boli localizate la nivelul genunchiului (3, 4). Tratamentul deosebit de complex şi desfăşurat pe perioade lungi de timp are ca scop combaterea durerii, a inflamației supraadăugate, diminuarea impotenței funcționale, menținerea funcției articulare, micşorarea handicapului şi, în general, ameliorarea calității vieții (1, 3, 4, 5).

MATERIAL ŞI METODE

Studiul clinic de tip descriptiv a fost efectuat pe un lot de 295 pacienți diagnosticați cu gonartroză în variate stadii de evoluție şi care au fost internați şi asistați terapeutic şi evolutiv în Spitalul Clinic de Recuperare Iaşi, în perioada 1 ianuarie – 31

decembrie 2010. Acest lot a cuprins şi reinternările, deci, studiul se referă la prevalența bolii.

Culegerea informațiilor s-a realizat prin evaluarea foilor de observație ale pacienților, precum şi a raportărilor statistice ale Spitalului Clinic de Recuperare, Iaşi. Criteriul de includere în lotul studiat s-a referit la codurile OMS pentru gonartroze ca diagnostic principal de externare, şi anume: M17.0 Gonartroză primara, bilaterală; M17.1 Alte gonartroze primare; M17.4 Alte gonartroze secundare, bilaterale; M17.5 Alte gonartroze secundare.

Pentru prelucrarea şi interpretarea datelor acumulate am utilizat programele computerizate *MS Excel*, *EpiInfo*, iar metodele biostatistice au inclus calcularea prevalenței globale, specifice, a probabilității, regresiei liniare şi tendinței.

REZULTATE ŞI DISCUȚII

1. Structura lotului studiat. Repartiția pe sexe a evidențiat predominanța femeilor (81,4%), cu un raport masculin / feminin de 0,23. Această valoare obținută de noi este în concordanță cu rezultatele altor studii privind predominanța gonartrozei la sexul feminin, cu o diferență semnificativă statistic ($p < 0,001$) (tab. I).

TABELUL I

Repartiția pe sex şi categorii de diagnostic

Diagnosticul - Coduri OMS	Sex feminin	Sex masculin	Total
Gonartroză primara, bilaterală - M17.0	220	46	266
Alte gonartroze primare - M17.1	8	4	12
Alte gonartroze secundare, bilaterale - M17.4	11	4	15
Alte gonartroze secundare - M17.5	1	1	2
Total	**240**	**55**	**295**

Repartiția pe medii de reşedință a pacienților cu gonartroză a fost în favoarea celui urban (51,8%), însă cu diferențe statistic nesemnificative, în toți anii luați în studiu ($p > 0,05$). Această constatare se regăseşte în datele din literatura de specialitate (2, 3, 4, 5), publicate în străinătate, cât şi în România (tab. II).

TABELUL II

Repartiția pe medii de reşedință şi categorii de diagnostic

Diagnosticul - Coduri OMS	Mediul urban	Mediul rural	Total
Gonartroză primara, bilaterală - M17.0	139	127	266
Alte gonartroze primare – M17.1	8	4	12
Alte gonartroze secundare, bilaterale - M17.4	4	11	15
Alte gonartroze secundare - M17.5	2		2
Total	**153**	**142**	**295**

Repartiția pe grupe de vârstă. Gonartroza a fost înregistrată la grupele de vârstă mare şi s-a remarcat faptul că, odată cu înaintarea în vârstă, creşte brusc şi incidența acestei patologii, mai ales după 50 ani, prin cumularea diferiților factori de risc implicați în etiopatogenia bolii. Calcularea regresiei liniare arată faptul că prevalența

cazurilor de gonartroză are o tendinţă crescătoare spre grupele de vârstă de peste 50 ani (90,1%). Date similare au fost menţionate şi în alte cercetări (2, 5), de unde rezultă necesitatea preocupării pentru depistarea precoce a gonartrozei, cu deosebire pentru femei şi grupele de persoane vârstnice (fig. 1).

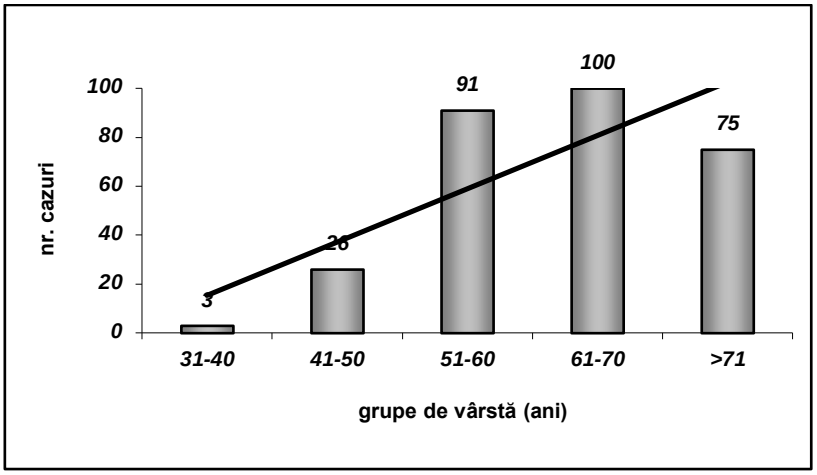

Fig. 1. Repartiţia pe grupe de vârstă a 295 pacienţi cu gonartroză, incluşi în studiul descriptiv

2. Aspecte ale comorbidităţilor la pacienţii din lotul studiat. Din analiza de statistică descriptivă, se constată că prevalenţa specifică a gonartrozei primare, bilaterale, are cea mai mare valoare în lotul studiat (fig. 2). Aceste rezultate nu le putem considera semnificative statistic, însă ele se suprapun peste rezultate asemănătoare ale diferitelor studii de prevalenţă a artrozelor, în variate regiuni geografice (2, 3).

Cele mai importante *patologii asociate* la cazurile cu gonartroză au fost următoarele: hipertensiunea arterială esenţială (HTAE) (10,9%); osteoporoza (9,5%); obezitatea (8,1%); traumatisme locale (gonartroza posttraumatică) (3,7%).

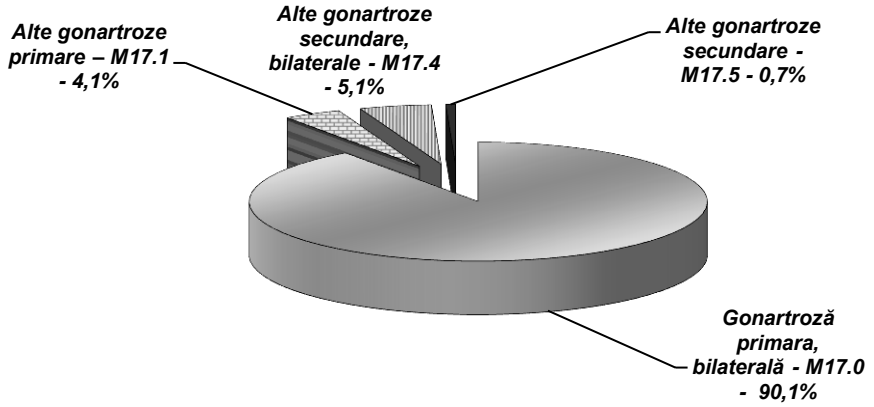

Fig. 2. Distribuţia cazurilor în funcţie de diagnosticul principal de externare

Altă asociere importantă etiologic, medical şi terapeutic, dar şi pentru criteriile de evaluare a calității vieții pacientului, a fost cea dintre gonartroză şi coxartroză (13,9%), fapt menționat şi în lucrări din literatura de specialitate (5, 6) (fig. 3). Datele obținute, referitoare la patologiile asociate gonartrozei, sunt în acord cu cele concluzionate de alți cercetători (2, 4, 5).

Dintre *factorii incriminați în producerea gonartrozei*, cei mai importanți sunt factorii endocrini, boala fiind mai frecventă la femeia după menopauză; obezitatea, deoarece studiile arată că două treimi din bolnavi sunt supraponderali; tulburări venoase, conform cu numeroase studii, mai mult de 35% din bolnavii cu gonartroză au varice la membrele inferioare. Menținerea unei greutăți corporale în limitele normale vârstei, taliei şi sexului pacientului, adoptarea unui regim de viață activ, bogat în exerciții fizice de întreținere a funcționalității articulare şi a tonusului muscular normal; evitarea exercitării unui stres fizic semnificativ si prelungit asupra articulației genunchiului reprezintă câteva din metodele prin care gonartroza poate fi prevenită (2, 3, 4).

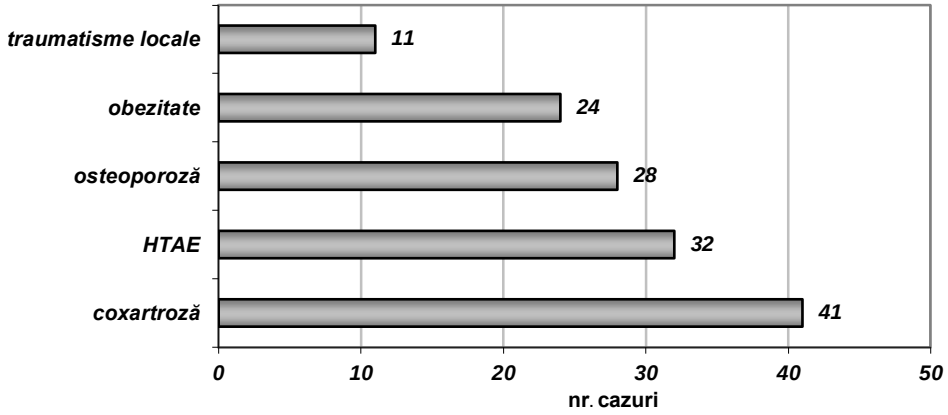

Fig. 3. Distribuția celor mai frecvente comorbidități la pacienții cu gonartroză

3. Investigațiile de laborator de tip biochimic au evidențiat faptul că, din cele 295 probe de sânge pentru glicemie, 19 (6,5%) au avut valori peste 120 mg/dl, din care 3 cazuri bărbați (5,5% din total bărbați) şi 16 cazuri femei (6,7% din total femei). Colesterolemia totală a fost investigată la un sublot de 45 pacienți din lotul total studiat; valori peste 200 mg% au fost observate la 5 cazuri din cei 7 pacienți de sex masculin incluşi în sublot (71,4%) şi la 27 din cele 38 femei (71,1%). Aceste aspecte relevă faptul că pacienții cu gonartroză, luați în studiu, prezintă multiple comorbidități care trebuie luate în considerație pentru alegerea celor mai potrivite metode terapeutice.

4. Unele aspecte privind procedurile terapeutice la bolnavii cu gonartroză. Din lotul de 295 cazuri de gonartroze, am remarcat faptul că la 180 (61,1%) s-a aplicat terapia cu ultrasunete; 173 (58,6%) cea utilizând curenți diadinamici; 150 (53,3%) – TENS, iar kinetoterapie la 49 pacienți (16,6%), dintre care 44 au primit proceduri la nivelul articulației genunchiului şi 21 pacienți la nivelul mușchilor membrelor inferioare (fig. 4).

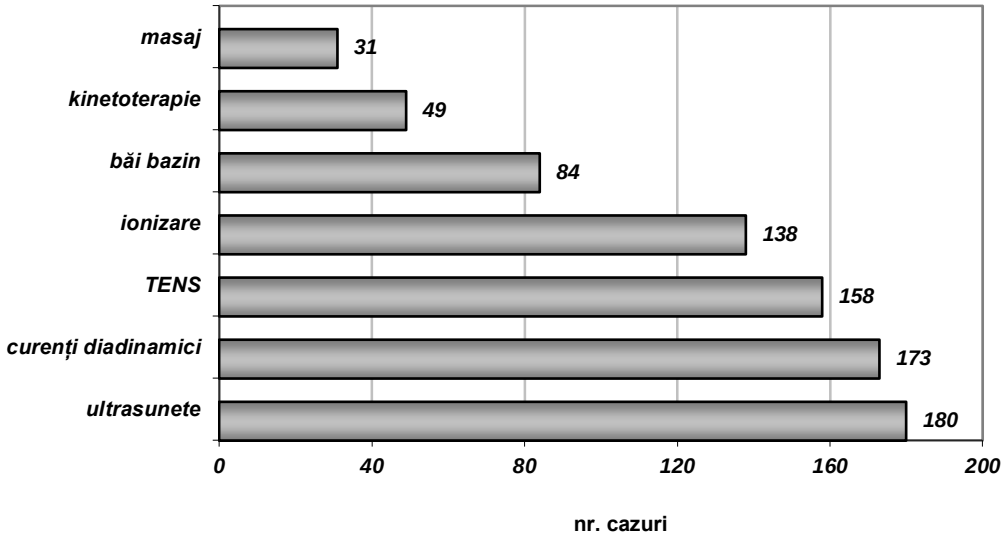

Fig. 4. Distribuția procedurilor fizice aplicate pacienților cu gonartroză primară bilaterală

Exercițiul aerob are indicații în reeducarea genunchiului artrozic. Mersul reprezintă un mijloc eficient pentru genunchiul stabil, urmărindu-se un program gradat, fiecare ședință fiind precedată de exerciții de încălzire, cu mişcări active din toate articulațiile, urmate de *stretching* (7). Mobilizarea articulațiilor învecinate şi tonifierea grupelor musculare ale membrului inferior reprezintă, de asemenea, o prioritate a kinetoterapiei la pacienții cu gonartroză.

Tolerabilitatea terapiei fizice este bună, cu efecte secundare tranzitorii şi uşoare, dar cu beneficii de lungă durată, pentru pacienții cu gonartroză, îmbunătățind semnificativ capacitatea funcțională a articulației şi scăzând simptomatologia dureroasă. Exercițiul fizic poate reprezenta o alternativă utilă la tratamentul farmacologic al gonartrozei, mai ales pentru pacienții care nu tolerează efectele adverse ale medicamentelor (5, 6).

Prognosticul pacienților cu gonartroză depinde foarte mult de evoluția bolii, anterior prezentării la medic şi începerii terapiei. Dacă afecțiunea a evoluat, există riscul ca evoluția să fie influențată de boala de bază, deoarece sunt limitate mişcările în una dintre cel mai importante articulații ale organismului. Din acest motiv, specialiştii recomandă pacienților să se adreseze unui medic, în cazul în care observă modificări locale, la nivelul articulației sau dacă aceasta a devenit dureroasă, iar funcționalitatea ei s-a redus (2, 5).

CONCLUZII

Rezultatele studiului a adus noi informații privind descrierea gonartrozei, utilizând criterii populaționale, dar şi clinice şi terapeutice, care caracterizează populația din nord-estul României. Pacienții incluşi în lotul de studiu au fost urmăriți clinic, imagistic şi evolutiv, pentru a observa, descrie şi analizează impactul metodelor terapeutice utilizate la aceştia asupra răspunsului biochimic şi imunologic. Prin acest studiu, continuat cu rezultatele legate

de corelațiile biostatistice ale răspunsurilor biologice evolutive, vom putea concluziona şi noi dacă variatele proceduri fizice au beneficii asupra gonartrozei, atât imediate, cât şi pe termen îndelungat.

De asemenea, nu trebuie neglijat impactul social, economic, dar şi emoțional familial, al acestei patologii, cu rezultate negative pentru bolnav, familia acestuia, dar şi referitor la costurile economice legate de concediile medicale, pensionări de invaliditate, precum şi scăderea importantă a indicelui de calitate a vieții, odată cu înaintarea în vârstă.

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PULMONARY ARTERIAL HYPERTENSION – A CHALLENGE FOR RESPIRATORY REHABILITATION

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PULMONARY ARTERIAL HYPERTENSION-A CHALLENGE FOR RESPIRATORY REHABILITATION (Abstract): Chronic pulmonary arterial hypertension (PAH) is a severe disease of the pulmonary vasculature, characterized by increased resistance at this level, and increased pulmonary artery pressure, ultimately leading to right heart failure and death. The current therapeutic strategy in PAH includes a stepwise approach, the first step being supportive therapy, supervised rehabilitation program, and psychosocial support, followed by pharmacological treatment according to the vasoreactive or nonreactive type of PAH and prognostic determinants. Small trials have shown that PAH patients experience reduced dyspnea and fatigue, as well as a higher level of physical activity, a greater distance to the 6-minute walk test, and improvement in the quality of life after completing a pulmonary rehabilitation program. Low-level graded endurance and resistance exercises, as well as inspiratory and peripheral muscles training, are recommended, along with careful patients' education regarding their own illness, nutrition, and safe level of physical effort. The optimal type of physical activity, intensity, and duration of training are issues still under debate. **Keywords:** PULMONARY ARTERIAL HYPERTENSION, RESPIRATORY REHABILITATION.

Chronic pulmonary arterial hypertension (PAH) is a severe disease of the pulmonary vasculature, characterized by increased resistance at this level and increased pulmonary artery pressure, ultimately leading to remodeling of the right ventricle, right heart failure, and death (1, 2, 3).

Incidence and prevalence of PAH are difficult to estimate because of the ongoing development of the definition of PAH, current data suggesting the rise of this life-threatening condition. The management of the disease is complex and requires the intervention of a multidisciplinary team including cardiologists, pulmonologists, rheumatologists, physiotherapists, and trained specialists in cardiorespiratory rehabilitation (4, 5).

The main symptoms are fatigue, exertional dyspnea, chest pain, dizziness, and

syncope. The current definition of PAH involves the presence of a mean pulmonary artery pressure ≥ 25 mmHg at rest, as measured by right heart catheterization.

Depending on pathophysiological mechanisms, PAH is classified into five main categories with clinical relevance (1, 6) (tab. I).

TABLE I
Clinical classification of pulmonary artery hypertension
(adapted according to 2015 ESC/ERS Guidelines) (6)

Main Categories	
I.	Pulmonary arterial hypertension
II.	Pulmonary hypertension due to left heart disease
III.	Pulmonary hypertension due to lung diseases and/or hypoxia
IV.	Chronic thromboembolic pulmonary hypertension
V.	Pulmonary hypertension with unclear etiology/multifactorial mechanisms

Therapeutic strategy – current recommendations

The therapeutic strategy in PAH has evolved over the past decades with the deepening of knowledge about this complex condition; the goal is to position and keep the patient in a lower risk class, associated with good exercise capacity, an acceptable function of the right ventricle, a good quality of life, and a low mortality risk. Currently, there is a stepwise approach to the treatment; three main strategies have been described (6, 7):

I. general measures, supportive therapy, patient orientation to centers with PAH expertise and vasoreactivity testing for indication of treatment with calcium channel blockers;

II. high-dose calcium channel blocker therapy (vasoreactive PAH) or other approved drugs for non-vasoreactive PAH, in accordance with the prognostic risk;

III. in case of inappropriate response to stage II treatment, the use of approved drug combinations for PAH and the lung transplant option.

General measures recommended as the first line in the management of patients with PAH involve physical activity and supervised rehabilitation, psychosocial support, patient and family education in the multiple aspects of the disease, increased adherence to treatment (5, 6, 7).

Adaptation to exercise in patients with pulmonary hypertension

The reduced physical activity level of patients with PAH results primarily from the decrease in peripheral and respiratory muscle strength, measured by the maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP), which are significantly lower in PAH patients compared to healthy subjects of comparable age and gender (8, 9).

Exercise intolerance in patients with PAH is a complex interplay between respiratory, cardiovascular, and musculoskeletal systems, altered hemodynamics of pulmonary circulation being the key element. Increased pulmonary peripheral vascular resistance affects the right ventricle (RV) and

results in a decoupling between these two anatomical elements. Over time, dilated RV compresses left ventricle (LV) and affects its distensibility and systolic flow, increasing pulmonary artery pressure (PAP) contributing in limiting exercise capacity. Another link is the increase in ventilatory demand and alteration of muscle contraction through low peripheral oxygen delivery, because of circulatory changes, resulting in lactic acid accumulation, effort limitation, and hyperactivation of the sympathetic vegetative system in response to cardiac output, like what happens in left heart failure (10).

Adaptation to physical training produces changes in the right ventricle and muscle strength, leading to increased systolic volume and the ejection fraction of RV, as well as reduced flux resistance in the pulmonary artery, simultaneously with pulmonary perfusion improvement, as MRI imaging demonstrated.

Respiratory rehabilitation finds its place in the first step of therapeutic approach, the main components of the training being represented by the respiratory muscles training, aerobic endurance training, and strength exercises, but the results of the studies, although promising, have not yet allowed the development of standardized training protocols (5, 6, 8, 9, 10).

Cardio-pulmonary rehabilitation in PAH has multiple beneficial respiratory, hemodynamic, and muscle effects (tab. II).

TABLE II

Adaptive mechanisms to physical training in pulmonary hypertension

(adapted according to Babu *et al.*, 2017) (10)

Effects on the right ventricle	Hemodynamic effects	Effectson the respiratory system	Muscle effects
Improved RV Function	Improved pulmonary perfusion	Increased forced expiratory volume (FEV_1)	Increased strength of diaphragm and quadriceps muscles
Increased RV stroke volume and ejection fraction	Decreased pulmonary flow resistance	Increased forced vital capacity (FVC)	Increased capillarization and oxidative capacity
	Increased cardiac output Decreased mPAP		

Positioning of the respiratory rehabilitation in the therapeutic strategy of pulmonary hypertension

Traditionally, the physical effort has been longtime contraindicated in patients with pulmonary hypertension because of the potential for an abrupt decrease in cardiac output during exercise and impairment of right ventricular function. Over the past decades, a growing body of evidence has demonstrated the safety and effectiveness of exercise dosing training, with benefits in terms of exercise capacity, peak oxygen consumption, and quality of life, but the findings are still not very extensive and there are many gaps concerning workout

for this category of patients (11).

Small trials have shown that PAH patients experience reduced dyspnea and fatigue, as well as a higher level of physical activity, a greater distance to the 6-minute walk test, and an improvement in the quality of life after completing a pulmonary rehabilitation program (tab. III).

TABLE III

The main effects of cardio-respiratory rehabilitation programs in patients with pulmonary hypertension of various etiologies

Main Effects
Improved exercise capacity (effort tolerance, endurance) (12-15)
Improved quality of life (12, 15, 16)
Decreased pulmonary artery pressure (12)
Improved peak oxygen consumption (17)
Hemodynamic improvement (right ventricular function, pulmonary perfusion) (17, 18)

Initial exercise effort assessment is of major importance, the most commonly used methods being the 6-minute walk test and cardiorespiratory exercise test, which is the gold standard and the most evidence-based in terms of diagnostic recommendations, prognosis, and evaluation of therapeutic efficacy (10).

Evaluation of a patient with PAH before the inclusion in a respiratory rehabilitation program should include several steps, feasible in a rehabilitation center equipped with multiple exploration facilities and access to a multidisciplinary team (19):

- Electrocardiogram, echocardiography – cardiologist;
- Right heart catheterization – interventional cardiologist;
- Diffusion capacity evaluations – pulmonologist/exercise physiologist;
- Pulmonary function tests (spirometry)-pulmonologist/exercise physiologist;
- Cardiopulmonary exercise testing-pulmonologist/ exercise physiologist / cardiologist (20);
- Six-minute walk test – exercise physiologist/physiotherapist/nurse (21);
- Muscle strength assessment (inspiratory and peripheral muscles) – exercise physiologist / physiotherapist (22, 23);
- Quality of life assessment – nurse;
- Assessment for depression – psychologist.

As a result, standard programs strictly supervised and hospital-based limit the accessibility of PAH patients to this important therapeutic intervention - pulmonary rehabilitation. Under these circumstances, even 45-minute sessions of walking, six times a week, at 65-75% of the heart rate reserve, plus L-arginine supplementation 6 g/day to maximize endothelial adaptation to exercise, have positive effects on exercise capacity, demonstrated by increasing of the distance walked, comparative to baseline (24).

European Society of Cardiology guidelines recommend supervised exercise training in patients with PAH, physically deconditioned but clinically stable, and under

optimal pharmacological therapy (class II a recommendation, level of evidence B) (6, 7). However, the optimal duration, intensity, frequency, and type of exercise for these patients as well as the mechanisms of positive effects remain an issue with incomplete or unclear answers (1, 6, 7). Among the training methods, skeletal peripheral muscles and inspiratory muscles training results in improvement of muscle strength, maximal inspiratory pressure, and peak oxygen uptake, without notable adverse effects (22). Consequently, low-level graded endurance and resistance exercises, as well as inspiratory and peripheral muscles training, are recommended, along with careful patients' education regarding their own illness, nutrition, and safe level of physical effort (7, 25).

CONCLUSIONS

Our study concludes that the exercise intolerance is a major cause of disability and decreased quality of life in patients with pulmonary arterial hypertension so that, careful evaluation of the causes and mechanisms of this intolerance is very important. Improving health care for patients with pulmonary hypertension increases their longevity, educating the patient and multidisciplinary approach being key elements. Although there are no standardized training protocols, pulmonary hypertension rehabilitation programs have an acceptable safety profile, with few serious side effects and should be encouraged as part of the multidisciplinary management of this complex disease.

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ASPECTE ETIOLOGICE ALE DURERII ABDOMINALE ACUTE LA COPIL

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THE ETIOLOGICAL ASPECTS OF ACUTE ABDOMINAL PAIN IN CHILDREN (Abstract): **Aim:** The study of the etiological aspects of acute abdominal pain in children, in order to perceive the clinical-etiological correlations and the disorders distribution related to age, gender and the origin of the patients. **Material and methods:** The criteria for including patients were age (between 0 and 18 years) and the presence of acute abdominal pain before or during the consultation with the physician. The research on acute abdominal pain in children was performed on the level of the Surgery and Pediatrics II clinical departments of the „Sf. Ioan" Children's Emergency Clinical Hospital in Galati, between 01.01.2009 - 01.01.2011. The clinical study performed on the patients registered in the studied groups focused on the identification, the evaluation of the symptoms of acute abdominal pain in children, diagnosing and treating it. The criteria for excluding patients were an age older than 18 years or the absence of acute abdominal pain as a symptom before or during the examination. The statistical analysis used the descriptive and analytical methods. The data was centralized and statistically processed in *M.S.EXCEL* and *S.P.S.S.* databases. **Results:** The patients with acute abdominal pain represent a percentage of 92.9% (2358 cases) of the total number of patients who suffer from abdominal pain (N=2537). The highest frequency of cases is represented by acute appendicitis (1056 cases – 44.8%). In the 5-18 years age group, acute appendicitis, mesenteric lymphadenitis, ovarian follicular cysts, acute pyelenophritis and salpingitis are predominant. In the 0-4 years age group gastroenteritis, acute pharyngitis, reactive hepatitis and lower digestive bleeding are predominant. **Conclusions**: In females, acute appendicitis, gastroenteritis, gastroduodenitis and cystitis are predominant, whereas in males, peritonitis, sepsis through *E. coli*, the contusion of the abdominal wall and acute pharyngitis are predominant. **Key-words**: ACUTE PAIN, CHILD, ETIOLOGY.

Durerea este definită de International Association for the Study of Pain (I.A.S.P.) (1) drept "o experienţă senzorială şi emoţională dezagreabilă, dată de o leziune tisulară veritabilă sau potenţială sau de o descriere cu termeni ce se referă la o asemenea leziune". În funcţie de durata ei, durerea se poate împărţi în două mari categorii: acută şi cronică (2). Durerea abdominală acută este de scurtă durată, fiind caracterizată de bruschețe (3,4). Datorită impactului durerii acute asupra calităţii vietii, ne-am propus să studiem aspectele etiologice ale durerii abdominale acute la copil, pentru a observa corelaţiile clinico-etiologice şi distribuţia afecţiunilor în raport cu vârsta, sexul şi mediul de reşedinţă al pacienţilor.

MATERIAL ŞI METODE

Criteriile de includere ale pacienților au fost vârsta (între 0 şi 18 ani) şi prezența durerii abdominale acute, înainte sau în momentul prezentării la medic. Cercetarea durerii abdominale acute la copil a fost efectuată în secțiile de Chirurgie şi Pediatrie II ale Spitalului Clinic de Urgență pentru Copii „Sf. Ioan" Galați în perioada 01.01.2009-01.01.2011. Studiul a inclus un lot de 2358 pacienți, urmărind recunoaşterea, evaluarea, diagnosticarea şi tratarea durerii abdominale acute. Criteriile de excludere ale pacienților au fost vârsta mai mare de 18 ani sau lipsa durerii abdominale acute drept simptom, înainte sau în momentul examinării. Pentru realizarea bazei de date am primit avizul comisiei de etică a Spitalului Clinic de Urgență pentru Copii „Sf. Ioan" Galați. Toate datele medicale au fost prelucrate şi evaluate statistic, folosindu-se programe specifice, *SPSS 13.0* şi *Microsoft Office Excel*, utilizându-se un interval de încredere de 95% şi obținându-se rezultate cu prag semnificativ p = 0,05.

REZULTATE

Dintre cei 2537 pacienți internați cu durere abdominală şi incluşi în studiu, 2358 (92,9%) au prezentat durere abdominală acută. În relație cu structura etiologică a durerilor abdominale acute, am constatat că apendicita acută a înregistrat cea mai mare frecvența (1056 - 44,8%), fiind urmată de apendicita acută cu abces peritoneal (187 - 7,9%), gastroenterită (218 - 9,2%), gastroduodenita acută (203 - 8,6%), şi limfadenita mezenterică (125 - 5,3%), tabloul etiologic fiind completat de afecțiuni semnalate într-un număr redus (<5%) (fig. I).

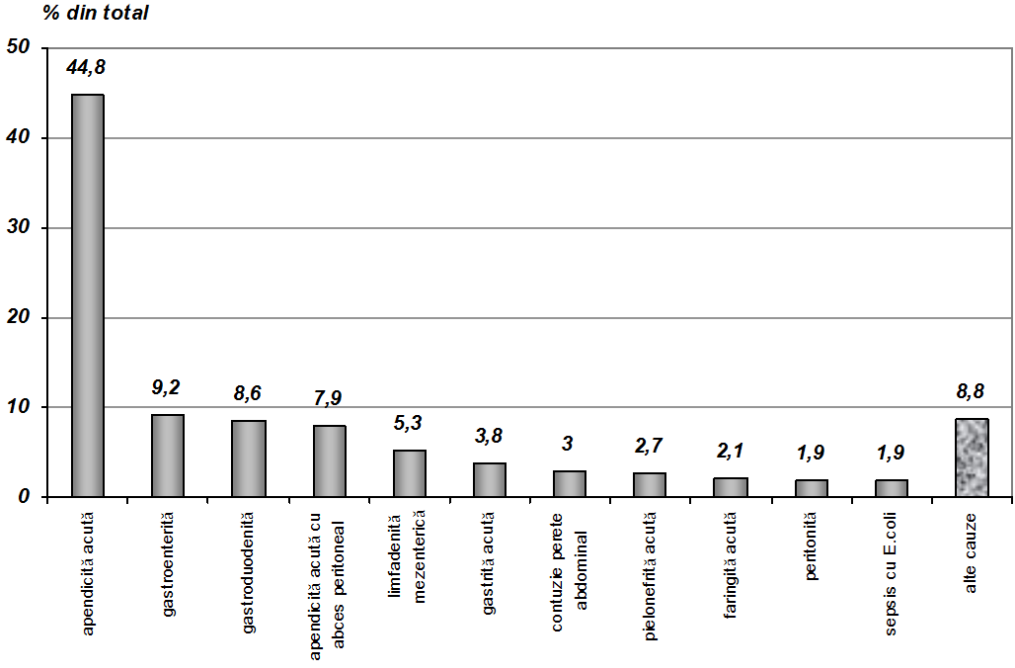

Fig. 1. Tabloul etiologic al copiilor cu durere abdominală acută

Repartiția pe grupuri de vârstă a cazurilor de durere abdominală acută a evidențiat un polimorfism etiologic semnificativ (tab. I, II).

TABELUL I

Tabloul etiologic, pe grupuri de vârstă, al copiilor cu durere abdominală acută

Etiologie	Grupul de vârstă (ani)					p
	0-1	1-4	5-9	10-14	15-18	
Apendicită acută cu abces peritoneal	-	3	42	100	42	**0,000006**
Limfadenită mezenterică	-	16	53	34	22	**0,0002**
Gastrită acută	-	4	25	32	28	0,054
Apendicită acută	-	6	260	540	250	**0,0000001**
Hemoperitoneu	-	-	1	-	-	-
Chist folicular ovarian	1	1	1	6	13	**0,0003**
Dureri intermenstruale	-	-	-	2	2	-
Peritonită	-	3	13	21	8	0,659
Litiază renală	-	1	5	8	9	0,289
Pielonefrită acută	4	8	21	14	16	**0,006**
Sepsis cu E. coli	-	2	13	20	4	0,184
Gastroenterită	26	126	51	13	2	**0,0000001**
Fisură anală	-	-	2	1	-	-
Salpingită şi oofarită	-	-	-	8	11	**0,0008**
Prolaps rectal	-	3	-	-	-	-
Hepatită reactivă	2	2	1	-	2	**0,009**
Contuzie perete abdominal	-	9	24	27	11	0,336
Corpi străini colon	1	6	1	2	-	**0,000002**
Ruptură de splină	-	-	-	2	2	-
Gastroduodenită	-	22	59	68	54	0,054
Contuzie lombară	-	1	-	-	1	-
Abces anal	1	1	-	-	1	-
Torsiune de ovar	-	-	1	4	1	0,715
Sindrom de şoc toxic	-	-	-	1	-	-
Pancreatită acută	-	-	-	-	1	-
Ocluzie prin invaginație	3	-	-	1	-	-
HDS	-	6	3	3	4	**0,008**
Faringită acută	1	11	20	13	4	**0,002**
Tetanie	-	-	-	1	-	-
HDI	1	4	3	-	1	**0,0009**
Ulcer gastric	-	-	1	1	4	0,117
Duodenită	-	-	1	-	-	-
Mucormicoză	-	1	-	-	-	-
Colecistită acută	-	-	-	2	-	-
Litiază biliară	-	-	-	-	2	-
Cistită	-	1	5	7	10	0,134
Ulcer bulbar	-	-	1	1	3	0,333
Bronşită acută	-	1	1	1	-	-
Hepatită cu CMV	1	-	-	-	-	-
Pneumonie	-	2	5	2	1	0,157
Hepatită virală A	-	-	1	1	-	-
Amigdalită	-	4	5	1	1	
Dop meconial	1	-	-	-	-	-
Corp străin stomac	-	1	1	-	-	-
Otită medie	-	1	-	-	-	-
Sarcină tubară	-	-	-	-	1	-
Iminență de avort	-	-	-	-	1	-
Retenție acută de urină	-	-	1	-	-	-

TABELUL II

Frecvența pe grupuri de vârstă a implicării unor afecțiuni în producerea durerii abdominale acute la copil

Grupul de vârstă (ani)	Nr. cazuri	Afecțiunea	% din total
0-1	**42**	gastroenterită	61,9
		pielonefrită acută	9,5
		ocluzie prin invaginație	7,1
1-4	**246**	gastroenterită	51,2
		gastroduodenită acută	8,9
		limfadenită mezenterică	6,5
		contuzie de perete abdominal	4,5
		pielonefrită acută	3,7
5-9	**621**	apendicită acută	41,9
		gastroduodenită acută	9,5
		limfadenită mezenterică	8,2
		gastroenterită	8,2
		apendicită acută cu abces peritoneal	6,8
10-14	**938**	apendicită acută	57,6
		apendicită acută cu abces peritoneal	10,7
		gastroduodenită acută	7,2
		limfadenită mezenterică	3,6
		gastrită acută	3,4
15-18	**511**	apendicită acută	48,9
		gastroduodenită acută	10,6
		apendicită acută cu abces peritoneal	8,2
		gastrită acută	5,5
		limfadenită mezenterică	4,3

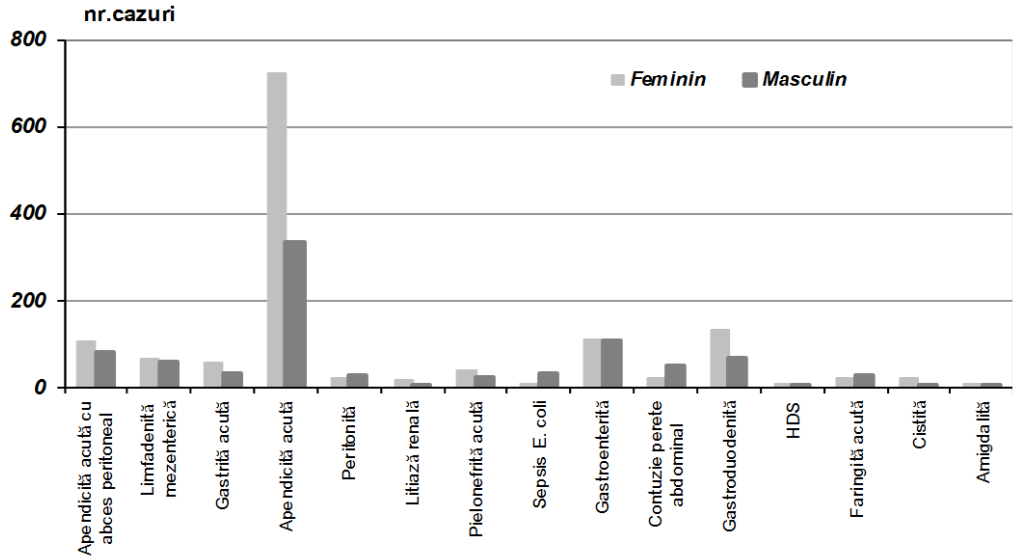

Fig. 2. Distribuția pe sex a pacienților, în relație cu etiologia durerii

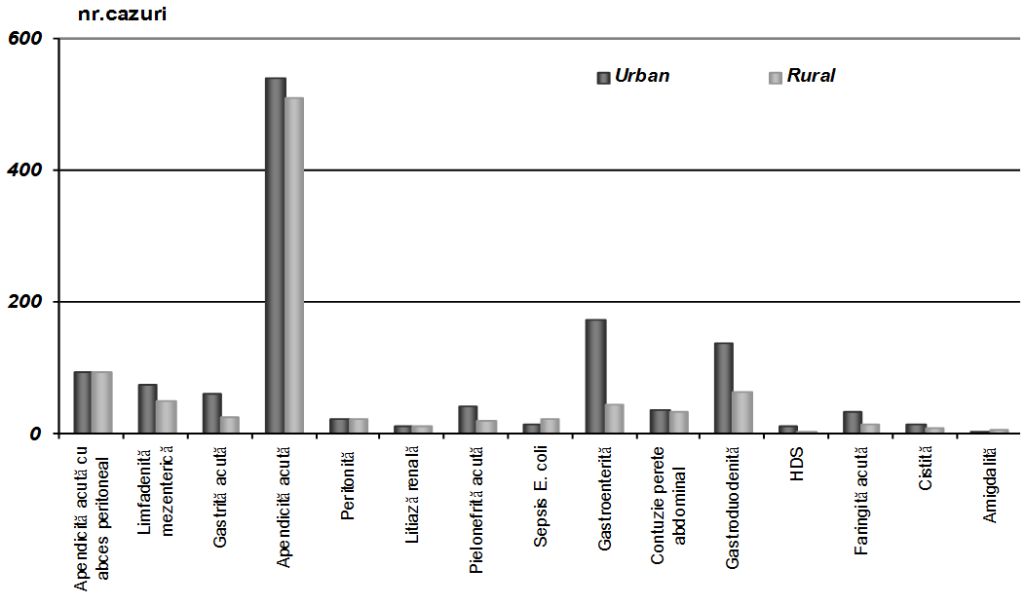

Fig. 3. Distribuția, pe medii, a pacienților, în relație cu etiologia durerii

Cercetările au evidențiat că durerea abdominală acută a fost înregistrată, cel mai frecvent, la grupul de vârstă 5-18 ani, în caz de apendicită acută, limfadenită mezenterică, chist folicular ovarian, pielonefrită acută şi salpingită, în timp ce la grupul de vârstă 0-4 ani, aceasta a fost prezentă în cadrul gastroenteritei, faringitei acute, hepatitei reactive şi hemoragiei digestive inferioare (tab. II).

La pacienții de sex feminin, s-a evidentiat frecvența crescută a următoarelor afecțiuni: apendicita acută, gastroenterita, gastroduodenita acută şi cistita, în timp ce la cei de sex masculin, a peritonitei, sepsisului prin *E. coli*, contuziei de perete abdominal şi a faringitei acute (fig. 2).

În relație cu mediul de reşedință şi etiologia durerii abdominale acute, s-a observat frecvența crescută a durerii abdominale acute la copiii proveniți din mediul urban, cu excepția sepsisului prin *E. coli* şi amigdalitei care au fost mai frecvente în mediul rural (fig. 3).

DISCUȚII

Studiile efectuate în România (5) arată că durerea abdominală acută la copil prezintă frecvent următoarea etiologie: apendicita acută, adenita mezenterică, gastroenterita, faringita, pneumonia de lob inferior drept, pielonefrita acută, abuzul alimentar.

Studiile efectuate în SUA (6,7,8,9,10) au evidențiat că apendicita acută, gastroenterita, limfadenita mezenterică, constipația şi traumatismul abdominal sunt cele mai frecvente afecțiuni care generează durere abdominală acută la copil. Detaliat, pe grupuri de vârstă, studiile (6,7,8,9,10) relevă că, la 0-1 an predomină colicile infantile, gastroenterita, constipația şi infecția de tract urinar; la 2-5 ani, gastroenterita, apendicita acută, constipația, infecția de tract urinar; la 6-11 ani, gastroenterita, apendicita acută, constipația, iar la 12-18 ani, apendicita acută, gastroenterita, constipația şi dismeno-

reea. Un studiu efectuat în Turcia (11) a arătat că apendicita acută este cea mai frecventă cauză de spitalizare a copiilor cu durere abdominală acută (44,1%), apărând la o vârstă medie de 11,2 ± 2,7 ani, mai frecvent la sexul masculin.

CONCLUZII

Studiul a evidențiat că apendicita acută estecea mai frecventă cauză a durerii abdominale acute la copil. La grupul de vârstă 0-4 ani, au predominat cazurile de gastroenterită, iar la 5-18 ani, cele de apendicita acută. În relație cu mediul de reședință al copiilor cu durere abdominală acută, s-a observat că sepsisul prin *E. coli* și amigdalita acută au fost mai frecvente în mediul rural, celelalte afecțiuni predominând la pacienții din urban. Datele obținute sunt în acord cu cele din literatură, evidențiindu-se existența unor variații geografice ale etiologiei durerii abdominale acute la copil.

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RESPIRATORY INVOLVEMENT IN THE GASTROESOPHAGEAL REFLUX DISESEASE

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RESPIRATORY INVOLVEMENT IN THE GASTROESOPHAGEAL REFLUX DISESEASE (Abstract)**:** The pathological gastroesophageal reflux disease may lead to the gastroesophageal reflux disease (GERD), manifested as a spectrum of conditions including erosive esophagitis, Barrett esophagus and has been linked to the development of adenocarcinoma of the esophagus. The gastroesophageal reflux has been incriminated in the occurence of a number of pulmonary symptoms and diseases, otolaryngologic symptoms, and other extraesophageal manifestations. Clinicians must be aware of the possibility of some extraesophageal reflux-related manifestations, even in the absence of heartburn and acid reflux, classic esophageal symptoms of GERD. Although the correlation between gastroesophageal reflux disease and the extraesophageal manifestations has been established, a cause-and-effect relationship has not been proved yet. In this article, we present the respiratory manifestations of gastroesophageal reflux disease, referring to epidemiology, pathogenesis, diagnosis and treatment. **Keywords**: BRONCHIAL ASTHMA, ESOPHAGITIS, GERD, LARYNGITIS, RESPIRATORY SYMPTOMS

The cardinal symptoms of gastroesophageal reflux disease (GERD) are heartburn and regurgitation. Heartburn is defined as a burning sensation in the retrosternal area, and regurgitation as the perception of flow of refluxed gastric content into the mouth or hypopharynx. Although the reflux of acid gastric content is usually responsible for the symptoms of GERD, non-acid reflux of substances such as bile may also be important in a minority of patients (1). It is extremely common that patients with GERD experience symptoms: approximately 20% of adults reporting heartburn, acid reflux or both at least once a week and approximately 40% reporting that such symptoms occur at least one a month (2).

The prevalence of GERD is estimated to be 10% to 20% in Europe and North America and at least 5% in Asia (3). The need for a patient-centered, symptom-driven approach to GERD assessment and diagnosis was addressed in the Montreal definition and classification published in 2006. Developed by an expert consensus group consisting of 44 primary and secondary care physicians from 18 countries, the Montreal definition is built on evidence-based medicine and systematic literature reviews. Montreal definition includes a measure of symptom severity by stating that GERD is "a condition which develops when the reflux of stomach contents causes

troublesome symptoms and/or complications"(4).

Classic reflux symptoms are not always present in patients with GERD. A significant number of patients with GERD present with atypical or extraesophageal symptoms. It has been shown that GERD is associated with pulmonary symptoms, as well as with lower airway diseases (asthma, chronic cough, bronchitis, aspiration pneumonia and idiopathic pulmonary fibrosis), otorhinolaryngologic signs/symptoms (hoarseness, laryngitis, subglottic stenosis, vocal cord granuloma and laryngeal carcinoma) and other extraesophageal manifestations (noncardiac chest pain, dental erosion, sinusitis, pharyngitis and sleep apnea) (5). Classic reflux symptoms are absent in 40 to 60 percent of patients with asthma, 57 to 94 % of patients with ear, nose, and throat symptoms, and 43 to 75% of patients with chronic cough in whom reflux is suspected as the primary etiology (6). Therefore, GERD should be strongly considered in the differential diagnosis of patients presenting with atypical symptoms when alternative diagnoses have been excluded (7).

Pathogenesis of respiratory symptoms in GERD

Two possible mechanisms have been proposed as underlying GERD-related extraesophageal symptoms: the microaspiration of gastric content into extraesophageal structures during reflux episodes and the stimulation by the gastric refluxate of a vagal reflex arch extending from the esophageal body to the bronchopulmonary and laryngeal systems (8).Both mechanisms have been supported by clinical and laboratory data documenting the injurious effects of esophageal acid on extraesophageal structures.

There are some defense mechanisms protecting against extraesophageal complications of GERD: junctional structures at the gastroesophageal interface include the lower esophageal sphincter, the crural diaphragm, the sling fibers, and the phrenoesophageal ligament. In addition to these potential pharyngoglottal mechanisms, intrinsic laryngeal reflex mechanisms play an important role in limiting the spread of aspirate and enhancing clearance. Such mechanisms would include the cough reflex and mucociliary action of the bronchotracheal surface (8). Any disfunctions in this defense mechanisms can lead to extraesophageal complications of GERD.

Diagnosis

Currently upper gastrointestinal endoscopy is the main clinical tool for visualizing esophageal lesions. Since the majority of GERD patients do not have endoscopic visible lesions other methods are required to document the abnormal acid exposure in the distal esophagus. For many clinicians ambulatory esophageal pH monitoring is the gold standard in diagnosing GERD since it quantifies distal esophageal acid exposure and allows the evaluation of the relationship between symptoms and acid reflux. Impedance is a new technical development that offers unexplored possibilities for investigating GERD. It allows the detection of the frequency, the height , and duration of reflux episodes independent of the pH of the refluxate (9). Combined with pH multichannel intraluminal impedance and pH (MII-pH) monitoring has become a clinically available tool which can detect gastroesophageal reflux independent of pH (10). This new techniques can quantify exposure to esophageal acid and identifying reflux events regardless of acidic

content to assess correlations with symptoms (11). The diagnostic sensitivity of multiple intraluminal impedance may correspond to that of the pH probe in untreated patients but is superior to the pH probe in patients treated with antacid medication (12). The availability of highly selective gastric acid suppressive therapy led to the introduction of short trials of PPI to diagnose GERD. PPI trials are often used as a first line diagnostic tool in clinical practice and in particular in the primary care settings (13).

Respiratory conditions

Bronchial asthma

Asthma is the pulmonary disorder most often associated with GERD, and acid reflux disease is found in 30% to 89% of asthmatics (14). Some studies have established not only the co-existence, but also the cause-and-effect relationship, establishing GERD as one of the significant causes of asthma in adults.

Two mechanisms have been incriminated in the pathogenesis of GERD-related asthma: microaspiration of the refluxed gastric contents and esophagobronchial reflex triggered by the stimulation of vagal receptors in the distal third of the esophagus. Through the mechanism of microaspiration, the pulmonary symptoms would result from the direct aggression of acids and enzymes present in the reflux gastric contents on the respiratory mucosa, thereby leading to reflex bronchospasm. On the other hand, some authors have suggested that the prevalence of GERD is higher in patients with chronic obstructive pulmonary disease, indicating that altered respiratory mechanics predisposes to GERD (15). Physiological alterations in asthmatic patients, such as increased intrathoracic pressure and hemidiaphragm rectification could debilitate the antireflux barrier and consequently predispose to GERD. Regarding the medication used in the control of asthma, some might favor reflux medications, such as theophylline and adrenergic beta-agonists, because they relax the smooth muscles of the esophagus and stomach (16). However, such medications cause reduced esophageal clearance capacity and delayed gastric emptying, resulting in a proreflux effect, especially in patients with mechanical defects of the lower esophageal sphincter.

The most commonly used exams for the diagnosis of GERD-induced asthma is the 24-h pHmetry but it presents less than satisfactory sensitivity and specificity, despite identifying and quantifying the pathological acid reflux, presents a high rate of false-negative results. Therefore, the 24-h pHmetry is only indicated for patients who did not respond to empirical treatment with acid secretion inhibitors (17). Endoscopy of the upper digestive tract remains an important exam in the evaluation of GERD. It makes possible the confirmation of the diagnosis, as well as detection of complications of the disease. Similarly, contrast-enhanced radiological examination of the esophagus, stomach and duodenum can detect alterations, although only in more advanced cases. However, these exams are incapable of objectively demonstrating the causal relationship between pathological acid reflux and respiratory symptoms (5). The empirical treatment used in the diagnosis of uncomplicated GERD is a good strategy for the evaluation of patients with GERD-induced asthma since it is relatively simple, inexpensive, and without the discomfort of invasive tests (18). In a cost-effectiveness analysis of the available di-

agnostic strategies it was concluded that the most cost-effective approach is empirical therapy with proton pump inhibitors (PPIs), such as omeprazole at 20-40 mg/day for three months. Therefore, the initial step in the diagnosis of GERD-induced asthma should be empirical treatment to suppress acid with a PPI (17).

In patients with uncomplicated GERD and presenting typical symptoms, the response to both clinical and surgical treatments, has been excellent (19). However, the therapeutic response in GERD patients presenting extra-esophageal symptoms is not very clear. Here is a study that evaluated 30 patients with asthma and GERD with the objective of determining the dosage and duration of omeprazole treatment needed to reduce respiratory symptom severity. The patients initially received a three-month course at 20 mg/day. From the fourth month onward, the dose was progressively increased until acid suppression, documented by 24-h pHmetry, occurred. In 73% of the patients, respiratory symptom severity was reduced or peak expiratory flow increased. In 57%, there was a significant reduction in symptom severity by the end of the third month of treatment. The mean omeprazole dose needed to obtain acid suppression was 27 mg/ day (20). On the other hand in their article on the use of esomeprazole in patients with poorly controlled asthma Mastronarde et al. conclude that treatment with proton-pump inhibitors does not improve asthma control in such patients (21).Although the use of proton-pump inhibitors diminishes the acidity of the reflux, it does not reduce either the number or proximal extent of reflux events, microaspiration and esophageal reflexes may still contribute to airway inflammation in patients with asthma who are receiving a high-dose proton-pump inhibitor, and this may be the explanation for the poor control of asthma manifestation (22). An alternative explanation for the lack of efficacy of esomeprazol is related to bacterial overgrowth in the stomach, promoted by acid suppression. A gastric pH of more than 4 favors the growth of predominantly gram-negative bacteria in the stomach (23). Although proton-pump inhibitors reduce the volume and acidity of gastric contents, they do not prevent pulmonary micro-aspiration. Recurrent microaspiration of bacteria enriched gastric contents might have sustained lung inflammation in the group of patients with poorly controlled asthma. Starting from this hypothesis it appears the interesting idea that bacterial colonization of the gastric contents and microaspiration in patients who are receiving proton-pump inhibitors might lead to worsening asthma. There were not found any convincing evidences that the use of proton-pump inhibitors leads to an increase in the rate of pneumonia or worsening of asthma. However, it is possible that there was a balanced trade-off between beneficial effects and harmful effects of acid suppression.

Since the causes of GERD are predominantly mechanical, the correction of the antireflux barrier through antireflux surgery controls two of the main etiological factors of GERD: the defective lower esophageal sphincter and the hiatal hernia. The currently recommended surgery is laparoscopic fundoplication.

It makes it possible to maintain the gastroesophageal junction in intraabdominal position and build a valve with the gastric fundus, safely re-establishing gastric cardia competence through the mechanical improvement of its function.

A group of authors conducted an in-

depth review of the literature analyzing the effects of the antireflux surgery on the symptom severity and the pulmonary function in patients with asthma and GERD. Of the 417 patients included in the analysis, a significant reduction in GERD symptom severity was obtained in 90% and in asthma symptom severity in 79%. In addition, 88% decreased their use of bronchodilators, and 27% presented improvement in their pulmonary function test results. The authors concluded that, although it has little effect on pulmonary function test results, antireflux surgery can significantly reduce GERD-related respiratory symptom severity and decrease the need for asthma control medications (24).

According to other studies evaluated respiratory symptoms in patients with GERD initially treated with a PPI and later with antireflux surgery, after six months of PPI treatment, respiratory symptom severity decreased in only 14% of the patients, and none of the patients remained asymptomatic during this period. After laparoscopic fundoplication, respiratory symptom severity decreased in 86% of the patients, and 67% remained asymptomatic until the end of the study. The results of these studies suggest that clinical treatment with a PPI should be the initial approach to GERD-related asthma. Surgical treatment is indicated for patients with GERD, especially for those who are under 50 years of age, who do not satisfactorily respond to clinical treatment, who suffer recurrence of symptoms within four weeks after the discontinuation of PPI therapy or who require continuous maintenance treatment with PPI (25,26).

Laryngitis

Laryngitis is the most common otorhinolaryngologic disease that has been associated with GERD (27). Although the prevalence of reflux laryngitis is unknown, it is estimated that 4% to 10% of the patients who seek the assistance of an otorhinolaryngologist present GERD-related symptoms or findings (28). It has been also shown that a significant portion of patients with laryngeal manifestations of GERD, including reflux laryngitis, do not present the typical symptoms of GERD (27).

Most gastroenterologists consider laryngopharyngeal reflux as an extraesophageal manifestation of GERD, while otolaryngologists belive laryngo-pharyngeal reflux and GERD are two completely different disease entities with different mechanisms and clinical manifestations

The symptoms of reflux laryngitis are nonspecific and cannot be differentiated from the symptoms of laryngitis with other causes. Hoarseness is the most common symptom; other symptoms can include cough, throat clearing, odynophagia, altered voice, globus and dysphagia. Laryngopharyngeal reflux is considered a causative role in 60% to 70% of the Western population with globus, hoarseness, chronic cough and frequent throat clearing (29-31). Symptoms such as globus or frequent throat clearing may be also caused by other factors such as smoking, voice abuse or allergy.

Laryngoscopy plays a fundamental role in the evaluation of chronic laryngitis. The classical abnormality described in reflux laryngitis is 'posterior laryngitis', which consists of the thickening of the mucosa accompanied by edema and erythema of the arytenoids. But none of those modifications are not specific to reflux laryngitis, arytenoid erythema and subglottic edema also can be found in healthy population.

The documentation of pathological gastroesophageal reflux is appropriate in some patients, particularly in those with symptoms refractory to PPI treatment. Currently, doublechannel esophageal and pharyngeal outpatient monitoring of esophageal pH is considered the most sensitive means of diagnosing laryngo- pharyngeal reflux (32).

Relating to treatment there are a lot of studies to evaluate the efficacy of PPI in patients with laryngopharyngeal reflux related to GERD. One study (33) evaluated 45 patients with possibly GERD-related otorhinolaryngologic symptoms. Patients were treated for two months with 20 mg of omeprazole twice a day or 30 mg of lansoprazole twice a day. The authors found that 47% of the patients presented improvement after two months, increasing to 64% after four months. The authors concluded that empirical treatment with PPI twice a day for four months is efficacious in the identification and treatment of most patients with otorhinolaryngologic symptoms caused by GERD. Unfortunately, six months after the discontinuation of PPI therapy approximately 80% of the patients suffer symptom recurrence.

An other study evaluated 82 patients with GERD and laryngopharyngeal reflux. It was tested rabeprazole efficiency in high doses (20mg twice daily) compared to placebo. It showed significant improvements in laryngopharyngeal reflux- related symptoms with high-dose rabeprazole within 12 weeks of PPI treatment in comparison with placebo. Relapse of symptoms was observed 6 weeks after stopping PPI therapy, indicating the requirement for longer treatment duration in patients with laryngopharyngeal reflux (34). Some sudies, comparing PPI treatment with placebo , showed similar response to both therapys , which suggest that other nonorganic factors such as anxiety and depression also may contribute to laryngo- pharyngeal reflux symptoms (35, 36). For example, patients with anxiety and depression were found to be more prone to exaggerate physical symptoms related to their laryngopharyngeal reflux conditions.

Antireflux surgery is effective in most patients with reflux laryngitis (37). Laparoscopic Nissen fundoplication have shown a response rate of over 70% among patients with GERD-related otorhinolaryngologic symptoms. It has been also proven that successful laparoscopic fundoplication is more cost-effective than is high-dose, long-term suppression therapy with a PPI (38). In conclusion the first-line treatment in patients with laryngo-pharyngeal reflux is the use of a PPI in moderate doses (e.g. omeprazole at 40 mg/day) for a minimum of three months. In case of recurrence of symptoms after this period, the reflux should be confirmed through complementary exams (24-h pHmetry) and the feasibility of performing laparoscopic fundoplication should be evaluated.

Chronic cough

Cough is the most common symptom for which patients seek medical attention (39).It is defined as persistent cough for a minimum period of eight weeks. Chronic cough is estimated to affect 11-20% of the population (40) which means it has a significant socioeconomic impact (41) and an important reduction in quality of life (42).

Patients with chronic cough accompanied by GERD present a typical profile that includes being a nonsmoker, presenting a normal chest X-ray and not using angiotensin-converting enzyme inhibitors.

Convergence of vagal afferents from the esophagus and respiratory tract in the brain suggested the idea of a possible esophageal-bronchial reflex. This is supported by the findings that acid infused into the distal esophagus of patients with chronic cough increased the frequency of coughing and cough reflex sensitivity (43). Sensitization of a central mechanism might explain why the reflux that was mainly confined to the distal esophagus appears temporally associeted with cough in a significant proportion of chronic cough patients in whom non-GERD causes were excluded (44). One study (45) evaluated the relation between cough and reflux. Twenty-four hour ambulatory acustic cough monitoring with simultaneous impedance/pH recording was carried out in 71 unselected patients with chronic cough. The results shown that 70% of patients exhibited temporal association, 48% having a positive symptom association probability for cough preceded by reflux, 56% a positive symptom association probability for reflux preceded by cough, and 32% both. In conclusion the study shown that irrespective of possible underlying cause reflux can temporally associate with cough. The lack of association with proximal extent of reflux and erosive disease, but link to increased cough reflex sensitivity, suggest that a sensitized central mechanism may be facilitating reflux-induced cough and cough-induced reflux.

Referring to the treatment of chronic cough associated to GERD most studies have shown that both clinical and surgical treatments are efficacious in reducing cough. One such study shown that a 4- to 6-week course of omeprazole at a dose of 40 mg, alone or combined with prokinetic agents (metoclopramide or cisapride) reduced GERD-related cough in 80% of the patients studied (46). Irwin et al, analyzing surgical treatment (laparoscopic Nissen fundoplication) of GERD-related chronic cough in adults, demonstrated a 100% response to treatment in two studies and a 56% to 85% rate of favorable response in another four studies (47). Currently, effective antitussive drugs are unavailable, and, although a number of agents have been shown to reduce transient lower esophageal sphincter relaxation, these have not been assessed in chronic cough (48). The first-line approach in the GERD-related cough is the PPI therapy even if a significant proportion of patients present recurrence of symptoms immediately after discontinuation of the treatment. The poor efficacy of traditional drugs, such as those targeting gastric acidity, may be explained by the presence of a sensitized central mechanism which facilitate reflux-induced cough and cough induced reflux.

GERD in lung transplanted patients

Patients with lung transplantation present alteration of gastrointestinal motiltiy. GERD is present in patients with chronic lung disease but may be encountered also after lung transplantation (49). GERD is connected to obliterative bronchiolitis, a factor causing rejection in lung transplantation (49). Preventing reflux in lung transplantation is improving the survival (50).

Oral changes

Respiratory involvement is not isolated in GERD. The refluxate may arrive in the oral cavity and produce there oral and dental alterations. Specific tooth erosion is generally associated with GERD symptoms. The importance of tooth loss is correlated to regurgitation frequency, and acid

pH oral modifications (51, 52). Therefore carefully teeth examination should be done in GERD with respiratory symptoms. The presence of glossitis has also been reported in GERD (53).

CONCLUSIONS

Extraesophageal manifestations of GERD constitute a heterogeneous disease group, but even so, some general characterizations can be made.

The diagnosis can be difficult because of the great prevalence of 'silent GERD' in the population. Acid reflux should be considered if signs of GERD are present, if extraesophageal symptoms are unexplained, or if these symptoms are refractory to treatment.

Traditional exams, such as endoscopy of the upper digestive tract and contrast-enhanced radiological examination of the esophagus, stomach and duodenum, can be useful in the diagnosis of GERD and are still important tools for the detection of esophageal complications. However, these exams might not confirm GERD, and they do not establish a causal relationship between GERD and extraesophageal symptoms. The most sensitive exam is the 24-h pHmetry, which is considered the gold standard in diagnosing GERD , and it plays an important role in the evaluation of patients with extra-esophageal manifestations. Nevertheless, 24-h pHmetry might not confirm the causal relationship between GERD and the extraesophageal symptoms. Therefore, the empirical test with PPI therapy can be useful in establishing the diagnosis. Atypical manifestations generally require more aggressive and longer-term antisecretion therapy than do typical GERD symptoms.

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DEPRESIA ŞI BOLILE CARDIOVASCULARE LA PACIENȚII VÂRSTNICI

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THE DEPRESSION AND THE CARDIOVASCULAR DISEASES IN THE ELDERLY (Abstract): The aim of this study was to reveal if there are connections between depression and cardiovascular disease. **Material and method**: We have studied 413 patients from Jorasti, Jud Galati on a two-years period (2008-2010). On these patients there was performed a careful history taking and they were physically examined (height, weight, body mass index, waist, blood pressure, heart rate). There was also performed an ECG (electrocardiogram), there were measured the levels of cholesterolemia, HDL-cholesterolemia, trygliceridemia and creatininemia. We used the Geriatric Depression Scale (GDS) in order to evaluate depression. **Results:** in these patients there was noticed a higher prevalence of dyslipidemias, obesity and depression in women. Out of the 46 women with mild and severe depression 32 are overweight, 7 have obesity, 29 a high cholesterol, 23 a high LDL-cholesterol; all of them had a high risk or the presence of cardiovascular disease. **Conclusions:** Depression is an independent risk factor for producing a cardiovascular event; the level of cholesterol and of the blood preessure was higher in patients with severe depression, proving that the level of the cardiovascular risk factors increased with the severity of the depression. Treating the elderly, which often associate depression, makes necessary a careful evaluation of all comorbidities and risks, in order to establish perfect individualized strategies of improving the quality of life. **Key words**: CARDIOVASCULAR DIASEASE, DYSLIPIDEMIA, DEPRESSION

Depresia este o tulburare mentală caracterizată printr-o modificare profundă a stării timice, a dispoziției, în sensul tristeții, al suferinței morale şi încetinirii psihomotorii, asociindu-se în general cu anxietatea. Ea întreține la pacient o impresie dureroasă de neputință globală, de fatalitate disperată, iar uneori antrenează ruminații subdelirante pe tema culpabilității, a indignității, a autoaprecierii, putând conduce la luarea în considerare a sinuciderii şi uneori, la realizarea acesteia (1). Sentimentul de inutilitate, de culpă poate cuprinde aprecieri negative asupra propriei valori care nu corespund realității. Tulburarea depresivă este legată de regresie, în aspectele ei de regenerare şi îmbogățire, în particular ea poate lua forma golului lipsit de viață, în asemenea circumstanțe noua dezvoltare este cea care a sustras energia din conştiință conducând la depresie (2). După Kielholz, sindromul depresiv constituie o

modalitate de reacție de bază la diferite condiții şi situații cu efect somatogen, psihogen sau endogen şi care se manifestă prin triada dispoziție depresivă, inhibiție psihomotorie şi afectarea funcțiilor psihomotorii (3).

Evoluția depresiei variază de la o persoană la alta. Pot fi prezente simptome uşoare sau severe de depresie, pentru o perioadă îndelungată de timp sau pentru o perioadă scurtă. Un procent mic de persoane se simt depresive în marea majoritate a vieții lor; acestea necesită un tratament de întreținere.

În cazurile severe de depresie, persoana respectivă poate ajunge la incapacitatea de a comunica, la incapacitatea de a efectua activitățile de rutină şi chiar la suicid. În aceste cazuri, consultul unui specialist şi urmarea unui tratament sunt esențiale.

Aspecte clinice ale relației riscului de boală arterială coronariană urmare a bolii depresive

Boala arterială coronariană este considerată ca fiind cauza principală de deces atât pentru bărbați cât şi pentru femei. Între cauzele care generează acest fenomen se numără şi factorii psihosociali care sunt implicați atât în etiologia, progresia cât şi prognosticul acestor evenimente (3). Cercetări recente sugerează că depresia este un indicator psihosocial particular de puternic, pentru deces în evoluția bolii arteriale coronariene.

Dovezi preliminare care susțin relația dintre depresie şi boala arterială coronariană (CAD) au fost furnizate de studii efectuate pe pacienți cu patologie ischemică coronariană, studiile respective au scos în evidență o prevalență disproporționat de mare a depresiei la pacienții cu boală ischemică coronariană față de (comparativ cu) populația generală. Astfel, prevalența depresiei la bolnavii cu patologie ischemică coronariană este între 19%-47% față de 4%-7% în populația generală, ceea ce a sugerat ideea că depresia ar putea contribui la apariția şi dezvoltarea bolii coronariene ischemice (4). Literatura medicală abundă în lucrări pe această temă, care în studii pe loturi mari de bolnavi obiectivează această idee în procentaje care variază de la autor la autor.

Tulburări somato - psihice ale bolnavilor cardiaci

Tabloul manifestărilor clinice psihosomatice propriu-zise este destul de bogat, de aceea vom aborda manifestările clinice unde raportul factor psihic - răsunet somatic este evident, ordonându-le astfel:

- simptome şi semne psihosomatice ca reacții normale la tensiuni psihice,
- tulburări psihosomatice ca dezvoltări psihofuncționale,
- boli psihosomatice ca organizări psihosomatice.

Manifestările psiho-somatice cardiovasculare sunt aproape o regulă în stările anxioase sub forma cenestopatiilor cardiace care pun reale probleme de diagnostic diferențial şi terapie, influențează calitatea vieții devenind uneori FR de mortalitate.

Simptome şi semne psihosomatice: reprezentate de manifestări clinice, componente normale asociate emoțiilor, care durează puțin şi sunt rapid reversibile.

Tulburări de ritm:

a) Tahicardiile şi bradicardiile regulate induse psihogen au la bază corelarea frecvenței cardiace cu reflexele de apărare sau de orientare către un stimul.

b) Extrasistolele (ES) reprezintă cea mai frecventă tulburare de ritm apărută în cursul SP.

c) Fibrilația atrială (FA) Riscul de atac vascular cerebral (AVC) al pacienților cu

FA este de 4,5% pe an. Episoadele tranzitorii de FA paroxistică, apărute în cadrul unui SP, sunt estimate la circa 3%. (5)

Tulburări de conducere. Sunt reprezentate de lărgirea complexului QRS şi scurtarea amplitudinii acestuia. Aceste valori ating în cursul unei excitaţii provocatoare de spaimă, 0,07 secunde şi, respectiv 1,38 mV, comparativ cu traseul de bază (0,01 secunde şi respectiv 1,68 - 1,83 mV) (3).

Dureri precordiale. Durerea toracică este un simptom ce caracterizează atât bolile organice, cât şi cele funcţionale. Peste jumătate dintre pacienţii cu durere precordială care se prezintă la medicul cardiolog sunt "non cardiaci".

Durerea precordială este un simptom relativ frecvent la pacienţii anxioşi.

Dispneea „cardiacă". Dispneea, simptom cardinal al bolilor cardiace şi respiratorii este frecventă la pacienţii cu anxietate. Acest simptom poate să apară brusc, fără nici o legătură cu un efort fizic şi în prezenţa unei frecvenţe normale a respiraţiei, gazele sangvine fiind evident normale.

Variaţii ale tensiunii arteriale. Reacţia catecolaminică de stres generează răspunsuri hipertensive chiar la subiectul normal

Modificări vasomotorii în diferite teritorii. Acestea pot fi apreciate direct prin observaţie (eritemul pudic, paloarea din stările de spaimă, groaza, sau din crizele vasospastice de tip Raynaud, opusă expresiei fizionomice de "roşu la mânie".

Importante sunt stările lipotimice produse de ischemia cerebrală apărută în cursul unui SP, soldat fie cu un răspuns hipotensor, fie cu diferite tulburări cardiovasculare menite să scadă irigaţia cerebrală (de exemplu tulburări paroxistice de ritm).

Tulburări psihosomatice. În acest caz modificările clinice survenite sunt mai ample ca intensitate şi durată şi sunt mai bine structurate (6).

Sindromul cardiac hiperkinetic

Acest sindrom pune în evidenţă vagi tulburări cardiace şi multe simptome paralele autonome. Pacienţii se simt slăbiţi, forţele lor scad şi unii îşi simt inima bătând mai rapid. Spre deosebire de forma fobică a nevrozei cardiace, anxietatea e totuşi rară şi frica de moarte mai rar întâlnită.

Tahicardia paroxistică supraventriculară (TPSV)

În vreme ce în nevroza cardiacă şi în sindromul cardiac hiperkinetic se consideră că tulburările sunt psihogene, această suferinţă cardiacă evidenţiază situaţia în care factorii de stres fiziogeni şi psihogeni apar simultan şi se completează reciproc. În TPSV există o predispoziţie la crize datorită particularităţilor inervaţiei autonome a atriilor. Deşi lipsite de pericol prin ele însele, extrasistolele supraventriculare şi ventriculare, împreună cu încordarea emoţională, pot în cele din urmă să producă întreaga configuraţie de simptome.

Hipotensiunea arterială primară

Legătura dintre hTAE şi SP este sugerată de caracterul de "sindrom" al afecţiunii în cadrul căreia TAS este < 100 mm Hg şi TAD < 60 mm Hg, fiind asociată cu o serie de alte simptome cu substrat psihopatologic: fatigabilitate, astenie, cefalee, vertij, irascibilitate, transpiraţii, labilitate afectivă etc, fapt ce a făcut pe unii autori să vorbească mai degrabă de un "sindrom al hipotensivului" (7).

Astenia neurocirculatorie (ANC). Pacientul prezintă simptome subiective dominate de palpitaţii şi dureri precordiale, însoţite de un substrat organic. În privinţa conţinutului simptomatic al sindromului, este de subliniat frecvenţa cea mai crescută (la peste 70% din bolnavi) a dispneei (78%), ameţelilor (78%) şi cefaleei (72%).

Simptomele subiective cardio-vasculare şi respiratorii sunt prezente, de regulă, concomitent.

C. Boli psihosomatice - Acestea permit înțelegerea raporturilor de coordonare şi subordonare a elementelor constitutive ale bolii: agentul psihosomatogen, personalitatea premorbidă, predispoziția de organ, amorsarea patogenă, imprimarea morfofuncțională, relevanța clinică, evoluția, terapia. Ceea ce pune eticheta de boală psihosomatică este evidențierea substratului organic şi strângerea manifestărilor clinice în entități bine conturate.

a) Hipertensiunea esențială

Studiul Framingham privind sistemul cardiovascular, aflat în desfăşurare încă din 1948, a publicat în Lancet (2004) un ultim raport în care arată că riscul de apariție a unei HTA autentice este asociat cu valorile inițiale ale TA şi cu vârsta. O analiză a altor eventuali FR ai instalării HTA a confirmat rolul greutății corporale, creşterea cu 5% a acesteia (4 kg pentru un bărbat şi 5 kg pentru o femeie de constituție medie) asociindu-se cu o creştere cu 20-30% a riscului de dezvoltare a HTA.

MATERIAL ŞI METODĂ

Lotul de studiu a cuprins un număr de 413 pacienți din mediul rural care s-au prezentat la C.M.I. Dr. Anghel Lucreția-Jorăşti.

Evaluarea pacienților s-a făcut prin:

I Anamneză – a cuprins întrebări privind:

1. vârsta, sexul, starea civilă, nivelul de instruire, ocupaţia;

2. antecedente heredo-colaterale: deces prin boală cardiovasculară sub 55 de ani, cancer diagnosticat sub 65 de ani, diabet zaharat, hipercolesterolemie familială;

3. antecedente personale patologice: existenţa de boli cronice, boli cu transmitere sexuală, intervenţii chirurgicale, medicație relevantă;

4. stilul de viaţă: alimentaţia, consumul de alcool, activitatea fizică, fumatul.

II. Examenul clinic obiectiv – inclusiv măsurarea înălţimii şi a greutăţii, a taliei, calcularea indicelui de masă corporală, măsurarea tensiunii arteriale (3 valori la interval de 5 minute) şi a pulsului.

III. Investigațiile au cuprins:

- Electrocardiograma;
- Analize de laborator: glicemie, colesterolul total, HDL-colesterol, trigliceride, creatinina serică, sideremie.

Pentru determinarea depresiei la lotul de pacienți am folosit Scala Depresiei Geriatrice YESAVAGE (GDS).

REZULTATE ŞI DISCUȚII

În urma analizelor efectuate am constatat că dintre cele 242 de femei cu vârsta peste 60 de ani: 67 de femei sunt cu boală cardiovasculară prezentă iar 72 de femei sunt cu risc crescut de boală cardiovasculară, restul de 103 fiind cu risc scăzut sau fără risc de boală cardiovasculară. Ţinând cont de valoarea normală a colesterolului total (120,00-200,00 mg/dl) şi de IMC (Interval normal - 20 - 24,9; Supraponderalitate, risc crescut - 25 şi peste; Obezitate, risc mare - 30 şi peste)

Menționez că: din aceste 242 de femei 159 sunt cu hipercolesterolemie, 96 sunt supraponderale, 44 prezintă obezitate, 39 de femei prezintă depresie uşoară şi 7 sunt cu depresie severă.

Dintre cei 171 de bărbați cu vârsta peste 60 de ani 43 de bărbaţi sunt cu boală cardiovasculară prezentă iar 86 sunt cu risc crescut de boală cardiovasculară, restul de 42 fiind cu risc scăzut sau fără risc de boală cardiovasculară.

Din cei 171 de bărbaţi 86 sunt cu hipercolesterolemie, 68 sunt supraponderali, 19 prezintă obezitate, un bărbat prezintă depresie uşoară şi unul depresie severă.

Dintre cele 46 de femei cu depresie uşoară şi severă se observă că un număr de 32 sunt supraponderale, 7 prezintă obezitate, 29 prezintă colesterol total crescut, 23 prezintă LDL - colesterol crescut şi toate sunt cu risc crescut de BCV sau cu afecţiune prezentă.

Cei 2 bărbaţi cu depresie uşoară şi severă prezintă şi celelalte afecţiuni.

Din repartiţia pe sexe a valorilor medii a celorlalte afecţini se observă că femeile prezintă valori mai mari decât bărbaţii atât în ceea ce priveşte IMC, colesterol total cât şi depresia iar barbaţii prezintă risc mai mare de boală cardiovasculară.

Din lotul de pacienţi studiaţi (413) majoritatea (88,37 %) prezintă un scor mediu al depresie de 7,4 încadrându-se în limite normale, restul de 11,63 % prezintă depresie uşoară şi severă.

CONCLUZII

Depresia este un factor de risc independent de producere a unui eveniment cardiac.

Deşi nu este obiectivul acestui studiu să recomande tratamentul depresiei pentru reducerea riscului cardiovascular, depresia este prezentă în procentaj mare în rândul populaţiei şi trebuie cu siguranţă să fie tratată chiar şi numai datorită îngrijorărilor privind calitatea vieţii.

Depresia, boala cardiacă ischemică şi relaţia lor cu colesterolul este una controversată.

Depresia ar putea duce la un profil nefavorabil al lipidelor rezultat dintr-o interacţiune a axelor catecolaminice şi steroide.

Alături de factorii de risc cardio-vascular, stresul psihic (factorii psiho-sociali, socio-profesionali, socio-familiali) are un rol extrem de important în declanşarea patologiei.

Dintre factorii de risc modificabili, cu importanţă în declanşarea şi evoluţia HTA şi sindromului depresiv remarcăm: stilul de viaţă negativ (sedentarismul, comportamentul adictiv de alcool, nicotina, cafea, obiceiurile alimentare nesănătoase, abuzul de sare) şi caracteristicile individuale (valorile oscilante ale tensiunii arteriale, indicele de masă corporală), obezitatea abdominală, toate acestea fiind asociate cu TA crescută.

Nivelul factorilor de risc cardiovasculari convenţionali asociaţi bolii depresive şi-au sporit valoarea în relaţie directă cu gravitatea depresiei, astfel în formele majore de depresie şi nivelul colesterolului şi valorile presiunii arteriale au fost mai mari în medie, ceea ce a dus la creşterea poverii riscului absolut de boală cardiovasculară.

Abordarea persoanei vârstnice presupune cunoaşterea şi determinarea riscurilor geriatrice cu scopul elaborarii de intervenţii la nivel individual concretizate prin servicii de îngrijire care vizeaza îmbunătăţirea calităţii vieţii şi la nivel populaţional sub forma politicilor de sănătate care vizează prevenţia şi o îmbătrânire de succes.

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DETECTAREA STERIGMATOCISTINEI LA PACIENŢII CU BOALĂ HEPATICĂ CRONICĂ

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HIGH LEVELS OF STERIGMATOCYSTIN IN PATIENTS WITH CHRONIC LIVER DISEASES (Abstract). Sterigmatocystin (STC) is a wide spread mycotoxin produced by *Aspergillus* fungi, with hepatotoxic and carcinogenetic proprieties. **Objectives:** To determine the STC concentration in blood and urine from patient with liver cirrhosis (LC) and hepatocellular carcinoma (HCC), with correlation with liver function parameters. **Material and methods:** The study enrolled 166 patients divided in three groups: control – 55 patients (27M, 28F); LC – 58 patients (31M, 27F); HCC – 53 patients (26M, 27F). 20 ml of blood and 50 ml of urine were collected from each patient and liver enzymes and alfa-fetoprotein (AFP) were measured. STC was determined by high performance liquid chromatography, with concomitant detection in ultraviolet and fluorescence. **Results:** STC was detected in 26.2% of samples, more frequently in LC and HCC groups ($p < 0.001$). STC mean values were 0.014 ng/ml and 0.005 ng/ml in blood, respective urine of controls, rising to 0.626 ng/ml ($p = 0.003$) respective 1.053 ng/ml ($p = 0.049$) in LC and 2.02 ng/ml in blood ($p < 0.0001$) and 9.39 ng/ml in urine ($p = 0.003$) in patients with HCC. There is a perfect correlation between serum and urinary levels of STC in controls ($r = 1$), that become weak in patients with LC ($r = 0.48$) and insignificant in HCC ($r = 0.15$). AFP values were significantly correlated with STC concentration in patient with HCC, in both blood ($r = 0.31$) and urine ($r = 0.84$). **Conclusions:** STC values in patients with LC and HCC were significantly higher compared to controls. Strong positive correlation of STC with AFP in patients with liver cancer suggested a possible role of this mycotoxin in pathogenesis of the disease. **Key words:** MYCOTOXIN, STERIGMATOCYSTIN, LIVER CIRRHOSIS, HEPATOCELLULAR CARCINOMA, ALFA-FETOPROTEIN

Micotoxinele sunt substanţe naturale care apar ca produşi secundari de metabolism ai fungilor filamentoşi care contaminează hrana animalelor şi oamenilor. Contaminarea cu micotoxine este considerată inerentă (1,2), eforturile depuse urmărind limitarea acesteia la niveluri considerate sigure pentru sănătatea oamenilor şi animalelor.

Clasele majore de micotoxine includ: aflatoxinele, tricotecenele, fumonizinele, zearalenone, ocratoxinele şi alcaloizii de ergot (1), majoritatea acestora fiind produse de fungi din genurile *Aspergillus*, *Penicillium* şi *Fusarium*. Principala micotoxină, atât din punct de vedere al cantităţilor produse, cât şi din punct de vedere al efectelor biologice (substanţa naturală cu cel mai puternic efect

TABELUL I
Valorile STC determinate la cele trei loturi de studiu

Lot	Număr probe pozitive		Procent probe pozitive		Valoarea maximă[a]		*p*[b]	
	Sânge	Urină	Sânge	Urină	Sânge	Urină	Sânge	Urină
Martor	2	2	3.63	3.63	0.38	0.13	-	-
CH	22	16	37.93	27.58	11.80	39.06	<0.0001	<0.0001
CHC	28	17	52.83	32.87	2.02	9.39	0.0005	<0.0001

a – ppm (părţi per milion) sau ng/ml ; b – test t-Student, comparativ cu lotul martor

TABELUL II
Valoarea AFP şi corelaţia cu STC

Lot	AFP	p[a]	Coeficient de corelaţie[b]	
			Sânge	Urină
Martor	4,42	-	0.004	0.004
CH	12,63	0.004	-0.02	0.05
CHC	1252,79	0.0002	0.31	0.84

a – testul t-Student ; b – coeficient de corelaţie (test Pearson)

carcinogen) este aflatoxina B_1 (AFB_1) (1,2).

Sterigmatocistina (STC) este o aflatoxină produsă de genul *Aspergillus* (*A. versicolor* – principalul producător de STC ; *A. nidulans*, *A. chevalieri, A. ruber*), şi de unele tulpini de *Penicillium, Bipolaris, Chaetomium, Emiricella* (1,2,3). Din punct de vedere chimic, STC este un precursor al AFB1, în care se poate transforma atât *in vitro* cât şi *in vivo* printr-o singură reacţie (1,2), producând aceleaşi efecte biologice.

Metabolizarea STC este predominant hepatică, rolul esenţial avându-l complexul CYP3A4, pentru care are o mare afinitate . Activarea STC este dependentă de enzimele citocromului P450, metabolizarea fiind iniţiată la nivelul dublei legături C_2-C_3 (1). Rezultatul este STC-2,3-epoxidul, care este instabil în mediul apos şi este rapid hidrolizat neenzimatic la dihidrodioli. Toţi aceşti compuşi au capacitatea de a forma adducţi cu ADN, acţionând la nivelul 7N al guaninei (1), similar cu AFB_1. STC este creditată cu 10% din capacitatea carcinogenetică a AFB_1 (3) şi declarat carcinogen de ordinul II (3), dar pare să fie mai toxică decât aceasta pentru rinichi (12).

OBIECTIVE

Studiul a urmărit detectarea prezenţei STC în sângele şi urina pacienţilor cu boală hepatică avansată, precum şi stabilirea corelaţiilor acesteia cu parametrii funcţiei hepatice.

MATERIAL ŞI METODĂ

Pacienţi. În studiu au intrat 166 de pacienţi divizaţi în : lot martor – 55 pacienţi (27M, 28F), fără afectare hepatică ; ciroză hepatică (CH) – 58 de pacienţi (31M, 27F) ; carcinom hepatocelular (CHC) – 53 de pacienţi (26M, 27F). Vârsta medie a participanţilor a fost de $57,9 \pm 8,4$ ani în lotul martor, $57,2 \pm 10,3$ ani în lotul cu CH ($p=0.33$) şi $63,7 \pm 10,2$ ani în lotul cu CHC ($p=0.001$ faţă de martor, $p=0.0006$ faţă de CH). Structura loturilor şi distribuţia pe medii de provenienţă este redată în Fig. 1. De la toţi pacienţii au fost recoltate în prima zi de la internare 20 ml de sânge periferic pe EDTA şi 50 ml de urină.

Teste biochimice. Folosind metoda chimiei umede (Hitachi), au fost determinate enzimele de citoliză hepatică (TGP, TGO) şi de colestază (FA, GGT). α-fetoproteina serică (AFP) a fost determinată prin electroche-

miluminiscenţă, pe aparat Modular E 170.

Cromatografia de lichide de înaltă performanţă (HPLC). Detecţia STC în probele analizate s-a făcut utilizând metoda HPLC. S-a folosit un cromatograf de lichide tip HP 1090 cu detecţie simultană în UV la 251 nm şi în fluorescenţă (excitaţie la 228 nm şi emisie la 423 nm) prevăzut cu o coloană C18 tip Zorbax (15 cm x 4,5 mm, 5 μm), cu o fază mobilă formată din amestec acetonitril : metanol : apă (99 : 99 : 2) la un debit de 0,7 ml/min, conform metodei descrise anterior (16), după precipitarea proteinelor din sânge cu acetonitril, purificare cu izooctan, extracţie cu cloroform şi reluarea reziduului obţinut, după evaporarea la sec, cu metanol. Folosind ecuaţia curbei de calibrare obţinută la studiul liniarităţii din cadrul validării metodei de analiză, utilizând soluţii de AFB1 şi STC în concentraţii cunoscute, s-a calculat concentraţia în micotoxine din probele de sânge recoltate de la pacienţi. Precizia metodei (RSD) este de 16,9629% cu o regăsire medie de 99,2%.

Analiza statistică. Rezultatele obţinute au fost incluse într-o bază de date Microsoft Excel 2010. Prelucrarea statistică s-a făcut folosind testul Fisher, testul *t-Student* şi testul de corelaţie Pearson, iar valoarea $p < 0.05$ a fost considerată semnificativă statistic.

REZULTATE ŞI DISCUŢII

Din totalul de 332 probe analizate, un număr de 87 de probe (26,2%) au prezentat nivele detectabile de STC, cele mai multe în lotul cu CHC (tab. I). Ambele loturi de pacienţi cu afectare hepatică au fost depistaţi pozitiv mai frecvent decât martorul, dar nu există diferenţe statistic semnificative între frecvenţa înregistrării probelor pozitive la pacienţii cu hepatocarcinom faţă de cei cu ciroză, atât în sânge ($p = 0.17$), cât şi în urină ($p = 0.68$). Recoltarea de sânge şi urină a fost efectuată în prima zi de internare, astfel încât valorile determinate reflectă gradul de ingestie al micotoxinei în alimentaţia obişnuită, de la domiciliu, a pacientului.

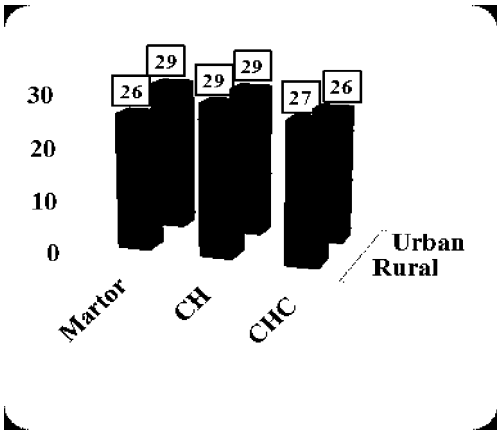

Fig. 1. Structura loturilor de studiu

Valorile medii ale concentraţiei STC în sângele şi urina lotului martor au fost de 0,014 ng/ml, respectiv de 0,005 ng/ml, fiind semnificativ statistic mai mari la pacienţii cu CH : 0,626 ng/ml în sânge ($p = 0.003$) şi 1,053 ng/ml în urina ($p = 0.049$). Concentraţia STC în probele recoltate de la pacienţii cu CHC a fost semnificativ mai mare decât a lotului martor, atât în sânge ($p < 0.0001$) cât şi în urină ($p = 0.003$), dar fără diferenţe statistic semnificative faţă de rezultatele înregistrate în lotul cu CH ($p = 0.17$, respectiv $p = 0.44$). Există o corelaţie perfectă între valorile serice şi urinare de STC la lotul martor ($r = 1$). Aceasta este semnificativă, dar mai slabă, pentru lotul cu CH ($r = 0.48$), şi se pierde la lotul cu CHC ($r = 0.15$).

Existenţa corelaţiei între valorile serice şi urinare ale STC la lotul fără afectare hepatică semnifică paralelismul între aportul şi eliminarea toxinei. Pierderea acestei corelaţii la loturile cu afectare hepatică avansată reprezintă probabil efectul perturbator pe care afectarea capacităţii de metabolizare hepatică îl are asupra biotransformării STC. Cum la pacienţii cu CH şi CHC numărul de probe pozitive este mai mare în sânge decât în urină, la aceste loturi capacitatea de metabolizare a STC să scadă, prelungind astfel acţiunile biologice ale acesteia.

Nu există diferenţe statistic semnificative între pacienţii provenind din medii diferite, nici între bărbaţi şi femei, pentru nici unul din loturile studiate.

Nu s-au înregistrat diferenţe statistic semnificative între valorile transaminazelor determinate în cele trei loturi. Valorile enzimelor de colestază au fost mai mari în lotul cu CH şi CHC, faţă de martor, dar fără să existe diferenţe semnificative între pacienţii cu boală hepatică cronică. Nu există corelaţii semnificative între valorile enzimelor de citoliză hepatică sau colestază şi concentraţiile de STC, pentru nici unul din cele trei loturi. Acest fapt sugerează că diferenţele sunt determinate de procesul fiziopatologic hepatic, fără a fi implicată ingestia de micotoxine.

Valorile AFP au fost maxime la pacienţii cu hepatom, diferenţele faţă de martor şi cirotici fiind înalt semnificative statistic (tab. II). La loturile martor şi cu CH, nu există o corelaţie între valoarea AFP (marker biologic al procesului de carcinogeneză hepatică) şi STC. La lotul cu CHC, această corelaţie este prezentă şi pozitivă, mai ales în cazul valorilor STC urinare, unde este foarte puternică ($r = 0.84$). Acest fapt sugerează existenţa unei corespondenţe între gradul intoxicaţiei cu STC şi procesul carcinogenetic hepatic, mai ales în contextul în care activitatea mutagenică a acesteia a fost dovedită experimental (17,18). Valorile mari ale STC urinare corespund unei detoxifieri hepatice mult scăzute. Ele pot semnifica rolul important al intoxicaţiei în procesul patogenia hepatomului la aceşti pacienţi, sau pot reprezenta consecinţa scăderii pronunţate a capacităţii ficatului de a metaboliza STC, astfel încât acesta este eliminată sub formă parentală.

CONCLUZII

Se remarcă în mod evident niveluri mai ridicate ale concentraţiei de micotoxină la pacienţii cu boli hepatice avansate în comparaţie cu lotul martor, ceea ce susţine rolul STC în afectarea hepatică şi mai ales în producerea cancerului hepatic. Creşterea semnificativă a valorilor STC urinare la pacienţii cu hepatocarcinom poate reprezenta atât cauza, cât şi efectul procesul carcinogenetic hepatic.

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CARDIOVASCULAR RECOVERY IN ELDERLY PATIENTS

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CARDIOVASCULAR RECOVERY IN ELDERLY PATIENTS (Abstract): Cardiac rehabilitation of elderly patients is becoming a growing necessity because, with aging, the prevalence of cardiovascular disease increases. The most vulnerable population, the elderly, might have greater needs for cardiac rehabilitation because the likelihood of being fragile is very high. After hospitalization for such an event as acute coronary syndrome or cardiac decompensation, all patients, but especially the elderly, are at increased risk for destabilization, being eligible for new cardiovascular events that may have a disabling impact on both physical and mental health. The efficacy and safety of cardiac rehabilitation has been also demonstrated in elderly patients with stable coronary artery disease, in those who underwent coronary intervention or in patients with chronic heart failure. Cardiac rehabilitation programs are designed to enhance recovery after an acute cardiovascular event and improve both quality of life and survival by measuring secondary prevention - controlling risk factors, physical activity, diet and optimizing medical treatment. Over the years, all the evidence has shown comparable benefits in elderly and younger patients. Particular attention should also be paid to frail elderly patients who need a personalized intervention. Frailty is defined as a complex of biological markers and clinical characteristics seen in a dynamic position between a healthy status and extremely severe clinical status. Frailty puts many elderly patients at greater risk of health destabilization and first it is necessary to prevent and to treat not only in an "acute" care setting, but also a "post-acute" recovery through home care. **Keywords:** CARDIOVASCULAR RECOVERY, CARDIOVASCULAR PATHOLOGY, CARDIAC DECOMPENSATION, FRAILTY, QUALITY OF LIFE, ELDERLY.

Cardiac Rehabilitation (CR) is defined World Health Organization (1) as a complex medical program primarily offered to patients with severe cardiovascular disease (acute myocardial infarction, heart failure, transluminal angioplasty, cardiac surgery) and aimed at improving cardiac function with the help of individually dosed exercise, lifestyle change with improvement of cardiovascular risk factors (diabetes mellitus, hypertension, smoking, obesity, dyslipidemias) and, last but not least, at managing daily life stress and stress induced by ongoing cardiac disease.

CR is represented by a multitude of comprehensive long-term programs that include medical assessment, physical activity prescription, changing risk factors, edu-

cation, and medical advice. These programs aim at limiting the physiological and psychological effects of heart disease, reducing the risk of sudden death and reinfarction, controlling cardiac symptoms, regression or stabilization of atherosclerosis, and improving the psychosocial and occupational status of patients. In the past, CR was indicated to young patients to facilitate their reintegration into society as soon as possible, to resume activities after surgery or after a stroke.

Cardiovascular disease is estimated to cause over 4 million deaths each year in Europe, with over 1.9 million cases in the European Union countries, thus placing cardiovascular disease as the leading cause of death (2).

Over the past 30 years we have witnessed a world population aging, with dramatic effects on the demand for medical services, with the highest increases for cardiovascular diseases. On January 1st, 2017 the population of Romania was 22.22 million people. The population aging phenomenon has intensified, the elderly population (65 years old and over) exceeding by 269,000 the young population aged 0-14 (3.55 million elderly versus 3.28 million young people). The demographic aging index has increased from 105, 8 (January 1st, 2016) to 108.2 elderly per 100 young people (January 1st, 2017) (3).

The mean age of the population was 41.1 years, 0.2 years higher than on January 1st, 2016. The median age was 40.8 years, up 0.4 years from January 1st, 2016.

Cardiovascular diseases remain the leading cause of death among elderly worldwide with combined prevalence rates of >50% of the following conditions: heart failure, cardiac arrhythmias, stroke, and peripheral vascular disease (4).

In Romania, among elderly people aged 65 and over compared to people aged 15-64, morbidity rate is double, with cardiovascular diseases being almost 4 times more common, followed by diseases of the nervous system, osteoarticular system, degenerative muscular disorders, neoplasms, mental and behavioral disorders. In addition, the almost universal risk of developing high blood pressure during life, coupled with the increasing rate of multiple cardiovascular risk factors, contributes dramatically to the increased incidence and prevalence of cardiovascular diseases with age (5).

This in the context of age-related normative changes in cardiovascular structure and function, which result in a phenotype that predisposes older people to cardiovascular disorders and amplifies their sequels. Consequently, although mainstream cardiology becomes de facto geriatric cardiology, in many places older adults with cardiovascular disease are often not cared for by cardiologists or geriatricians, but rather by primary care providers and allied medical professions.

Under these conditions, CR in the elderly patient becomes an imperative necessity, even more so since the studies conducted over the past 20 years show that physical training in rehabilitation programs for the elderly has similar effects to those achieved in the adult. Studies in elderly patients with coronary artery disease and/or heart failure show that CR offers similar benefits to younger subjects through physical rehabilitation, increased functional capacity, and reduced cardiovascular risk. Research has shown that there may be even greater increases in aerobic capacity following a personalized physical exercise program in this age category (6).

Starting an CVR program in the elderly patient will need to consider a number of peculiarities encountered at this age:

- occurrence/increase/worsening of frailty, manifested by: decrease in muscle mass (sarcopenia), nutrition disorders, progressive worsening of osteoporosis, increased risk of falling (7, 8);

- concomitant presence of other diseases than the existing cardiovascular ones might have a significantly impact on CR: osteoarticular, neurological, respiratory, ophthalmic diseases;

- occurrence of cognitive disorders, depression and delusional episodes - caused by inexorable changes in the emotional, social and emotional life of the elderly and aggravated by the presence of cardiovascular and concomitant diseases.

Before initiating a CR program, it must be considered that the diseases of the elderly patients are chronic, long-lasting, requiring high consumption of medical services (consultations, medical treatments, repeated and long-term hospitalizations) and permanent medical-social care, domestic and personal hygiene, institutionalization). Loneliness, impairment of sexual activity and chronic metabolic disorders are some of the causes that can lead to emotional disturbances and decreased quality of life I the elderly (9, 10) (fig. 1).

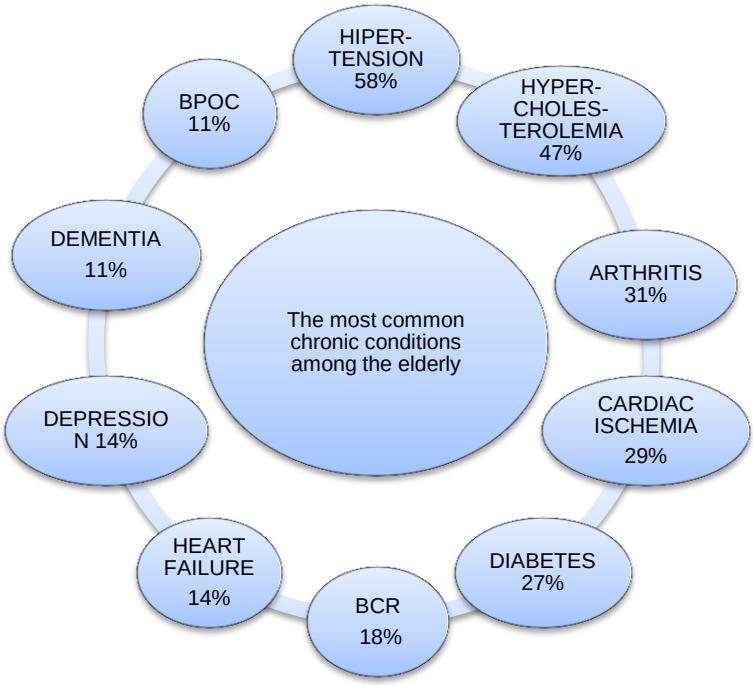

Fig. 1. The most common chronic conditions among the elderly

Elderly people need to be encouraged and helped to live healthy, productive, participative, dignified and independent lives, and in this respect three major general objectives must be met: prolonging active life, promoting social and welfare

participation for the elderly, and achieving more independence and safety for people in need for long-term care.

Farzianpour *et al*. (9) concluded in one of their studies that the elderly who have an active lifestyle automatically have a better quality of life, are less depressed, more satisfied and more socially active. Older people who can carry out their daily activities are also less likely to become depressed, and thus the decompensation rate is lower. Similar results are reported by Prescott *et al*. (11) who support that many patients with cardiovascular impairment, physical, social limitations, and unstable health, become anxious or depressed as the state of incapacity and frailty is prolonged.

The goal of the CR is to help the elderly to regain their physical form before the disease, to prevent physical deconditioning and subsequent vascular accidents. CR provides the opportunity to fight predictable geriatric complications in elderly patients with cardiovascular disease (CVD), including:

1) co-morbidity (multiple cardiac and non-cardiac diseases occurring in combination);

2) polymedication (multiple new drugs in the context of age-related changes in pharmacokinetics and pharmacodynamics predisposing to undesirable side effects),

3) iatrogenic processes (harmful effects of polypragmasy, repeated and prolonged hospitalizations and transitions including delirium, deconditioning, further institutionalization, and occurrence and/or worsening of sarcopenia and poor quality of life) (12).

CR is one of the few standard therapeutic options that aims to improve physical function as a primary outcome and which therefore has the potential to improve prognosis of heart disease but also to reduce age-related vulnerabilities such as frailty and disability, and which are usually exacerbated by CVD.

The study by Harada *et al.*, (13) suggests that the CR, together with good nutrition control, risk prevention and drug treatment, can be a useful strategy for the prevention and treatment of sarcopenia in patients with CVD.

Recovery in cardiovascular disease is not only a physical exercise program but also a lifestyle education and change program designed specifically to increase the quality of life of the elderly patient with cardiovascular pathology (myocardial infarction, after surgery for CVD, arterial, and peripheral venous disease). Finally, rehabilitation programs have the role of increasing the chance of survival of patients.

Attention should be paid to increasing the quality of life of the elderly patient to the level of his/her expectations. The quality of life of patients with CVD is significantly affected, especially in the elderly, due to several factors, including the high risk of recurrence of cardiovascular events, admission to hospital, physical disability, co-morbidity and general frailty. With physical and social limitations and unstable health, many patients become anxious or depressed.

In a recent study, Vigorito *et al*. (14) warn that the prevalence, clinical and prognostic relevance of frailty in a cardiac rehabilitation setting has not yet been well characterized, although many studies have reported frailty in patients with coronary syndromes in intensive care or cardiology units. At the same time, frailty complicates the recovery and treatment of elderly patients as it can affect the type and timing of

diagnostic procedures and pharmacological and non-pharmacological treatment (5). The assessment of the basic physical function must be adapted to physical conditions and the CR program must be individualized based on functional compromise and disability (tab. I).

TABLE I
Indications of cardiovascular recovery

INDICATIONS OF CARDIOVASCULAR RECOVERY		
CLASS I	CLASS II	CLASS III
Certain indications for rehabilitation	Possible indications with probable but still controversial effectiveness	Contraindications or non-indications for rehabilitation

Cardiovascular damage is a major cause of disability and decreased quality of life, regular physical activity under the guidance of professionals representing the main non-medical factor - and thus without adverse effects - that can effectively help in the secondary and tertiary prevention of this pathology (15).

Currently, due to improved programs and medical monitoring, CR has become accessible to people of any age who have cardiovascular disease. Menezes *et al*. (16, 17) claim that, as the population ages, a growing proportion of inactive adults, aged at least 85, will be candidates for the CR. Specific care processes (both in terms of physical exercise and wider goals of CR) will need to be better suited to the needs of an increasing population of vulnerable elderly people.

Exercise has beneficial effects on physical state and on cardiovascular risk factors. Physical endurance training increases exercise capacity and more effectively influences the risk factors for ischemic heart disease. It reduces the percentage of fat in the tissues and reduces the risk of obesity, increases bone mineral density, thus reducing the risk of osteoporosis, the risk of falls and fractures, decreases insulin response to glucose loading and prevents diabetes, lowers serum lipids, increases HDL cholesterol, reduces dyslipidemia and atherogenic risk, lowers heart rate and blood pressure, improves cardiac output and cardiac performance, improves basal metabolism, improves sleep quality, increases quality of life, reduces mortality in post-myocardial infarction patients, relieves symptoms in patients with angina pectoris, increases exercise capacity, quality of life improves mental health and mood and decreases depression rate, reduces hospital readmission rate and increases survival. In cardiac failure, physical training increases exercise capacity (VO_2 max), improves cardiac output, increases the size and density of mitochondria, improves skeletal muscle metabolism and function, reduces endothelial dysfunction, and lowers plasma catecholamine levels; in patients with lower limb arteriopathy obliterans, exercise is very effective, helping to increase the walking distance and maximally tolerated pain in elderly patients (18, 19) (fig. 2).

To support the above statements, Schopfer *et al*. (12) concluded in a review-

type study that CR is more than a program, but a complete lifestyle that can have a benefit for elderly patients with CVD and that the maintenance of physical function (cardiorespiratory fitness, as well as strength and balance) helps to overcome progressive disease and deconditioning as well as associated vulnerabilities.

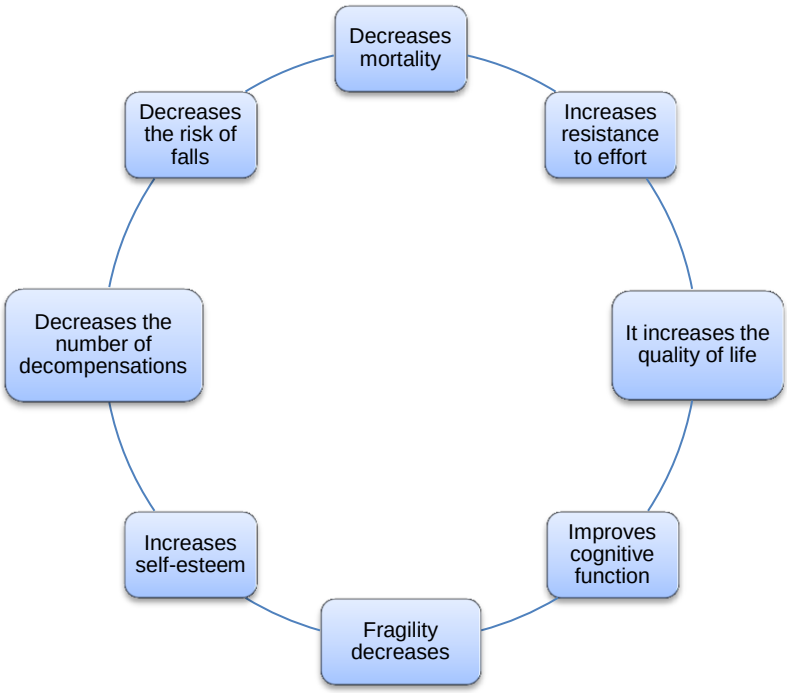

Fig. 2. Benefits of cardiovascular recovery in elderly patients

Baldasseroni *et al*. (19) also concluded in their study, which enrolled patients aged 75 years and older who presented themselves to an outpatient treatment unit following an acute coronary event (unstable angina, acute myocardial infarction) or heart surgery (coronary bypass), that a CR program based on physical exercise was associated with the improvement of all areas of physical fitness even in older adults, especially those who had lower baseline performance.

Nishant *et al*. (20) believe that in the present state of knowledge additional investigations are needed to better understand the impact of CR on heart failure and that recent literature presents its benefits in these patients, although much of the evidence is limited to patients with systolic dysfunction; also, it encourages the exploration of strategies to increase the use of CR.

CONCLUSIONS

In our opinion, based on the researches made, the rehabilitation process for the elderly should be implemented in accordance with the exercise-related risk assessment criteria and adapted to the different disease-related patterns and disabilities.

Disability tends to coincide with a state of complexity, comprising a broad spectrum of cardiovascular and non-cardiovascular diseases associated with underlying cardiac disease characterized by varying degrees of impairment of functional capacity. This comes from a disability that causes varying degrees of frailty, thus limiting the individual's participation in social life.

Therefore, rehabilitation intervention can be guided by a multidimensional assessment configured in patients with moderate cardiovascular disease in a classical physical training program, while patients with severe cardiovascular disease and more limited functions may be subjected to physical activity that, even if not an effective stimulus for training, helps prevent total disability. In any case, CR configures an operational framework that involves different reasons from many professions, depending on the degree of disability and the different patient needs. The process of rehabilitation of these patients will be strictly individualized in relation to their functional impairment/admission disability and normal functional capacity. The proposed Protocols do not follow the traditional approach to rehabilitation in cardiology (different protocols for different events or acute or chronic illnesses) but instead propose a cross-sectional criterion for different clinical scenarios based on different levels of patient disability.

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NEWS

BRONCHIAL HYPER-RESPONSIVENESS: A TEHNICAL UPDATE

Bronchial hyper-responsiveness (BHR) is often regarded as a "hallmark" of asthma, and bronchoprovocation testing is frequently performed to support a diagnosis of asthma. The European Respiratory Society (ERS) and American Thoracic Society (ATS) have recently updated their technical standards and guidelines for performing methacholine challenge testing (MCT), the most commonly performed clinical test of BHR. The new guidelines recommend that methacholine challenge testing be performed using tidal breathing, not deep inspirations with breath holding. Results should be reported as the PD20 (cumulative dose causing a 20% fall in forced expiratory volume in 1 s), rather than PC20 (concentration causing a 20% fall in forced expiratory volume in 1 s). Manufacturers of nebulizes and other delivery systems should provide performance characteristics to allow calculation of PD20 values. The clinicians who utilize and evaluate BHR testing are encouraged to understand the differences among various MCT protocols. Of particular importance is an appreciation of the relatively high likelihood of false negative tests in mild or asymptomatic asthmatics, and the difficulty of interpreting MCT over time when testing methods are not held constant. Finally, there is hope that clinicians will encourage their hospitals and pulmonary function laboratories services to more actively adopt the recent guidelines (Borak J, Lefkowitz RY, Linde B. Bronchial hyper-responsiveness: a technical update. *Occupational Medicine* 2018, kqy122, https://doi.org/10.1093/occmed/kqy122).

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HEREDITARY HEMOCHROMATOSIS IN NORTH-EASTERN ROMANIA

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HEREDITARY HEMOCHROMATOSIS IN NORH-EASTERN ROMANIA (Abstract): Hereditary hemochromatosis is a genetic disturbance of iron metabolism resulting in iron overload in several organs and their functional failure. Early diagnosis is necessary to start a simple and effective therapy: bleeding. It is the most frequent genetic disease in some populations. Our **objectives** were 1. An estimate of the expectancy of the disease in the population of our geographic region, and 2. To diagnose the disease by applying the established methods and estimate the efficiency of our diagnosis. **Methods**: 1.To estimate the expectancy, we genotyped 200 persons for the most frequent mutations causing the disease: HFE-C282Y and HFE-H63D by PCR-RFLP. 2. To diagnose the disease phenotypically we determined plasma iron level, ferritin level and transferrin saturation index in 549 patients previonsly diagnosed as chronic hepatitis or cirrhosis and genotyped those with hemochromatosis phenotype. **Results**: 1. We found allelic frequencies of 1.75% and 13.25% for the HFE-C282Y and H63D mutant alleles respectively. From these frequencies we calculated that a severe case caused by a C282Y/C282Y homozygote can arise in 816 people and a mild case caused by a C282Y/H63D compound heterozygote can arise in 100 people 2. Among 549 patients we found 10 to have the phenotype of hemochromatosis and 3 out of the 10 were found to carry mutations: two in the HFE gene (one homozygous C282Y and one compound heterozygous C282Y/ H63D) and one in the hemojuvelin (HJV) gene (a G320V). **Key words**: PREVALENCE, HFE-C282Y, HFE-H63D, HJV-G320V

Hereditary hemochromatosis is the most frequent genetic disease in some regions of Europe such as Scandinavia, Ireland, Britain (UK) and Brittany (France). In these regions the disease is a public health problem. In other European and American regions its frequency is lower but its severe consequences and its curability makes early diagnosis very important. This is also our reason for initiating its study in our region. Some of our results have been published elsewhere (1) or are being prepared to be published. This is a summing-up report of what we have done to date.

The disease has been described by Trousseau in 1865 as 'bronze diabetes with cirrhosis' and coined as hemochromatosis by Recklinghausen in 1889. In 1996 the HFE gene and its mutations C282Y (a cysteine to tyrosine substitution at codon 282) and H63D (a histidine to aspartic acid substitution at codon 63), were described by Feder and colleagues (2). About 83% of patients genotyped by Feder were C282Y homozygous. Since the HFE gene discovery, new genes have been discovered to be involved in hemochromatosis and their encoded proteins to be involved in iron metabolism.

Brief presentation of iron-overload pathophysiology. Hemochromatosis is due to iron overload in tissues, resulting in death of cells. The outcome of iron overload can be hepatic cirrhosis, heart failure, diabetes, arthroses, hypogonadotropic hypogonadism etc. Iron overload is due to a systemic deregulation of iron metabolism. The central regulator of iron homeostasis is hepcidin, a polypeptide of 25 aminoacids secreted by hepatocytes into plasma (as a hormone). Almost all iron overloads are dues to a hepcidin deficiency, except one caused by the ferroportin excess. Ferroportin is a protein expressed by enterocytes, hepatocytes and macrophages and is inserted into those cells membranes where it facilitates iron export. The ferroportin expression is negatively regulated by hepcidin. Extracellular hepcidin binds membrane ferroportin causing its internalization and proteolysis (3).

Hepcidin's expression and secretion by hepatocytes is upregulated by several proteins organized into at least two pathways. One pathway is acted by Fe-transferrin (Fe-Tf), transferrin receptor 1 (TfR1), transferrin receptor 2 (TfR2), and HFE. HFE, a protein of the major histocompatibility complex I (MHC) superfamily, can form complexes with both TfR1 and TfR2 transferrin receptors, but in different manners. Association of TfR1 with Fe-TF or HFE is mutually exclusive (i.e. TfR1 associates with either HFE or Fe-Tf), while TfR2 associates with both Tf and HFE. The ternary complex TfR2-Tf-HFE is the positive transcription regulator of the HAMP gene encoding hepcidin's precursor. Fe-Tf abundance in plasma releases HFE from TfR1 in hepatocytes, making it available for the ternary complex TfR2-Tf-HFE which increases the hepcidin synthesis and secretion. Hepcidin negatively regulates ferroportin activity and a retention of iron in enterocytes and macrophages (main sources of plasma iron) occurs, maintaining a constancy of plasma iron–transferrin concentration (4).

A second pathway regulating the hepcidin level is acted by hemojuvelin and the members of the bone morphogenetic proteins (BMP) signaling pathway. This pathway assures an influence of infection/inflammation and hypoxia over the iron metabolism via hepcidin secretion (4). Hemojuvelin deficiency is associated with a very severe iron overload.

Type 1 of hereditary hemochromatosis is due to mutants inactivating HFE. Type 2A, 2B and type 3 hemochromatosis are dues to inactivating mutations in hemojuvelin, hepcidin and transferrin receptor 2 (TfR2) respectively. Type 4 hemochromatosis is due to mutations in the ferroportin encoding gene that preclude ferroportin to be negatively regulated by hepcidin.

Our approach to hemochromatosis in our geographical region. We had two objectives in our study : 1) To estimate the expectancy of the disease by genotyping a sample of normal population for the most frequent HFE mutations, C282Y and H63D, and 2) To use and optimize methods of discovering the disease among patients.

MATERIALS AND METHODS

Population samples : For the prevalence of the C282Y and H63D alleles in the healthy population the sample consisted of 200 individuals. Forty were healthy blood donors and 160 were ambulatory and hospitalized patients with diagnoses not suggesting hemochromatosis, liver diseases, diabetes or rheumatism. We included these patients in the sample in order to vary its geographical origin. Eighty -five originated from the Iasi district (including the blood donors), 33, 19, 23, 24 and 16 individuals originated from the districts of Vaslui, Bacau, Botosani, Suceava and Neamt respectively. 105 were males and 95 were females. All the subjects were genotyped for C282Y and 146 subjects were genotyped for H63D.

TABLE I
Mutant genotypes of HFE and their frequency in Moldavia

Genotype	Nr of subjects tested	Nr of subjects with genotype	Percentage
C282Y/+	200	7	3.5
C282Y/ C282Y	200	0	0
H63D/+	140	28	20
H63D/H63D	140	4	2.9
C282Y/ H63D	140	0	0

For searching the disease among patients, the sample consisted of 549 patients hospitalized at the Institute of Gantroenterology and Hepatology Iaşi, diagnosed as chronic hepatitis or cirrhosis. The ages were : under 30 : 28 patients ; 30-39 : 79 patients ; 40--49 : 177 patients ; 50-59 : 235 patients ; over 60 : 30 patients ; 208 were men and 241 were women. The patients were tested for plasma iron level, transferrin saturation index, and ferritin level in addition to other investigations (including liver biopsies in some cases) specific to their pathology. Ten patients having high cumulated levels of blood iron, transferrin saturation index and ferritin or a hepatic iron overload, discovered at the liver biopsy were genotyped for C282Y and H63D and one of them was sequenced for mutations in ferroportin, hepcidine and hemojuveline genes. Informed consent was obtained from all the subjects in the two groups mentioned.

Biochemical investigations : Iron level and the total capacity of iron binding were determined colorimetrically by using commercial kits. Transferrin saturation index was calculated from the two values aforementioned. Ferritin was determined by using an immunochemical method with a commercial kit.

Genotyping : The C282Y and H63D mutations were tested by a PCR- RFLP analysis as described (1,5). Parts of the HFE gene were PCR amplified using appropriate primers and the amplicons obtained were digested with restriction enzymes : RsaI for C282Y and MboI for H63D. The fragments obtained were analyzed for their sizes after electrophoretic separation in agarose gels.

The sequencing of ferroportin, hepcidin and hemojuvelin genes was carried out in the molecular biology laboratory of the Lille University Hospital (France) by one of us.The genomic DNA was extracted with a Qiagen kit, The entire coding regions and splice sites of all these genes were sequenced using a terminator cycle sequencing reaction kit and a 3130 XL Genetic Analyzer (Applied Biosystems),

RESULTS

Our first goal was to evaluate how frequently we expect a hemochromatosis case in our population. A population sample of 200 persons from the Moldavian territory was organized, consisting of individuals not suspected of hemochromatosis. The distribution of HFE mutant alleles in our population group designed to evaluate prevalence of mutations in HFE gene are presented in Table I.

The allele frequency (nr. of chromosomes carrying the mutant allele x 100 / total nr of chromosomes) calculated from the data (tab. I) are 1.75 (95% confidence interval : 0.7 – 3.7) for the C282Y mutant allele and 13.25 (95% confidence interval 10.4 – 16.5) for the H63D allele. Also from the data, there is an expectancy of one homozygous (possibly ill) C282Y/C282Y person out of 816 persons. This would be so if penetrance of the mutant allele was 1. However, penetrance is lower than 1 and varies in different populations (6). As we do not know yet penetrance (nr of persons with phenotype / nr of persons with genotype) in our population, we can only assess that we expect at most one person suffering from hemochromatosis among 816 persons. As to the compound heterozygote C282Y/H63D its expectancy

is much higher (one out of 100 individuals), but this genotype very rarely causes hemochromatosis (generally a mild iron overload with little clinical consequences) (6).

Our second goal was to apply and optimize a selection method in order to discover among clinically suspects the persons who really suffered from hemochromatosis. As genotyping is more expensive and do not always discover the disease, we tested 549 patients, whose clinical status suggested possible hemochromatosis for iron-overload parameters generally recommended (6): plasma iron level, transferrin saturation index and ferritine level. We selected eventually 10 patients cumulating iron blood levels higher than 160 mg/dL, transferrin saturation index higher than 70 and a ferritin level higher than 500 ng/mL for genotyping. Three of them were found to carry mutations: one was a HFE C282Y/C282Y homozygote, another one was a HFE C282Y/H63D compound heterozygote and the third one had no mutations in HFE. After sequencing other genes (ferroportin, hepcidin and hemojuvelin genes) a homozygous G320V mutation was finally found in the hemojuvelin gene.

DISCUSSION

In this study we aimed to diagnose hemochromatosis among our patients. First we wished to estimate the status of the most frequent mutations causing hereditary hemochromatosis, the HFE C282Y and H63D mutant alleles, in the broad population of our region. We established a population sample of 200 people from most part of the districts of the region. Subjects composing the sample were healthy blood donors and (in majority) patients with various diseases, but not the ones possibly suggesting hemochromatosis. We think that did not cause a bias of the data since the mutations we found were in heterozygous condition, known as not having a phenotype. The allelic frequency of the HFE- C282Y found in our population, 1.75%, can be compared with allelic frequencies in other regions in Eastern Europe: northwestern Poland 4.73% (9), southern Poland 3.11 % (10) , Moscow region 3.4 % (11), Czech Republic 3.45 % (12), Hungary 3.40 % (13), Slovenia 3.61 % (7), Croatia 3.30 % (8) , Serbia-Montenegro 1.60 %(14), Bosnia-Herzegovina 2.25 % (15) Bulgaria (Sofia Region) 0 % (16), Greece 0.3 % (17). Our figure fits approximately the interesting (but not very well understood) pattern of northwest-to-southeast decline of the frequency available in Europe. This may account for the population migrations in the past. The allelic frequency of H63D in our population (13.25) is close to the frequencies in almost all European and non European populations.

From our figures a maximal expectancy of one individual affected by severe hemochromatosis in approximately 800 people can be calculated, which makes hemochromatosis still a rare disease, but the most frequent genetic disease in our region and justifies special attention from the medical services.

We also tested a practical method to discover hemochromatosis in patients who, we had reasons to suspect, were suffering from it. We applied the established rules as to discover the phenotype (6) and we found 10 patients having a hemochromatosis phenotype in a sample of 549 patients. They were genotyped for the HFE mutations and one of them was a C282Y/C282Y homozygote. This was close to expectancy in the general population, although we expected more in a group of pathology selected patients. This could be due to the lower than 1 penetrance of the mutant allele as well as to flaws in the phenotype or genotype determination, resulting in missing of some cases. A similar evaluation in a group of patients from southwestern Romania, four C282Y homozygotes were found among 21 cases of iron overload (18). As to the other

7 cases of phenotypic hemochromatosis in our group, some may have been secondary hemochromatosis (one of them had a myelodysplasic syndrome) and others may have had mutations in other genes. This was proved in one of them by sequencing other genes and finding a homozygous G320V mutation in the hemojuvelin gene, the second case to carry this mutation in Romania, after another one recently published (19). We have not yet discovered the cause of iron overload in the remaining 5 patients.

CONCLUSIONS

Allelic frequency of the HFE mutant allele C282Y in Moldavia is about 1.75% and the expectancy of a severe form of hemochromatosis is one in about 800 people.

Three cases of hereditary hemochromatosis were found by looking for its phenotype among 549 patients: one was a HFE--C282Y homozygote, a second was a HFE-C282Y/H63D compound heterozygote and a third was a HJV-G320V homozygote.

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NOUTĂŢI

Noradrenalina mai eficientă Şi mai bine tolerată decât dopamina în Şocul cardiogen

Administrarea unui vasopresor ca primă intenţie în tratamentul şocului este controversată. Atât în şocul septic cât şi în cel cardiogenic dopamina este cel mai des folosită. În absenţa unor studii clinice randomizate comparative, o serie de studii observaţionale recente subliniază obţinerea unor rezultate mai bune cu ajutorul noradrenalinei. Este şi motivul pentru care autorii, reanimatori din Belgia şi Spania, prezintă cel mai vast studiu clinic ce compară dopamina şi noradrenalina în tratamentul de primă intenţie al şocului. Diagnosticul a fost stabilit pe baza prezenţei unei hipotensiuni arteriale (presiunea arterială – PA medie <70 mm Hg, sau PA sistolică <100 mm Hg), asociată cu semne de hipoperfuzie tisulară (alterarea stării mentale, paloare tegumentară, scăderea debitului urinar, nivelul lactatului >2 mmol/L). În total, 1679 de subiecţi au fost randomizaţi în dublu orb între o perfuzie de dopamină sau de noradrenalină. În caz de rezultate insuficiente asupra PA cu doza maxim admisă (20 microg/kg/min pentru dopamină, respectiv 0,19 microg/kg/min pentru noradrenalină), adrenalina, dopamina sau vasopresina au fost administrate repetitiv. Toate celelalte tratamente (în special hidrocortizonul sau proteina C activată în cazul şocului septic) au fost administrate conform deciziei clinicienilor pentru fiecare caz în parte. Criteriul principal de evaluare a fost nivelul deceselor după 28 de zile de evoluţie. Caracteristicile celor 2 loturi au fost comparabile, cu un total de 62,2% de şocuri septice, 15,7% şocuri hipovolemice (hemoragice) şi 16,7% şocuri cardiogenice. Mortalitatea globală la 28 de zile a fost apropiată în cele 2 loturi, 52,5% decese în lotul cu dopamină, faţă de 48,5% în lotul cu noradrenalină ($p=0,10$). Toleranţa la noradrenalină a fost mai bună, cu 12,4% evenimente aritmice, faţă de 24,1% în cazul dopaminei ($p<0,001$). Nu au fost semnalate diferenţe pentru alte efecte secundare. Deşi s-ar putea aduce o serie de critici (cum ar fi asocierea în studiu a şocului cardiogen cu cel necardiogen, cele două având etiologii atât de diferite, sau faptul că o serie de metode terapeutice din şocul cardiogen – revascularizaţia percutană, contrapulsiunea aortică, asistenţa ventriculară stângă – nu sunt detaliate, alături de evaluarea administrării de vasopresină), prezentul studiu demonstrează că administrarea de noradrenalină ca medicaţie de primă intenţie în şocul cardiogen, departe de a creşte mortalitatea, este mai bine tolerată şi mai eficace (DeBacker D et al. Comparison of dopamine and norepinephrine in the treatment of shock. *N Engl J Med* 2010 ; 362 : 779-789).

Al.D. Ciochină

THE INFLUENCE OF FRAILTY ON PATIENTS UNDERGOING MITRAL SURGERY

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THE INFLUENCE OF FRAILTY ON PATIENTS UNDERGOING MITRAL SURGERY (Abstract): The current tendency to ageing populations leads to increased degenerative pathology. Frailty, defined as a low capability to recover from pathogenic stressors due to aging related deterioration, plays a role in cardiovascular diseases as it increases operative risk and alters the postoperative prognosis and quality of life. Degenerative valve diseases occur more frequently in the elderly and register a clinical course in this group of patients. The aim of the current review is to evaluate the frailty scoring systems and their utility in cardiac surgery but also to highlight the particularities of surgical care in degenerative mitral valve diseases occurring in frail patients. Rigorous preoperative selection and functional status improvement is mandatory in order to maintain a relatively low complication rate. Long-term benefits regarding the duration and quality of life makes the operational risk acceptable by correcting a pathology that threatens the life expectancy of the elderly patients on short term. The geriatric patient's approach is multidisciplinary and involves creating a heart team based on the collaboration of geriatric doctors, anesthetists, cardiologists, internal medicine, cardiac surgeons and related specialties for early diagnosis, treatment and management of the complications. **Keywords**: FRAILTY, ELDERLY, MITRAL VALVE, SURGERY.

Nowadays, more elderly patients address to cardiac surgery clinics than never due to the increased life expectancy over the last century and because of population getting older. The aging process is not homogeneous, the biological age being different from the chronological age. One of the most problematic expression of population aging is frailty, a biological syndrome characterized by a decrease in physiological reserve and increased vulnerability to stress factors. Even if the prevalence is increasing with age, frailty is not synonymous with disability and comorbidity (1).

According to studies, the prevalence of frailty is higher in women than in men, women also having higher level of frailty compared to men of the same age (2, 3, 4). This finding can be explained by the greater burden of chronic disease, disability and poorer health status (5). It is well known that frailty is associated with a higher risk of death and that women, despite of having higher levels of frailty, live longer than men (4). This is called the 'sex-frailty paradox' and it is explained by the fact that

women have greater physiological reserve than men and it would enable women to acquire more deficits in multiple organ systems (6).

Aging is an evolutionary process characterized by extreme heterogeneity, with 3 categories of elderly people being delimited (7):

- Robust individuals with minimal or absent alteration of physiological functions without chronic or acute disease. This category represents 60-70% of the total elderly and should be assimilated to the general population;
- Dependent patients with severely progressive, complicated or disabling illnesses who require home assistance or those who live in an institutionalized setting - 5% of the elderly;
- Pre-fragile or fragile people in a precarious state of health and disease, autonomy and dependence; balance that can be disturbed at any time by stressors. The latent evolutive frail process becomes manifest or is accentuated by events such as illness, partner death, institutionalization or hospitalization. Thus, a cascade of events is initiated and exhausts the physiological reserves of the body, reduces its autonomy and speeds up death. Pre-fragile or fragile people account for about 25% of the elderly.

The phenotype of frailty

Frailty, the most problematic expression of population aging, is a widely debated concept in recent international literature as an approximate assessment of biological age. Frailty is defined as a biological syndrome that reflects a clinical condition characterized by a decrease in physiological reserve and vulnerability to stress factors, generically classified as iatrogenic (e.g. cardiac surgery), acute or chronic disease (7).

Disability is a term used to define the ability to perform day-to-day functional (ADL) and instrumental (IADL) activities. Frail patients exposed to stressors have an increased risk of decompensation, adverse clinical events, procedural complications, prolonged recovery, functional decline, disability, and increased mortality. In our opinion, frailty syndrome (fragility) precedes secondary disability and is a clinical status often associated with comorbidities typical to advanced age (8, 9).

The prevalence of frailty among elderly patients varies between 10 and 60% depending on the study, recent epidemiological research demonstrating a 2-fold increase in mortality and morbidity risk in a range of chronic cardiovascular diseases, acute coronary syndromes, heart failure, surgery, and percutaneous interventions (2).

In the last decade, frailty has been assessed as a predictor of unfavorable prognosis after cardiovascular interventions, with evidence of increased risk of morbidity, mortality, and institutionalization of frail patients compared to those of the same age group. This risk is not due to surgery. Conditions associated with long-term hospitalization such as prolonged immobilization have a negative impact on the development of complications compared to the actual intervention (10, 11, 12).

The pathobiology of frailty

Chronic inflammation is the common pathophysiological base of frailty syndrome and cardiovascular diseases (CVD) (9, 13, 14). In both instances, inflammatory markers such as neutrophils and monocytes, C-reactive protein, IL-6 and thrombotic markers including factor VIII and D-

dimers are identified. Proinflammatory status determinants, both in frailty and CVDare prolonged exposure to antigens, angiotensin 1 activation, obesity, insulin resistance, imbalance between antioxidants and pro-oxidants (15). The common biological pathway and mutual determinism in CVD and frailty syndromes are characterized by etiopathological and clinical elements that can determine and explain unfavorable cardiac or non-cardiac prognoses such as disability, prolonged hospitalization, increased mortality and morbidity (14). A frail patient is at risk of unfavorable outcome in case of invasive cardiovascular procedures and unsuccessful cardiac rehabilitation interventions as a counterpart to physical and mental exhaustion. Decreasing the physiological functional reserve of the human body with age increases the clinical picture of underlying conditions and accelerates the progression of the disease to multiple system organ failure (MSOF) syndrome development. Thus, the issue of biological age raises as a determinant of the operator's risk, regardless of the type of surgery. It is currently considered that chronological age is a major risk factor for postoperative mortality and morbidity in cardiovascular surgery even though the number and severity of comorbidities, that usually increase with chronological age, are factors that compete with perioperative prognosis on the mid and long-term in this high-risk group. Changes in age-related cardiac morphology concern alterations in intracellular and biochemical molecular pathways (16). They may affect the protein function, mitochondrial oxidative phosphorylation, excitatory coupling with cardiac muscle fibrillation, calcium ion kinetics, myofilament activation, cell matrix composition and regeneration (17).

Functionally, cardiac aging is characterized by decreased mechanical efficiency, reduced myocardial contractility, degenerescence of the valvular connective tissue, decreased myocyte number, increased myocyte size, accelerated myocardial cell apoptosis rate, decreased inotropic and adrenergic-mediated contractile response. The aging process is associated with functional and structural changes in coronary vascularity, with an impact on myocardial perfusion. Reduced coronary artery reserve with aging may interfere with reflex vasodilation and determine decreased cardiac output and myocardial blood flow (18). Decreased vasodilatation may be due to alteration of epithelial endothelial component, increased resistance in coronary territory, decreased stimulation and release of nitric oxide in coronary endothelium, but also to the vasoconstrictive effect of increased endothelin 1 (ET-1) plasma levels in the elderly (19). From a morphological point of view, large arteries expand and increase their parietal thickness mainly due to changes in the parietal matrix (fig. 1).

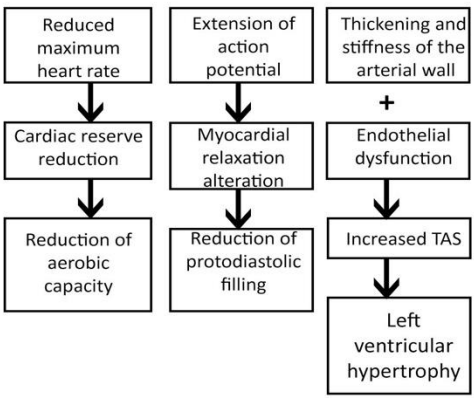

Fig. 1. The pathobiology of frailty syndrome

These changes increase vascular stiffness and act as a trigger factor for a high systolic blood pressure and pulse wave velocity. The final stage is the increase of aortic impedance and mechanical loading, which seems to diminish the cardiac performance (18).

Frailty scores

There are several factors contributing to the exacerbation of frailty syndrome in patients with CVD. In the literature there are described up to 20 assessment tools, most of them being adjacent to the phenotypic domain of frailty syndrome.

Despite of being a widely debated concept, a consensus in defining frailty has not been reached. According to Fried's frailty phenotype, known as the Cardiovascular Health Study Index (CHSI), frailty is defined by the presence of three or more of the following criteria:

- Weight loss: unintentional loss of more than 4.5 kg in the past year or weight at examination <10% of the weight registered at the age of 60 years;
- Exhausting: self-reported fatigue or felt unusually tired or weak in the past month;
- Low physical activity;
- Slow walking speed: 4 m >7 s if height <159 cm or >6 s if height >159 cm;
- Weakness: low grip strength (20).

Pre-frail is defined as scoring one or two out of the five criteria. This definition does not include the measures of mental and cognitive health which are important parameters in frailty. Compared to Fried's model of frailty, the Study of Osteoporotic Fractures (SOF) frailty index added the domain of cognitive decline. Frailty is classified as the presence of more than 2 components from shrinking, exhausting and low mobility (21).

In cardiac surgery, the operative risk is commonly assessed using the European System of Cardiac Operative Risk Evaluation (Euro SCORE)and the Society of Thoracic Surgeons (STS) score (22).

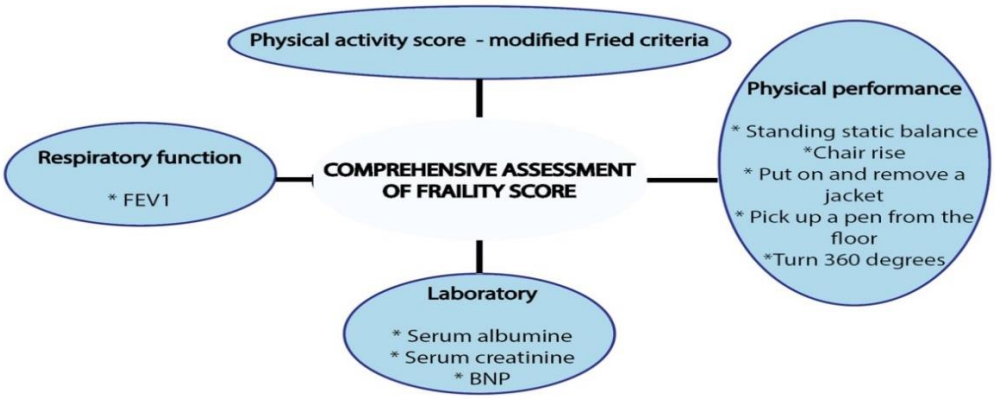

Fig. 2. Comprehensive Assessment of Frailty score

To measure the perioperative risk of elderly patients receiving elective cardiac surgery, there is a "Comprehensive Assessment of Frailty" (CAF) score that is used concurrently with conventional risk scores in cardiovascular surgery as a predictive factor in clinical practice. The CAF should be used during the pre-operative

period, minimum 5 meters walking test, being a simple and powerful tool for evaluating the frailty syndrome. The score incorporates physical ability, patient phenotype, the Fried criteria and laboratory values (fig.2) (22). The score shows significant statistically correlation within 30 days mortality. The disadvantages of CAF score are that it takes about 10 to 20 minutes to perform it and needs special equipment (23). The same authors described FORECAST, a score that includes the most predictive items of the CAF-score like 'chair rise', 'weakness', and 'creatinine' and takes maximum 5 minutes to perform. After one-year follow-up, in the estimation of one-year mortality risk, the FORECAST score had better results than the Euro SCORE and the STS score (24).

Ageing mitral valve in frail patients

Almost 10% of patients over 75 years register significant mitral valve regurgitation because of the natural aging process of the mitral valve. It has a very high incidence, being classified as second, after the aortic valvular disease (11). There are many uncertainties regarding the benefits of mitral valve surgery in elderly patients, but recent studies show that it can improve their general health status, with a positive impact on the quality of life.

The etiology of mitral regurgitation in the elderly is primarily degenerative. These lesions are therefore usually accessible to repair. Some studies have reported a mortality rate associated with mitral valve surgery in elderly patients greater than 15%. However, recent articles show that this surgery can be performed in patients over 80 years with good results and mortality less than 5% (25).

Peri and postoperative complications also vary depending on the type of surgery. They are less important in mitral valve repairment compared to replacement, due to better preservation of left ventricular function and to prevention of specific problems with different types of valve substitutes. The choice of valve prosthesis in case of repairment must consider the existence of potential contraindications to oral anticoagulation. The use of bio prostheses is recommended after 70 years on the one hand, because of their durability not exceeding the long-term survival of these patients and on the other hand, because of the potential risk of hemorrhage under anticoagulants in this population.

The surgical risk in frail patients is counterbalanced by good functional results on the long term and by obtaining a survival time close to the life expectancy observed in the general population of the same age (26).

Nowadays, modern therapeutic approaches have emerged such as implants for mitral reconstruction (Mitra Clip) and implants for mitral replacement (Transcatheter Mitral Valve Replacement - TMVR). From a technical point of view, Mitra Clip has been designed for the treatment of secondary (or functional) mitral regurgitation; in clinical practice, however, it is increasingly used in cases of primary (structural) mitral regurgitation in frail and very old patients (27).

The current use of transcatheter aortic valve implantation (TAVI) should serve as an example for the further development of transcatheter techniques for treating mitral valve diseases. These innovations will most likely result in improved and faster management of mitral insufficiency. In a second step, depending on the results, the corresponding recommendations will be

adapted to allow the planning of the most appropriate possible treatment at an early stage of the disease. A Heart Team is mandatory for selecting the most appropriate treatment.

CONCLUSIONS

Our research draws attention to comorbidities such as frailty, that occurs very commonly in elderly patients and requires a different surgical course. Degenerative damage to heart valves occurs in all elderly patients, but the need for surgical interventions is only needed in patients with class IV heart failure. The important outcomes of the frailty patients after cardiac surgery are to improve the functional survival and the quality of life. It is well known the association between frailty and the rising number of complications, longer length of stay in hospital and mortality. Therefore, it is important to develop a practical standard score in order to identify the frailty patients at high risk of poor postoperative outcomes and to improve the patient's selection for cardiac surgery.

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NEWS

MACROPHAGE-MEDIATED NUTRITIONAL IMMUNITY AGAINST *RHIZOPUS* SPP

A study by Andrianaki AM *et al*. aims to describe the pathogenic mechanisms of mucormycosis, a severe respiratory infection caused by *Rhizopus* spp. The study shows that *Rhizopus* spp. can persist inside alveolar macrophages. This is explained by melanin-induced inhibition of phagosome maturation. The mechanism permits the microorganism to evade phagocytosis. Iron assimilation from the host plays an important role in the life cycle of *Rhizopus* spp. The study shows that host immunity against *Rhizopus* is mediated by iron limitation inside macrophages, a defense strategy known as nutritional immunity. Dual RNA-sequencing technique and functional studies were used to identify both the fungal and host modulators of iron homeostasis in macrophages that play a role in promoting invasive fungal growth. (Andrianaki AM, Kyrmizi I, Thanopoulou K, *et al*. Iron restriction inside macrophages regulates pulmonary host defense against Rhizopus species. *Nat Commun* 2018; 9(1): 3333).

IMPACT OF ACROMEGALY ON THE CARDIOVASCULAR SYSTEM

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IMPACT OF ACROMEGALY ON THE CARDIOVASCULAR SYSTEM (Abstract): Cardiovascular manifestations occur in 60% of patients with acromegaly. The severity of the structural and functional heart abnormalities mainly depends on the levels of growth hormone and insulin-like growth factor 1 and the time between disease onset and diagnosis. Long-lasting elevated hormone levels are responsible for acromegalic cardiomyopathy, valvular disease, arrhythmias, hypertension, coronary artery disease and even congestive heart failure. We highlight the tremendous impact of hormone imbalance upon the heart structure and function in order to raise awareness upon the fact that cardiovascular disease has become the leading cause of mortality in patients with acromegaly. We draw attention to the fact that acromegaly is more than an endocrine disease and we emphasize the need for a multidisciplinary approach of the acromegalic patient, of which the cardiologist must be part.
Keywords: ACROMEGALY, CARDIOMYOPATHY, ARRITHMIAS, HYPERTENSION.

Acromegaly is a rare endocrine disorder caused by prolonged and excessive secretion of growth hormone (GH) by the pituitary gland. GH exerts its effects either directly or indirectly, through its peripheral effector insulin-like growth factor 1 (IGF-1). Long-lasting high levels of these hormones lead to enlargement of organs and soft tissues.

Cardiovascular manifestations occur in 60% of patients. The cardiovascular impact of hormonal imbalance manifests as cardiomyopathy, valvular disease, arrhythmias, hypertension, coronary artery disease and even congestive heart failure if acromegaly is uncontrolled. Cardiovascular disease has become the leading cause of mortality in patients with acromegaly (1), especially in the presence of diabetes mellitus and obstructive sleep apnea, which are highly prevalent in these patients.

Acromegalic cardiomyopathy

Cardiac involvement in the absence of other identifiable causes of cardiomyopathy defines acromegalic cardiomyopathy. It

affects 3% of patients with acromegaly. The severity of the structural and functional heart abnormalities mainly depends on the levels of GH and IGF-1, and the period of time when the heart was exposed to elevated hormone levels (the time between disease onset and diagnosis) (2).

Acromegalic cardiomyopathy evolves in three stages (3). The characteristics of the first stage are enhanced myocardial contractility, increased cardiac output and decreased peripheral vascular resistance. It is the so called "hyperkinetic syndrome" (4). IGF-1 increases Ca^{2+} influx and raise peak Ca^{2+} levels in cardiomyocytes and also increases the sensitivity of myofilaments to Ca^{2+}, thus resulting in increased inotropism and a hyperkinetic heart (5).

The intermediate stage is characterized by biventricular hypertrophy, interstitial fibrosis, diastolic dysfunction and impaired systolic function during exercise (4, 6). The ventricular walls are concentrically thickened due to a relative increase in myocyte size. Usually, hypertrophy of the left ventricle is more significant, but the right ventricle can be equally involved. Hypertrophy without ventricular dilation is an early feature of the disease. Studies show that up to 54.5% of young patients (<40 years old) with active acromegaly of 3-7-year duration had echocardiographic evidence of left ventricular hypertrophy. This percentage rises up to 72.2% in patients aged 41-60 years and disease duration of 5-15 years (7). Interstitial fibrosis, consequence of a markedly increased myocyte apoptosis (495 fold higher than normal) is associated with extracellular collagen deposition and myofibrillar derangement (8). The damaged heart architecture has a negative impact on ventricular function. Ventricular relaxation is impaired, and compliance is reduced. These changes lead to diastolic dysfunction. Left ventricular filling is insignificantly impaired at rest but is associated with reduced exercise tolerance.

In the final stage, severe enlargement of cardiac chambers and systolic and diastolic dysfunction occur (4). The peripheral vascular resistance increases. This late stage ends in congestive heart failure.

Successful treatment of acromegaly is associated with a decrease in left ventricular mass and with the improvement of diastolic and systolic function (9). A beneficial effect of treatment has also been reported in patients with acromegalic cardiomyopathy and congestive heart failure (10). Cardiac function improvement is greatest in young patients with short disease duration, in which the early diastolic changes are completely reversible under treatment.

Arrhythmias in acromegaly

Patients with acromegaly have a high incidence of arrhythmias (11). Cardiac arrhythmias and sudden cardiac death are the most common causes of increased mortality in acromegaly. Abnormal heart architecture, with areas of hypertrophied myocytes separated by fibrosis and cellular infiltration provide de substrate for the structural uncoupling of cardiac myocytes. Other mechanisms like phenotypic changes in membrane proteins and conduction system disease are also involved (12), but fibrosis is mainly responsible for the disorganized and asynchronous electrical activity in ventricles, favoring reentrant events.

The electrophysiological abnormalities have a wide range of manifestations, from ectopic beats, paroxysmal atrial fibrillation,

paroxysmal supraventricular tachycardia, sick sinus syndrome, to ventricular tachycardia and bundle branch blocks (6,13). One study reported conduction disorders in up to 41% of patients with acromegaly (14).

Arrhythmic events are present at rest, but especially during exercise. The frequency and severity of ventricular arrhythmias correlate with clinical activity score, duration of acromegaly and left ventricular mass, but not with hormone levels (12). This is supported by several studies (14,15) that showed that ventricular arrhythmias were as frequent before as after treatment of acromegaly, implying that fibrous tissue infiltration had resulted in permanent irreversible scarring.

Hypertension in acromegaly

Hypertension is the cardiovascular disease most frequently encountered in acromegaly patients, with a reported prevalence ranging from 25 to 50% (5). The diastolic blood pressure is usually elevated (16). The pathogenesis of HTA in acromegaly is multifactorial, including increased vascular resistance, increased plasma volume, alterations in renin-angiotensin-aldosterone system and insulin resistance (9). Long-lasting high GH levels stimulate smooth muscle cell growth, thereby increasing peripheral vascular resistance and blood pressure (5). Moreover, GH blocks insulin actions at several levels, leading to insulin resistance (17), which is associated with low nitric oxide production and high activity of the vascular renin-angiotensin-aldosterone system (9). The impaired endothelium-dependent vasodilation, the increased vascular response to angiotensin II and the exaggerated sympathetic-mediated vasoconstrictor response contribute in various degrees to the increase of arterial peripheral resistance (18, 19).

GH can cause sodium retention through three mechanisms: activation of Na^+-K^+ ATP-ase pump in the renal tubules (20), activation of the renin angiotensin system and inhibition of atrial natriuretic peptide release (21). Due to its antinatriuretic action, GH increases extracellular volume and by consequence blood pressure.

It is important to highlight the fact that hypertension is an independent risk factor for cardiac morbidity in acromegalic patients and an important negative prognostic factor for mortality in these patients.

Valvular disease in acromegaly

Few data are available regarding valvular disease in acromegaly because it rarely occurs (11). Mitral, aortic and tricuspid regurgitation have been reported in the late stages of disease, usually as a result of ventricular remodeling. One small study (22) including 32 patients with acromegaly found aortic regurgitation in 31%, mitral regurgitation in 47%, and tricuspid regurgitation in 37% of the cases. Another study (23) described mitral and aortic valvular annulus fragility and leaflet disarray, accompanied by functional regurgitation.

Coronary artery disease in acromegaly

Data regarding coronary artery disease (CAD) in acromegaly are limited and conflicting (11,24). There is much evidence that acromegaly per se does not increase the risk of CAD (25). Calculations based on the European Society of Cardiology risk score and Framingham risk score showed a

low 10-year CAD risk (9). These findings were supported by studies using coronary artery calcium (CAC) score with the purpose of identifying early-stage coronary atherosclerotic disease in asymptomatic patients, in which low scores have been obtained.

Conversely, a study (26) that evaluated the risk factors and CAC score showed that 41% of acromegalic patients are at risk for coronary atherosclerosis and that coronary calcifications were evident in about half of cases. These findings are consistent with the fact that acromegaly is an insulin-resistant state, associated with a high prevalence of diabetes mellitus and hyperlipidemia, which contribute to the cardiovascular risk.

CONCLUSIONS

Our research shows that acromegaly is far more than an endocrine disease. We highlight the major influence of GH and IGF-1 on cardiovascular system. Morphological and functional heart abnormalities induced by long-lasting high hormone levels have a tremendous impact upon mortality, morbidity and quality of life in acromegalic patients. We encourage the close collaboration between endocrinologists and cardiologists in order to ensure an early diagnosis and adequate monitoring of cardiovascular consequences of acromegaly. Since many parameters of cardiac function change with acromegaly treatment, we emphasize the need of periodical cardiovascular assessment.

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CERCETĂRI CLINICO-EPIDEMIOLOGICE ASUPRA COMPLICAŢIILOR CARDIOVASCULARE APĂRUTE LA PACIENŢII CU SINDROM METABOLIC

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CLINIC AND EPIDEMIOLOGIC RESEARCHES ON CARDIOVASCULAR COMPLICATIONS IN PATIENTS WITH METABOLIC SYNDROME (Abstract): Our study aims to identify correlations between the metabolic syndrome and cardiovascular diseases determinism. **Material and methods**: A retrospective study was carried out on a sample of 1463 patients of which 734 patients met the diagnostic criteria of cardiometabolic syndrome, admitted in the Clinic of Cardiovascular Rehabilitation, Rehabilitation Hospital, Iasi, between 01.01. 2009-31.12.2009. **Results**: The incidence of cardiometabolic syndrome has a soft predominance on the group 40-59 years (61.66%). The high level of hypertension was found in the patients group 48-69 years (53.70%) and lowest in the patients group 25-38 years (11.35%) (p=0.001). The coronary heart diseases showed a significant difference between age groups: 48-69 years (41.80%), compared to 25-38 years (7.55%) (p=0.003). Heart failure is part of the same age distribution curve (45%, 6.8%) (p=0.02). **Conclusions**: The metabolic syndrome is a predictor of the occurrence and development cardiovascular disease. **Key words**: METABOLIC SYNDROME, CARDIOVASCULAR DISEASE, INCIDENCE, HYPERTENSION, HEART FAILURE

Sindromul metabolic reprezintă o tulburare complexă a metabolismelor organismului ce însumează o combinaţie de factori precum obezitatea, rezistenţa la insulină, hipertensiunea arterială şi dislipidemiile, precum hipertrigliceridemie, creşterea acizilor graşi liberi, scăderea HDL colesterolului şi creşterea LDL colesterolului.

De-a lungul timpului, sindromul metabolic a primit diverse definiţii. Prima încercare a fost realizată în 1923 de către suedezul E. Kylin, care a definit sindromul cardiometabolic ca o afecţiune la care coexistă hipertensiunea arterială (HTA), hiperglicemia (HG) şi guta, numindu-l „sindrom de hipertensiune-hiperglicemie-hiperuricemie". Raven în 1988 considera sindromul metabolic ca o interrelaţie între stilul de viaţă sedentar, dieta hipercalorică şi factorii genetici, acestea determinând apariţia dezechilibrelor manifeste prin: hiperglicemie, obezitate abdominală, hipertrigliceridemie, reducerea nivelului seric al HDL şi hipertensiune arterială (HTA). Ulterior definiţia a fost completată, updatată de diverse forumuri precum OMS (Organizaţia Mondială a Sănătăţii) în 1998, WHO – (World Health Organization), 1999, ATP III (Third Report of the National Cholesterol Education Program's Adult Treatment Panel), în Executive Summary of the NCEP, 2001. În octombrie 2009, a fost adoptată o definiţie ce include toate criteriile menţionate anterior, la care au conlucrat Comitetului Operativ pentru Epidemiologie şi Prevenţie al International Diabetes Federation (IDF), National Heart, Lung and Blood Institute

(NHLBI), Federaţia Mondială a Inimii, Societatea Internaţională de Ateroscleroză, American Heart Association (AHA) şi Asociaţii Internaţionale de Studiu a Obezităţii, în încercarea de a elimina câteva din confuziile apărute în identificarea pacienţilor cu sindrom cardiometabolic (SCM).(1, 2)

Deşi sindromul metabolic este o afecţiune cunoscută şi facil de diagnosticat, complicaţiile ce derivă sunt tot mai numeroase şi cu un impact deosebit în creşterea incidenţei afecţiunilor cardiovasculare. Bolile cardiovasculare (BCV) rămân pe primul loc în determinismul morbimortalităţii la nivel mondial. La sfârşitul anului 2000, BCV reprezentau cauza directă a peste 4.35 mil decese la nivel european (43% bărbaţi, 57% femei). Mai mult de 300 mil. persoane din lumea întreagă prezintă un indice de masă corporal (IMC) > 30 kg/m^2, iar procentul acestora este în continuă creştere (2,3).

Ghidul european al ESC (European Society of Cardiology) de prevenţie şi recuperare al BCV atrage atenţia asupra comorbidităţilor determinate de factorii de risc tradiţionali şi non-tradiţionali cardiovasculari şi metabolici.

MATERIAL ŞI METODĂ

Studiul nostru îşi propune identificarea corelaţiilor existente între sindromul cardiometabolic şi determinismul afecţiunilor cardiovasculare pe o populaţie reprezentativă pentru zona Moldovei. Prin acest studiu se doreşte atragerea atenţiei asupra necesităţii de evaluare a pacientului cu sindrom metabolic şi din punct de vedere al afectării cardiovasculare.

Studiul s-a desfăşurat în cadrul Clinicii de Recuperare Cardiovasculară, a Spitalului de Recuperare Iaşi în perioada 01.01.2009--31.12.2009 şi a inclus un număr de 1463 pacienţi, din care 752 (51.40%) au îndeplinit criteriile.

Criteriile de includere în studiu au fost

- perimetrul abdominal >94 cm la bărbaţi, >80 cm la femei
- trigliceridemie >150 mg/dl sau tratament specific ;
- HDL-colesterolemie <40 mg/dl la bărbaţi, <50 mg/dl la femei sau tratament specific ;
- TAS >130 sau TAD >85 mmHg sau tratament specific ;
- glicemia á jeun >100 mg/dl sau DZ tip 2 în diagnostic.

Studiul de faţă aduce argumente cu privire la creşterea incidenţei sindromului metabolic în populaţia generală – 735 (50, 23%) pacienţi din numărul total – 1463 pacienţi adresat Clinicii de Recuperare Cardiovasculară.

Prelucrarea şi interpretarea datelor a fost realizată prin metode epidemiologice (ancheta), statistico-matematice (p, deviaţie standard (DS), tendinţă) şi computerizate.

REZULTATE ŞI DISCUŢII

1. *Repartiţia pe sex*. În cadrul studiului nostru se păstrează aceeaşi tendinţă globală de creştere a obezităţii în populaţia generală, cu predominanţa la sexul feminin (58.37%). Diferenţa dintre sexe este uşor semnificativă. Datele clinico-epidemiologice existente în literatură confirmă afectarea preponderentă a sexului feminin, obezitatea fiind influenţată atât de factorii socio-economici, comportamentali, factorii de mediu cât şi de factorii naturali.

Mai mult, cercetările recente au relevat existenţa unei legături între insulinorezistenţă (IR) şi dereglările hormonale la femei. Astfel, unele studii au demonstrat incidenţa mai mare a dezvoltării DZ tip 2 la femeile cu oligomenoree, aproximativ 82% din femeile cu DZ tip 2 prezentau polichistoză ovariană. În premenopauză femeile prezintă un risc crescut de dezvoltare a HTA, iar în perioada climacterică frecvenţa dezvoltării HTA creşte până la 52,4%. Această creştere s-ar datora diminuării efectului pozitiv al estrogenilor asupra funcţiei endoteliale, care

TABELUL I

Parametrii estimaţi în testarea asocierii sexul pacienţilor vs. diagnostic

	Chi-pătrat χ^2	**df**	**p** *95% interval de încredere*
Chi-pătrat - χ^2	1,535857	df=1	p=0,21524
Coeficient de contingenţă	0,0347956		
Coeficient de corelaţie	0,0875777		p=0,21555

TABELUL II

Parametrii estimaţi în testarea asocierii mediul de provenienţă vs. diagnostic

	Chi-pătrat χ^2	**df**	**P** *95% interval de încredere*
Chi-pătrat - χ^2	6,136817	df=1	p=0,01324
Coeficient de contingenţă	0,4704490		
Coeficient de corelaţie	0,31836212		p=0,01192

TABELUL III

Valorile medii ale gradelor de severitate a HTA în funcţie de grupa de vârstă

GRUPA VÂRSTA	**HTA - Means**	**HTA - Std.Dev.**
20-39 ani	1,826087	0,576208
40-59 ani	2,115942	0,607217
60-79 ani	2,034483	0,700013
80-99 ani	2,085714	0,631145
Toate grupele	2,055319	0,634752

corija spectrul lipidic, prin creşterea HDL colesterolului şi scăderea LDL colesterolului, precum şi ameliorarea reactivităţii acetilcolinice vasculare, inclusiv în arterele coronare, stimularea sintezei NO–sintazei şi creşterea nivelului bazal al NO (4). Karelis et al. au demonstrat că descreşterea sensibilităţii insulinice este asociată cu creşterea graduală a numărului de caractere ale SM (5).

2. *Repartiţia pe medii de provenienţă* a evidenţiat o diferenţă uşor semnificativă între mediul urban (60.25%) şi mediul rural. Mediul urban, prin implicaţiile stilului de viaţă şi a factorilor de risc ridicaţi (condiţii de viaţă şi muncă, factori nutriţionali, poluarea, stresul, etc.) face preponderentă dispunerea în mediul urban vs. rural a obezităţii.

3. *Repartiţia pe grupuri de vârstă*. La pacienţii cu sindrom cardiometabolic (SCM) de notat este ponderea semnificativă în cadrul criteriilor de încadrare a circumferinţei abdominale, colesterolului, a HTA (cel mai frecvent grad I-III) şi a hiperglicemiei a jeun. Îngrijorător este faptul că procentul cel mai mare de pacienţi cu sindrom metabolic este pe intervalul de vârstă 40-59 ani, deci la nivelul populaţiei active (61,66%).

Rezultatele trialurilor clinice realizate de Women's Health Initiative (WHI) au evidenţiat existenţa hiperinsulinismului la 75% din cazuri. Nivelele înalte ale insulinemiei bazale au fost corelat atât pozitiv cu creşterea vârstei, IMC, perimetrul abdominal, cât şi negativ cu nivelele mai înalte de activitate recreaţională. Manco et al. a observat nivele mai joase ale sensibilităţii la insulină şi nivele mai înalte de insulinemiei bazale la femeile în premenopauză, direct proporţională cu creşterea în vârstă, a masei adipoase şi nivelurile TG (factori de risc determinanţi pentru BCV) (5, 6)

4. Impactul HTA. Datele din literatură conform cărora România este pe primul loc prin procentul de 40% al hipertensivilor în populaţia generală sunt confirmate şi în cadrul studiului nostru. Astfel, se constată ponderea importantă a HTA de orice grad şi pentru toate grupele de vârstă. Aproximativ 50% hipertensivi au hiperinsulinism şi până la 75% indivizi cu DZ tip 2 sunt hipertensivi. Esenţa legăturii între HTA,

TABELUL IV
Analiza comparativă a severităţii HTA în funcţie de grupa de vârstă prin metoda One Way ANOVA

	SS - Effect	MS - Effect	SS - Error	MS - Error	F	p
HTA	1,553957	0,388489	92,72689	0,403	0,963	0,001

insulinorezistenţă (IR) şi BCV este complicată. Explicaţia acestui procent ridicat ar putea fi mecanismul hipertensinogen al hiperinsulinismului, prezent la pacienţii cu sindrom cardiometabolic. Hiperinsulinismul determină o creştere a retenţiei de Na şi apă în organism, prin creşterea reabsorbţiei tubulare, are efect vasodilatator asupra peretelui vascular, vasoconstrictor la nivelul capilarelor, reglator asupra metabolismelor Na şi Ca prin acţiune asupra sistemului renină-angiotensină. Această supresie a natriurezei indusă de insulină are ca rezultat creşterea volumului sanguin, deci a tensiunii arteriale. De asemenea, hiperinsulinismul poate induce o hiperexpresie a endotelinei I. Pacienţii cu hiperinsulinism prezintă un tonus simpatic crescut, care va determina vasoconstricţie, deci creşterea rezistenţei vasculare periferice, toate acestea contribuind la apariţia şi menţinerea HTA. Deci, HTA este multifactorială şi faptul că HTA este inclusă printre criteriile de descriere este din cauza prezenţei extrem de răspândite a HTA în cadrul SCM (6).

În cadrul studiului nostru, de subliniat ponderea importanta a HTA grad I şi III nediagnosticată la grupa de vârstă 26-50 ani cu implicaţii în dezvoltarea comorbidităţilor. Diferitele grade ale nivelului TA pot afecta profilul de risc cardiovascular al acestor pacienţi, care evident va fi mai înalt la indivizii cu nivele tensionale mai înalte (tab. III).

Un rol dominant în corelaţia dintre HTA şi obezitate îl are hiperactivitatea sistemului nervos simpatic (SNS). Legăturile dintre etiopatogenia obezităţii şi SNS sunt multiple şi în multe cazuri nu sunt elucidate până la capăt. Leptina, un hormon secretat de adipocite nu doar reglează apetitul ci joacă şi un rol important în cheltuielile de energie prin activarea SNS. Leptinemia este înaltă la pacienţii obezi, ceea ce are un rol important în cauzarea hiperreactivităţii SNS. Din contra, la pacienţii obezi receptorii apetitului sunt rezistenţi la reglarea centrifugă a sistemului nervos central (SNC), ceea ce duce la creşterea leptinemiei. Surplusul de norepinefrină, ca o consecinţă a hiperactivităţii SNS la obezi, implică afectarea rinichilor ca efectori majori ai activităţii SNS. Creşterea acţiunii SNS asupra rinichiului, duce de la sine la creşterea reţinerii de sare şi apă şi respectiv la HTA. Comprimarea rinichiului de către depozitele de grăsimi, modifică structura acestuia, care poate duce după sine la HTA prin stimularea nervilor simpatici renali aferenţi şi prin inducerea HTA renovasculare (7).

Relaţia cauzală dintre HTA şi obezitate poate fi biderecţională. La indivizii cu predispoziţie la HTA, creşterea activităţii SNS este asociată cu reglarea centrifugă a receptorilor ß-adrenergici, ce determină afectarea termoreglării şi tendinţa de creştere a circumferinţei abdominale (CA).

5. Impactul SCM asupra frecvenţei cardiace. Sindromul metabolic, denumit şi sindromul X, sindromul rezistenţei la insulină, sindromul hipertensiunii sau sindromul excesului de catecolamine prin mecanismul său central de creştere a tonusului simpatic determină creşterea frecvenţei cardiace - factor major al creşterii consumului de oxigen şi a necesităţilor metabolice. Deci, influenţa hiperinsulinismului se reflectă şi asupra frecvenţei cardiace. Aceasta creştere este direct proporţională cu creşterea debitului cardiac.

Studiile anterioare au demonstrat că activitatea metabolică, debitul cardiac şi frec-

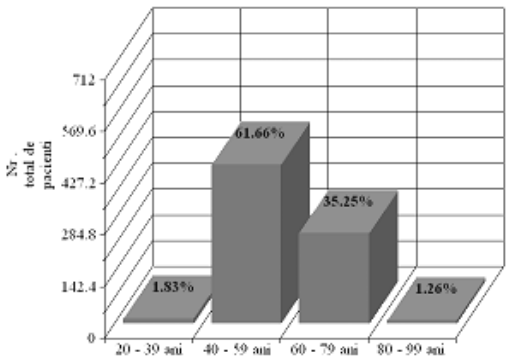

Fig. 1. Repartiţia pe grupe de vârstă a SCM

venţa cardiacă sunt crescute la pacienţii obezi, proporţional cu surplusul de greutate. Deci, creşterea frecvenţei cardiace s-ar datora acţiunii sinergice a celor doi factori implicaţi în evoluţia sindromului metabolic : hiperinsulinismul şi obezitatea (8).

În cadrul studiului nostru, se confirmă ipotezele anterioare, numărul pacienţilor cu sindrom metabolic şi frecvenţă cardiacă ridicată FC > 80/min fiind semnificativ.

6.Impactul SCM asupra aritmiilor cardiace. Cercetările recente anatomopatologice, macro- şi microscopică asupra cordului la pacienţii obezi relevă o creştere a greutăţii cordului, cu dilatare şi hipertrofie concentrică de ventricul stâng. Aceste modificări predispun la apariţia aritmiilor ectopice, în parte datorate unei disfuncţii vegetative cardiace. Având în vedere factorii de risc asociaţi sindromului metabolic FC > 80/min şi masa VS > 270 g, ruptura plăcii de aterom este o complicaţie bine cunoscută, la pacienţii cu boală coronariană. În cadrul studiul nostru, la eşantionul de pacienţi cu vârsta cuprinsă între 26-50 ani 9,52%(4 bărbaţi) şi 7,83%(4 femei) au fost diagnosticaţi cu aritmie. În eşantionul de pacienţi cu vârsta cuprinsă între 51-75 ani 24,60% (47 bărbaţi) şi 21,72% (58 femei) au fost diagnosticaţi cu aritmie. Aceeaşi tendinţă a fost observată la eşantionul de pacienţi cu vârsta peste 75 ani 55,55% (5 bărbaţi) şi 59,09% (13 femei au fost diagnosticaţi cu aritmie (9).

7. Impactul SCM asupra insuficientei cardiace. Studii realizate prin cateterism cardiac la pacienţii obezi fără HTA arată că deşi se înregistrează creşteri ale presiunilor de umplere, telediastolice, a dimensiunilor cavităţilor cordului, precum şi a presiunii capilare pulmonare, performanţa ventriculului stâng este în limite normale. La pacienţii cu obezitate de grad II - III sau la cei ce asociază HTA, este afectată atât funcţia sistolică cât şi diastolică a ventriculului stâng, atingând grade diferite de insuficienţă cardiacă reversibilă cu scăderea în greutate. Cardiomiopatia obezilor se datorează iniţial creşterii volumului circulant şi a debitului cardiac pentru ca ulterior să fie agravată de HTA, insuficienţă coronariană şi tulburări respiratorii. Hipertrofia ventriculului stâng reduce complianţa cordului cu creşterea presiunilor de umplere ale ventriculului stâng şi risc de edem pulmonar acut. Deşi în condiţii de repaus performanţele ventriculului stâng sunt în limite normale, adaptabilitatea la efort este slabă, creşterea debitului cardiac nu se poate face decât pe seama creşterii frecvenţei cardiace întrucât volumele de ejecţie nu se pot adapta (8, 9).

În cazul studiului nostru, la eşantionul de pacienţi cu vârsta cuprinsă între 26-50 ani 14,28% (1 bărbat) şi 12,5% (1 femeie) aflaţi sub tratament hipolipemiant au fost diagnosticaţi cu insuficienţă cardiacă. În eşantionul de pacienţi cu vârsta cuprinsă între 51-75 ani 31,41% (60 bărbaţi) şi 21,72% (58 femei) au fost diagnosticaţi cu insuficienţă cardiacă. În eşantionul de pacienţi cu vârsta cuprinsă peste 75 ani toţi bărbaţii şi 66,66% (2 femei) au fost diagnosticaţi cu insuficienţă cardiacă.

CONCLUZII

Sindromul metabolic reprezintă o problemă de mare actualitate prin creşterea alarmantă a prevalenţei acestuia, precum şi datorită complexităţii mecanismelor fiziopatologice, riscului de apariţie a compli-

caţiilor şi abordării terapeutice. O pondere importantă a pacienţilor cu HTA, gradul I - III, asociază SCM, precum şi alţi factori de risc pentru evoluţia BCV. Cel mai mare impact în apariţia şi dezvoltarea BCV îl are insulinorezistenţa, care poate avea diferite grad de manifestare – de la ascunsă sau latentă până la slab pronunţat, la care se adaugă prezenţa altor tulburări metabolice precum obezitatea, hiperleptinemia, dislipidemia, hiperuricemia, tulburarea sistemului hemostazei (hipofibrinoliză şi hipercoagularea), afectarea sistemului imun prin imunodeficienţă secundară şi influenţa postmenopauzei la femei (10,11).

Studiul nostru îşi propune identificarea corelaţiilor existente între sindromul cardiometabolic şi determinismul afecţiunilor cardiovasculare pe o populaţie reprezentativă pentru zona Moldovei. Astfel, se remarcă o creştere a incidenţei insulinorezistenţei şi a sindromului cardiometabolic pe 2 intervale de vârstă: 25-50 şi 51-75 ani (la aceştia din urmă, este necesară introducerea în cadrul programelor terapeutice şi de recuperare).

Grupul ţintă de evaluat şi introdus în programe de prevenţie şi recuperare, aşa cum o arată procentele, se situează între 25-50 ani la care schimbarea stilului de viaţă, diminuarea factorilor de risc, reevaluarea periodică în cadrul unui program de specialitate va duce nu numai la diminuarea severităţii comorbidităţilor, ameliorarea prognosticului in timp, ci şi la îmbunătăţirea semnificativă a calităţii vieţii pacientului.

Asocierea tulburărilor metabolice într-un lanţ comun în care insulinorezistenţa tisulară nu numai că înrăutăţeşte manifestarea clinică a lor, dar şi le oferă un caracter specific determină apariţia şi dezvoltarea comorbidităţilor asociate sindromului cardiometabolic, din care afectarea cardiacă are o pondere majoritară. Deci, diagnosticul precoce al SCM la pacienţii hipertensivi cu prezenţa măcar a unuia din factorii de risc metabolici ai BCV este extrem de necesară şi raţională pentru aprecierea ulterioară a riscului sumar la pacienţii respectivi şi posibilitatea normalizării valorilor tuturor componentelor de bază ale SCM.

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DIFFERENCES IN TUBERCULOSIS BASED ON GENDER PECULIARITIES

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DIFFERENCES IN TUBERCULOSIS BASED ON GENDER PECULIARITIES (Abstract)**:** The relationship between the gender peculiarities and tuberculosis is under-recognized due to a complex of factors. The **aim** of the study was to assess the impact of the gender peculiarities over tuberculosis features and treatment outcome in the Chisinau city. **Material and methods:** A retrospective, selective, descriptive study targeting social, demographic, economic and epidemiological peculiarities, case-management, radiological aspects and microbiological characteristics of 467 patients registered with tuberculosis in the Chisinau city, the Republic of Moldova (MD) during 1.1.2015-31.12.2015 was performed. **Results**: the distribution based on gender identified the predominance of men 285 (61.03%) compared to women 182 (38.9%). Women statistically predominated in the group of 18-34 years old and men in the group of 35-54 years old. Every tenth patient from both groups was a hard-to-reach case. The microbiological positive cases constituted one third of both groups and multidrug resistance was identified in every tenth patient. Pulmonary infiltrative tuberculosis was diagnosed in most of the patients while severe forms such as disseminated, generalized and fibro-cavernous tuberculosis - more frequently in men. TB-HIV co-infection was established in a similar proportion in both groups. Social-vulnerable patients, from high risk groups, with associated diseases were identified at a similar rate in both groups. Treatment success rate was higher in women and lower in men. **Conclusions:** Women aged 18-34 years and men aged 35-54 years should be included in high risk groups for annual screening. Despite the lack of differences in the microbiological patterns severe forms such as disseminated, generalized and fibro-cavernous tuberculosis were more frequently diagnosed in men and as a consequence the treatment success was low. **Keywords**: TUBERCULOSIS, GENDER, SECTIONAL STUDY.

Tuberculosis (TB) represents a social disease (1, 2, 3, 4). It is the consequence of gross defects in social organization and errors in individual behavior (1). WHO placed the Republic of Moldova (MD) in the list of high burden countries for TB in the post-2015 era (3, 4). Social determinants that are increasing the susceptibility for tuberculosis are: poverty, low level of education, gender inequality, rapid urbanization, overcrowding, migration, malnutrition, smoking, alcoholism, HIV-infection and male sex (5, 6, 7). The relationship between male sex and tuberculosis risk is under-recognized due to highly complex range of factors (6, 7). These was identified a strong relation between notification rates, prevalence, mortality and male sex due to behavioral and physiological differences between men and women (7, 8). Although

epidemiological factors are important for spreading of the disease among men, specific issues that contributed to the increased risk of low treatment outcomes are underrecognized.

The aim of the study was to assess the impact of gender differences on the tuberculosis features in patients from the Chisinau city. The objectives were: 1. Assessment of the main epidemiological indices in the Chisinau city. 2. Assessment of general, socioeconomic and epidemiological risk factors of tuberculosis patients by gender distribution. 3. Evaluation of case-management, diagnosis, radiological aspects and microbiological characteristics of patients according to the gender distribution.

MATERIAL AND METHODS

There was performed a retrospective selective, descriptive study targeting social, demographic, economic and epidemiological peculiarities, case-management, radiological aspects and microbiological characteristics of 467 patients. The study included the patients registered in the Chisinau city (MD) in the period 1.1.2014-31.12.2014 as new tuberculosis cases. Inclusion criteria were: age > 18 years old, new case of tuberculosis and signed informed consent. Patients were managed in the clinical subdivisions of the Clinical Municipal Hospital of Phtysiopneumology of the Chisinau city according to the National Clinical Protocol. As a rule, the patients suspected with tuberculosis are investigated using the microscopic and bacteriological methods. Two smear samples are investigated after the staining by the Ziehl-Neelsen method and examined using the optical microscopy. Positive smears for acid-fast-bacilli are graded as 1+, 2+ and 3+. After the decontamination with N-acetyl-L-cysteine sodium hydroxide the sputum sample is inoculated on two slopes of solid medium Lowenstein Jensen. The slopes are incubated at 37°C and examined weekly for evidence of growth of colonies till 8 weeks. The growth was graded according to the number of the colonies. The drug susceptibility testing is performed for identification of the resistance against the first line antituberculotic drugs: isoniazid, rifampicin, ethambutol and streptomycin using the absolute concentration method on Lowenstein Jensen medium. The liquid culture methods, such as MGIT (Becton Dickinson), MB/BacT (BioMerieux) or Bactec 460 TB are compulsory used for testing the sputum samples of patients suspected with tuberculosis in association with solid Lowenstein Jensen medium. It confirms that tuberculosis in less than two weeks and drug susceptibility test for first-line drugs are obtained in the following two weeks. Additionally, the radiological examination is performed in all suspected with tuberculosis patients. Statistical analysis was carried out using the quantitative and qualitative research methods. For the comparative epidemiological assessment of major epidemiological indices in MD and Chisinau there were used the published data by the National Statistical Bureau and the National Centre for the Health Management (1).

RESULTS

According to the published data by the Moldovan National Centre for the Health Management in the period 2013-2015 there was registered an important decline of all epidemiological indices (1). The global incidence - which included new and relapsed cases) in MD decreased by 21.9% between 2013 and 2015 and by 22.1% - in Chisinau. A similar vector was established in respect of tuberculosis prevalence which decreased between 2013-2015 by 26.3% in MD and by 25.5% - in Chisinau. Less evidently decreased the incidence of new cases by 18.4% in MD and by 18.2% - in Chisinau. During the studied period the mortality decreased from 11.3 to 10.0 / 100,000 population in

MD, and from 10.8 to 6.9 / 100,000 population in Chisinau (1). The men and women ratio among new cases remains at the same level in MD and Chisinau demonstrating that men were two times more affected than women (tab. I).

In 2014, in the Clinical Municipal Hospital of Phtysiopneumology of Chisinau 467 cases were managed. The distribution according to gender identified the predominance of men 285 (61%) compared to women 182 (39%), $p<0.0001$. The male-female ratio was 1.56:1. The distribution by age groups identified the predominance of women in the 18-34 years old group and men in the 35-54 years old group. Distribution according to the demographic characteristics identified that two third of both groups were residents of urban districts of Chisinau. The patients from hard-to-reach groups that included citizens with lack of residence card, homeless or migrants were every tenth patients of both groups (tab. II).

By assessing the microbiological characteristics of the selected patients, it was established that the molecular genetic test GeneXpert MTB/Rifampicin contributed to the diagnosis of tuberculosis in most of the patients from both groups. GeneXpert MTB/Rifampicin resistant strains of *Mycobacteria* were identified in a similar rate of both groups. Smear microscopy identified a similar rate of microscopic positive results for acid-fast-bacilli in men and women groups. A similar rate of patients from both groups had culture positive results. Drug susceptibility testing on Lowenstein-Jensen solid either liquid (BACTEC MGIT 960 system) medium identified a higher rate of multidrug-resistant than mono- and poly-resistant strains of *Mycobacteria* in both groups (tab. III).

TABLE I

Comparative epidemiological indices of tuberculosis

Index	2013		2014		2015	
	Abs.	100,000 population	Abs.	100,000 population	Abs.	100,000 population
Global incidence MD	4488	110.3	4061	99.7	3599	88.4
Global incidence Chisinau	755	94.0	660	81.8	580	71.9
Incidence of new cases MD	3604	88.5	3275	80.4	2854	70.1
Incidence of new cases Chisinau	579	72.1	509	63.1	435	53.9
Prevalence MD	5351	131.5	4812	118.3	4278	105.2
Prevalence Chisinau	990	123.4	867	107.4	806	99.9
Mortality MD	459	11.3	510	12.5	406	10
Mortality Chisinau	87	10.8	81	10.0	56	6.9

Gender Rate

Index	2013		2014		2015	
	Abs.	%	Abs.	%	Abs.	%
Men rate from the total number of new cases in MD	2548	70.7	2292	70.0	2028	71.1
Women rate from the total number of new cases in MD	1056	29.3	983	30.0	826	28.9
Men rate from the total number of new cases in Chisinau	371	64.1	309	60.7	304	69.9
Women rate from total number of new cases in Chisinau	208	35.9	200	39.3	131	30.1

TABLE II

Distribution by demographic data

Indices	Age Residence	No. of cases 467		p value
		Men N=285 n (%)	Women N=182 n (%)	
Age groups	18-34 years	102 (35.79)	97 (53.29)	**<0.001**
	35-54 years	126 (44.11)	53 (29.12)	**<0.01**
	>55 years	57 (20.00)	32 (17.58)	>0.05
Residence	Urban	210 (73.68)	142(78.02)	>0.05
	Rural	75 (26.32)	40(21.98)	>0.05
Other categories	Lack of residence card	24 (8.42)	21(11.54)	>0.05
	Homeless	22 (7.72)	8(4.49)	>0.05
	Labor migrants	28 (9.82)	12(6.59)	>0.05

Note: Applied statistical test: paired simple T-test.

TABLE III

Distribution of patients by microbiological features

Characteristics		No. of cases 467		P value
		Men N=285 n (%)	Women N=182 n (%)	
Microbiological results	GeneXpert MTB/ Rifampicin positive	124 (43.5)	69 (37.9)	>0.05
	GeneXpert MTB/Rifampicin resistant	33(11.6)	27 (14.8)	>0.05
	Microscopic positive	80 (28.1)	45(24.7)	>0.05
	Culture positive	93 (32.6)	48(26.4)	>0.05
Resistance type at DST	Mono-resistance	4 (1.4)	1(0.54)	>0.05
	Poly-resistance	2 (0.7)	3 (1.7)	>0.05
	MDR-TB	31 (10.9)	20 (10.9)	>0.05

Note: Applied statistical test: paired simple T-test, DST-drug susceptibility testing. MDR-TB multidrug-resistant tuberculosis.

By assessing the case-management it was identified that the general practitioners detected more frequently men-187 (65.61%) comparing with women - 99 (54.39%). High risk groups screening was used more frequently for the detection of women 114 patients (40.00%) than of men-107 patients (58.79%). Addressed directly to the specialized clinical services were more frequently men (tab. IV).

By assessing the clinical radiological forms of tuberculosis, it was established that pulmonary infiltrative tuberculosis was diagnosed in most of the patients. Disseminated tuberculosis, generalized tuberculosis, lung destructions and involvement of both lungs predominated in men. A similar rate of extra-pulmonary tuberculosis was identified in both groups. The most frequently were diagnosed tuberculosis pleurisy and intrathoracic tuberculosis. TB-HIV co-infection was diagnosed in a similar proportion in both groups 16 (5.6%) cases in men and 10 (5.5%) cases in women) (tab. V).

When distributing the patients according to the economic status, it was established that women were more frequently employed and had health insurance policy. Students statistically predominated in the group of women due to a higher rate of early adult-

hood (ages 18-35) cases. Disease disabled were more frequently men, but women were more frequently retired. From infectious clusters there were more frequently women, but the statistical threshold was not achieved. Co-morbid status of TB patients was identified in a similar rate in both groups, as well as HIV-infection (tab. VI).

Treatment outcomes were assessed using the standardized indices. The highest success rate was established in the women group and the rate of patients with low treatment outcomes was higher in the men group. The low outcomes included: dead, failed and lost to follow-up patients represented 50 (17.5%) men and 12 (6.6%) women. Still continuing the treatment were patients treated according to the DOTS-Plus regimen for multidrug-resistant tuberculosis (tab. VII).

TABLE IV

Case-management and case-detection ways

Health level	Detection ways	No. of cases 467		p value
		Men N=285 n (%)	Women N=182 n (%)	
PHC	Detected by GPs-symptomatic	99 (34.7)	29(15.9)	>0.05
	Detected by GPs – screening of HRG	88 (30.9)	70(38.5)	>0.05
Ambulatory specialized level	Detected by SP-symptomatic	27 (9.5)	28(15.5)	>0.05
	Detected by SP-screening of HRG	26 (9.1)	37(20.3)	>0.05
Hospital	Direct addressing	45 (15.8)	18(9.9)	>0.05

Note: Applied statistical test: paired simple T-test. GPs-general practitioners, HRG- high risk groups.

TABLE V

Radiological characteristics of Moldovan TB patients

Parameters	Types	No. of cases 467		p value
		Men N=285 n (%)	Women N=182 n (%)	
Pulmonary TB	PIT	269 (94.4)	158(86.8)	>0.05
	NT	2 (0.7)	4(2.2)	>0.05
	FCVT	0	2(1.1)	N/A
	DT	14(4.9)	4(2.2)	>0.05
	Lung destructions	114(40.0)	56(30.8)	>0.05
	2 lungs involved	26(9.1)	7(3.9)	>0.05
Extrapulmonary TB	Total cases	15(5.3)	14(7.7)	>0.05
	Pleurisy	6(2.1)	3(1.7)	>0.05
	ITTB	4(1.4)	2(1.1)	>0.05
	Generalized TB	1(0.4)	0	N/A
	OAT	1 (0.4)	6 (3.3)	>0.05
	Other localizations	3(1.1)	3(1.7)	>0.05

Note: Applied statistical test: paired simple T-test, N/A-non available.
PIT- pulmonary infiltrative tuberculosis. FCVT fibro-cavernous tuberculosis.
DT- disseminated tuberculosis. ITTB-intrathoracic tuberculosis.
OAT – ostheo-articular tuberculosis.

TABLE VI

Socio-economic status and risk groups

Economic indices	State	No. of cases 467		p value
		Men N=285 n (%)	Women N=182 n (%)	
Stable	Employed	74 (25.9)	62 (34.1)	>0.05
	With health insurance	185 (64.9)	130 (71.4)	>0.05
	Students	10 (3.5)	29 (15.9)	**<0.001**
	Disable	18 (6.3)	7 (3.9)	>0.05
	Retired	22 (7.7)	22 (12.1)	>0.05
Vulnerable	Unemployed	155 (54.4)	62 (34.1)	**<0.001**
Epidemiological risk factors	History of detention	24 (8.4)	2 (1.1)	>0.05
	From TB cluster	20 (7.0)	20 (10.9)	>0.05
Co-morbid status	Associated diseases	71 (24.9)	39 (21.4)	>0.05
	Chronic alcoholism/abuse	18 (6.3)	6 (3.29)	>0.05
	Diabetes mellitus	5 (1.8)	2 (1.1)	>0.05
	Psychiatric diseases	2 (0.7)	3 (1.7)	>0.05
	Neoplastic diseases	1 (0.4)	0	
	Illicit drug use	2 (0.7)	1 (0.5)	N/A

Note: Applied statistical test: paired simple T-test.

TABLE VII

Final treatment outcomes

	Results	No. of cases 467		p value
		Men N=285 n (%)	Women N=182 n (%)	
Standardized outcome	Success	228 (80.0)	155 (85.2)	>0.05
	Died	23 (8.1)	4 (2.2)	>0.05
	Treatment failure	7 (2.5)	2 (1.1)	>0.05
	Lost to follow-up	20 (7.02)	6 (3.3)	>0.05
Other	Still continuing	1 (0.4)	15 (12.1)	>0.05

Note: Applied statistical test: paired simple T-test.

DISCUSSION

The actual study established an important decline of the epidemiological indices in the Republic of Moldova as well as in Chisinau. There are a lot of causes that determined this statistical improvement of the epidemiological situation, such as: low rate of high risks groups investigated in the frame of the active way of screening (annual chest radiological examination), high rate of migrants that are inaccessible for screening procedures, poor health care seeking behavior of the high risk groups and low accessibility to health care services of social vulnerable patients (7). Globally, men were more frequently diagnosed with tuberculosis than women with a male-to-female ratio 1.6:1 (4). Differences in active tuberculosis, according to gender sex distribution represented an important problem considering the high rate of men established in our research as well as in other studies (6, 7, 8). Multiple

gender-related factors that depend on regions were studied. In South Asia tuberculosis affected predominantly young women due to the high rate of the TB-HIV coinfection and pregnancy-related conditions (8). In our study, we also confirmed the predominance of young aged women and middle-aged men. A randomized meta-analysis of 3.1 million patients with tuberculosis from 28 countries established that men were more frequently microbiologically positive that were associated with social disadvantage in screening for detection and ensuring gender equity in health care (6). The most prevalent risk factors were established: alcohol abuse, imprisonment, drug use and associated diseases, such as chronic obstructive pulmonary disease, HIV infection and previous anti-tuberculosis treatment (6). The social and economic vulnerability was linked with the male sex in our research, as well as in a large number of international studies (5, 6, 7). Eastern Europe and Eurasia regions are confronted with numerous high risks, all being similar with those identified in our study: socioeconomic vulnerability, migration, detention, chronic alcoholism and therapeutic incompliance more expressed in the treatment of MDR-TB patients (4, 5, 6, 7, 9, 10). Connected to the detection and microbiological diagnosis, our study, as well as some foreign studies registered a low level of microbiological positive cases (5, 6, 7, 8). It could be explained by the low quality of the specimen collection procedure, disease-related factors and the low quality of the screening (11). One of the prevalent gender differences was higher rate of death in men comparing to women, however the statistical threshold was not achieved. The biological differences predisposed to a higher treatment outcome in the women group, without achieving the statistical threshold (8). Cigarette smoking was established as a high-risk factor for tuberculosis in the population of men from the Northern California (12). A cohort study conducted in Portugal for 4 years established that the male-female ratio was 2.1. The most affected age group in men was 40-49 years old and in women of 20-29 years age group. However, in the 50-59 years old group the male-to-female ratio constituted 3.4 (13). Conforming to the Czech health statistics the male-female ratio was 2.3:1 in the general population. According to the prevalence the most affected age group was constituted from patients aged more than 80 years old (14). Epidemiological data offered by Poland established that the incidence in men was 2 times higher than in women and the highest difference between sex groups was identified in patients aged 50-59 years old (15). In Romania the male-to-female ratio was 2.2:1 in 2012, with the highest incidence in the group 45-64 years old (16). Considering all related information, we can conclude that our study obtained similar results with other researches from high tuberculosis burden and low- or middle-income countries. So, we confirm gender inequality in patients with tuberculosis due to biological, socioeconomic vulnerability and harmful habits which were more frequently in men population.

CONCLUSIONS

The profile of patients enrolled in our study demonstrated that TB was more frequently diagnosed in women in the early adulthood and in men in the period of middle adulthood, due to the high rate of risk factors.

Urban residents constituted two thirds of our selected samples due to overcrowding of Chisinau and to criteria of the patients' selection.

Smear positive state as the criteria of the epidemiological danger was identified in every third patient and multidrug resistance

was identified in every tenth patient, demonstrating the tense epidemiological situation in Chisinau.

Men were more frequently detected as symptomatic patients and women - by high risk group screening. Social-economical high-risk factors in men were those associated to unemployment and in women - student and retired person's status.

The history of detention was more frequently met in men and infectious contacts, as being the resident of a disease cluster, it was more frequently met in women. Severe forms: disseminated, generalized and fibrocavernous tuberculosis was established more frequently in men.

Treatment success rate was higher in women and low outcomes (death and lost to follow- up) was established more frequently in men.

The present study indicates that the evaluation of the risk factors in men and women is important for screening programs which target susceptible subpopulations and establish the recommendations for the subsequent follow-up.

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EMBOLIC STROKE OF UNDETERMINED SOURCE – THE TOP OF AN ONGOING ICEBERG

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EMBOLIC STROKE OF UNDETERMINED SOURCE–THE TOP OF AN ONGOING ICEBERG (Abstract): Even though the incidence of cerebrovascular disease has decreased worldwide, stroke remains an important epidemiological concern due to its still high mortality and morbidity rates. Approximately one third of ischemic strokes are of undetermined cause according to the TOAST classification. From these cryptogenic strokes, ESUS is a separate entity caused by an underlying embolic mechanism. ESUS usually affects younger men and the severity of focal deficits is comparable to those of large-artery atherosclerosis. The most frequent cause of this non-lacunar cryptogenic stroke is covert atrial fibrillation and other atrial dysrhythmias. The aim of the present study is to provide up to date information regarding diagnosis and management of ESUS. Even though the current standard of ESUS treatment remains antiplatelet drugs, ongoing clinical trials address the need for chronic oral anticoagulation. **Keywords:** ESUS, COVERT ATRIAL FIBRILLATION, ANTIPLATELETS, ANTICOAGULANTS.

Even though stroke remains worldwide one of the most important disorders in terms of incidence, prevalence, mortality and morbidity, in the past years there has been a positive epidemiological change in developed countries due to efficient implementing of specific pharmacological and nonpharmacological prophylactic measures. According to recent reports of the American Heart Association, the incidence of both de novo and recurrent stroke has a tendency of declining. Also, in the last decade, the overall death by stroke has significantly decreased to a point that stroke has become only the fifth cause of death in the United States (1). The reported prevalence of cerebrovascular disease is 794 of hundred thousand people (2).

In spite of these positive tendencies, stroke remains one of the major health problems, especially in developing countries where it is responsible with 75.2% of deaths and 81.0% of stroke-related disability adjusted life years lost (3).The difference in incidence, prevalence and mortality rates vary along with geographical and demographical characteristics of populations, including specific associations of cardiovascular risk factors. For instance, according to Cuciureanu *et al.* who studied

70 patients diagnosed with ischemic stroke with spontaneous hemorrhagic transformation, the pathogeny of stroke in this specific population relied on interaction between atrial fibrillation, high blood pressure and dyslipidemia (4).

It is a general accepted fact that ischemic strokes represent the majority, comprising of approximately 87% of all strokes. These are followed by primary hemorrhages in a percent varying from 10% to 25%, with the highest prevalence among populations with Asian, Latin American and African descent compared to European descent (5). Only 3% to 5% of acute cerebrovascular disease is represented by subarachnoid hemorrhages (6).

Ischemic stroke, this black mark in the mortality and morbidity of cerebrovascular diseases, has been the focus of study throughout decades in terms of pathology, pathogeny, management and prophylaxis. Regarding etiology, nowadays more than 100 causes are known to be involved in its pathogenesis (7). In order to provide a much more accurate etiology and therefore a more efficient management, several classifications of ischemic stroke have emerged. These tend to be either phenotypic or causative. In the first category several classifications are included such as Baltimore-Washington or Causative Classification of Stroke System (CCS) (8, 9). All these phenotypic classifications grade the probability of every possible stroke etiology and, therefore, their main disadvantage is the lack of explicitly stating the most probable cause of stroke. On the other hand, causative ischemic stroke classifications use generally accepted inclusion criteria in order to segregate patients in specific etiological subgroups.

One of the most widely used causative classifications of ischemic stroke is the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification which divides ischemic stroke into five etiological subtypes: large-artery atherosclerosis, cardio embolism, small-vessel occlusion, stroke of other-determined etiology and stroke of undetermined etiology (10). According to Adams *et al*., the last category, also known as a cryptogenic stroke, is defined by impossibility of stating the etiology of the stroke. This occurs either despite extensive paraclinical examinations or in cases of scarce evaluation, or when at least two potential causes are discovered but the physician cannot point towards a single one (10). When using TOAST criteria, cryptogenic stroke seems to affect approximately one third of the elderly patients and up to 62.4% of young patients aged 15 to 45 years (11, 12). The incidence of cryptogenic stroke varies also with the inclusion criteria used in different classifications. For instance, the newer and improved version of the TOAST classification, the Stop-Stroke-Study TOAST (SSS-TOAST) classification which provides newer epidemiological and neuroimaging criteria has managed to decrease the incidence of cryptogenic stroke to 4 % when applied (13).

Starting from the general known fact that most non-lacunar ischemic strokes are embolic and from the observation that patients diagnosed with cryptogenic stroke have embolic pattern brain imaging, Hart *et al.* introduced the concept of embolic stroke of undetermined source (ESUS) which they defined as a cryptogenic stroke of thromboembolic nature (14). Thus, in 2014 a new stroke entity was recognized worldwide.

The importance of classifying a stroke as ESUS resides not only in lack of knowledge regarding recurrence rate but also in the management of this specific kind of stroke. Logically, if no cause of stroke is discovered then no proper secondary prevention treatment can be applied. Given these arguments, ESUS is one of the main focuses of the current studies worldwide, in terms of etiology, risk factors, recurrence predictors, extensive paraclinical evaluations and treatment. Therefore, the aim of the present review is to provide up to date information regarding diagnosis and management of ESUS.

ESUS DEFINITION, EPIDEMIOLOGY AND DIAGNOSIS

Despite intensive research towards etiology and management, a standardized definition of ESUS has not yet emerged. However, ESUS definition can be simplified to that of a subtype of cryptogenic stroke highly probable caused by an underlying embolic mechanism. If one takes into account its initial diagnosis criteria proposed by Hart *et al*, ESUS can be defined as a subtype of non-lacunar cryptogenic stroke characterized by absence of more than 50% occlusive atherosclerosis in arteries supplying the ischemic area, absence of major-risk cardioembolic source and absence of other specific stroke causes such as non-atherosclerotic arteritis, dissection or vasospasm (14).

Currently, there are scarce population-based studies regarding epidemiology, clinical and paraclinical characteristics and outcome of ESUS. According to the ESUS Global Registry study published in 2016, ESUS comprises of 16% of all ischemic strokes from the cross-sectional global sample of patients included (15). On the other hand, Ntaios *et al*. reported that ESUS represented 10% of ischemic stroke patients registered in the Athens Stroke registry between 1992 and 2011 (16). A major systematic review by Hart *et al* published in 2017 states that the average reported frequency of ESUS is 17%, in other words, one of every six strokes is of undetermined embolic source (17).

Even though incidence of ESUS may vary from one population to another due to several populational characteristics, there are some features that remain constant. For example, ESUS tends to appear at a younger age, with a mean age at presentation varying from 62 to 65 years compared to 68 years among non-ESUS cryptogenic stroke (15, 17). ESUS appears to affect men more than women, 58% compared to 42% (17). Patients with ESUS tend to experience symptoms at onset of low to moderate severity with a mean National Institute of Health Stroke Scale score of 5 (17). In other words, in terms of stroke severity, ESUS is reported to be similar to strokes due to large artery atherosclerosis and less severe than strokes caused by a major cardioembolic source (18).

Cryptogenic stroke is an exclusion diagnosis after extensive paraclinical evaluation. As new etiological and pathogenically theories emerge, several investigations can be added to the ongoing list. However, ESUS is diagnosis-criteria based and in accordance to the initial diagnosis criteria, in order to positively identify an ESUS, a minimum of tests must be performed: brain computer tomography (CT) or magnetic resonance imaging (MRI) that excludes lacunar infarcts, invasive or non-invasive imaging of extracranial and intracranial

arteries (catheter angiography or CT / MRI angiography or cervical duplex plus transcranial Doppler ultrasonography), 12-lead EKG, transthoracic echocardiography and minimum 24 hour cardiac monitoring with automated rhythm detection (14).Regarding brain imaging, in most countries, CT is the most used diagnosis tool in ischemic stroke unlike neurodegenerative disorders such as Parkinson's disease in which MRI is the adequate (19). In spite of this fact, recent studies reveal that contrast injected MRI is the golden standard in early diagnosing acute ischemic stroke, whereas use of certain contrast agents can point the real ischemic penumbra (20).

In addition to these basic investigations, recent studies concur that transesophageal echocardiography is mandatory in correctly diagnosing a cryptogenic stroke due to its superiority in analyzing aortic arch, left atrium and interatrial septum (11). Even though in their initial assessment, Hart *et al* did not recommend systematic proximal aortic arch imaging, a recently published multicentric study which assessed 677 cryptogenic stroke patients by using transesophageal echocardiography concluded that aortic arch plaque larger than 4mm, with ulcerative or mobile components increases the risk of ESUS (21).

Regarding detection of minor-risk potential cardioembolic sources such as covert paroxysmal atrial fibrillation or other atrial dysrhythmias, the role of prolonged cardiac rhythm monitoring is currently debated. The American Stroke Association recommends in its guidelines a 30 days prolonged cardiac monitoring in cryptogenic strokes whereas, European Society of Cardiology recommends a minimum 72 hours monitoring in all strokes with the possibility of exceeding this duration (22, 23). In the last decade a series of clinical trials have focused on the role of prolonged cardiac rhythm monitoring in diagnosing cryptogenic stroke and ESUS. The overall conclusion is that the longer the monitoring the higher are the chances to diagnose a paroxysmal atrial dysrhythmia. For instance, a study conducted on 441 cryptogenic stroke patients revealed that after a cryptogenic stroke, the use of an implantable monitoring device increases the rate of atrial fibrillation detection at 6 months to 8.9% compared to 1.4% by conventional management (24).

Several biomarkers are starting to be followed in several west-European countries in order to predict the presence of covert atrial fibrillation. Elevated levels of either atrial natriuretic peptide, brain natriuretic peptide and D-dimmersare predictors for asymptomatic atrial fibrillation (25).

ESUS – ETHIOPATHOGENY

ESUS patients appear to have fewer cardiovascular risk factors compared to non-cryptogenic stroke patients, from which hypertension, diabetes and previous stroke are in the top 3 (15, 25). ESUS is caused by several conditions which favor the formation of brain emboli. The most frequent source of emboli appears to be the heart in accordance to several studies which analyzed thrombus composition obtained from patients suffering from various types of ischemic stroke. The general conclusion was that the histology of thrombi is remarkably similar among cryptogenic strokes and cardioembolic ones, with higher proportions of fibrin, leucocytes and lower number of erythrocytes (26).

According to several trials dedicated to

prolonged heart rhythm monitoring, the most prevalent diagnosed cause after recurrence of non-lacunar ischemic stroke or during follow up is atrial fibrillation (24, 27). Das *et al.* who documented risk factors associated with silent cerebral infarcts in an updated sample of Framingham cohort showed that atrial fibrillation is significantly associated with silent stroke with an odds ratio of 2.16 (28). In less than 40% of ESUS cases atrial fibrillation is eventually detected.

Hence, new cardioembolic sources emerge, as covert atrial fibrillation and atrial high-rate episodes. An asymptomatic atrial high-rate episode is defined by an atrial heart rate of minimum 190 beats per minute with no clinical expression. Covert or subclinical atrial fibrillation is defined as atrial high-rate episodes lasting more than 6 minutes but less than 24 hours which have no clinical expression and are therefore detected only fortunately during continuous EKG monitoring (25). According to ASSERT study which followed 2780 patients with either pacemaker or implantable defibrillator, detection of atrial high-rate episodes lasting minimum 6 minutes is associated with an increased risk of developing atrial fibrillation and cryptogenic stroke (29). However, the causative and temporal relationship between atrial high-rate episodes and thromboembolic events has not been totally elucidated yet. Currently, it is not known whether atrial-high rate episodes and covert atrial fibrillation are certain causes of ESUS or simply markers of high thromboembolic risk (25).

A recent model was proposed for explaining the complex bidirectional relation between atrial fibrillation and ischemic stroke including ESUS. According to this model, systemic vascular risk factors induce in time an atrial cardiopathy that can manifest through atrial fibrillation which, in turn, will further cause remodeling of the atrium and worsen the cardiopathy (30). An ischemic stroke of any etiology can cause an autonomic malfunction including cardiac dysrhythmias by affecting certain autonomic regions of the brain. For example, it has been demonstrated that in right-handed patients, right middle cerebral artery infarction is associated with elevated heart rate while left middle cerebral artery infarction is associated with an enhanced parasympathetic control of the heart rate (31, 32). All these autonomic changes induced by ischemic stroke will aggravate the left cardiac disfunction (30). In resume, a vicious heart-brain-heart circle emerges.

More recent population-based studies have questioned the heart as the primary source of brain emboli in ESUS and placed nonstenotic, but potentially vulnerable atherosclerotic plaques situated in the carotid system or the aortic arch. Emerging data have shown that CHADS2 and CHA2DS2-VASc scores allow stratification of the risk of stroke recurrence and death in ESUS in association not only to atrial fibrillation in all of its forms but also in atherosclerotic plaques of the aortic arch. Specifically, aortic arch plaque larger than 4mm, with ulcerative or mobile components associate with a CHA2DS2-VASc score of minimum 2. However, so far, the associations of underlying embologenic diseases in ESUS with CHADS2 and CHA2DS2-VASc scores have remained essentially unknown (21).

According to the ESUS Global Registry, only 35% of patients had at least one minor-risk potential embolic sources and

no carotid artery plaques whereas, the majority of 79% of patients had no stenotic plaques in the cervical carotid arteries (15). These data suggest that the classical stenosis measurements which divide stroke risk and guide primary and secondary prevention of stroke are insufficient. Developments in plaque imaging investigations such as ultrasonography with and without contrast medium, micro emboli signal detection and 18Fluorodeoxyglucose positron emission tomography have discovered new high-risk plaque features. Among the latter, the most relevant are intraplaque hemorrhage, plaque ulceration, plaque neovascularization, fibrous cap thickness, the presence of a lipid-rich necrotic core, and evaluation of plaque inflammatory activity (33). Several small studies have proposed certain nonstenotic carotid artery aortic plaques complication as potential candidates but there remains an uncertainty whether these plaques are the cause of ipsilateral ischemic strokes (34).

In the case of paradoxical embolism as another brain emboli mechanism, potential sources are represented by the venous system in correlation with a patient with *foramen ovale* or *atrial septum* defect. Also, the existence of a pulmonary arterio-venous fistula can be a rare cause of ESUS (14). One must also not forget tumor emboli from unknown cancer or even marantic endocarditis (14).

ESUS-TREATMENT

The intense research regarding causes and mechanisms of ESUS is justified by the imperious need of implementing clear secondary prevention treatment guidelines. Even though the existence of brain emboli which defines ESUS is a clear indication for anticoagulants, in the absence of demonstrating the actual source of emboli, most physicians treat ESUS with antiplatelet drugs. According to Hart, 86% of ESUS were treated with antiplatelet therapy and the average annualized rate of recurrence was quite elevated, at 4.5% (17). These data point towards use of oral anticoagulants in the secondary prevention of ESUS.

Three recent trials evaluated the use of novel oral anticoagulants in the prevention of recurrent ESUS (34, 35, 36). From these, RE-SPECT ESUS was a multicenter randomized double-blind phase III trial which compared the effects of dabigatran twice daily with aspirin once daily in terms of ESUS recurrence and major bleeding in two equal lots which had a median follow-up of 19 moths (35). Even though dabigatran in any of the two generally available dosages did not associate with higher major bleeding events, there was no statically significant differences regarding stroke recurrence between this direct oral anticoagulant and aspirin. However, more no major bleeding events associated more frequently with dabigatran when compared to aspirin. NAVIGATE ESUS was also a multicenter randomized double-blind phase III trial which instead compared the effects of rivaroxaban 15 mg daily with aspirin once daily in terms of ischemic/hemorrhagic stroke or systemic-embolism recurrence and major bleeding in two equal lots which had a median follow-up of 11 moths (35). The results revealed that not only rivaroxaban is not superior to aspirin in stroke prevention but also is associates with a higher risk of bleeding (34). ATTICUS is an ongoing multicenter randomized double-blind phase III trial which compares the effects of apixaban versus aspirin once daily ad-

ministered in ESUS patients in terms of ESUS recurrence (36). The first two trials are comparable in terms of lot dimensions and time interval follow-up and they both conclude in favor of antiplatelet treatment. The third study, however, is using much smaller lots due to the fact that one of the mandatory inclusion criteria is represented by the already existence or the planned implantation of an insertable cardiac monitor. Therefore, the aim of the ATTICUS study is not only to compare effectiveness of apixaban versus aspirin but also to investigate the impact of atrial fibrillation (36).

ESUS-A GLIMPSE OF THE FUTURE

As advances in technologies occur, new diagnosis criteria will add to the list of ESUS. For instances, advanced imaging of carotid aortic plaque such as MRI and 3d ultrasound are currently used in certain laboratories. 3D ultrasound is superior to Doppler ultrasound in terms of image quality and accuracy, with an increased rate of small ulceration detection (12).

One of the current concerns in terms of ESUS is to determine new clinical, biological and cardiological predictors for primary diagnosis or recurrence in order to prevent these events. Apparently, ischemic stroke recurrence after ESUS is corelated with high values of nontraditional lipid parameters such as total cholesterol/high density lipoprotein-cholesterol and low-density lipoprotein-cholesterol/ high density lipoprotein -cholesterol ratios (37). According to Chung *et al.* who measured the levels of plasma free fatty acids during the acute phase of ESUS or cardioembolic stroke in 254 patients, high levels of free fatty acids associate with more severe focal deficits at onset, increased volume of the ischemic lesion and increased prevalence of large cortical infarcts. Therefore, one possible way to reduce ischemic lesion volume and clinical severity is to lower the plasma level of free fatty acids (38).

Meisel *et al.* who studied the shape and function of the left atrium in18 ESUS patients discovered that the left atrium has both at rest and exercise dysfunction due most likely to a spherical remodeling (39). Thus, one possible way to identify high risk-ESUS patients is to analyze the left atrium and to determine certain at rest and during exercise functional parameters such as end-diastolic volume and dejection fraction.

Unfortunately, diagnosis of subclinical atrial fibrillation in stroke patients remains a challenge for most clinicians. One direction, as stated previously, is to increase as much as possible the duration of cardiac rhythm monitoring. Other modalities are following certain EKG parameters such as P wave dispersion, P wave duration and PR interval duration's wave dispersion is defined as the difference between the maximum and the minimum P wave duration detected in a 12-lead ECG and it is a known predictor for atrial fibrillation (40). P wave dispersion, PR interval longer than 200 ms and a P wave duration over 120 ms reflect atrial disfunction secondary to atrial remodeling which can be a cause for atrial fibrillation (40). Regarding biological markers of atrial fibrillation, according to Acampa *et al.* inflammatory markers such as high-sensitive C-reactive protein are related to presence and persistence of atrial fibrillation and, therefore, can be used as suggestive parameters in ESUS patients (41). In order to even better individualize

ischemic strokes caused by emboli formed in the heart secondary to yet undetected atrial fibrillation, a new stroke category recently emerged, SURF or Stroke with Underlying Risk of Atrial Fibrillation (42).

CONCLUSIONS

ESUS is a quite novel entity with important implications in its management. Although its main defining criteria are established since 2014, new paraclinical criteria emerge as new potential causes and mechanisms are discovered. Future studies are required in order to identify new etiologist – potential targets for specific prophylactic treatments.

Despite high prevalence of covert atrial fibrillation and other high risk atrial tachyarrhythmias, the current standard of ESUS treatment remains antiplatelet drugs.

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NEWS

LUTEOLIN, A FLAVONOID AS AN ANTICANCER AGENT

Diet and consumption of bioactive compounds from natural sources (fruits and vegetables)have been recognized for their preventive role against a variety of human diseases including cancer. The inhibitory effects of phytochemicals found in plants against major diseases are well documented in literature. In this context, luteolin, a flavonoid found in different fruits and vegetables has been known as an anticancer agent through inducing apoptosis and cell cycle arrest and trough inhibiting metastasis and angiogenesis in multiple cancer cell lines such as breast, colon, pancreatic among other. Luteolin can be an important complementary medicine for the prevention and treatment of different types of cancers, owing to its natural origin safety and low cost relative to synthetic cancer drugs. However, since most of the findings are based on in vitro and vivo studies, which do not necessarily represent the effect on humans, more work on different pharmacokinetic parameters and may involve human subjects is needed in the future before this substance becomes a prescribed drug. Luteolin (3,4,5,7 tethrahidroxi flavone) is an natural flavonoid, extensively present in many plant species. It is particularly present in fruits and vegetables such as celery, chrysanthemum flowers, sweet bell pepper, carrots, onion leaves, broccoli and parsley (Muhammad Imran, Abdur Rauf, Tareq Abu-Izneid, Muhammad Nadeem, Mohammad Ali Shariati, Imtiaz Ali Khan, Ali Imran, Ilkay Erdogan Orhan, Muhammad Rizwan, Muhammad Atif, Tanweer Aslam Gondal, Mohammad S. Mubarak, *Biomedicine & Pharmacotherapy*, April 2019,Volume 112).

EDITORIAL

ESTABLISHMENT AND CONSOLIDATION OF THE SUPERIOR MEDICAL EDUCATION SCHOOL IN IAȘI. ROLE OF THE SOCIETY OF PHYSICIANS AND NATURALISTS OF IAȘI

1830-1833: ***The Society of Physicians and Naturalists of Iași, a ferment of Moldavian medical education***. Designed on January 11th, 1830, in the form of a *Medical Reading Circle* within the *Spiridonia* Hospital, and having the representatives of the Doctoral Council in the capital of Moldavia among its first members, the Society of Physicians and Naturalists of Iasi (SMN) was fully recognized on March 18th, 1833. Its founders were doctors Iacob Cihac, the city's chief obstetrician, with a doctorate at Heidelberg, and Moldavia's protomedicus, Mihail Zotta, with a doctorate in Vienna, the first Romanian licensed physician in Bucovina. As an association of academic type, SMN was the first scientific society in the Romanian Lands, and in South-East Europe as well. It gathered not only doctors and pharmacists from all over the Principality, but the intellectual, political and religious elite of the country, of neighboring regions inhabited by Romanians, along with many outstanding scholars from different European countries. Almost all the founders of the Iasi medical school were SMN members and/or leaders, benefiting from what that Little Academy had founded in national première: the Library (1830), the Museum (1834), the Botanical Garden (1856), all of public use. Against the background of emulation that the introduction of the Organic Law (1832) and the 1848 Revolution in Moldavia aroused, things finally started moving in this forgotten corner of the world, also due to the particularly rich scientific and cultural life SMN generated. Concurrently, this context facilitated the founding of the Michaelian Academy (1835), the University (1860) and finally, the Faculty of Medicine (1879). Iacob Cihac, the author, in 1837, of the first treatise on natural history published in Romanian at the *Albina Institute* in Iasi, delivered a Natural History course at the Michaelian Academy. Dreaming of a Faculty of Medicine, in his speech at the Congress of the Naturalists in Freiburg in 1838, he already talked about the "the hope of opening a course of surgery in Iasi". His initiative was going to become true only after the "By-laws for the reorganization of public education.." were enacted in 1851, and especially after the 1859 Union (1).

1852-1864: The premise of higher medical education in Iasi. In the second half of the XIX-th century, the process of medicalization of the society and professionalization of health care institutions was intensified in the Romanian Countries as well. Following the promulgation in 1851, by the Prince Grigore Alexandru Ghica, SMN president in 1834, of the document "By-laws for the reorganization of public education in the Principality of Moldavia", the establishment within the Michaelian Academy of a Medicine Faculty was considered, however, a process that had to be prepared by taking intermediate steps, because "the means and the needs of the country do not allow the immediate creation of a Medi-

cal Faculty". Thus, in this Province, too, similarly to Muntenia, a School of Midwifery – *The Gregorian Institute*, in 1852, and two *Schools of Surgery*, one in 1859 and another in 1863, were established in the beginning. These preliminary structures, of secondary medical instruction, owed much to the efforts made by Dr. Gheorghe Cuciureanu (Cuciuran), protomedic of Moldavia during the years when the Gregorian Institute was designed, and by Dr. Anastasie Fătu, chief doctor, first manager and professor, animator and tutor of the Institute, and by Dr. Neculai Negură, a close friend of Cuza Vodă, and Dr. Ludovic Russ senior, for the surgical art, respectively. (2) In fact, Dr. Negură, a SMN member after 1862, submitted in October 1859 to the Ministry of Cults and Public Instruction the *Statutes of the Medical Faculty to be created in Iasi*. On December 3rd, 1859, he obtained, by a princely ordinance issued by Alexandru Ioan Cuza, the right to open a *School of Surgery* at the Michaelian Academy. Nicolae Negură's S*chool of Surgery and Medicine* (November 30th, 1859-1861) included the *Surgery Course* of this enthusiastic initiator, together with a *Pharmacy Course*, held by the pharmacist Iosif Mihailovici. Both courses were free and benevolently offered to the participants. The *Course of Surgery* dealt with notions of Anatomy and Dissection, Principles of Physiology and Surgical Pathology (3). It was the first medical university course in Romanian (4). If Anastasie Fătu already wrote in 1852 a *Handbook for the teaching of midwives* (*Buciumul Român* Printing House, Iasi) and a *Handbook of Popular Practical Medicine* (*D. Gheorghiu* Printing House, Iasi, 1871), Dr. Negură only translated Josef Hyrtl's Anatomy, failing to publish it. From August 1st, 1863, Dr. Ludovic Russ senior, guardian of the SMN Museum and chief physician of the Surgery Section of the "St. Spiridon" Hospital, gave a free *Course of topographic anatomy, upper and operative surgery, bandages and instruments*, teaching 14 chapters. For this purpose, the "St. Spiridon" Hospital of Iasi, where Russ was chief medical officer of the Surgical Department, placed its clinical basis at his disposal. (5)

Meanwhile, in 1859, the School of the Sanitary Company opened its doors in Iași, due to Dr. Iacob Cihac, deputy protomedic of Moldavia and chief physician of the Moldavian army, the one who printed that very year (1859), in the *Bee* Printing House [Tipografia Albinei] of the reputed scholar Gheorghe Asachi, a *Handbook for teaching the soldiers of the sanitary company of the Moldavian-Romanian army*, the first medical-military book in the Romanian literature (6).

The first attempts to start pharmaceutical education in Iași date back to 1830, accompanying the organization of the Society of Physicians and Naturalists of Iasi (SMN), among whose members there were numerous chemists, pharmacists and doctors interested in characterizing and valorizing the flora, fauna and mineral resources of Moldavia. The fruits of these efforts were noticed only in 1857, when the Pharmaceutical Guild, - founded in the Moldavian capital in 1854, - set up the *Preparatory School of Pharmacy*, under the administration of Dr. Ludovic Steege, protomedic of Moldavia and Cuza's ally, and of the pharmacist Theofil Stenner. Intended for the apprentices in the last year of pharmaceutical practice, the School operated within the "St. Spiridon" Establishment, where, according to the Guild Statutes, a laboratory and a library were created. The teaching language was Romanian. The School of the Guild of

Pharmacists functioned from 1857 to 1859. Graduates were pharmacy assistants (7).

All these were precursory forms of higher medical education, expected to be implemented in Iasi once the conditions,- involving the degree of training of both masters and candidates, as well as the optimal spaces and equipment for the didactic process-, would be met. Thus, Romania was aiming at attaining Western-European standards, not risking to laurel second-hand doctors. The founding of the University of Iași, the first high-ranking academic forum in the Romanian Principalities, officially celebrated on October 26th, 1860, illustrated such a favorable circumstance. If, sacrificing itself on the altar of National Union in 1859, Iași had lost the status of Capital of the small country of Moldavia, it had instead to "become the seat of science, the focal point of Romanian intelligence", a capital of the nation's progress and culture, as Mihail Kogălniceanu emphasized, and as Prince Cuza wanted it to be, too. Both Kogălniceanu and Cuza, leading unionists closely linked to the life and role of the SMN, were directly involved in the attempt to establish the Faculty of Medicine in Iasi. On September 16th, 1860, during the meeting of the School Council – gathering the General Inspector of Schools, the Department Director, the Director of Education and three professors elected among the secondary and primary school teachers -, it was Dr. Iacob Cihac to read the *Programme of the Faculty of Medicine* that the Ministry of Public Instruction had sent (Statement No.10976). The Council decided that for that year, the Faculty of Medicine should appoint only professors of Theoretical Anatomy, Surgery and Pharmacy. For other medicine-related sciences, medical students had to attend the Faculty of Philosophy, section I (of sciences). (8) However this approach found no echo. It was a mere mirage: the project had to wait for other 19 years.

December 1, 1879: From fiction to university reality. Predicted *de jure* to exist first within the Michaelian Academy, inaugurated in 1835, then within the University of Iasi, opened in 1860, the Faculty of Medicine began its activity *de facto* only on December 1st, 1879, when the Parliament of the Kingdom of Romania voted the budget and the syllabus of the institution. The decree no. 11389 of September 27th, 1879 was signed by the Minister of Public Instruction of that time, Dr. Nicolae Kretzulescu, eminent surgeon, politician and patriot. The Faculty of Iași was particularly urged to train the local youth, who by their knowledge, dedication and devotion, had to face the severe medico-social problems that had always plagued the country.

The first professors of the Faculty of Medicine were substitutes, and in Iași the title of doctor of medicine and surgery could only be granted since 1887. The Ministry had accepted Dr. Leon Logothety Sculy, a skilled surgeon specialized in Paris, and a student of Ludovic Russ sr in Iasi, to teach Anatomy and Histology, whereas pharmacist Samuel Konya, doctor of the University of Vienna, a valuable SMN representative, a former student of the Michaelian Academy and of the School of the Iasi Guild of Pharmacists, was responsible for teaching Chemistry. (9) The Faculty was assigned two rooms in the University headquarters, which itself had an inadequate premises. At that moment (1875-1880), its rector was Prof. Dr. Petru Suciu, a lawyer. Without previous festivi-

ties, the courses started on December 1st, 1879. The inaugural lecture, that of Anatomy delivered by Prof. Dr. Sculy at only 26 years of age, in his position as the first dean (1879-1881) of the Faculty, addressed a number of only 14 students, registered in the Ist year. In the meantime, Dr. Konya was replaced by another famous SMN member, chemist Petru Poni, scientist of international notoriety, professor at the Faculty of Sciences of the Iasi University and academician. The newspapers of that time welcomed the event with great restraint, speaking of an "imaginary Faculty" (10).

If the SMN contributed to the establishment and consolidation of the Medical Faculty, the Faculty gave soon consistency to the first scientific periodical that the pioneering academic association ever edited: *The SMN Bulletin*, published in a bilingual edition - Romanian and French - on January 1st, 1887. It followed the SMN first short lived editorial attempts - *The Guide to Health and Economy. Leaflet for the Romanian people* (1844-1845) and *Leaflet of the Society of Physicians and Naturalists of the Principality of Moldavia* (1851-1853), publications disseminating sanitary education and domestic medicine. In 1924, *The SMN Bulletin* was renamed *The Medical-Surgical Journal. Bulletin of the Society of Physicians and Naturalists in Iași*, a title that it retained until today. *The Medical-Surgical Journal* was also the periodical of the Faculty of Medicine and of the Spiridonia Hospitals, as well as of the University of Iasi. But it was only when the university medical-pharmaceutical activity intensified that the SMN periodical could gain real value, surviving hard times. Now it is printed in English and is included in the Web of Science Core Collection - Clarivate Analytics. In 2019, this journal of the medical academic staff reached its 123rd volume.

Set up with huge difficulty 140 years ago, the Faculty of Medicine, cornerstone of higher medical education in Iasi, proved to be resilient and fertile, supporting the dynamic evolution of an ever more flourishing and fruitful *alma mater*.

The Tree of Medical Education in Iași at the present time

As a consequence of the Education Reform in 1948, the Faculty of Medicine separated from the University of Iasi and was transformed into an Institute of Medicine and Pharmacy, enjoying a much larger autonomy. Later, in 1991, the Institute turned into a University of Medicine and Pharmacy, placed under Professor Grigore T. Popa's protective genius.

The history of the three other Faculties the University includes today, besides the classic **Faculty of Medicine** – its nucleus, is also an interesting one.

The Faculty of Pharmacy. After 1879, the year when the Faculty of Medicine began its activity, the Professor Council insisted on the establishment of a *Pharmacy School*, too, starting even on October 1st, 1898 (11). The request was not accepted, although the pharmacists were extremely sought after, and their important role in the life of the society justified the existence of a specialized higher school. The attempt was repeated in 1908, when in the business meeting of February 29th, 1908, the University Senate unanimously admitted the establishment of a *School of Pharmacy* belonging to the Faculty of Medicine (12). The same failure occurred. Only between 1913-1934, a *Pharmacy Education Section*

could be attached to the Iași Faculty of Medicine. The section reopened between 1948-1952, whereas in 1965 it was resized as a Faculty of Pharmacy, which has brilliantly developed since then on. Prof. Dr. Gheorghe Popovici was its first dean.

The Faculty of Dental Medicine. Dental education could hardly find its way in Iași. Responding to the needs of medical-health care, immediately in the post-war period, in 1945, Dentistry was introduced as a compulsory study subject, in the form of a Conference / Chair / Section of the Faculty of Medicine. It was the first time that the medical students of Iasi learned the concepts of dental medicine. If between 1945-1946, it was a section of the Faculty of Medicine, in 1948 the Faculty of Stomatology was created, which resisted until 1952, when the difficulties related to specialized chairs forced the cessation of its activity. It was coordinated by a skilled surgeon, Prof. Dr. Nicolae Dutescu. On october 25th, 1965, the Faculty of Stomatology was reestablished, and after 1990, it was renamed the Faculty of Dental Medicine. Soon it imposed itself as a meritorious institution, which to this day has plenary contributed to the renown of university medical education in Iasi. Its first dean was Prof. Dr. Gheorghe Timoșca, a specialist in oro-maxillo-facial surgery (13).

The Faculty of Medical Bioengineering. Continuing the avant-garde initiatives of the Iasi medical school, along with SMN traditions that combined art, technique and science at all times, in 1994, within "Grigore T. Popa" University of Medicine and Pharmacy, the Faculty of Medical Bioengineering could be inaugurated, due to its first dean's efforts, Prof. dr. Florin Topoliceanu. Later the Specialization of *Balneophysiokinetotherapy and Rehabilitation*, with bachelor's studies, was equally added to its initial structure.

Visibility. Furthermore, the international recognition -that SMN and the Faculty of Medicine have always promoted- is currently completed, substantially improved and amplified by the study programmes in English and French languages and the Erasmus programmes that the University of Medicine and Pharmacy carries out, by the *Medical-Surgical Journal*, and many other international publications and collaboration agreements the University teaching staff is promoting.

On September 5th, 2017, "Grigore T. Popa" University of Medicine and Pharmacy became the first institution of higher medical education in Romania listed in the *Times Higher Education World University Rankings*. As to the international academic visibility indicator, it ranked first in Romania and among the first 500 universities in the world (428th place).Thus the successful beginnings have constantly represented prerequisites for future achievements, synchronizing "Grigore T. Popa" University to continually updated professional priorities. Now its overall academic activity undoubtedly illustrates the main development directions of globalized contemporary medicine.

On the other hand, due to the flourishing Faculties within the University of Medicine and Pharmacy, the SMN sections and activities have significantly grown. Moreover, while most of the SMN presidents have always been professors of the Faculty of Medicine, in recent years, rectors of the University of Medicine and Pharmacy have been elected SMN presidents, increasing the chances of coherent programmes, both institutions can undertake in partnership.

Here are the fruits borne over time by the fragile branches once visionary forerunners had sprouted with confidence and hope!

To them grateful thoughts are unfailingly addressed on each succeeding anniversary of those days.

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DUREREA TORACICĂ ŞI REFLUXUL GASTROESOFAGIAN

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NONCARDIAC CHEST PAIN AND GASTROESOPHAGEAL REFLUX DISEASE (Abstract): Gastroesophageal reflux disease (GERD) may manifest typically with heartburn and regurgitation or may have atypical manifestations as laryngitis, asthma, chronic cough or noncardiac chest pain (NCCP). While typical GERD is easy to be recognized, the atypical extraesophageal symptoms of the disease make the diagnosis difficult because most patients do not have heartburn or regurgitation. Most common atypical manifestations include ear, nose and throat (ENT), pulmonary or cardiac symptoms. GERD should be included in the differential diagnosis of patients with atypical symptoms, especially when alternative diagnoses are excluded. NCCP is defined as recurring angina-like substernal chest pain of noncardiac origin. We present the most recent epidemiologic data, path physiology, diagnosis and treatment of NCCP. The major causes of NCCP are GERD and esophageal dysmotility. By far, GERD has been demonstrated to be the most frequent source of NCCP. After a complete cardiac evaluation, the patient with NCCP will be referred to a gastroenterologist. All recent studies suggest the use of PPI test as the first diagnostic tool in patients with NCCP. The invasive diagnostic tests (especially, the 24-hour pH monitoring and esophageal manometry) are used only in those cases who do not respond to PPI therapy. Patients with GERD-related NCCP require long-term treatment with a PPI. **Key words**: GASTRO-ESOPHAGEAL REFLUX DISEASE, NONCARDIAC CHEST PAIN

CUT-OFF VALUES OF ANTHROPOMETRIC MEASUREMENTS, HANDGRIP STRENGTH, PHYSICAL ACTIVITY AND GERIATRIC SCORES FOR THE MALNUTRITION RISK AMONG OLDER PATIENTS WITH CHRONIC KIDNEY DISEASE

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CUT-OFF VALUES OF ANTHROPOMETRIC MEASUREMENTS, HANDGRIP STRENGTH, PHYSICAL ACTIVITY AND GERIATRIC SCORES FOR THE MALNUTRITION RISK AMONG OLDER PATIENTS WITH CHRONIC KIDNEY DISEASE (Abstract): **The aim** of the study is to describe the prevalence of malnutrition and to determine the prognostic role of geriatric assessments and anthropometric measurements in patients with malnutrition. **Materials and methods**: Eighty patients with chronic kidney disease hospitalized in a geriatric clinic from north-eastern Romania were included. A geriatric evaluation was performed [Mini Nutritional Assessment-MNA, Geriatric Depression Scale-GDS, daily activities (Activities of Daily Living-ADL, Instrumental Activities of Daily Living-IADL), cognitive impairment scores (Mini-Mental State Examination-MMSE) and frailty (Fried criteria)]. We also evaluated muscle strength and calf/arm circumference. Using the area under the curve (ROC) analysis, cognitive status, depression, frailty, physical activity, handgrip strength and anthropometric measurements were evaluated as possible predictors for malnutrition. **Results**: MNA reported malnutrition in 8.75% of cases and 58.75% were at risk of malnutrition. ROC curve analysis showed that the arm and calf circumference predict nutritional status with very good accuracy, while muscle strength, GDS and Fried criteria have also good accuracy (specificity, sensitivity). **Conclusions**: Our research has found a strong correlation between nutritional status, anthropometric measurements, and handgrip strength. Malnutrition should be carefully evaluated in frail or depressed older patients with CKD. **Keywords:** MALNUTRITION, GERIATRIC EVALUATION, CHRONIC KIDNEY DISEASE, FRAILTY.

Chronic diseases and aging are major global public health problems correlated with increased life expectancy. One of the common disorders that appears with aging is chronic kidney disease (CKD). After the age of 30, glomerular filtration rate decreases progressively, Australian Diabetes, Obesity and Lifestyle Study (AusDiab) suggesting

that over one-third of people over 65 years-old have an estimated glomerular filtration rate below 60 mL/min/1.73 m² (1, 2). This population group is often at risk of frailty and malnutrition.

Food intake decreases with age. Older people tend to lose hunger sensation, eat smaller meals, have fewer snacks between meals and also eat slower (3). Most individuals lose weight, in varying proportions, as they age (4). Loss of muscle tissue is associated with decreased strength, bone mass and cognitive function, anemia, immune system dysfunction, slower recovery after surgery and, consequently, an increase in both morbidity and mortality (5). In addition to "aging anorexia", there are a multitude of physical, social, cultural, environmental, psychological and financial factors for an inadequate diet (6-8).

Often considered to be a late complication in CKD, malnutrition occurs from the early stages of kidney disease (9,10), with direct implications in the evolution of the disease.

The prevalence of malnutrition among older patients with CKD differs depending on the methods used (anthropometric parameters - arm circumference, tricipital fold, body mass index - biochemical or nutritional status assessment questionnaires), suggesting a wide variety of tests and methodology applied, with multiple predetermined cut-offs (11).

The problem of malnutrition is even more complex due to the heterogeneity of the geriatric population, with various symptoms, disorders and treatments that affect their nutritional status (12-14).

The ultimate goal is to improve the quality of life and ensure successful aging. In order to achieve this goal, the risk and predisposing factors leading to the development of malnutrition have to be recognized. As a result, both clinicians and researchers should explore the possible associations between nutritional status and various predictors and outcomes, to determine clear cut-offs.

In Romania, to date, there has been no estimation of the prevalence and even less of the incidence of malnutrition in older patients with CKD, making it more difficult to maintain a correct record of those who need an early therapeutic approach.

Our research aimed to study the interplay between nutritional status and the main components of the geriatric evaluation (cognitive status, depression, functional status, and frailty) and to establish the prognostic role of several assessments for patient's malnutrition status.

MATERIALS AND METHODS

Participants

The retrospective study included 80 patients (28 men and 52 women) admitted to a geriatric clinic from Iasi, Romania. Patients younger than 65 years old, with severe comorbidities (neoplasia, recent bone fractures), severe cognitive impairment, without CKD, or those who refused to participate in the study were excluded. In the remaining 80 patients, several measurements and scores were performed.

Demographic data and physical performance

Demographic data were obtained from the case history and the observation sheet. For the anthropometric measurements (arm/calf circumference) we used standard techniques and an electronic dynamometer was used to evaluate muscle strength. Physical function was assessed through the Short Physical Performance Battery

(SPPB) that combines the results of the gait speed, chair stand and balance tests. The scores range from 0 (worst performance) to 12 (best performance). The test has been shown to have predictive value showing the risk for disability, nursing home admission and mortality. The cut-off point we used for the statistical analysis was < 8 points.

Geriatric evaluation

Mini-Nutritional Assessment (MNA) was used as a screening tool to evaluate the nutritional status. Patients with a score between 24-30 points were considered well-nourished, those between 17-23.5 points were at risk of malnutrition and the older patients with a score bellow 17 points were considered malnourished. We also evaluated the cognitive status (by the Mini-Mental State Examination – MMSE) and the frailty syndrome (Fried criteria). The capacity of performing the activities of daily living was assessed through Activities of Daily Living (ADL) and Instrumental Activities of Daily Living (IADL) scores.

All the patients completed the questionnaires individually, to avoid the subjective factor. Signed informed consent was obtained from all the study participants before data collection.

Statistical analyses

Means, standard deviations and percentages were used for descriptive analysis. Comparative analysis was performed using Independent Samples t-test and One-way ANOVA. For correlational analyses we used Pearson and Spearman correlations. Cut-off value was determined between each anthropometrical index and geriatric evaluation parameters by the receiver operating characteristic (ROC) curve, which was quantified by the area under ROC curve (AUC). The Youden's index (sensitivity+specificity-1) was used to determine the optimal cut-off point of each index. P-value < 0.05 was considered to be statistically significant. Statistical analysis was performed using *SPSS Statistics version 21.0.*

RESULTS

Demographic data

The mean age was 76.74±5.93 years, higher among women (77.79±5.64 years versus 74.79±6.05 years, p=0,03). The prevalence of malnutrition was 8.75% (n=7) and 58.75% (n=47) were at risk. Women had a higher prevalence of malnutrition than men (tab. I).

Hypertension was the most prevalent comorbidity in our study group, followed by cardiac failure and diabetes mellitus. No statistical difference in demographic characteristics could be found between stages of malnutrition, except age (p=0.007).

Anthropometric measurements and physical activity

Patients with malnutrition had a smaller arm circumference (21.14 cm versus 28.19 cm in well-nourished patients, p=0.002) and a smaller calf circumference (29.29 cm versus 36.23 cm in well-nourished patients, p<0.001). Muscle strength was also decreased (tab. II).

Geriatric evaluation

A geriatric evaluation revealed that 36.25% had moderate cognitive impairment. All patients needed help (partially/totally) to carry out the activities of daily living. IADL was significantly lower in patients with malnutrition (p=0.01). Also, malnourished patients were more depressed and frailer (women more than men) (tab. III).

TABLE I

Demographic characteristics of the study group

	Well nourished (24-30) n=26	At risk (17-23.5) n=47	Malnutrition (<17) n=7	p
All patients (n[%])	26 (32.5%)	47 (58.75%)	7 (8.75%)	0.15
Female (n[%])	14 (53.84%)	32 (68.08%)	6 (85.71%)	
Male (n[%])	12 (46.15%)	15 (31.92%)	1(14.29%)	
Age	73.96±4.33	77.89±6.46	79.29±3.63	0.007
Marital Status	17 (65.38%)	27 (57.44%)	5 (71.42%)	0.68
Family support	23 (88.46%)	42 (89.36%)	7 (100%)	0.65
Drugs*	2.23±0.71	1.89±0.78	2.29±0.95	0.07
Hypertension (n[%])	20 (76.92%)	37 (78.72%)	5 (71.42%)	0.86
Diabetes (n[%])	10 (38.46%)	16 (34.04%)	3 (42.85%)	0.87
Cardiac failure (n[%])	17 (65.38%)	32 (68.08%)	3 (42.85%)	0.91
Comorbidities	1.81±0.74	1.83±0.67	1.43±0.53	0.90

*number of drugs from chronic prescription

TABLE II

Nutritional status, anthropometric measures, and physical activity

MNA				
	Well (24-30) n=26	At risk (17-23.5) n=47	Malnutrition (<17) n=7	p
Arm circumference (cm)	28.19±5.06	24.57±4.29	21.14±1.77	<0.001
Calf circumference (cm)	36.23±4.18	32.44±4.14	29.29±2.43	<0.001
Muscle strength (kg)	21.37±7.94	18.84±7.67	12.97±6.76	0.03
Physical activity score (SPPB)	6.35±2.13	5.74±2.89	4.29±2.56	0.22

TABLE III

Nutritional status and geriatric evaluation

MNA				
	Well (24-30) n=26	At risk (17-23.5) n=47	Malnutrition (<17) n=7	p
MMSE	25.42±3.58	24.36±4.21	24,.7±4,429	0.45
ADL	5.27±1.00	5.30±0.85	4.43±0,787	0.33
IADL	7.12±1.47	6.19±1.84	4.71±2,690	0.01
GDS	5.54±4.06	7.47±3.38	8.71±3,684	0.03
FRIED	3.38±1.38	3.70±1.14	4.71±0,756	0.03

ROC curve

Anthropometric measurements, geriatric evaluation scores and physical performance were explored as possible predictors for malnutrition. Furthermore, to determine the cut-off point with optimal specificity and

sensitivity for each tool, we performed cut-off analysis (fig. 1, tab. IV).

Only arm (AUC = 0.832) and calf circumference (AUC = 0.842) had very good accuracy in identifying patients with malnutrition. A value < 23 points in the arm circumference offers 100% sensitivity and 61.64% specificity, while a value below 32 at the calf circumference offers 100% sensitivity and 60.27% specificity.

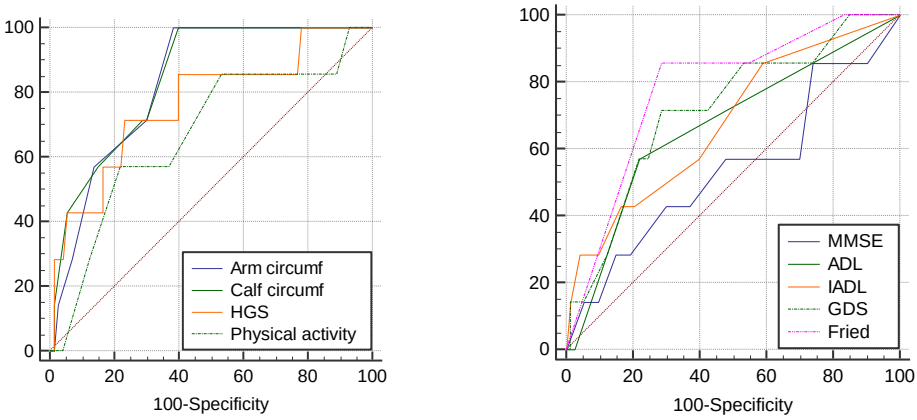

Fig. 1. ROC curves according to different cut-off values for arm circumference, calf circumference, handgrip strength and physical activity score.

Muscle strength, GDS and frailty score had also a good accuracy in identifying patients with malnutrition. Physical activity, cognitive status and the ability to perform the activities of daily living had no predictive power for malnutrition in our study group.

Linear correlations between geriatric evaluation scores and different demographics and anthropometric parameters are presented in Table V. Univariate correlations showed that only age and calf circumference have a significant correlation with MMSE ($p < 0.05$). MNA was significantly correlated with ADL, IADL and GDS.

TABLE IV

The area under the curve (AUC) and cut-off analysis

	AUC	95% CI	Cut-off (sensitivity /specificity)
Arm circumference	0.832*	0.732-0.906	<23 (100%, 61.64%)
Calf circumference	0.842*	0.744-0.914	<32 (100%, 60.27%)
Muscle strength	0.766**	0.658-0.854	≤13.5 (71.43%, 76.71%)
SPPB	0.668	0.554-0.770	≤3 (57.14%, 78.08%)
MMSE	0.541	0.426-0.653	≤20 (28.57%, 84.93%)
ADL	0.668	0.554-0.770	≤4 (57.14%, 78.08%)
IADL	0.679	0.565-0.779	≤7 (85.71%, 41.10%)
GDS	0.716**	0.604-0.811	>8 (71.43%, 71.23%)
FRIED	0.778**	0.671-0.863	>4 (85.7%, 71.2%)

* – very good accuracy ** – good accuracy

TABLE V
Linear correlations between anthropometric, malnutrition scores and geriatric assessment

	MMSE		ADL		IADL		GDS	
	r	p	r	p	r	p	r	p
Age	-0.55*	0.029	-0.051	0.662	-0.107	0.366	0.068	0.563
Arm circumference	0.059	0.616	-0.025	0.832	-0.015	0.896	0.186	0.114
Calf circumference	-0.230*	0.049	-0.020	0,865	0.023	0.845	-0.103	0.383
Muscle strength	0.190	0.105	0.083	0.481	0.126	0.285	0.064	0.587
SPPB	-0.054	0.649	0.062	0.601	-0.043	0.715	-0.095	0.420
MNA	0.218	0.062	0.114	0.334	0.269*	0.021	-0.267*	0.022
Frailty	0.091	0.441	-0.106	0.370	-0.058	0.622	0.152	0.197

* values indicate significant correlation ($p<0.05$)

DISCUSSION

The assessment of nutritional status remains a challenge in medical practice. Although an essential part in the complete evaluation of older patients, it is often overlooked, clinicians focusing on other aspects. Malnutrition as an independent factor in the development of other disabilities, has a negative impact on the general health status and quality of life (15).

In our study, malnutrition was evaluated through the Mini Nutritional Assessment. 8.75% of the patients were malnourished, a lower number compared to other studies that showed different percentages in older patients with CKD, between 28-65%, depending on the criteria used for the diagnosis. Most of them included end-stage renal disease (ESRD) patients who were already on dialysis and only a few pre-dialysis patients (16, 17). However, 58.75% of our patients were considered to be "at risk", a very important aspect of our findings. Patients at risk of malnutrition have in most cases normal weight or are overweight, which is why they are often overlooked, and nutritional tests are not performed. The risk of developing malnutrition is high, with a significant impact (lower quality of life, increased risk of hospital admissions, higher mortality rates). Furthermore, this is the reversible stage of the disease, in which early intervention can change the overcome.

Malnutrition correlated with age, anthropometric measurements and muscle strength. These findings were similar to previous studies (18) suggesting that a decrease in anthropometric measurements is associated with increased malnutrition. Patients with poor nutritional status had lower muscle strength. Recent studies regarded handgrip strength as an independent predictor and the one of the earliest markers of malnutrition (19). Although all the patients included in the study were right-handed (dominant hands were significantly stronger compared to nondominant hands) the results were still lower than those of other studies (20). Possible causes may be the financial difficulties, diets rich in carbohydrates and fat and low in vitamins and proteins and sedentarism. Studies show that malnutrition and physical inactivity may progress together and are independent predictors of mortality (21).

The geriatric evaluation revealed that malnourished patients were frailer, more

depressed and performed with difficulty or were unable to perform without assistance the IADL. These findings underline the importance of the complete assessment in older patients, as normal psychological status is essential for most activities in daily living (planning and preparing meals, food intake, and physical exercise). Other studies also observed an association between malnutrition and depression, but the relationship is complex, and it can be questioned whether depression is the cause or the consequence (22).

Malnutrition and frailty, two related syndromes, are frequently found in older patients with CKD (23). Many frailty scores include weight loss and also weight loss is considered to be a modifiable factor in frailty (24).

Regarding this objective of our study, arm and calf circumference were found to have the best power of prediction on malnutrition: AUC of 0.832, respectively 0.842, while muscle strength, depression and frailty had good accuracy also.

The consequences of poor nutritional status include low quality of life, depression, erythropoietin low-responsiveness, increased susceptibility to infections and aggravation of other chronic disorders (25). All these consequences sustain the importance of geriatric evaluation as part of managing older patients with CKD, even in the early stages of the disease.

CONCLUSIONS

Routine screening of nutritional status in older patients with CKD enables early detection and timely intervention. However, some other parameters/tests draw attention to a possible case of malnutrition (anthropometric measurements, degree of fragility, muscle strength, depression). Besides identifying patients with nutritional problems, effective strategies for the prevention and treatment of malnutrition in the increasing number of old patients at risk are needed.

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THE RELEVANCE OF EUROPEAN LEUKEMIA NET RECOMMENDATIONS IN ACUTE MYELOID LEUKEMIA: A ONE CENTER THREE-YEAR EXPERIENCE

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THE RELEVANCE OF EUROPEAN LEUKEMIA NET RECOMMENDATIONS IN ACUTE MYELOID LEUKEMIA: A ONE CENTER THREE-YEAR EXPERIENCE (Abstract). **Aim:** The cytogenetic and molecular features of leukemia cells are chief predictors of survival in acute myeloid leukemia (AML). However, treatment outcomes in AML differ between low-, medium and high-income countries. We thus aimed to investigate the relevance of the European Leukemia Net (ELN) prognostic system in Romanian AML patients. **Material and methods:** We investigated the clinical significance of the ELN recommendations in 130 newly diagnosed AML patients treated at the Iasi Regional Oncology Institute. **Results:** Favorable, intermediate and adverse risk ELN classes had a similar distribution and retained their prognostic relevance (median overall survival of 18, 11 and 2 months respectively at a median follow-up duration of 18 months) when compared with literature data. However, in both the chemotherapy eligible and ineligible patients a higher prevalence of comorbid conditions and treatment related mortality could be identified. **Conclusions:** The management of Romanian AML patients poses significant challenges mainly due to higher incidence of comorbid conditions and infections. However, growing access to the allogeneic stem cell transplant procedure in our country will significantly improve the outcome of these patients. **Keywords:** ACUTE MYELOID LEUKEMIA, EUROPEAN LEUKEMIA NET, CYTOGENETICS, CHEMOTHERAPY.

Acute myeloid leukemia (AML) is an aggressive myeloid neoplasm characterized by extensive bone marrow (BM) infiltration with immature myeloid precursors and subsequent BM failure (1). AML diagnosis requires BM aspirate and thorough morphological, immunophenotypic, cytogenetic and molecular analysis of tumor cells (2). Furthermore, AML cases are classified in different subgroups as proposed by the 2016 revision of the World Health Organization classification of myeloid neoplasms

(3). Since AML therapy responses are highly heterogenous, ranging from deep and sustained molecular remissions in acute promyelocytic leukemia (APL) to primary refractory AML, the prognostic evaluation of patients is highly important in order to determine eligibility for more intensive therapeutic interventions, such as allogeneic stem cell transplantation (4-6). When evaluating the prognosis of AML patients, clinicians must take various factors into account, such as the antecedent hematologic disorders, radiation or cytotoxic drug exposure, hyperleukocytosis, therapy response and minimal residual disease, but also patient age, comorbidities and their performance status. However, the cytogenetic and molecular features of AML blasts are currently the strongest predictors for therapy response and long-term survival (7-10). The impact of cytogenetic and molecular anomalies is highlighted by the 2017 update of the European Leukemia Net (ELN) prognostic classification that defines three prognostic subgroups (favorable, intermediate and adverse risk) with different complete remission (CR) rates, progression and disease-free survival (11). Noteworthy, the ELN risk stratification does not take into account patient-related factors, such as age or comorbidities (12). Furthermore, recent studies revealed that prognosis in AML patients differs from high- to middle and low-income countries, probably related to inherent socio-economic issues (13, 14).

The relevance of the ELN classification in AML patients from Romania has not yet been systematically investigated. However, recently, a national program that ensures widespread access to the immunophenotyping, cytogenetic and molecular tests necessary to formulate the ELN prognostic prediction has been successfully implemented. In a recent report from the World Health Organization (15), Romanian patients have been shown to have higher incidences of cardiovascular diseases, chronic obstructive lung disease, asthma, tuberculosis and depression, all of which are known to have a negative impact on AML patient outcome (16). Furthermore, it is to be expected that the other factors reported by the WHO, such as the inequalities in patient education, the occasionally difficult access to public health services, especially in patients from rural areas, the deficiencies in the primary care system and the lowest levels of public health expenditure among the European Union member countries will likely influence the prognosis of AML in these patients (15).

The aim of our study is to evaluate the impact of the 2017 ELN classification on the prognosis of AML patients treated at the Iași Regional Oncology Institute, in North-Eastern Romania.

MATERIAL AND METHODS

A number of 130 patients diagnosed with *de novo* AML between 2017 and 2019 at the Hematology Department of the Iași Regional Oncology Institute, Romania were included in this retrospective study. AML diagnosis was established based on the 2016 WHO classification of myeloid neoplasms and patients were risk stratified according to the 2017 ELN recommendations. The cases with APL were excluded. This study was approved by the local research ethics committee. Fresh BM samples were collected at diagnosis. Immunophenotypic diagnostic of AML was established according to Euro Flow standards (17). Cytogenetic G-banding

analysis was performed using standard methods. We further examined 4 chimeric gene transcripts (BCR-ABL1 p210, PML-RARA, RUNX1-RUNX1T1, CBFB-MYH11) by reverse transcriptase-mediated quantitative PCR. Patients were tested for NPM1 and FLT3-ITD mutations. We screened for chromosome 11 rearrangements using fluorescence *in situ* hybridization techniques. In case of a dry tap, a BM biopsy and immunohistochemistry tests were performed, and cytogenetic tests were done on peripheral blood samples. Patients <65 years were treated according to the national protocol with "7+3" therapy (Cytarabine 150 mg/m^2 for 7 days in continuous i.v. administration and Idarubicine 12 mg/m^2 for 3 days). Patients > 65 years were treated with "7+3" therapy in selected cases (depending largely on the absence of comorbid conditions and an ECOG (Eastern Cooperative Oncology Group) performance status less than 2), Decitabine, low-dose cytarabine (ARA-C) or best supportive care (BSC). Post-induction response was evaluated by BM morphologic examination and flow cytometry on day 21-28 following "7+3" chemotherapy.

The statistical analysis was performed using the *IBM SPSS Statistics 21.0 Software*. The Chi square and Mann Whitney tests were used to analyze the associations between different variables. A p value <0.05 was considered to be statistically significant. Overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan Meier method. The log rank test was used for comparisons of Kaplan-Meier curves. OS was defined as the time from diagnosis to death from any cause. Early death was considered when occurring in the first 28 days following diagnosis. For the patients who achieved complete remission, DFS was defined as the time from CR to relapse or death by any cause.

RESULTS

We studied 130 patients with newly diagnosed AML. Baseline clinical and laboratory data of the patients with respect to the 2017 ELN risk classification are summarized in table I. The mean age at diagnosis was 60 years. The median follow-up duration was 18 months. 87.8% of patients presented with *de novo* AML, while 9.1% and 3.1% had secondary and, respectively, therapy-related AML. According to the WHO 2016 classification, patient distribution was the following: AML with recurrent genetic abnormalities (21.2%), therapy-related AML (3.1%), AML associated with myelodysplasia-related changes (9.1%) and AML not otherwise specified (NOS) (66.7%). 28.4% of cases presented with an ECOG (Eastern Cooperative Oncology Group) score greater than 2 due to extensive comorbidities or life-threatening complications at diagnosis.

Cytogenetic data was available in 69.2% of patients. A normal karyotype was detected in 46.7% of cases. The distribution of the cytogenetic anomalies is shown in figure 1A. 41.5% of patients had detectable molecular anomalies that are detailed in figure 1B. In 25.4% of cases we found a FLT3-ITD mutation, which co-existed with a NPM1 mutation in 21.5% of cases. An isolated NPM1 mutation was found in 30% of patients.

Following the analysis of cytogenetic and molecular data, cases were classified according to the 2 017 ELN risk classification, as follows: favorable 20.8%, intermediate 51.5%, adverse 27.7% (fig. 1C).

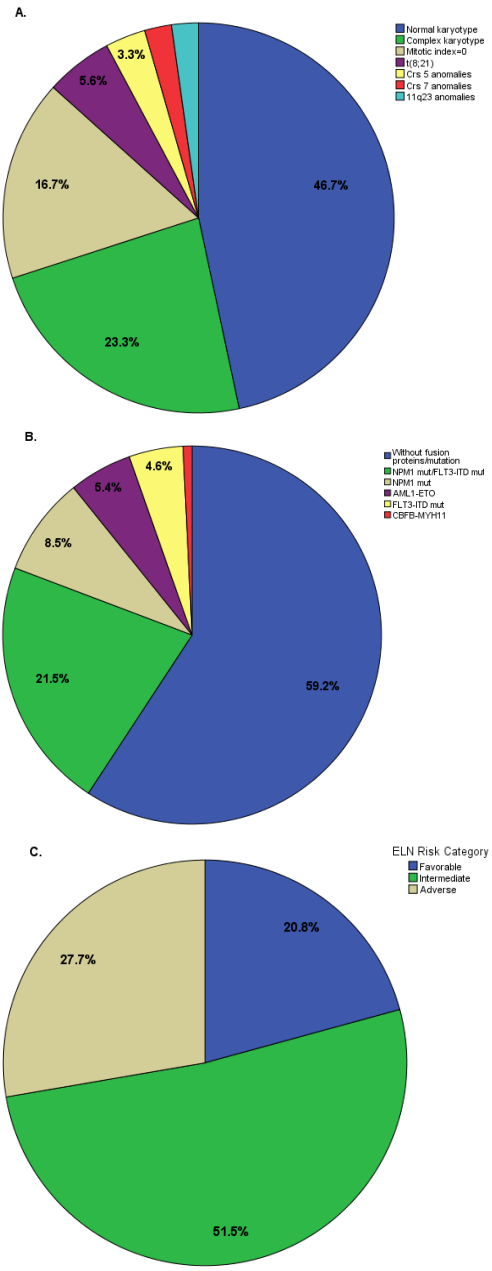

Fig. 1. A. Distribution of cytogenetic characteristics in AML patients
B. Molecular findings in AML patients
C. Distribution of ELN risk subgroups.

66.2% of patients were deemed eligible for intensive ("7+3") chemotherapy. Out of these, 81.4% completed induction chemotherapy and 58.8% achieved CR. The median OS for all patients was 12 months and the median DFS was 9 months. On ELN risk category analysis, the median OS for favorable, intermediate and adverse risk subgroups was 18 (95% CI: 12.5-23.4 months), 11 (95% CI: 7.1-14.8 months) and 2 months (95% CI: 0.8-3.1 months), respectively (fig. 2). Favorable and intermediate risk patients had DFS of 73.4% and 57.2%, respectively, while patients within the adverse category displayed the highest risk of early relapse, with a relapse rate of 75% within 3 months after achieving CR.

41.2% of chemotherapy-eligible patients had refractory disease and a median OS of 4 months. 36% of chemotherapy-eligible patients had an indication for allogeneic bone marrow transplant (ASCT). However, this procedure could be performed in only 8.1% of our patients. All patients who underwent ASCT remained disease-free at 24 months.

Out of the patients deemed ineligible for standard chemotherapy, 27.3% followed Decitabine therapy, 27.3% low dose-cytarabine (LDAC) and 45.4% received exclusively palliative care. 16.7% of the patients receiving Decitabine achieved a complete hematologic response. None of the patients treated with LDAC responded to therapy.

Furthermore, we investigated the presence of correlations between the main clinical and laboratory characteristics of patients and their ELN risk, chemotherapy completion and therapy response.

Extramedullary involvement was more frequent in the *de novo* AML-NOS subtype (p=0.008) and splenomegaly was more

frequent in patients with M7 phenotype (66.6%, p=0.009). An M5 phenotype was more frequently associated with hyperleukocytosis (33%, p=0.005) (tab. I).

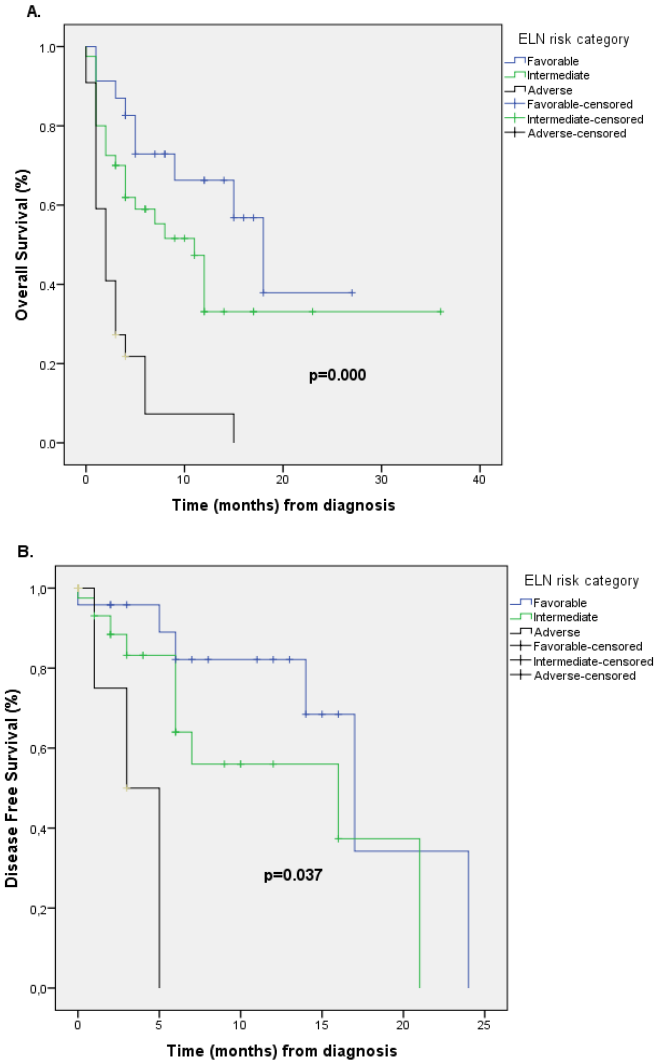

Fig. 2. Probability of overall survival (A) and disease-free survival (B) in AML patients according to the ELN prognostic system.

70.8% of patients had various associated comorbid conditions, of which the majority were represented by cardiovascular (49.2%) and metabolic (13.8%) comorbidities. 28.5% of patients had more than two associated comorbid conditions. The presence of comorbidities had a negative impact on the therapeutic response. Thus, 67.5% of patients achieving CR had comorbidities, while in patients with refractory disease their prevalence was much higher, reaching 82.9%. Moreover, the presence of infection

during or following induction chemotherapy had a detrimental effect on patient outcome. Thus, the presence of infection, irrespectively of the etiology, was associated with a 58.7% probability of refractory disease (p=0.022, Chi-Square Test). Treatment related mortality (TRM) in chemotherapy eligible patients was 16.3%. In older patients treated with Decitabine or LDAC the TRM reached 46.2%. Deaths were mainly due to infection (63.2%) or hemorrhagic complications (13.6%) (tab. I).

TABLE I

Demographic, clinical, biological, cytogenetic, molecular and therapy response data of AML patients

Patient characteristics	
Sex, n (%)	
Male	69 (53.9%)
Female	61 (46.9%)
Age	
Median	63
<65/ >65yrs., n (%)	71 (54.6%)/ 59 (45.4%)
Extramedullary disease n (%)	41 (31.8%)
Splenomegaly	15 (11.4%)
Hepatomegaly	27 (20.8%)
Adenopathies	20 (15.4%)
Gum hypertrophy	8 (6.2%)
CNS involvement	1 (0.8%)
WBC at diagnosis	
Median	9100/µL
<100000/100000, n (%)	19 (14.6%)/ 111 (85.4%)
BM blasts (%)	
Median	42%
Hb level (median/range)	8.3 g/dL/ 3.5-12 g/dL
Platelet level (median/range)	35000/µL/ 1000-1138000/µL
AML type, n (%)	
De novo	110 (84.6%)
Therapy-related	5 (3.8%)
Secondary AML	15 (11.5%)
WHO 2008 classification, n (%)	
AML with recurrent genetic abnormalities	28 (21.5%)
AML with multilineage dysplasia	12 (9.2%)
t-AML	6 (4.6%)
AML-NOS	84 (64.6%)
AML-NOS type, n (%)	
M0	4 (4.5%)
M1	17 (17%)
M2	13(14.8%)
M4	25 (28.4%)
M5	21 (21%)

Patient characteristics	
M7	3 (3.4%)
Acute basophilic leukemia	2 (2.3%)
ELN 2010 cytogenetic risk, n (%)	
Favorable	27 (20.8%)
NPM1 mut/FLT3 WT	11 (8.5%)
t(8;21)	4 (3.1%)
Intermediate	67 (51.5%)
NPM1 mut/FLT3 mut	28 (21.5%)
NPM 1 WT/FLT3 mut	6 (4.6%)
NPM1 WT/FLT3 WT	72 (55.4%)
t(9;11)	1 (0.8%)
Adverse	36 (27.7%)
-7	2 (1.6%)
-5	3 (2.4%)
Complex	21 (16.2%)
Induction therapy, n (%)	
"3+7"	84 (64.6%)
Decitabine	12 (9.2%)
Low dose cytarabine	13 (10%)
Best supportive care	3 (2.3%)
Therapeutic response, n (%)	
CR	40 (58.8%)
RD	28 (41.2%
Death in induction	14 (16.3%)

AML= acute myeloid leukemia, t-AML= therapy related acute myeloid leukemia, BM= bone marrow, CNS= central nervous system, CR= complete response, ELN= European Leukemia Net, FLT3= FMS-like tyrosine kinase, NOS= not otherwise specified, NPM1=nucleophosmin 1, RD= refractory disease, WBC= white blood count, WT= wild type

We found no further statistically significant correlation between classic risk factors such as the total white blood cell count and the WHO AML types or the response to therapy.

The log rank test was used for comparisons of Kaplan-Meier curves.

DISCUSSION

All AML patients who achieve CR are facing the likelihood of relapse (7). Thus, an accurate prognostic assessment is necessary to identify patients with high and intermediate ELN risk, who would benefit immediately from consolidation by ASCT, and patients with favorable risk, for whom high dose consolidation chemotherapy is rather indicated (11).

However, multiple reports indicated that the prognostic value of the cytogenetic and molecular features of AML can be questioned in patients from developing countries such as Romania because of local socio-economic factors (13,14). Large scale national reports regarding the outcome of Romanian patients with AML are missing and a recent WHO analysis has identified multiple issues in Romania's public healthcare system that could influence the outcome of AML patients (15). Thus, we

aimed to investigate the relevance of the ELN risk classification system in the "real-life" setting of patient1s treated at the Iași Regional Oncology Institute.

We analyzed the data of 130 newly diagnosed AML patients and we identified cases with favorable, intermediate and adverse risk with frequencies similar to those reported by other groups (18). In our study group, the ELN risk categories demonstrated survival probabilities comparable to literature data (fig. 2).

TRM in AML is defined as death within four weeks following completion of induction therapy. Literature data reveals that TRM rates vary from 10 to 30% or even more in elderly AML patients (12). Current advances in supportive care in developed countries have reduced TRM rate below 10%, even in the elderly patient population (19, 20), but death rates remain higher in developing countries. In our study group, TRM was 16.3% in chemotherapy eligible patients. However, in elderly patients treated with Decitabine or LDAC, mortality was much higher, reaching 46.2% (data not shown). The high TRM rates in our study group are mainly due to infectious and hemorrhagic complications, but also to associated comorbid conditions. Poor education, inadequate hygiene habits, especially in patients from rural areas, irrational antibiotic use in the general population and colonization with multidrug resistant bacteria all contribute to a higher incidence of infection among our patients when compared to reports from more developed countries (18). Comorbidities, especially cardiovascular ones, which have a higher prevalence among Romanian patients (15), further contribute to even a higher mortality among patients with infectious complications. Moreover, the presence of infection at diagnosis delays the administration of induction therapy and the development of severe, life-threatening sepsis during induction chemotherapy forfeits its completion, which compromises therapeutic response and leads to a dismal prognosis. Thus, the median survival in patients who did not complete induction therapy was 5 months (data not shown). Moreover, the higher incidence of infection also reduces survival in the group of AML patients with favorable ELN risk.

Although the majority of patients were classified as intermediate or adverse risk, allogeneic stem cell transplant was performed only 8.1% of patients. Timely access to this procedure is vital for the survival of AML patients (21). Widespread availability of this procedure remains a major unmet need for Romanian AML patients, even if, in the last years, Romania is cited by reports from the European Bone Marrow Transplantation Society as one of the European countries with a rapid increase in transplanted cases per year (22, 23). Thus, it is expected that the access to this procedure will improve drastically in the near future.

We can conclude that the ELN risk assessment system is relevant for Romanian AML patients, but infections, more frequent comorbidities and the lack of timely access to the bone marrow transplant procedure have a detrimental influence on their short- and long-term prognosis.

CONCLUSION

In our study we examined the relevance of the European Leukemia Net prognostic system in AML patients treated at the Regional Institute of Oncology from North-Eastern Romania. Briefly, we conclude that the ELN risk classes have similar distribution when compared to literature data. Fur-

thermore, despite a higher incidence of comorbidities among Romanian patients and higher treatment related mortality rates in both the chemotherapy eligible and ineligible patients, the ELN prognostic system retains its significance. However, more prevalent comorbid conditions, poor hygiene habits, treatment discontinuation and low availability of the bone marrow transplant procedure can pose major challenges in the management of AML and impact the long-term prognosis of patients.

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NEWS

THE INTERPLAY BETWEEN VAGINAL MICROBIOTA AND INTRAUTERINE ADHESION USING NEXT GENERATION SEQUENCING METHODS

Intrauterine adhesion represent a disease that is a very common cause for female infertility. Since it has an increased recurrence rate after surgical interventions, its pathogenesis and recurrence causes are important to understand better this condition. Liu *et al*. (2019) analysed, using high-throughput sequencing method, the vaginal microbiota of females diagnosed with intrauterine adhesion and healthy patients` vaginal secretion. The results shown that intrauterine adhesion does not bring any important changes for vaginal microbiota, having a slighty effect on the number of vaginal bacterial species. The patients with intrauterine adhesion presented at the phylum level a reduced percentage of Firmicutes and increased percentage of Actinobacteria whereas in the healthy females group the rate was more lower ($P<0.05$). Moreover, in about half of the intrauterine adhesion vaginal samples was identified at the genus level a remarkable decreased number of Lactobacillus and enhanced growth of Prevotella and Gardnerella ($P<0.05$). The interplay between synechiae and vaginal microflora can reveal remarkable changes in the vaginal microbiota diversity in patients with intrauterine adhesion. The increase of Lactobacillus population may bring interesting benefits in the endometrial regeneration process but also may help to decrease de recurrence rate of intrauterine adhesion (Liu Z, Kong Y, Gao Y *et al*. Revealing the intraction between intrauterine adhesion and vaginal microbiota using high-throughput sequencing. *Mol Med Rep*. 2019. 19(5),4167-4174).

EVALUATING PREDICTIVE FACTORS FOR DISEASE ACTIVITY AMONG PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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EVALUATING PREDICTIVE FACTORS FOR DISEASE ACTIVITY AMONG PATIENTS WITH INFLAMMATORY BOWEL DISEASE (Abstract) **Aim**: this study focused on evaluating several potential predictive factors for an increase in disease activity at 6 months for patients with inflammatory bowel disease (IBD). **Material and methods**: We performed a prospective cohort study, including patients who were evaluated at baseline and at 6 months. We assessed disease activity, clinical and biochemical parameters, anxiety and depression using Hospital Anxiety and Depression Scale (HADS) and sleep disorders using Pittsburgh Sleep Quality Index (PSQI) both at baseline and at 6 months. **Results:** Among the 59 patients who completed the follow-up, there was a statistically significant correlation between the presence of hyposideremia (p=0.023), hypercholesterolemia (p= 0.015) and altered psychological state (p=0.006) at baseline with an increase in disease activity at 6 months. Moreover, among the studied parameters, hypercholesterolemia (OR 5.18; 95% CI, 1.25-21.44), the neutrophil to lymphocyte ratio (OR 3.83; 95% CI, 1.87-7.83) and the depression score (OR 1.56; 95% CI, 1.22-2.01) were predictors for increase in disease activity at 6 months. **Conclusions:** Beyond usual inflammatory markers, neutrophil to lymphocyte ratio could have potential predictive value for systemic inflammation in IBD patients and could be easily assessed at routine evaluations. An integrative management, addressing several comorbidities, such as dyslipidemia and alteration of psychological distress could contribute to diminishing the risk for future disease flares in this patient category. **Keywords**: INFLAMMATORY BOWEL DISEASE, PREDICTIVE FACTORS, HADS.

Inflammatory bowel disease (IBD) represent chronic inflammatory disorders which evolve with a relapsing-remitting pattern and include two main subtypes, namely ulcerative colitis (UC) and Crohn's disease (CD). Although with increasing prevalence in the past decades, there is still no curative treatment and relatively few information on the factors which could predict disease evolution. After diagnosis, disease monitoring is essential via clinical, biological and endoscopic criteria but also through evaluation of disease activity scores, the most commonly used being Mayo score for UC and Crohn's disease activity index (CDAI) for CD.

Although non-invasive disease monitoring following various biological parameters

reflecting inflammation such as C reactive protein (CRP), fibrinogen, fecal calprotectin is available (1) less information is available on noninvasive parameters predictive for future disease activity. Identifying predictors of unfavorable clinical course are useful, since they could facilitate treatment decisions, indicate requirement for tight monitoring and avoid overtreatment (2). Our aim was to identify potential predictive factors for increased disease activity at 6 months evaluation among several accessible parameters in the clinical practice.

MATERIAL AND METHODS

This prospective study assessed the prediction value of several parameters for disease activity at 6 months and included IBD patients from the Institute of Gastroenterology and Hepatology from "Sf. Spiridon" County Clinical Emergency Hospital, Iași, Romania between September 2019 and February 2020, who were evaluated at baseline and at 6 months.

Inclusion criteria were age above 18, written informed consent, diagnosis of IBD based on clinical, endoscopic and histological criteria. The exclusion criteria included refusal to participate in the study, active infections and various aspects which might influence psychological status and sleep quality (9): pregnancy, history of neoplasia, previously diagnosed psychiatric disorders, history of alcohol or drug use, sleep apnea, BMI > 35, significant major associated illness (active malignancy, significant cardiac or pneumological comorbidities), night shift workers, and associated medications (analgesics, muscle relaxants, antineoplastic agents, thyroid medication).

For the enrolled patients, clinical and anamnestic data were collected during patients' evaluations, including information related to extraintestinal manifestations, current and previous medication, IBD- related surgery. For the evaluation of disease activity, Crohn's Disease Activity Index (CDAI) was used for patients with CD and Mayo score for UC. The biochemical evaluation included not only markers of systemic inflammation, namely C reactive protein (CRP), erythrocyte sedimentation rate (ESR) and fibrinogen, but also evaluation of the complete blood count (CBC) with calculation of neutrophil-/to-lymphocyte ratio (NLR), iron level, ferritin, albumin, cholesterol, triglycerides. Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI) and psychological distress was evaluated through the Hospital Anxiety and Depression Scale (HADS). Impaired sleep was defined as a PSQI ≥5 (3), while altered psychological status was considered when either of the 2 HADS sub scores, namely HADS-depression (HADS-D) and HADS-Anxiety (HADS-A) were ≥8 (4). All these parameters have been evaluated at baseline, while at 6 months clinical and anamnestic data, CBC, inflammatory markers (CRP, fibrinogen) were recorded and disease activity scores (CDAI, MAYO) calculated.

The data was statistically analyzed using *SPSS version 25*. Variables were reported as mean values and standard deviation, or as median with 25th-75th percentiles, after testing for normality of the distribution using Kolmogorov-Smirnov test. To evaluate the homogeneity of variance, Levene test was used. The comparisons between the analyzed groups were performed using Student's t-test for normally distributed continuous variables.

The qualitative variables were presented as absolute (n) and relative (%) frequencies and the comparison among the groups was based on the results of Chi-square tests. The

bivariate correlation analysis was completed based on Pearson Correlation test. Predictive value for several parameters was evaluated using logistic regression and variables with significant results were afterwards included in the multivariate analysis. ROC curve analysis was used to evaluate the prediction power of some variables. For all tests applied, the calculated significance level (p-value) was considered for $p < 0.05$.

The study protocol was in accordance with the ethical standards of the institutions' Ethical Review Boards and with the 1964 Helsinki declaration and its later amendments and the study was performed after obtaining approval from the institutions involved.

RESULTS

Among the 82 eligible patients with IBD, 59 completed the study; 6 did not consent for participation in the study, 14 could not complete the follow-up, 2 had active infection, 1 had history of neoplasia. For the 59 patients included in the study several characteristics were evaluated, with no statistically significant differences between these parameters depending the disease subtypes (Crohn's disease and ulcerative colitis) (tab. I).

TABLE I

Descriptive statistics of the evaluated parameters

Patient characteristics	Study Group n=59 (100%)			Statistic test	*p-value* (*)
	Ulcerative colitis n=41 (69.5%)	Crohn's Disease n=18 (30.5%)			
Active disease, n (%)	13 (22%)			0.133 ↑	0.716
	8 (19.5%)	5 (27.8%)			
Anemia, n (%)	19 (32.2%)			2.864 ↑	0.091
	16 (39%)	3 (16.7%)			
Disease activity scores median (Q25;Q75)	Mayo score 3 (1;7)	CDAI score 129.5 (40.75; 198.5)		NA	NA
Hemoglobin, median (Q25;Q75)	13.1 (11.9; 14.3)			1.669 #	0.101
	12.6 (11.7;14.25)	13.8 (12.45; 14.52)			
Seric iron level median (Q25;Q75)	59 (23; 86)			1.029 #	0.308
	51 (23; 80)	66.5 (20.75; 97)			
Ferritin median (Q25;Q75)	75 (36; 174)			-0.167 #	0.868
	75 (32.5; 182.5)	62 (39.5; 172.25)			
CRP median (Q25;Q75)	0.82 (0.24; 2.09)			0.743 #	0.461
	1.07 (0.2; 2.07)	0.61 (0.33; 2.84)			
Albumin (mean ± SD)	4.02 ± 0.51			0.410 #	0.683
	4 ± 0.55	4.06 ± 0.41			
Fibrinogen (mean ± SD)	419.49 ± 67.1			1.173 #	0.246
	412.41 ± 59.62	435.88 ± 81.65			
ESR median (Q25;Q75)	5 (3; 10)			-0.377 #	0.708
	5 (3.25; 9.5)	6 (3; 11)			
Cholesterol (mean ± SD)	179.71 ± 47.21			-0.95 #	0.346
	183.84 ± 45.81	171 ± 50.25			

Patient characteristics	Study Group n=59 (100%)			Statistic test	*p*-value (*)
	Ulcerative colitis n=41 (69.5%)	Crohn's Disease n=18 (30.5%)			
Triglycerides median (Q25;Q75)	86 (70.25; 120.5)			0.354 #	0.725
	89.5 (69.75; 120.75)	81.5 (69.75; 121)			
Extraintestinal manifestations n (%)	12 (20.3%)			0.133 ↑	0.481
	11 (26.8%)	1 (5.6%)			

- T-test, ↑ Chi-Square test, (*) marked effects are significant for $p < 0.05$

In order to identify potential predictive value for increased disease activity at 6 months, reflected by increased values of CRP and fibrinogen, we evaluated potential correlations with parameters such as anemia (defined as Hb<12 g/dL for female patients and <13 g/dL for male patients), hyposideremia (seric iron level <50 mg/dL), hypercholesterolemia (Cholesterol level>200 mg/dL), extraintestinal manifestations, impaired sleep (PSQI ≥ 5), altered psychological status (HADS-A and/or HADS-D ≥ 8). Pearson test revealed a statistically significant correlation between the presence of hypercholesterolemia (p=0.015) and altered psychological status (p=0.06) at baseline with increased values of CRP at 6 month evaluation; moreover, hyposideremia (p=0.023), hypercholesterolemia (p=0.01) and impaired sleep (p=0.039) at baseline were correlated with increased fibrinogen value at 6 month evaluation IBD patients (tab. II).

TABLE II

Correlations between various parameters at baseline and increased inflammatory markers (CRP, fibrinogen) at 6 months

Parameters evaluated at baseline	Increased CRP value at 6-month evaluation IBD patients (p value)	Increased fibrinogen value at 6-month evaluation IBD patients (p value)
Anemia	0.118	0.852
Hyposideremia	0.054	0.023*
Hypercholesterolemia	0.015*	0.01*
Extraintestinal Manifestations	0.198	0.274
Impaired sleep	0.960	0.039*
Altered psychological status	0.006*	0.256

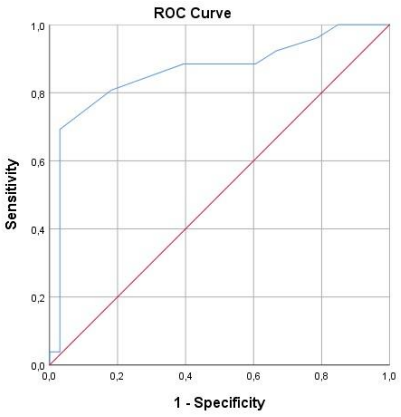
Pearson correlations, (*) marked effects are significant for $p < 0.05$

Considering the previously mentioned findings, logistic regression was used to identify predictors of sleep quality and examine the effect of various parameters at baseline for increase in disease activity at 6 months. In the study group, the univariate analysis showed that the presence of hyposideremia (OR: 13.09; 95% CI: 1.57-109.11), hypercholesterolemia (OR: 5.18; 95% CI: 1.25-21.44), the values for NLR (OR 3.835; 95% CI, 1.87-7.83) and HADS-D (OR 1.56; 95% CI, 1.22-2.01) at baseline were predictors for an increase of disease activity at 6 months. In the multivariate analysis, however only hyposideremia was identified as a predictive factor (tab. III).

TABLE III
Evaluation of the prediction power of various parameters on increase in disease activity at 6 months

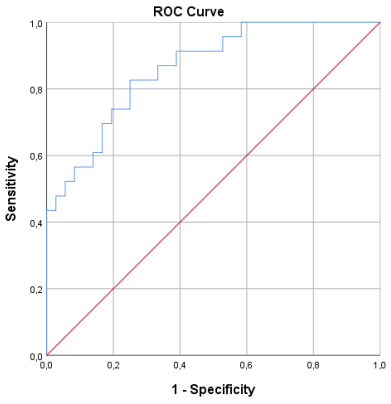
Parameter	Univariate analysis Study Group OR 95% CI	Multivariate analysis Study Group OR 95% CI
Anemia	1.77 (0.42-7.39)	
Hyposideremia	**13.09 (1.57-109.11)**	**9.63 (1.05-87.75)**
Hypercholesterolemia	**5.18 (1.25-21.44)**	3.38 (0.71-15.95)
Extraintestinal manifestations	3.77 (0.44-32.36)	
Impaired sleep	2.47 (0.59-10.31)	
NLR	**3.83 (1.87-7.83)**	0.54 (0.25-1.15)
CRP / ALB	0.31 (0.04-2.08)	
HADS-D	**1.56 (1.22-2.01)**	0.99 (0.79-1.25)
HADS-A	0.96 (0.79-1.15)	
PSQI	1 (0.85-1.18)	

Increased disease activity was defined as an increase in MAYO and respectively CDAI score at 6 months compared to baseline since NLR and HADS-D might have predictive value on the increase of the inflammatory parameters, we used ROC curve analysis in order identify a cut-off value for these parameters, which could predict active disease at 6 months. Consequently, we identified a cut-off value of 2.7 for NLR and a cut-off value of 8.5 for HADS-D at baseline to be predictive for an increase in disease activity at 6 months (fig. 1).



(a)

Cut off value	2.7
AUC	0.862
Sensibility	0.826
Specificity	0.722
p value	0.001



(b)

Cut off value	8.5
AUC	0.853
Sensibility	0.808
Specificity	0.82
p value	0.001

Fig. 1. ROC curve evaluating the prediction power of (a) NLR and (b) HADS-D at baseline on disease activity at 6 months

DISCUSSION

Considering that IBD represent diseases with a relapsing-remitting pattern, with high disease burden, but also with significant socio-economic impact (5), there are efforts in identifying potential predictors of disease severity and disease flares. In the current study, we searched for parameters which could be easily used in the clinical practice to predict future disease flare and consequently to identify patients who require tight monitoring, for better disease control.

We have identified several factors that could contribute to predicting disease activity at 6 months in IBD patients, among parameters that are or could be routinely evaluated. Among the biochemical parameters we identified increased NLR, low seric iron level and hypercholesterolemia as predictors for active disease at 6 months. An interesting finding was that the presence of hyposideremia, but not necessarily the presence of anemia constituted predictive factors for disease activity, which highlights once more the importance of diagnosing and correcting the low iron levels in patients with IBD, as this influences disease-related outcome (6). This could be due to the coexistence of inflammation (either clinical or subclinical) with histologic changes, which play a role in iron deficiency, probably mediated by the hepcidin-ferroportin axis (7).

As far as the other biochemical markers are concerned, NLR has been investigated as marker of subclinical inflammation and it can be easily available from the CBC. A study performed by Acaturk G *et al.*, investigated the utility of NLR in evaluating disease activity in IBD patients and identified an NLR cut-off point for active CD and UC was 3.2 and 3.1. In our study, a cut-off NLR of 2.7 was a predictor for disease activity at 6 months (8).

Regarding other parameters identified as potential predictors for active disease at 6 months, we identified that psychological distress correlated with the presence of inflammatory syndrome at 6 months. Our findings also included that increased depression, but not anxiety score is predictive for active disease at 6 months. Psychological comorbidity is important in the evolution of IBD, as it can promote inflammation and it can be associated with sleep impairment which could enhance the effects of psychological distress on the proinflammatory status (9). Moreover, there is a potential bidirectional relationship between psychological status and IBD activity, with inflammation driving depression and vice versa (10). Considering that several studies have highlighted that the presence of depression and anxiety generates a subsequent worse course of disease compared to IBD patients without psychiatric comorbidity (11-14), early identifying and targeting the associated psychological impairment could contribute to improving disease control (10). Considering that, we evaluated a potential cut-off value for HADS-D of 8.5 as predictive for disease activity at 6 months. This aspects could support the role of screening for depression among IBD patients, so that the psychological comorbidity could be addressed and consequently to improve disease outcome.

Although the univariate analysis highlighted significance for all the abovementioned parameters, when using multivariate analysis to study the parameters, we did not obtain significant results; however, this is most probably due to low sample size and is the main limitation of our study. Therefore, prospective cohort studies with large number of patients should be per-

formed in order to further evaluate easily available markers with predictive value for disease activity and lead to improving IBD patients' management.

CONCLUSIONS

There is an increased need for identifying predictive factors for disease activity in IBD patients in order to optimally tailor the disease monitoring and adjust treatment. Among commonly assessed parameters, hyposideremia, hypercholesterolemia, increased NLR and the presence of depression symptoms show promising value as predictive factors and could be easily followed in the daily clinical practice.

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URINALYSIS UNDER THE MICROSCOPE: FAST, CHEAP AND COMMON BUT STILL RELEVANT

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URINALYSIS UNDER THE MICROSCOPE: FAST, CHEAP AND COMMON BUT STILL RELEVANT (Abstract) **Aim:** The complete urine examination consisting of the analysis on the strip and the manual microscopic examination is one of the most important and used tests in current medical practice. The purpose of this study was to compare the efficiency of the strip vs the microscopy, but also the results obtained from the hospital compared to those from the ambulatory. **Material and methods:** The present study is a retrospective study conducted on a batch of 103 patients (53 hospitalized, 50 outpatient) between July and August 2019, with samples processed in the Clinical Laboratory of Medical Analyses of the Clinical Emergency Hospital for Children "Louis Țurcanu" Timișoara. Of the 103 patients, 63 (61.2%) were female, while 40 (38.8%) were male. **Results:** The red blood cells (RBCs) reading with the help of the strips and the bio-analyzer revealed negative results in 85.4% of the samples, while the microscopic examination revealed only 59.2% negative results. The same trend could also be observed in the case of leukocytes: 81.6% negative results on the strip, 20.4% results negative on microscopic examination. The *p* values for both examinations were <0.0001. Comparing hospital patients and outpatients, 5 entities (epithelia, bacteria, leukocytes, RBCs, and salts) were chosen, which were then divided by frequency (absent, very rare, rare, relatively common, common, numerous and deposit). Percentage values between the two groups were similar. *ANOVA* tests revealed the no difference either, *p* values being over 0.9277 when considering the absent values and over 0.1956 when excluding absent values. **Conclusions:** The difference between strip and microscope analyses for RBCs and leukocytes has proved to be statistically significant. No major percentage differences were identified between hospital and ambulatory for the number of positive samples for the epithelial variables, salts, leukocytes, RBCs, or bacteria. **Keywords:** URINALSYS, URINARY STRIPS, MICROSCOPY.

The urinalysis is one of the most important habitual tests that can be performed in a medical analysis laboratory and is used for prevention, screening, monitoring and diagnosis. It is widely used for early diagnosis but also for empirical treatment of urinary tract infections (UTI) (1).

There are two parts that can be distin-

guished in performing an urinalysis. These are the automatic reading with an analyzer of urinary dipsticks and the microscopy of the centrifuged urine. The advantages of using the urinary strips are the low cost, the ease of interpretation as well as the short effectuation time. In addition, this procedure can be performed by minimum qualified personnel using an analyzer, while the view under the microscope is performed by higher trained personnel (2).

The efficiency of strips has been proven amongst pediatric population, including under the 2 years old as a screening method of UTI as well as other pathologies (3). The examined parameters are as follow: pH, concentration, leukocyte esterase, presence of red blood cells, nitrite levels, urobilinogen, ascorbic acid, glucose levels, ketone bodies, bilirubin, or proteins (especially albumin) (4).

The microscopy of centrifuged urine has the advantage that it can give both a quantitative but also qualitative result for the number of leukocytes and red blood cells, but it is also useful for the objectification of bacteria, salts, and epithelia (5, 6).

The urinalysis represents a useful test in diagnosing of different renal-urinary pathologies. Although the microscopic examination contains a subjective characteristic represented by the examiner (based on training, experience), the fact that the test executes a qualitative analysis (7, 8), not only quantitative of the elements that can be found in the urine sample, makes it have a higher value of examination than in the case of strips reading (9, 10). Given this hypothesis, we study if the microscope examination leads to more accurate results than strips analysis.

MATERIAL AND METHODS

This study is retrospective, observational and includes the pediatric patients hospitalized in Emergency Hospital for Children "Louis Țurcanu" Timișoara and the outpatients, who underwent a complete urine examination (strips and microscope) between July-August 2019. After the identification of relevant patients, demographic (sex, age) and paraclinical (quantitative and qualitative data from previously performed tests) data were collected from the electronic archive of the hospital. Besides the above criteria, the main inclusion criterion was represented by the age below 16 years.

The examined group includes 103 patients distributed relatively equal in two subgroups: hospitalized patients and outpatients. Whereas it is reasonable to expect that the hospitalized patients to be suffering from more serious pathologies than the outpatients, we can assume the hypothesis that their urinalysis results would agree with the severity of their pathologies (11-14).

The results that are the subject of this study were obtained using strips with parameters, urine bioanalyzer *LabURreader Plus* for reading the strips and for microscopy it has been used a Nikon microscope. The microscope glass slides were viewed with a 40x magnification objective. All the samples were processed in the laboratory of medical analysis of the Emergency Hospital for Children "Louis Țurcanu" from Timisoara, which is accredited by RENAR.

Statistical analysis

The data obtained were processed statistically using *Microsoft Excel version 14.0* and IBM *SPSS Statistics 25.0*. We have also calculated a 95% confidence interval (C.I. 95%) for means by using the Wilson method. Due to having a small sample, *Fisher's* test with two tails was performed for comparison between strips and microscopy finding. For analysis between hospital patients and outpatients, an *ANOVA* test

was performed for each entity (epithelia, bacteria, leukocytes, RBCs, salts)

RESULTS

During the given period, there were identified 103 patients, which met the criteria for inclusion in the study. Of them, 63 (61.2%) were females and 40 males (38.8%). The median age of the group was 8 years old, with a minimum age of one month and a maximum age of 16 years old (tab. I).

TABLE I
Distribution of the pediatric population

Sex	No.	Percentage	C.I. 95%
Female	63	61.2%	51.52 - 70.02
Male	40	38.8%	29.98 - 48.48

Microscopy *vs.* Strips

Following the descriptive analysis of the database, we obtained the second table, which shows a remarkable difference between the results of the centrifuged samples seen under the microscope and those of the strips, read in the analyzer. The presence or absence of red blood cells and leukocytes were described in the table because they are common elements between the two parts of the complete urine examination.

For the positive results, we do not consider that the detail regarding the number of leukocytes or red blood cells is relevant because the microscopic reading measures the number of elements present in the field while the strip performs a semi-quantitative analysis and has as a unit of measure mg/dL (tab. II). *Fisher's test* returned p value of < 0.0001 for both RBCs and leukocytes, resulting in a statistically significant difference between the two methods.

TABLE II
Difference between the results of the strips read in the analyzer and those of the centrifuged samples seen under the microscope

	Strip reading with analyzer						Urinary sediment by microscopy						p
	Negative		C.I. 95%	Positive		C.I. 95%	Negative		C.I. 95%	Positive		C.I. 95%	
RBCs	**88**	85.4%	77.36-90.97	**15**	14.6%	9.03-22.64	**61**	59.20%	49.56-68.21	**42**	40.8%	31.79-50.44	< 0.0001
Leukocytes	**84**	81.6%	72.97-87.86	**19**	18.4%	12.14-27.03	**21**	20.4%	13.74-29.17	**82**	79.6%	70.83-86.26	< 0.0001

Hospital *vs.* Ambulatory

We identified two sublots consisting of outpatients (n = 50) and patients hospitalized (n = 53). Starting from the hypothesis that the hospitalized patients should have worse results than in the ambulatory, we wanted to see if there are differences between the percentages of negative and posi-

tive samples for the variables of leukocytes, red blood cells, epithelia (both flat and round), salts (whether we are talking about amorphous salts, Ca oxalates, amorphous urates, ammonia-magnesium phosphates) and bacteria within the two groups.

The number of negative samples for epithelia, blood vessels and salts had similar percentage values between hospital and outpatient groups. The same is true for leukocytes with a major difference between the two percentages. The only variable that is more common in the hospital than in the outpatient setting is the presence of bacteria. Thus, no significant differences were found between the two sublots, but the small differences of several percentage points tilt the balance in favor of outpatients regarding the number of positive samples on the studied variables (tab. III).

TABLE III

The number and the percentages of negative and positive samples for the variables investigated within the two groups

		Hospital			Ambulatory				
		No.	**Percentage**	**C.I. 95%**	**No.**	**Percentage**	**C.I. 95%**	**p1**	**p2**
EPITHELIA	Absent	26	49.1%	36.13-62.12	21	42.0%	29.38-55.77	0.9307	0.9250
	Very rare	8	15.1%	7.85-27.05	18	36.0%	24.14-49.86		
	Rare	12	22.6%	13.45-35.52	5	10%	4.35-21.36		
	Relatively Common	7	13.2%	6.55-24.84	3	6.0%	2.06-16.22		
	Common	0	0%	0-6.76	2	4.0%	1.1-13.46		
	Numerous	0	0%	0-6.76	1	2.0%	0.35-10.5		
	Deposit	0	0%	0-6.76	0	0%	0-8.89		
BACTERIA	Absent	44	83.0%	70.78-90.8	46	92.0%	81.16-96.85	0.9623	0.1956
	Very rare	1	1.9%	0.33-9.95	0	0%	0-8.89		
	Rare	3	5.7%	1.94-15.37	1	2.0%	0.35-10.5		
	Relatively Common	3	5.7%	1.94-15.37	1	2.0%	0.35-10.5		
	Common	1	1.9%	0.33-9.95	2	4.0%	1.1-13.46		
	Numerous	1	1.9%	0.33-9.95	0	0%	0-8.89		
	Deposit	0	0%	0-6.76	0	0%	0-8.89		

		Hospital			Ambulatory				
		No.	Percentage	C.I. 95%	No.	Percentage	C.I. 95%	p1	p2
LEUKOCYTES	Absent	15	28.3%	17.96-41.57	6	12.0%	5.62-23.8	0.9277	0.8532
	Very rare	20	37.7%	25.94-51.2	27	54.0%	39.45-67.94		
	Rare	11	20.8%	12-33.45	10	20.0%	11.24-33.04		
	Relatively Common	4	7.5%	2.45-17.86	3	6.0%	2.06-16.22		
	Common	2	3.8%	1.04-12.75	4	8.0%	3.15-18.84		
	Numerous	1	1.9%	0.33-9.95	0	0%	0-8.89		
	Deposit	0	0%	0-6.76	0	0%	0-8.89		
RBCs	Absent	32	60.4%	46.94-72.41	29	58.0%	44.23-70.62	0.9444	1.0000
	Very rare	11	20.8%	12-33.45	15	30.0%	19.1-43.75		
	Rare	7	13.21%	6.55-24.84	3	6.0%	2.06-16.22		
	Relatively Common	1	1.9%	0.33-9.95	3	6.0%	2.06-16.22		
	Common	2	3.8%	1.04-12.75	0	0%	0-8.89		
	Numerous	0	0%	0-6.76	0	0%	0-8.89		
	Deposit	0	0%	0-6.76	0	0%	0-8.89		
SALTS	Absent	41	77.4%	64.48-86.55	35	70.0%	56.25-80.9	0.9543	0.6994
	Very rare	6	11.3%	5.29-22.58	5	10.0%	4.35-21.36		
	Rare	3	5.7%	1.94-15.37	2	4.0%	1.1-13.46		
	Relatively Common	0	0%	0-6.76	3	6.0%	2.06-16.22		
	Common	3	5.7%	1.94-15.37	4	8.0%	3.15-18.84		
	Numerous	0	0%	0-6.76	1	2.0%	0.35-10.5		
	Deposit	0	0%	0-6.76	0	0%	0-8.89		

p1: result of ANOVA test taking “absent” values into consideration;
p2: result of ANOVA test excluding “absent” values;

Two *ANOVA* tests were done. The first considered the "absent" values and the p values are recorded into column p1 from third table. The second excluded the "absent" values and the p values are recorded into column p2 from third table. Both p1 and p2 values are more than 0.05, revealing the fact that there are no statistically significant differences.

DISCUSSION

Countless studies have shown an increased incidence of UTI in the female population compared to the male population (15). The predominance of the female sex in the studied group is not random because it is known that women have more UTIs than men due to the anatomical differences of the genitourinary system. However, in the first months of life there were no significant differences regarding this incidence.

Although the reading of the urinary sediment from the centrifuged urine obtained positive results regarding the variables of the red blood cells and leukocytes in higher percentages than the reading of the strips by the urine bio-analyzer, we must also discuss the differences between the two test methods. On the one hand, the reading on the strip is done by interpreting the color change reaction that the reagent on the strip paper has at the time of contact with the sample, thus obtaining a semi-quantitative reading expressed in mg/dL. In addition, it should be mentioned that the strip reacts to leukocyte esterase and then estimates the number of leukocytes (16). On the other hand, the results given by microscopy are subjective, depending on the examiner's skill (17) and for the number of elements present in the field, a standardization table of the number of elements is used as follows: very rare (0-2 elements per field), rare (2-5 items per field), relatively common (5-10 items per field), common (10-40 items per field), numerous (26-40 items per field) and deposit (more than 40 items per field) (18).

Our hypothesis that it is reasonable to expect that the results of patients admitted to the hospital to be more frequently modified than normal than those presented in the outpatient setting cannot be supported by the results obtained on our batch of patients. The complete urine examination is a routine test and most likely, the large number of pathologies for which pediatric patients in our hospital are admitted far exceeds the reno-urinary sphere for which this examination is mostly relevant. Thus, one can explain the large number of samples from the hospital that did not necessarily have elements of pathogenicity (19). At the same time, patients sent to the outpatient laboratory by the primary care services (family medicine, outpatient offices of specialist doctors) with a referral ticket are often targeted for the pathologies of the genitourinary sphere with changes in the urinary sediment, being thus used to diagnose or at least to make a correct differential diagnosis (20).

CONCLUSIONS

Our results showed that microscopic examination revealed more positive results than the test on the strip and that the difference was statistically significant. The hypothesis according to which the admitted cases show more frequent and more serious changes than those in the outpatient turned out to be false, since the results obtained by us showed similarities between the two sublots.

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PARTICULAR ASPECTS IN PATIENTS WITH PARANEOPLASTIC DEEP VEIN THROMBOSIS: FROM A SHADOWY DIAGNOSIS TO A ROUTINELY MET PATHOLOGY

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PARTICULAR ASPECTS IN PATIENTS WITH PARANEOPLASTIC DEEP VEIN THROMBOSIS: FROM A SHADOWY DIAGNOSIS TO A ROUTINELY MET PATHOLOGY (Abstract). The oncological patient presents many pathophysiological particularities, including a hypercoagulable state, which causes frequent thromboembolic complications. Furthermore, the side effects of chemotherapy, laborious surgical interventions and the poor performance status favor the occurrence of deep vein thrombosis (DVT). The aim of this retrospective study is to identify the prevalence of paraneoplastic DVT in patients admitted in the Cardiology Department of the "Sf. Spiridon" County Clinical Emergency Hospital from Iași and to establish the correlations between the DVT, type of neoplasia and certain particularities of the patients. **Material and methods**: The current retrospective study was conducted over a two-year period and enrolled 100 patients divided into two groups based on the presence or absence of a neoplasia (25 patients having a tumoral pathology and 75 patients without such a comorbidity). All study participants underwent a complete clinical examination and were assessed through biochemical and echocardiographic parameters. **Results:** Various tumor locations were identified in the 25 patients with a malignancy, the most prevalent being the colorectal cancer (6 cases, 24% of total), followed by the lung cancer (4 cases, 16%) and, with a similar occurrence, the brain, ovarian and breast cancer (2 cases each, 8%). Among the patients with neoplasia, 13 had a history of cancer (52% of cases from the neoplasia group), while for the remaining 12 patients it was a *de novo* diagnosis. **Conclusions**: Patients with DVT showed a significant cancer prevalence, either previously diagnosed or incidentally discovered. The increased risk of DVT in cancer patients is multifactorial and represents a diagnosis and therapeutic challenge. In our study, we recorded a slightly increased prevalence of cancer in women (56% vs. 44%), who had cervical cancer as the most common etiology (35.7%). On the other hand, male sex was associated with an increased frequency of colorectal cancer (36.4% of all cancers diagnosed in men), followed by lung cancer (27.3%). **Keywords:** DEEP VEIN THROMBOSIS, CANCER, PARANEOPLASTIC SYNDROME.

Deep vein thrombosis (DVT) is an underdiagnosed and serious, yet preventable medical condition that can cause disability and death. DVT is the formation of a blood clot usually in the lower leg, thigh, or pelvis venous networks, but they can also

occur in the arm (1, 2).

The incidence of DVT increases sharply with age and appears steady over the last years, despite extensive preventive strategies. Women are more often affected at younger ages, but this ratio is reversed in the case of elderly patients. It is predicted that the number of DVT will continue to increase, and by the year 2050, the prevalence is expected to reach approximately 1.8 million cases per year in the United States (3). Recent European population studies reported a DVT incidence of 70-140 cases/100,000 person/year (4).

Cancer and thrombosis are connected through a well-established interdependence. DVT contributes significantly to the morbidity and mortality of cancer patients, with a fatal pulmonary embolism (PE) being three times more common in cancer patients compared to non-cancer patients. In a similar way, approximately 15% to 20% of patients with malignancy will develop venous thrombosis, whereas 18% to 20% of idiopathic venous events are caused by an occult neoplasia, (5). Annually, 0.5% of cancer patients will experience a thrombotic event, compared to a 0.1% incidence rate in the general population, furtherly confirming the mutual relationship between these two pathologies (6).

Studies have shown that many patients who had developed DVT were diagnosed with a type of neoplasia in the 12 months following the thromboembolic event. It is also estimated that cancer patients who develop DVT have a 94% probability of a fatal event in the 6 months following the episode. Therefore, DVT can be considered a negative predictive marker of survival in cancer patients (7).

The evolution of both DTV and a neoplasm can be unpredictable, a sufficient reason for an adequate approach of these fragile patients, with a careful assessment of the risks and benefits of each intervention, in order to prevent major complications.

The aim of this retrospective study is to identify the prevalence of DVT in patients admitted in the Cardiology Clinic of the "Sf. Spiridon" County Clinical Emergency Hospital from Iasi and to establish the correlations between the type of neoplasia and the patients' characteristics. Therefore, we assessed the prevalence of DVT and analyzed the included patients according to gender, age, tumor location, type of onset and we also identified some particularities concerning the paraclinical profile or other investigations used to detect the neoplastic etiology of DVT.

MATERIAL AND METHODS

The current study was conducted over a two-year period (2018-2020) and included 100 consecutively enrolled patients, admitted in the Cardiology Clinic of the "St. Spiridon" Emergency Clinical Hospital with DVT. The patients were divided into two categories, based on the presence of a neoplasia (25 patients with a neoplasia and 75 patients without a neoplasia).

For all of the patients, a detailed medical history, physical examination, blood pressure measurement, electrocardiogram recording, and echocardiography evaluation were performed. The diagnosis of DVT was established based on the clinical manifestation, biochemical parameters, and ultrasound imaging. A complete blood count was the initial step of the laboratory diagnosis, followed by D-dimers determination (a value over 5 microgram/ml was considered significant). Venous Doppler ultrasound of the affected lower limb re-

vealed the presence and location of the thrombus. Moreover, various paraclinical investigations aiming to identify the etiological tumor were performed: chest X-ray, abdominal ultrasound, uro-genital examination, PSA, and abdominal and pelvic computed-tomography.

Additionally, the patients were examined for potential complications or comorbidities which may interfere with an appropriate therapeutic management. By far, the most severe complication of DVT is represented by the pulmonary embolism (PE); consecutively, an electrocardiogram, an echocardiography, a chest X-ray, and a computed pulmonary angiography were performed. In order to identify the comorbidities, the anamnesis and personal files represented important aspects, as well as the usual paraclinical investigations (regarding the renal function, glycemia, lipid profile and urinalysis).

We excluded the patients with venous thrombosis located in the upper limbs, lower vena cava or mesenteric veins and the patients who refused to have a complete cardiological evaluation or who did not agree with some exploratory investigations.

Statistical analysis was carried out using the *SPSS 20.0* software and *Microsoft Excel 2010*. Data were expressed as mean ± standard deviation or number of cases with percentage, for continuous and ordinal variables. For all data, a two-sided p value < 0.05 was considered statistically significant.

RESULTS

For the age-based comparative analysis we divided our patients into three groups: aged < 50 years (19%), aged 50-70 years (51%) and aged > 70 years (30%). Their mean age was 60.52 years. Of the entire cohort, 52% were males. The statistical analysis included several parameters, either derived from the characteristics of DVT (location, mode of onset, first episode or reoccurrence) or a selection of some usual laboratory markers.

Concerning DVT location, 56% of the patients presented a thrombosis of the right lower limb, while 52% of the entire cohort suffered from PE, which is the main complication of DVT.

Anemia represents a negative prognosis factor in cancer patients; therefore the immediate assessment of serum hemoglobin is a step of utmost importance. The presence of anemia in 38% of the included patients (expressed as a Hb<12 g/dL for women and <13 g/dl for men) required additional investigations in order to highlight the DVT etiology and, eventually, the tumor existence. In our study, we observed no significant gender-based differences concerning the presence of anemia, PE or cancer (tab. I). Very interestingly, statistical analysis revealed a significant correlation between a Hb>14 g/dL and the diagnosis of PE (p = 0.05).

Various tumor locations were identified in the 25 patients with malignancy, colorectal cancer having the highest percentage (6 cases, 24%). In the second place came the cervical cancer, diagnosed in 5 patients (20%), followed by the lung cancer (4 cases, 16%) and, in equal percentage, by the brain, ovarian and breast cancer (2 cases each, 8%). A detailed report can be found in first figure.

Among female patients, cervical cancer was the most commonly documented neoplasia (35.7%), a situation distinct from male patients, who were predominantly suffering from colorectal neoplasm (4 cases, 36.4%). More information can be found in first table.

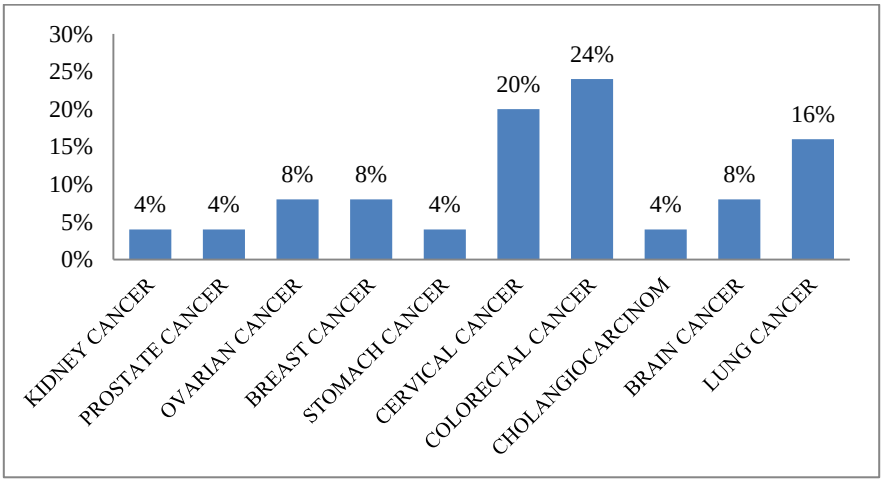

Fig. 1. The prevalence of different type of neoplasia in the study group

TABLE I

Gender-based differences between the presence of anemia, PE or cancer

Pathology	Men	Women	*p*
Pulmonary embolism	30 (57.7%)	22 (45.8%)	0.235
Anemia	23 (44.2%)	15 (31.3%)	0.181
Cancer	11 (21.2%)	14 (29.1%)	0.355

TABLE II

Cancer type distribution according to gender

Gender	Type of cancer	Number of cases	Percentage (%)
Female	Lung cancer	1	7.1 %
	Brain cancer	1	7.1 %
	Cervical cancer	5	35.7 %
	Colorectal cancer	2	14.3 %
	Stomach cancer	1	7.1 %
	Breast cancer	2	14.3 %
	Ovarian cancer	2	14.3 %
Male	Lung cancer	3	27.3 %
	Brain cancer	1	9.1 %
	Cholangiocarcinoma	1	9.1 %
	Colorectal cancer	4	36.4 %
	Prostate cancer	1	9.1 %
	Kidney cancer	1	9.1 %

The proportion of cancers discovered following the paraclinical investigations was 12%. In patients with newly diagnosed neoplasia, we observed the high incidence

of colorectal cancer (33.3%), lung cancer (25%), and cervical cancer (16.6%).

By performing the full extension assessment of the malignancy, we found that 40% of cancer patients were showing metastases. Although the proportion of cases with disseminated cancer was higher in the group with previously diagnosed cancer, it was not a statistically significant difference ($p = 0.288$).

Concerning the specific oncological treatments that could determine the occurrence of DVT in our study, 23.07% of cancer patients underwent surgery and radiotherapy, 30.07% - surgery and chemotherapy and 46.15% - surgery and both radio- and chemotherapy.

The established treatment strictly individualized according to the pre-existing comorbidities, the location of DVT, the presence of associated pulmonary embolism, hemodynamic status, hematological balance, the existence of a history of neoplasia and the presence of metastases followed one of these pathways: unfractionated heparin (65 cases), low molecular weight heparin (23 cases) and thrombolysis + subsequent heparin therapy (12 cases).

DVT of the popliteal vein was significantly associated with the need for urgent thrombolysis (performed exclusively in patients with DVT complicated with PE) (p=0.015). The effectiveness of the treatment was monitored during hospitalization and at discharge through venous doppler ultrasound, in order to assess the resumption of blood flow. Moreover, we recorded no fatalities during hospitalization or at the follow-up performed one month after discharge.

DISCUSSION

As we mentioned above, cancer patients are generally associated with a hypercoagulable state, as they usually have several associated abnormalities that are influencing each component of Virchow's triad (stasis of blood flow, endothelial injury, and hypercoagulability), thus leading to an enhanced prothrombotic activity. The cancer-associated thrombosis is a general term that covers a plethora of mechanisms and pathways involved in the imbalance between pro- and anticoagulant elements. It is noteworthy that the various data cited in literature regarding the prevalence or outcome of DVT in cancer patients are related to multiple variables (e.g., tumor site, stage, histological subtype, time since diagnosis, inclusion criteria and study design) (6). In the same manner, even if there is a systemic prothrombotic environment in all cancer types, the tumor location greatly influences the anatomical site of the thrombosis. Generally, DVT of the lower extremities or pelvic vein thrombosis are associated with colorectal cancer or other genito-urinary malignancies due to their proximity (8, 9) while a hepatocellular carcinoma determines particularly a portal or hepatic vein thrombosis (10), the latter can even migrate into the right cardiac chambers (11).

It is universally accepted that the incidence of DVT is not equivalent in different cancer types and, in fact, the rates of increased DVT in cancer patients may reflect the frequency of cancer within the general population and not necessarily some specific tumor-related prothrombotic effects. Nevertheless, multiple studies revealed that the highest incidence rates for DVT occur in certain adenocarcinomas of the lung and gastrointestinal tract (notably pancreas and colorectum) (6).

Usually, the cancer determines an up-regulation of the coagulation cascade. In

healthy individuals, the tissue factor (TF)- the initiator of the extrinsic clotting pathway- is expressed by sub-endothelial cells such as myocytes or interstitial cells. In many cancers, TF is abnormally expressed on tumoral cells' membrane, its presence being strongly correlated with a poor clinical outcome (high TNM stage, presence of metastases, decreased survivability) (9).

Very interestingly, TF expression not only induces prothrombotic effects *per se*, but it also accelerates cancer progression via clot formation, which are engulfing metastatic cells in the blood stream, allowing them to escape immune surveillance and to form circulatory islands of metastatic cells. In addition, TF upregulates intracellular signaling, acting via protease-activated receptor (PAR)-2, which is a promoter to key malignant processes including proliferation, migration, angiogenesis, and metastasis (9, 12). This mutual relationship rose the possibility to use the coagulation cascade as a potential therapeutic target in cancer patients. Multiple studies investigate the LMWH and direct oral anticoagulants (e.g., rivaroxaban) as well as direct TF inhibitors for their anticancer effects, showing beneficial effects on the survivability (13).

There is scarce data in literature concerning the overall effect of the gender in a cancer cohort and the potential risk of developing DVT. Some studies show that females are at a greater risk for VTE, while other authors failed to confirm this aspect (6, 14). The epidemiological data collected in our study regarding the etiology of DVT and gender distribution revealed similarities with other international studies, but also some particular aspects. Jara-Palomares *et al.* conducted one of the largest researches regarding paraneoplastic DVT, including 5864 patients. Of these, only 7.6% presented a neoplasm, a significantly lower percentage compared to our study. The same authors found that most common malignancies in men were lung cancer (25.9%) and prostate cancer (17.07%), while in women colorectal cancer (19.19%) and breast cancer were predominant (11.61%) (15). In contrast to these findings, in our study we observed a different distribution of cancer types according to sex, the colorectal cancer being the most prevalent among men, whilst cervical cancer was the leading etiology in the women. This pattern is multifactorial, being probably correlated with certain local specific risk factor, either hereditary, socio-economic, geographic, or dietary.

In a recent study, Rouzaud *et al.* found similar results to our research. Thus, the mean age was calculated to be 63.6 years, while the incidence of neoplasia was roughly the same in the two studies (29.6% vs. 25%) (16). Compared to our study, other data from literature have shown a lower incidence of neoplasia (9.71% and 7.5% respectively), but with a similar pattern of cancer distribution (17, 18).

A previous episode of DVT predicts well the occurrence of a new DVT episode. Cancer patients with a history of thrombosis are up to 7 times more prone to a DVT recurrence when compared to cancer patients without previous thrombotic events. Hettiarachchi *et al.* showed that 6.56% of patients had a recurrence of DVT, of which 7.96% had a neoplasm, a significantly lower percentage than those in our group (20.9%) (19). On the other hand, Prandoni *et al.* reported a significant association between recurrent thrombotic episodes and the subsequent diagnosis of malignancy (20).

Another interesting aspect from our study is regarding the significant association between the presence of metastases and the onset of PE (p= 0.048). This finding is in line with the results from several studies, which emphasize the association between advanced (stage III to IV) or metastatic cancer and the risk of PE (21, 22). This is due to the lower clot time and higher plasminogen activator inhibitor (PAI-1) level in patients with end-stage or metastatic cancer compared to those with stage I/II ($p < 0.05$), which could explain the higher risk of PE in these patients (23). Besides this, advanced stage cancers are associated with a poor-performance status in affected patients, the immobilization representing an important risk factor to the development of PE (6).

Anemia represents another well-known negative prognosis element not only in cancer patients (21), but also in patients with DVT (24). In our study, more than 30% of cases showed a variable degree of anemia, regardless of the presence of cancer. However, a very interesting issue was highlighted by Wang J *et al.* who demonstrated in a multivariate analysis that also an increased serum hemoglobin > 14 g/dL represents a high risk for the development of PE in lung cancer patients (OR, 2.10; 95% CI: 1.44-3.08, p = 0.03), explained by the stasis of blood flow due to higher blood viscosity (22). This controversial aspect was furtherly confirmed by the results from our study, a Hb>14 g/dL being significantly correlated with the occurrence of PE (p=0.05).

The proportion of DVT onset in our study was similar to that conducted by Carrier *et al.*: 67.4% had only DVT, 32.6% had only PE and 12.3% had a clinical presentation of both DVT and PE. However, the incidence of a neoplasm was only 3.9%, compared to 25% in our study. This may be due to a significantly larger number of included patients (854 cases) and the origin country of the study group- Canada- a developed country, where there is an improved accessibility and increased adherence to the medical system than in the North-Eastern Romania and, consequently, an increased probability for an early diagnosis (25).

This study presents certain limitations. First, the group size was relatively small – 100 patients with DVT, 25 of whom had a malignancy. In addition, DVT presence was assessed only in the lower extremities, without considering its occurrence in the upper limbs, pelvic veins, or inferior vena cava. Finally, some details regarding complete staging information of cancer were lacking due to various reasons, in addition to other aspects regarding its local extension or metastases that could influence the occurrence of DVT.

CONCLUSIONS

In conclusion, cancer patients represent a special category with a significantly higher risk of developing DVT as compared to non-cancer patients. This is due to a combination of tumor-related, treatment-related, and patient-related factors. Basically, the clinician should always maintain a certain degree of suspicion for thrombotic events when managing a cancer patient. In recent years there have been significant advances in the therapeutic approach of venous thrombosis and its most life-threatening complication-PE, by using the full spectrum antithrombotic medication, as stated in the last ESC guidelines (1).

The importance of the simultaneous cancer-DVT approach is confirmed by the

epidemiological data: the prevalence of paraneoplastic DVT in our group was 25%, with the proportion of incidentally discovered cancer in patients admitted for DVT being a worrying 12%. These results confirm the symbiotic relationship between these two pathologies, the specific incidence of DVT in cancer patients and vice versa being likely underestimated in the general population.

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NEWS

HIGH RIDING JUGULAR BULB - IMAGING EXAMINATION

High riding jugular bulb is a condition which appears when the roof of the jugular bulb extends superiorly in the petrous temporal bone than usual. The transverse level above which a jugular bulb is considered high riding has been variably defined as the following: floor of the internal auditory canal, 2 mm below the floor of the internal acoustic meatus, inferior rim of the round window niche or basal turn of the cochlea. Depending on the definition, the prevalence in different studies varies between 6 and 34%. The jugular vein bulb is almost always larger than the contralateral bulb. The associated enlargement of the jugular foramen can simulate a mass lesion on non-contrast-enhanced computed tomography (CT), although the diagnosis is made easily on contrast-enhanced CT or flow-sensitive magnetic resonance imaging (MRI). The sigmoid plate normally separates the jugular bulb from the hypotympanum of middle ear. This can only be appreciated on thin slice bone algorithm CT and is too thin to appreciate on MRI. If the sigmoid plate is deficient, the bulb protrudes into the middle ear and is known as a dehiscent jugular bulb, a common cause of a retrotympanic vascular mass. (Deng F, Gaillard F, *et al.* High riding jugular bulb. https://radiopaedia.org/articles/high-riding-jugular-bulb).

FUNCTIONAL HINDRANCE, COMORBID DEPRESSION AND EARLY DEVELOPMENT OF RESTRICTIVE LUNG DISEASE ARE LINKED TO ANKYLOSING SPONDYLITIS PATIENTS' WITHDRAWAL FROM THE WORKFORCE

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FUNCTIONAL HINDRANCE, COMORBID DEPRESSION AND EARLY DEVELOPMENT OF RESTRICTIVE LUNG DISEASE ARE LINKED TO ANKYLOSING SPONDYLITIS PATIENTS' WITHDRAWAL FROM THE WORKFORCE (Abstract): Ankylosing spondylitis (AS) is a debilitating inflammatory rheumatic condition that has been associated with young patients' early retirement from the workforce. The present study aimed to examine the characteristics of professionally active patients with AS who were applying for work disability. **Material and methods:** We conducted a prospective observational study on working age patients with AS. The inclusion criteria were confirmed AS diagnosis, professionally active status, and stable treatment for at least 6 months. We assessed functional impairment using BASFI (Bath Ankylosing Spondylitis Functional Index). **Results:** The study group included 114 patients with AS. Functional hindrance (based on BASFI scores) was a major factor leading to work disability. Work impairment was more frequent in male patients, yet females were the only ones to retire from the workforce in the first 5 years of illness. We obtained statistically significant results regarding the association between work disability and restrictive lung disease as well as depression. Moreover, the relationship with restrictive lung disease remained statistically significant in subjects under 40 years of age as well as in the subgroup with a disease duration under 10 years. **Conclusions:** Our results indicate that, together with functional impairment, comorbid depression and an early development of restrictive lung disease may impact the work status of AS patients. Further longitudinal studies are needed to define and monitor the factors that lead to work disability in AS.
Keywords: ANKYLOSING SPONDYLITIS, DISABILITY, RESTRICTIVE LUNG DISEASE, DEPRESSION, OBESITY.

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic condition that has been associated with a notable economic burden on the health system (1, 2). Importantly, the onset of AS most frequently occurs in young working-age patients, frequently causing them to retire due to disease-related manifestations (3).

As the disease progresses, patients may develop potentially severe extra-articular manifestations (uveitis, kidney disease, cardiopulmonary or neurological involvement), spinal restriction and deformity, all leading to significant physical disability and decreased quality of life (4-6). Together with pain and fatigue, different aspects of AS-related functional impairment have been shown to demonstrate strong relationships with absenteeism and early withdrawal from the workforce (5-9).

The present study aimed to analyze the features of professionally active patients with AS who were applying for work disability. Secondary objectives included the characterization of relationships between the factors contributing to subjects' functional hindrance and work abandonment.

MATERIAL AND METHODS

We conducted a prospective observational study on working-age AS subjects who were either hospitalized or were referred to our department as outpatients. The inclusion criteria were confirmed AS diagnosis (according to the New York criteria), working age according to the local legislation, professionally active status, and stable treatment for at least 6 months. We excluded patients who were not professionally active (patients who had never worked, were already receiving work disability benefits for either AS or comorbidities, or had retired due to other reasons), persons who worked under special conditions, as well as subjects who had incomplete data.

We assessed disease activity using BASDAI (Bath Ankylosing Spondylitis Disease Activity Index) and ASDAS scores (Ankylosing Spondylitis Disease Activity Score). We evaluated the patients' functional hindrance using BASFI (Bath Ankylosing Spondylitis Functional Index). Certain characteristics such as HLA-B*27 positivity (Human Leukocyte Antigen), disease duration, treatment, comorbidities, and BMI (Body Mass Index) were taken from the patients' files. Walking impairment was defined as the inability to walk without support.

The statistical analysis of the data was performed using IBM *SPSS Statistics version 23.0* for Windows. Depending on variable distribution, we presented the descriptive analysis as Mean ± SD (Standard Deviation) or Median : IQR (Interquartile Range). Crosstabulations were analyzed using X^2 or Fisher's exact test (where the proportion of expected frequencies less than 5 was more than 20%, we chose the Fisher test).When comparing numeric values between different subgroups, we performed either parametric (student t-test) or non-parametric tests (Mann-Whitney U test) according to variable distribution. The statistical significance threshold was set at $p \leq 0.05$.

RESULTS

We examined 167 consecutive patients who fulfilled the inclusion criteria for the present study. Following the elimination of subjects who met the exclusion criteria, the final group consisted of 114 patients aged between 23 and 56 years with a mean disease duration of 14.88 years, male subject being more numerous than females (M:F=2.7:1) (tab. I).

More than half of the study group applied for work disability (60.5%), whereas 39.5% chose to remain professionally active. Work impairment was significantly more prevalent in male patients (67.5% *vs.* 41.9%; X^2, p=0.013) and those residing in rural areas (72.6% *vs.* 46.2%; X^2, p=0.004). We found notable associations between work disability and disease duration as well

as age (t-test; p=0.004, p<0.001). Nevertheless, age and duration of symptoms did not vary significantly according to gender (t-test; p=0.123, p=0.143) or the area of residence (t-test; p=0.118, p=0.891).

TABLE I

Characteristics of the study group

Parameter	Characteristics Number (%); Minimum-Maximum (Mean±SD/Median: IQR)		
Age (years)	23-56 (46.82±7.51)		
Gender	Male	83 (72.8%)	
	Female	31 (27.2%)	
Area of residence	Urban	52 (45.6%)	
	Rural	62 (54.4%)	
HLA-B*27	Positive	109 (95.6%)	
	Negative	5 (4.4%)	
Disease duration	2-30 (14.88±5.95)		
Professional activity	Applying for work disability	69 (60.5%)	
	Not applying for work disability	45 (39.5%)	
Smoking	Smokers	29 (25.4%)	
	Non-smokers	85 (74.6%)	
BMI (kg/m^2)	16.61-38.05 (27.02±4.79)		
Comorbidities and disease-related complications	Uveitis	8 (7.0%)	
	Chronic kidney disease	4 (3.5%)	
	Depression	10 (8.8%)	
	Diabetes mellitus	9 (7.9%)	
	Cardiovascular involvement	Arterial hypertension	33 (28.9%)
		Ischemic heart disease	1 (0.9%)
		Heart failure	2 (1.8%)
		Rhythm/conduction abnormalities	1 (0.9%)
	Respiratory involvement	Pulmonary fibrosis	3 (2.6%)
		Restrictive lung disease	47 (41.2%)
	Digestive involvement	Gastritis/Ulcer	12 (0.5%)
		Crohn's disease	1 (0.9%)
CRP (mg/l)	0.10-123.30 (15.21±22.35)		
ESR (mm/1h)	2.00-107.00 (24.51±18.85)		
BASDAI	0.40-8.40 (3.55:1.48-6.1)		
ASDAS-CRP	1.00-6.00 (2.75±1.19)		
ASDAS-ESR	0.77-5.55 (2.69±1.25)		
BASFI	0.50-9.60 (5.25:2.40-7.1)		
Walking impairment	2 (1.8%)		
Treatment	NSAIDs	69 (79.3%)	
	Sulfasalazine	12 (10.5%)	
	Biologics	Total	46 (40.3%)
		TNF inhibitors	42 (36.8%)
		IL-17 inhibitor	4 (3.5%)

Most patients had tested positive for HLA-B*27 (95.6%) (tab. I). However, HLA-B*27 positivity, although more prevalent, was not significantly associated with work disability in our study group (61.5% *vs.* 40%; Fisher's exact test, p=0.382).

ESR (Erythrocyte Sedimentation Rate) and CRP (C-reactive protein) levels, ASDAS-ESR as well as ASDAS-CRP did not differ significantly in patients with work impairment compared to those who remained professionally active (t-test; p=0.293, p=0.206, p=0.995, p=0.927).

Restrictive lung disease (78.7% *vs.* 47.8%) and depression (90% *vs.* 57.7%) were significantly associated with work impairment (X^2, p=0.001 and p=0.046), while uveitis, diabetes, renal and cardiovascular manifestations did not show a notable relationship with disability (p>0.05). Work impairment was also more likely to be found in the subgroup presenting with gastritis and gastroduodenal ulcers (90% *vs.* 57.7%; X^2, p=0.003).

Whereas BASDAI values did not vary significantly with respect to work disability (Mann-Whitney, p=0.467), we found significantly higher BASFI scores (Mann-Whitney, p<0.001) in patients who expressed the intent to retire from the workforce (6.3:3.05-7.45) compared to professionally active individuals (3.1:1.90-5.70) (fig. 1).

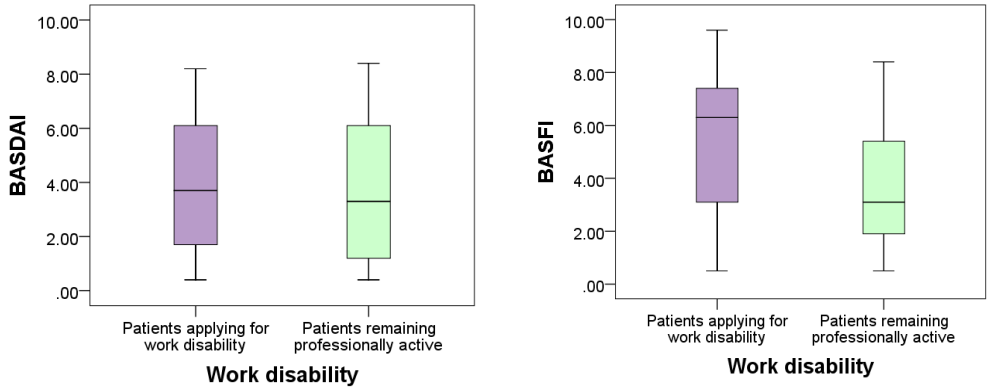

Fig. 1. BASDAI and BASFI values with respect to work disability.

Certain actions such as looking over the shoulder, reaching up to high shelves and picking up an object from the floor were found to be impossible (scored as 10) in patients with work disability more often than in professionally active subjects (X^2, p=0.034, p=0.042 and p=0.005).

Total BASFI scores over 5 were more likely to be noted in the work impairment subgroup (75.9% *vs.* 44.6%; X^2, p=0.001). Similarly, scores higher than 5 were found more frequently in the work disability category with respect to reduced mobility in the cervical spine (78.8% *vs.* 35.4%; X^2, p=0.001), difficulty picking up an object off the floor (75% *vs.* 46.6%; X^2, p=0.002) and putting on socks (75.9% *vs.* 44.6%; X^2, p=0.001), as well as greater effort performing physically demanding actions (73.5% *vs.* 41.3%; X^2, p=0.006) or common daily activities (70.6% *vs.* 45.7%; X^2, p=0.008), climbing stairs (70% *vs.* 50%; X^2, p=0.029), reaching up to high shelves (75% *vs.* 44.4%; X^2, p=0.001), getting up from the floor (70.8% *vs.* 46.9%; X^2, p=0.010), and standing up from an armless chair

without help (76.7% *vs.* 40.7%; X^2, p=0.006). While scores over 5 remained more prevalent in subjects applying for work disability (67.9% *vs.* 54.1%), results did not reach statistical significance with regard to standing unsupported (Fisher's exact test, p=0.179). However, scores higher than 4 were more frequent in the subgroup with work impairment (69.2% *vs.* 49%; X^2, p=0.029).

Young patients (≤ 40 years old) who applied for work disability did not demonstrate higher BASFI scores (total scores as well as for each question) compared to professionally active patients in the same age group (Mann-Whitney, p>0.05). Nevertheless, the presence of restrictive lung disease as well as depression requiring chronic treatment showed notable associations with work impairment (p<0.001 and p=0.030). In this age category, we did not identify a connection between gender and work disability (Fisher's exact test, p=0.376), despite the latter being more frequent in female patients (44.4% *vs.* 23.1%).

In the subgroup with a disease duration under 5 years, work disability was only found in female patients. However, these results were not statistically significant (Fisher's exact test, p=0.464). The presence of restrictive lung disease was significantly associated with work impairment in patients with a disease duration under 10 years (100% *vs.* 26.3%; X^2, p<0.001) but not in those displaying symptoms for over a decade. In these cases, BASFI remained the most notable factor linked to the patients' withdrawal from the workforce (6.3:3-7.57 *vs.* 3.7:1.9-6.2; Mann-Whitney, p=0.008).

Neither underweight nor excess weight were significantly more frequent in patients with disability. However, obese subjects demonstrated increased median BASFI scores compared to eutrophic and underweight individuals (6.15:4.37-7.17 *vs.* 4.05:2.00-6.95; Mann-Whitney, p=0.022). Moreover, these patients had higher difficulty bending down to put on socks (6:4-8.25 *vs.* 3:2-7; Mann-Whitney, p=0.002) or to pick up an object of the ground (6:4-8 *vs.* 4:2-7.75; Mann-Whitney, p=0.011), standing up from an armless chair (4.5:3-6 *vs.* 3:1-6; Mann-Whitney, p=0.038), getting up from the floor without support (6.5:4.75-8 *vs.* 4.5:2-8; Mann-Whitney, p=0.022), as well as greater effort performing daily activities (6:4.75-7 *vs.* 5:2.25-7; Mann-Whitney, p=0.041) and physically demanding actions (6:5-8 *vs.* 5:2-7; Mann-Whitney, p=0.026).

Whereas NSAIDs and Sulfasalazine did not demonstrate a notable connection to work disability, patients under biological therapy were more likely to retire (71.7% *vs.* 52.9%; X^2, p=0.044). Moreover, there was no association with older age in this subgroup (47.37±6.88 *vs.* 46.87±8.44 years; t-test, p=0.728). Although the mean disease duration did not differ between patients with work disability compared to the rest of the subjects in the group treated with biologics (15.30±5.77 years *vs.* 14.59±6.07 years; t-test, p=0.526), individuals who expressed their intent to retire were more likely to exhibit a disease duration of over 10 years (78.4% *vs.* 44.4%; X^2, p=0.043) as well as restrictive lung disease (94.1% *vs.* 58.6%, X^2, p=0.010).

Patient-reported fatigue was not significantly associated with the retirement from the workforce in our study group irrespective of age, gender, or disease duration (Mann-Whitney, p>0.05). Nonetheless, work disability was associated with more severe neck, back and hip pain in female patients (7:3.5-8 in women with work disa-

bility *vs.* 2:1-7 in women who remained professionally active; Mann-Whitney, p=0.028) and in those with a disease duration under 10 years (6:4-8 in patients with work disability *vs.* 2:1-4.25 in persons in the same subgroup who remained professionally active; Mann-Whitney, p=0.009).

DISCUSSION

AS remains a debilitating disease despite the recent advancements in therapeutic management. Importantly, BASFI continues to be a reliable indicator of functional hindrance and correlates with absenteeism and early retirement from the workforce in AS (10). Similar to our findings, previously published research states that older age and longer disease duration may impact both patient-reported functional impairment as well as their work status (3, 11, 12).

Our results showed a statistically significant relationship between work disability and male gender, the latter demonstrating a clear predominance in the study group. The currently available literature reports discrepant findings regarding the link between gender and work capacity in AS (13). A Swedish study indicated that the prevalence of work impairment could be reduced in women with AS (14). Moreover, it has been shown that female patients may exhibit higher rates of work abandonment during the first years of illness (15). We obtained similar results in this respect, only women with a disease duration under 5 years applying for work disability.

In AS, restrictive lung disease can influence certain functional parameters as well as the patients' quality of life (4, 16). We obtained statistically significant results regarding the association between restrictive lung disease and work disability. Moreover, this relationship remained statistically significant in subjects under 40 years of age as well as in the subgroup with a shorter disease duration (under 10 years). In contrast, restrictive lung disease was not associated with work impairment the group exhibiting symptoms for more than a decade where work abandonment was rather correlated with impaired functionality.

The relationship between depression and absenteeism in AS has been studied previously, research reporting discrepant results in this respect (3,17-19). Depression may be frequent and has been correlated with higher rates of work abandonment in AS (20-22). A recent study found a significant association between absenteeism and the mental component of SF-36 (the 36-item Short Form health survey) (19). Comorbid depression was linked to work disability in our study group including in patients under 40 years of age. These data further support the inclusion of psychologists and psychiatrists in the multidisciplinary teams that manage the care of AS patients (17-19).

Pain, fatigue and disease activity have been shown to impact AS patients' presenteeism, absenteeism and early retirement (5-7). Disease activity and fatigue were not associated with work disability in our group, while back pain was only linked to work impairment in women and in persons with a shorter disease duration.

Contrary to the results described in other studies that highlight the improvement of functionality under biological therapy, we obtained a high rate of work disability among these patients compared to those receiving conventional treatment (23-27). Nonetheless, the subjects who were treated with biologics and expressed their intent to retire were more likely to exhibit a disease duration of over 10 years and restrictive lung disease compared to their profession-

ally active counterparts, thus explaining the discrepancy. Additionally, the differences between countries regarding the inclusion criteria for biological therapy (BASDAI and ASDAS scores, inflammatory marker levels and other factors) may result in patients starting the treatment either earlier or later in evolution.

Obesity has been shown to be more frequent in persons with axial SpA compared to the general population and may influence clinical outcomes, particularly patients' response to anti-TNF therapy (28, 29). Interestingly, it has been stated that excess weight may play a role in the development of structural damage and radiographic progression. Moreover, the expression of inflammatory adipokines is increased in obese patients and it has been suggested that leptin and resistin promote new bone formation in AS (30-33). The currently available literature reports inconsistent results regarding the link between obesity and the burden of symptoms, BASFI values and work disability in AS (34, 35). Whereas body composition was not significantly associated with work abandonment, obesity was associated with functional disability in our study group, impacting such actions as bending down, standing up from an armless chair and getting up from the floor. Moreover, obese patients reported having higher difficulty performing daily activities and physically demanding actions, Thus, albeit without a direct relationship between obesity and early retirement, our results indicate that AS patients' functional impairment may be aggravated by excess weight.

CONCLUSIONS

Work impairment continues to be a major issue in AS. We aimed to analyze the characteristics of professionally active patients who were applying for work disability, our results indicating that functional hindrance is one of the main contributors to the patients' early retirement in these cases. HLA-*B27 positivity, fatigue, disease activity, and most comorbidities or complications did not exhibit significant relationships with work impairment in our study cohort. Nonetheless, restrictive lung disease and comorbid depression were significantly correlated with AS patients' work abandonment. Moreover, the relationship with restrictive lung disease remained statistically significant in subjects under 40 years of age as well as in the subgroup with a disease duration under 10 years. Additionally, obesity has been found to contribute to the aggravation of functional impairment in working age AS patients. Further longitudinal studies are needed to define and monitor the factors that may lead to AS patients' functional hindrance and work disability.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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ANTIPLATELET THERAPY AND INFLAMMATORY STATUS ASSOCIATED WITH INTRA-STENT RESTENOSIS AFTER PERCUTANEOUS CORONARY INTERVENTION

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ANTIPLATELET THERAPY AND INFLAMMATORY STATUS ASSOCIATED WITH INTRA-STENT RESTENOSIS AFTER PERCUTANEOUS CORONARY INTERVENTION (Abstract): The present study analyzed the existence and quantification of inflammatory status and antiplatelet medication associated with intra-stent restenosis (ISR) in coronary artery disease (CAD) patients after percutaneous coronary intervention (PCI). **Material and methods**: an observational cross-sectional study was conducted in a high-volume PCI centre over a 2-year period. A total of 235 consecutive patients diagnosed with angina or acute coronary syndrome treated by PCI were included in the study. Diagnosis of ISR was documented by coronary angiography and the patients were divided into groups (with and without ISR). All patients underwent clinical and laboratory examination, the administration of antiplatelet medication and the type of drug administered to each patient. **Results**: The mean time until ISR was 33 ± 34 weeks. The lower use of single or dual antiplatelet therapy was associated with increased risk of ISR. Aspirin alone [risk ratio (RR)=1.13; 95% confidence interval (95% CI): 0.882-1.462], clopidogrel alone (RR = 1.41; 95% CI: 0.879- 0.323), ticagrelor alone (RR = 1.98; 95% CI: 1.746-2.252) or dual antiplatelet therapy (DAPT) (RR = 0.82; 95% CI: 0.637-1.065) had a significant role in estimating the risk for ISR. Bare-metal stents (BMS) presented an associated risk of ISR higher as compared to drug-eluting stents (DES) (0.88 *vs.* 0.74). **Conclusions**: Inflammatory status is associated with ISR; the type of stent used, time to ISR and antiplatelet medication were found to be associated with ISR.
Keywords: ANTIPLATELET THERAPY, INTRASTENT RESTENOSIS, PERCUTANEOUS CORONARY INTERVENTION.

Cardiovascular diseases (CVD) remain the leading cause of death worldwide and atherosclerotic coronary artery disease (CAD) are mainly involved in this patholo-

gy (1). After percutaneous coronary intervention (PCI) patients are still at risk of developing new stenosis and in some cases intra-stent restenosis (ISR). The introduction of coronary drug-eluting stents (DES) has substantially decreased the incidence and prevalence of ISR, but it is still encountered in 5 to 15% of PCI procedures (2-5). The increasing use of percutaneous revascularization in complex and long lesions has likely contributed to the continued episodes of restenosis and the need for repeated interventions.

Despite the safety and efficacy of DES, bare-metal stents (BMS) are still used (6). The incidence of ISR is also significant because inflammatory responses after PCI lead to abnormal neointimal healing and generate an increased risk for unfavorable outcomes (7, 8). Such data suggest that the type of stent is only one factor to consider when searching for additional promoters of ISR. Thus, the aim of the current study is to analyze the risks of ISR associated with the administration of antiplatelet medication after PCI.

MATERIAL AND METHODS

Patient selection and study design: An observational, cross-sectional, over a 2-year period study was conducted and included 235 patients diagnosed with angina pectoris or acute coronary syndrome (myocardial infarction with or without ST-segment elevation) with intra-stent restenosis documented by coronary angiography hospitalized in the Cardiology Clinic of the "Prof. Dr. George I. M. Georgescu" Institute of Cardiovascular Diseases Iasi. Our population was divided into 2 groups: the ISR group (119 patients) that presented ISR documented by coronary angiography (> 50% stenosis of a previously stented segment) and the control group (116 patients) without angiographic ISR, but with different other culprit lesions or no significant angiographic stenosis.

The selection of patients was made on the following inclusion criteria: age over 18 years; diagnosis of angina pectoris/acute myocardial infarction with or without ST-segment elevation treated by PCI with stent implantation at the time of study; free informed consent. The exclusion criteria were refusal of treatment; failure to report or abandon treatment; patients who belonged to vulnerable categories, such as coma patients or pregnant women. The study was approved by the "Grigore T. Popa" University of Medicine and Pharmacy Iasi Research Ethics Committee and all subjects had initially agreed and signed an informed consent to take part in this study.

Clinical and paraclinical characteristics: To prevent the onset of ISR after PCI, all patients underwent antiplatelet therapy according to the guidelines. Initially, all stent patients received two antiplatelet agents. The duration of double antiplatelet treatment varied according to diagnosis at the time of initial presentation; type of DES/BMS implanted stent; the presence of medical conditions or special situations that required individualization of the duration of antiplatelet treatment (hemorrhagic complications, combination of oral anticoagulant treatment, surgery that required stopping one or even both antiplatelet drugs). At the end of the treatment period in double combination, the patients most frequently received the recommendation to continue with a single antiplatelet agent, aspirin being the most frequently used. Regarding the double combination, there were patients who received aspirin with ticagrelor and aspirin with clopidogrel. Most commonly, however, the combination of aspirin and clopidogrel has been used.

Diagnosis of ISR was documented by coronary angiography in patients with suggestive coronary symptoms and ischemic changes in non-invasive or invasive paraclinical investigations. Depending on the drug used, a significant difference in the rate of intra-stent restenosis was observed.

Statistical analysis: Data analysis was performed using *SPSS version 23.0* (Statistical Package for the Social Sciences; IBM Corp.). Comparison between the 2 groups of patients was performed using *Chi-square* test for the categorical data and one-way *ANOVA* and *Student's t-test* for continuous data. To estimate the strength of the association between treatments and outcome (with or without ISR) relative risk (RR) with 95% confidence interval (CI) was used in the statistical analysis of the data. A p value < 0.05 was considered statistically significant.

RESULTS

The mean age of the patients was 61.84 ± 11.12 years (63.08 years, ISR group; 60.57 years, non-ISR group; p=0.084). The male was 83(70%) in ISR group and 81(70%) in non-ISR group(p<0.989) and the repartition of female was 36(30%) in ISR group and 81(70%) in the other group(p<0.522). The CRP > 2 mg/dL was significantly higher in the ISR group: 94(87%) *vs*. 83(77.6%, p<0.05).

In our study the antiplatelet treatment was composed of single aspirin, single ticagrelor, single clopidogrel alone and double therapy (DAPT). As stated in first table, only aspirin was administered in 51.3% of ISR group and 44.8% of non-ISR group (p = 0.324). In the ISR group, regarding DES patients, aspirin was given in 53.1% and in BMS patients in 50.6%; similar percentages were identified in the non-ISR group (41.7% in DES patients and 48.2% in BMS patients). Ticagrelor and clopidogrel were not so frequently administered, with 0.8% in ISR group and 0% in non-ISR group for ticagrelor (p = 0.322) and 4.2% in ISR group and 1.7% in the non-ISR group for clopidogrel (p = 0.264). Double combination was used in 43.7% of ISR patients and 53.4% of non-ISR patients (p = 0.135). In the ISR group, regarding DES patients, double combination was given in 43.8% while in BMS patients in 43.7%; higher percentages were identified in the non-ISR group (55.0% in DES patients and 51.8% in BMS patients).

Furthermore, our study aimed to identify if there is a different relative risk (RR) regarding ISR depending on the drug and type of stent used (tab. II). Therefore, single aspirin regime has a relatively 1.13-fold increased risk of ISR (RR = 1.13; 95% CI: 0.882-1.462). In patients with DES the risk of restenosis is 1.34 times higher (RR = 1.34; 95% CI: 0.771-2.362) while in patients with BMS is 1.03 (RR = 1.03; 95% CI: 0.798-1.350). Aspirin was part of the adjuvant treatment in both groups, with a higher proportion in ISR group (51.3%) especially after the implantation of DES (53.1%). In our study, ticagrelor treatment has been used only in patients with DES, identifying a risk of ISR approximately 3-fold higher (RR = 2.93; 95% CI: 2.206-3.907). Clopidogrel has been used in both groups in varying proportions and is associated with a 1.68-fold increased risk of ISR in patients with BMS (RR = 1.68; 95% CI: 1.466-1.932) and 55% higher in patients with DES (RR = 1.55; 95% CI: 1.331-1.809).

In patients with BMS, double antiplatelet therapy is noted as a protective factor (RR = 0.88; 95% CI: 0.673-1.150), a result that has been also noticed in patients with DES (RR = 0.73; 95% CI: 0.423-1.312). Overall, the results indicate that double antiplatelet combination involves a lower risk of ISR (RR = 0.82; 95% CI: 0.637-1.065).

TABLE I

Statistical description of the frequency of antiplatelet therapy administrated in the two population groups depending on the type of stent

Antiplatelet/stent type	ISR group		Non-ISR group		p X^2 test
	n/N	%	n/N	%	
Aspirin					
Administered at:	61/119	51.3	52/116	44.8	0.324
DES	17/32	53.1	25/60	41.7	0.293
BMS	44/87	50.6	27/56	48.2	0.783
Ticagrelor					
Administered at:	1/119	0.8	0/116	0	0.322
DES	1/32	3.1	0/60	0	0.169
BMS	-	-	-	-	-
Clopidogrel					
Administered at:	5/119	4.2	2/116	1.7	0.264
DES	0/32	0	2/60	3.3	0.296
BMS	5/87	5.7	0/56	0	0.068
Double combination					
Administered at:	52/119	43.7	62/116	53.4	0.135
DES	14/32	43.8	33/60	55.0	0.304
BMS	38/87	43.7	29/56	51.8	0.343

ISR, intra-stent restenosis; DES, drug-eluting stents; BMS, bare-metal stents

TABLE II

Relative risk and confidence intervals corresponding to the antiplatelet treatment associated with ISR (depending on the type of the stent used)

Antiplatelet/stent type	ISR group / n/N	Relative Risk	95% Confidence Interval
Aspirin			
Restenosis	119/235	1,13	0.882-1.462
DES	42/92	1.34	0.771-2.362
BMS	71/143	1.03	0.798-1.350
Ticagrelor			
Restenosis	119/235	1.98	1.746-2.252
DES	1/92	2.93	2.206-3.907
BMS	-	-	-
Clopidogrel			
Restenosis	119/235	1.42	0.879-2.323
DES	2/92	1.55	1.331-1.809
BMS	5/143	1.68	1.466-1.932
Double combination			
Restenosis	119/235	0.82	0.637-1.065
DES	47/92	0.74	0.423-1.312
BMS	67/143	0.88	0.673-1.150

ISR, intra-stent restenosis; DES, drug-eluting stents; BMS, bare-metal stents

Assessing the presence of restenosis events in patients that went PCI and the type of drugs used in our patients, BMS implantation was directly linked to restenosis events, considering the time related to ISR, in comparison with DES implantation, regardless of the type of antiplatelet therapy. Furthermore, late ISR in DES patients were observed only in those patients with a double combination of drugs (figs. 1-4). Moreover, the mean time until ISR was 33 ± 34 weeks.

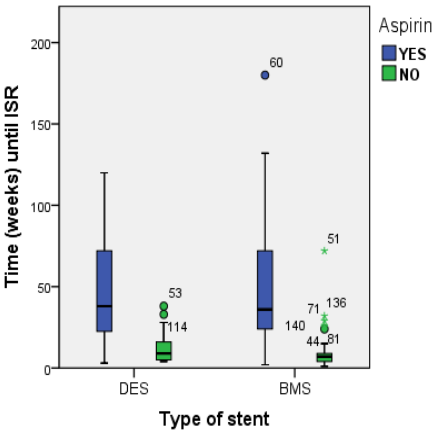

Fig. 1. Time until ISR depending on type of stent and single aspirin

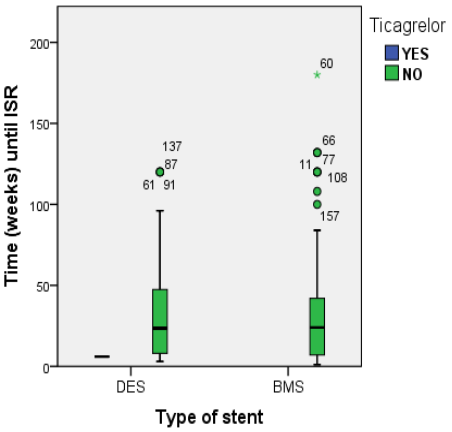

Fig. 2. Time until ISR depending on type of stent and single ticagrelor

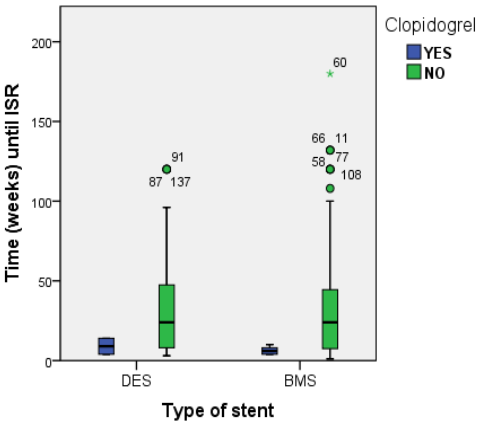

Fig. 3. Time until ISR depending on type of stent and single clopidogrel

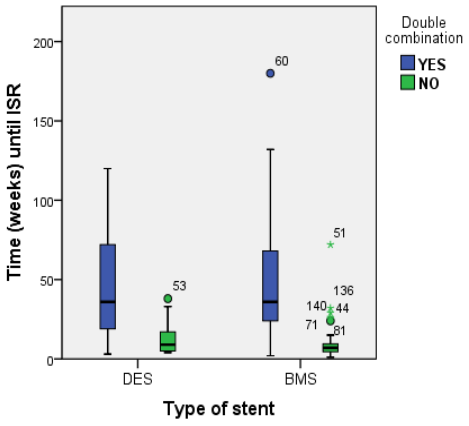

Fig. 4. Time until ISR depending on type of stent and double antiplatelet therapy

DISCUSSION

The main finding of the present study is that ISR in patients following PCI is associated with the type of antiplatelet medication and the type of stent. Patients were screened for clinical and paraclinical characteristics, coronary-lesion related factors and stent-related factors. Studies are con-

troversial concerning the risk of ISR in patients after PCI, thus further research in larger cohort studies is still encouraged.

An important aspect derived from our retrospective study is related to the time until ISR appear in our cohort of patients. The mean time until ISR was 33 ± 34 weeks (approximate 231 ± 238 days). In practice, because of differing pathophysiology and risk factors it is useful to dichotomize events into two major categories (9): early ISR is defined as thrombosis within the first 30 days after PCI and late ISR occurs beyond 30 days. According to RESTART investigators (9), early ISR is more common, accounting for 50 to 70% of all cases depending on the overall time frame of reference (10). In our study, according to this classification, late ISR was more common that early ISR meaning that other risk factors are associated with ISR (11-14). Moreover, in our cohort, serum C-reactive protein levels are statistically significant for ISR.

Recently, important progress has been made to reduce the incidence of ISR. Large-scale registries show that with contemporary antithrombotic therapies and modern generation of DES stents the rate of early ISR is low (15). Furthermore, a recent systematic review of randomized trials with DES reporting results at 9–12 months has shown a median incidence of ISR of 0.61% (16). The advantages of DES over BMS in preventing ISR have been proven by literature and are also confirmed in our study. A systematic review concerning the treatment of coronary ISR confirmed a higher rate of ISR after BMS implantation (20-35%) *vs.* DES implantation (5-10%) (17). In addition, Zbinden *et al.* showed a higher risk of ISR in segments with a BMS compared to segments with a DES (5.4% *vs.* 0.76% after 2 years) in 2,278 patients (18). Moreover, randomized trial data in the setting of stent implantation for acute myocardial infarction suggests the reduction of early ST segment with polymer coated *vs.* BMS (19). In our analysis, BMS presented an increased risk of ISR as compared to DES (RR 0.88 *vs*. 0.74 in case of dual antiplatelet therapy).

The key for ISR prevention is the prescription of dual antiplatelet therapy (DAPT) after PCI with an appropriate duration. In patients presenting with acute coronary syndrome, the cardiovascular risk remains substantially elevated beyond the first year, even if successful revascularization has been achieved (20). In this setting, intensified antiplatelet therapy on top of aspirin has been shown to be an effective therapeutic strategy to prevent recurrent ischemic events. In our study DAPT was the most administered scheme in non-ISR group (53.4%) and second scheme (43.7%) in ISR group. Also, the relative risk of ISR was the lowest in DAPT group (0.82; 95% CI: 0.637-1.065) in comparison with aspirin alone (RR = 1.13; 95% CI: 0.882-1.462), clopidogrel alone (RR = 1.41; 95% CI: 0.879-2.323) or ticagrelor alone (RR = 1.98; 95% CI: 1.746-2.252). Single aspirin regime has a relatively 1.13-fold increased risk of ISR. This can be explained by the fact that patients were late diagnosed with ISR when patients had already switched from the DAPT regimen to a single antiplatelet drug. According to the DAPT trial (21), a DAPT regimen is more effective than aspirin monotherapy in reducing the incidence of very late stent thrombosis. Moreover, it suggests that DAPT mitigates the risk of stent thrombosis across the whole spectrum from acute to very late events. From another point of view, although DAPT can reduce the risk of stent thrombosis (22), it had no impact on the

ISR risk, thus raising a question over the role of initial thrombus formation in the subsequent development of neointima (23). The lack of relationship on clinical or imaging endpoints between platelet inhibition and neointima formation was subsequently confirmed with other antiplatelet agents (24). The newer P2Y12 inhibitors, prasugrel and ticagrelor, have proven more efficacious than clopidogrel in the prevention of early and late stent thrombosis in patients with ACS, even with higher bleeding liability (25, 26). In our study ticagrelor was not very frequently used due to local socio-economic reasons at the study time.

Our retrospective study has several limitations. Firstly, the small sample size may overestimate the magnitude of an association or even induce false-positive results. Secondly, our patients were not divided into acute or chronic angina subgroups. Also, the study reported an increased number of BMS implantations, and the socio-economic factor had a high influence in the treatment decision on the baseline of CAD.

CONCLUSIONS

Inflammatory status is associated with ISR. Further on, the type of stent used, time to ISR, but especially antiplatelet medication was found to be associated, to varying degrees, with ISR in patients following PCI. Therefore, a close follow-up should be targeted in these patients.

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CONFLICT OF INTEREST AND FUNDING

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VARIATIONS OF THE EFFICIENCY OF BIOLOGICAL THERAPY IN VULGAR PSORIASIS

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VARIATIONS OF THE EFFICIENCY OF BIOLOGICAL THERAPY IN VULGAR PSORIASIS (Abstract): With the rapid understanding of the pathogenesis of psoriasis, the number of treatment options available for moderate to severe form has also increased. In this paper, we focus on the efficacy and safety profile of biological products in patients with psoriasis. In addition, we discuss the impact of biologics on comorbidities caused by psoriasis, considering several factors associated with the patient and the condition, and aim to review therapeutic opportunities in terms of biologic therapies and their variety. **Material and methods:** A retrospective study that has as a sample a group of 113 patients diagnosed with psoriasis vulgaris, under investigation by the Dermato-Venerology Clinic at the "Sf. Spiridon" County Clinical Emergency Hospital Iași. All these patients are under biological therapy and were admitted to the clinic between 2017-2021. **Results:** Of the 113 patients proposed for the study, 63% were male (n = 71) and 37% female (n = 42). Of the total sample, the preponderance of the age range was that of the '> 40 years' range with a total of 85% (n = 96) consisting of 61 men and 35 women (p = 0.019, statistically significant result). **Conclusions:** With the introduction of new biological substances and the discontinuation of others, an update of biological substances in psoriasis is delayed. Understanding the benefits, risks and monitoring of patients with biologic therapies is becoming increasingly important for the dermatologist**.** **Keywords:** PSORIASIS, BIOLOGICAL THERAPY, THERAPEUTIC VARIETY.

Psoriasis is a chronic condition with several systemic and immune manifestations that affect over 125 million people worldwide (1). It is associated with multiple comorbidities, which may complicate the management of the condition for these patients (2, 3). With the rapid understanding of the pathogenesis of psoriasis, the number of treatment options available for moderate to severe form has also increased. Moreover, factors related to the patient and pathology must be considered when initiating biological treatment in clinical practice (3, 4). A variety of biological agents for the treatment of psoriasis are available today. However, characteristics such as rapid onset, long-term efficacy, safety profile and effects on comorbidities are different (5). A

better understanding of these characteristics leads to the correct individualized choice for the patient, resulting in greater persistence, longer drug survival, greater patient satisfaction and minimizing the impact of the disease (6). Randomized clinical trials provide important information on the safety and efficacy of biological agents, however, the data in these studies have no practical implications for personalized therapy for patients with psoriasis (7, 8).

Psoriatic arthritis is a progressive inflammatory arthritis present in up to 30% of patients with psoriasis, with a higher risk of more extensive psoriasis. Where possible, a single biologic should be used in the treatment of both psoriasis and psoriatic arthritis. Most of the currently available biological agents in psoriasis have been approved for the treatment of psoriatic arthritis with 2 exceptions: guselkumab and brodalumab (8, 9).

In this paper, we focus on the efficacy and safety profile of biologics in patients with psoriasis, including plaque psoriasis and psoriatic arthritis. In addition, we discuss the impact of biologics on comorbidities caused by psoriasis, considering several factors associated with the patient and the condition, and aim to review therapeutic opportunities in terms of biologic therapies and their variety.

MATERIAL AND METHODS

A retrospective study that has as a sample a group of 113 patients diagnosed with moderate, moderate-severe and severe form of psoriasis vulgaris, under investigation by the Dermato-Venerology Clinic at the “Sf. Spiridon” County Clinical Emergency Hospital Iași. All these patients are under biological therapy and were admitted to the clinic between 01/01/2017-31/05/2021. In establishing their profile, the following were taken into account: gender, age, which was subsequently divided into age ranges, associated pathologies, special conditions of psoriasis, current biological therapy and year of initiation, PASI score, DLQI at the initiation of current biological therapy and to the re-evaluation of the scores at present, the previous biological therapies (therapeutic switches), the number of their presentations per clinic (both day and continuous hospitalizations) in the interval proposed for the study. All patients included in the study signed their consent to the processing of personal data.

The modification of the PASI and DLQI score was used as a clinical marker of disease progression, the comparative values between the initial and current scores establishing the activity of the disease and the patient's response to treatment. Statistical analysis of patient data was processed in *SPSS Statistics version 17* and *Microsoft Excel*. In *SPSS*, t-test and χ^2 were used, as follows: $p < 0.05$, the statistical link is significant (S, 95% confidence); $p < 0.01$, the statistical link is significant (S, 99% confidence); $p < 0.001$, the statistical link is highly significant (HS, 99.9% confidence); $p > 0.05$, the statistical link is insignificant (NS).

RESULTS

A total of 113 clinically diagnosed and histopathologicaly confirmed patients with plaque psoriasis were enrolled. The gender distribution includes 42 female patients (37%) and 71 male patients (63%). From the total sample, it appears that with age, the cases of psoriasis increase, the preponderance of the age range being ’over 40 years' with a total of 85% (n = 96) consist-

ing of 61 men and 35 women. (p = 0.019, statistically significant result).

As a place of origin, 62% (n = 70) with 57% men and 43% women belong to the urban area and 38% are from the rural area (n = 43) with 72% men and 28% women. The number of men is higher in both urban and rural areas. (p= 0.00016 statistical link is highly significant). We can see that the predominance of patients in urban areas is predominant, a fact explained in accordance with the literature through a more open access to education, new and updated information, where a greater emphasis is placed on the physical aspect, this being an important element for the profession and for socialization.

From the point of view of special impairment, the touch of the nail, palms and soles, scalp, face, genital area and folds was considered, and we highlight the fact that 66% of patients (n = 75) have special impairment and 34% (n = 38) are without. The most common touch was the scalp with 37% (n = 52), followed by 29% (n = 41) nails, 13% (n = 19) palms and soles, 10% (n = 14) on the face, 6% (n = 9) inverted and 5% (n = 7) genital with a predominance of male involvement except genital - 71% (n = 5) were women and 29% (n = 2) men, a situation also found in a study by Burlando *et al*, published in October 2019 (9), (p = 0.0007 the statistical link is highly significant). While in classical literature we are shown the onset of lesions on traditional areas (lumbosacral, elbows, knees), today, in a new approach we can see that the most interested areas were the scalp, nails, palms and soles, either from the beginning or along the way.

Regarding the comorbidities of the patients, 31% have associated cardiovascular pathology (n = 53) and also 31% (n = 53) have metabolic damage, where the preponderance was male on all comorbidities (p = 0.049 significant). Let's not forget that the metabolic syndrome is closely related to the cardiovascular damage and that the association of psoriasis with these two conditions increases the risk of each disease by creating a vicious circle, a risk that not only endangers the quality of life but also the patient's vital prognosis. Being a chronic inflammatory disease with both skin and systemic damage, it becomes absolutely necessary to control its activity, which has the consequence of reducing cardiovascular and metabolic complications.

Referring to biological therapies, 59% of patients are in the first biological therapy (n = 67) of which 66% are men (n = 44) and 34% (n = 23) are women, 31% are in the first therapeutic switch (n = 35) of which 63% men (n = 22) and 37% (n = 13) women and 10% are at the 2nd therapeutic switch (n = 11) of which 55% men (n = 6) and 45% women (n = 5) (p = 0.0005 significant high).

In terms of current biologic therapy, 58% of patients are being treated with anti-TNF α agents (58%) of which 68% are men (n = 64) and 32% are women (n = 41) and 42% are anti-interleukin agents (n = 48) of which 56% men (n = 27) and 44% women (n = 21). Of the 46 patients with previous biologic therapies, 52% (n = 24) switched from an anti-TNF α therapy to an anti-TNF α agent, 40% (n = 18) switched from an anti- TNF α on anti-IL, 4% (n = 2) an anti-IL on anti-TNF α and 4% (n = 2) an anti-IL also on anti-IL.

In terms of severity, PASI at the time of initiation of current biological therapy had a score between '2-10' in 19 patients, between '11-20' in 30 of them, between '21-30' in 56 patients and 8 people had '> 30'

with a direct ratio proportional to severity in both men and women up to the range '21-30'. Most of those with '> 20' also had special impairments - (scalp, nails) with significantly higher PSSI and NAPSI scores. Regarding the current PASI, most of the patients had a significant improvement of the disease, 50 patients with PASI '<2' and 49 with a score between '2-10'. At the initial DLQI, the score was between '21-30' in a total of 66 patients (%) of whom 38 were men and 28 women. At the current DLQI, 43 patients are in the range '0-1' and 38 between '2-5' with a significantly higher ratio in favor of men, but at a score of '21-30' there is an increased ratio of the number of women (n = 8) compared to men.

TABLE I

Distribution of patients according to gender, provenance, special impairment, comorbidities and biological agent

Parameter		Total	Lot 1	Lot 2	P
			Male	Female	
Gender		113(100%)	71(63%)	42(37%)	0.0019
Provenance	urban	70(62%)	40(57%)	30(43%)	0.00016
	rural	43(38%)	31(72%)	12(28%)	
Special impairment	nail	41(29%)	27(66%)	14(34%)	0.046
	inverted	9(6%)	5(56%)	4(44%)	
	face	14(10%)	11(79%)	3(21%)	
	scalp	52(37%)	34(65%)	18(35%)	
	genital	7(5%)	2(71%)	5(29%)	
	palms and soles	19(13%)	10(53%)	9(47%)	
Comorbidities	infections	13(8%)	9(69%)	4(4%)	0.049
	cardiovascular	53(31%)	29(55%)	24(45%)	
	metabolic	53(31%)	29(55%)	24(45%)	
	pneumology	10(6%)	5(50%)	5(50%)	
	neurology	9(5%)	4(44%)	5(56%)	
	atopy	4(2%)	3(75%)	1(25%)	
	gastroenterology	17(10%)	11(65%)	6(35%)	
	psychiatric	9(6%)	5(60%)	4(40%)	
Therapeutic switch	no previous biological therapies	67(59%)	44(61%)	23(56%)	0.00097
	1 switch	35(31%)	22(31%)	13(32%)	
	2 switches	11(10%)	6(8%)	5(12%)	
Biological agent	anti TNF- α	62(56%)	41(66%)	21(34%)	0.000054
	anti - IL	48(44%)	27(56%)	21(44%)	

Regarding those with a previous therapeutic switch, most of them had the PASI score at the initiation of the current biological therapy between '21-30' (n = 15), and those with 2 previous biological therapies totaled 5 people in this interval. In those without previous biological therapies, the '21-30' interval had the highest proportion with 36 patients (p = 0.46 - insignificant). Actual PASI score in those

with a single switch, at most - 49% (n = 17) '<2', two switches - 46% (n = 5) '<2' and without previous biological therapies most 48% (n = 32) had interval '2-10'. Although there were many therapeutic switches, it was still correlated with good disease activity. We evaluated the survival of drugs in second-line biologic therapies and estimated the risk of recurrent discontinuation due to adverse events or inefficiency in patients with psoriasis who failed a first biologic therapy Cause of discontinuation of previous biologic therapy, patients with a single switch: 4 cases with adverse reactions and 41 cases based on inefficiency, and for those with 2 previous biological therapies: 1 case of adverse reaction and 10 cases of therapeutic inefficiency. There are 31 patients with arthropathic psoriasis -15 women and 16 men, most of them (75%) being in the age range '> 40'. All patients in this category also associate nail touch.

As a number of hospitalizations, of those with a single biological therapy so far, 51% of patients (n = 34) required <5 hospitalizations throughout the study period, 25% (n = 17) between 5-10 hospitalizations, 12% (n = 8) needed more than 20 hospitalizations, 6% (n = 4) between 11-15, 6% (n = 4) between 16-20 hospitalizations. Of those with a therapeutic switch, 29% (n = 10) needed more than 20 hospitalizations, 23% (n = 8) between 16-20 hospitalizations, 20% (n = 7) between 5-10, 20% (n = 7) with less than 5 hospitalizations and 8% (n = 3) with hospitalizations between 11-15. Of those with 2 therapeutic switches, 37% (n = 4) are those with more than 20 hospitalizations, 27% (n = 3) between 16-20 hospitalizations and 18% (n = 2) between 11-15 and 18% (n = 2) between 5-10 hospitalizations.

DISCUSSION

The emergence of biological agents in the last two decades has dramatically improved the treatment of psoriasis and psoriatic arthritis (10). Although approved biological agents differ from country to country, biological agents commonly used for the treatment of psoriasis are classified into three groups: TNF - alpha inhibitors, IL -23 inhibitors and IL-17. However, the fact that there are so many options available has also caused confusion (11). The simpler idea that more of the available older biological agents are outdated may not be true. In practice, the variations of the clinical challenges faced by the clinician are vast. Given this, a biological agent considered to be obsolete may prove to be the best agent for a particular patient. For example, some experts now believe that etanercept, which was approved in 2004, is no longer worth considering because of its limited effectiveness. However, it is good to remember that etanercept has one of the best 'geriatric claims' of the FDA attesting to its safety in elderly patients, stating in essence that the risk for elderly patients is almost the same as the risk for younger patients (12). Other biological agents report opposition (i.e., older patients are at higher risk than younger patients, such as adalimumab) or do not have geriatric statements due to a lack of data in this population (12, 13). Another example is certolizumab, which is also an older agent. This agent can be easily forgotten with the assumption that it probably does not have a better effectiveness than other older biological agents for psoriasis. In reality, the PASI 75 achievement rate of certolizumab is close to that of newer IL-17 agents. Moreover, being an anti-TNF drug, its effectiveness for psoriatic arthritis is one of the best, given its high

performance for the treatment of psoriatic arthritis, which may not match the latest IL-23 agents. The authors' recommendation is therefore not to abandon the currently available biological agents, based on superficial assessments (13, 14).

Ellis *et al.* evaluated two network meta-analyzes that evaluated systemic therapies for moderate to severe psoriasis (15). They concluded that newer biological agents targeting the IL-12/23 and IL-17 axes appear to be more effective than older biological agents and oral agents. Sawyer *et al.* analyzed 17 studies that reported results at 40-64 weeks to evaluate long-term efficacy (16). Four 52-week randomized controlled trials showed that brodalumab was significantly more effective than secukinumab, ustekinumab and etanercept. Secukinumab was also more effective than ustekinumab and both exceeded etanercept. Evidence from 13 additional studies and four additional therapies (adalimumab, apremilast, infliximab and ixekizumab) showed that brodalumab was most effective, followed by ixekizumab and secukinumab, followed by ustekinumab, infliximab and adalimumab. Etanercept had the lowest long-term efficacy. They concluded that brodalumab is associated with a higher probability of a sustained PASI response, including complete clearance, at week 52 than the comparators (17, 18).

Sbidian *et al.* according to the meta-analysis of 140 reported studies (17), in terms of the percentage of patients who achieved PASI 90, infliximab, ixekizumab, secukinumab, bimekizumab, brodalumab, risankizumab and guselkumab were more effective than ustekinumab, adalimumab, certolizumab and etanercept. Furthermore, adalimumab and ustekinumab were more effective than certolizumab and etanercept. No significant differences were observed between any of the interventions and placebo for the risk of severe adverse events. In general, we can expect a rapid onset of efficacy on IL-17 inhibitors, especially brodalumab and ixekizumab (17). IL-17 inhibitors and IL-23 inhibitors, especially brodalumab, ixekizumab and risankizumab, have shown high long-term efficacy (18, 19, 20).

These biologics are able to produce a long-term remission of psoriasis, however, the disease usually recurs after stopping treatment. Drug survival is the time to discontinuation of the drug and is considered to be an important measure of real-world biological success of treatment (21, 22, 23). Biologicals with prolonged drug survival rates are associated with superior efficacy and safety profiles (24, 25, 26). In addition, prolonged adherence to treatment is noted as a surrogate measure for the success of treatment (26, 27).

CONCLUSIONS

However, because psoriasis is a chronic disease and some patients have to receive biologics for a long time, it could affect their long-term efficacy and safety. A longer observation is needed to clarify this. With the introduction of new biological substances and the discontinuation of others, an update of biological substances in psoriasis is delayed. Biological therapies have limits in terms of their performance. Perhaps the issue of the therapeutic switch and its timing should be reviewed. Understanding the benefits, risks and monitoring of patients with biological substances is becoming increasingly important for dermatologists. Although recent scientific advances have led to highly effective treatments for moderate to severe psoriasis, lower efficacy of biological treatments has been reported in those with

previous biological exposure and /or failure. Knowing the factors that predict the persistence or discontinuation of biological treatment is also important to understand the interaction between biological and certain cohorts of psoriasis patients.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

REGENERATING THE CORNEAL NERVES WITH AN INNOVATIVE TREATMENT

In this prospective, interventional, before-and-after case series an evaluation is made regarding the efficacy of recombinant human Nerve Growth Factor (rhNGF) eyedrops for the regeneration of structure and function of the corneal nerves in a cohort of patients with neurotrophic keratopathy (NK) (N=18). All patients were evaluated at baseline, 4 and 8 weeks of treatment, and underwent a clinical assessment of corneal sensitivity, regeneration, and in vivo corneal nerve morphologic examination. The authors suggest that topical treatment with rhNGF increases sub-basal nerve density and corneal sensitivity thus promoting healing of corneal ulcers and persistent epithelial defects in patients with NK. (Mastropasqua L *et al*. In vivo evaluation of corneal nerves and epithelial healing after treatment with recombinant nerve growth factor for neurotrophic keratopathy. *Am J Ophthalmol* 2020; 217: 278-86).

IMMUNE DEFICIENCY AND INFECTION IN PEDIATRIC CHRONIC RESPIRATORY PATHOLOGY

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IMMUNE DEFICIENCY AND INFECTION IN PEDIATRIC CHRONIC RESPIRATORY PATHOLOGY (Abstract). The relationship between infection and the immune system is certain, and the infection has a negative impact on the functionality of the respiratory system in children with chronic pathology. **Material and methods**: The study group consisted of 107 children with chronic respiratory pathology, diagnosed and followed in the IInd Pediatric Clinic of the "Sf. Maria" Emergency Clinical Hospital for Children Iasi Romania with asthma or cystic fibrosis, subsequently associated with immune deficiency or hyper IgE. **Results**: 94 patients were diagnosed with asthma and 13 with cystic fibrosis. Most hospitalizations are noted in children aged 3-10 years (61.68). 79 cases associated pathologies that can be considered as factors favoring recurrent infections in children with chronic respiratory distress. 70 patients had IgA deficiency. Of these, 30 children (42.86%) had selective Ig A deficiency, 31 (44.29%) Ig G deficiency, 6 (8.57%) hyper Ig E, and 3 (4, 29%) also associated class M immunoglobulin deficiency. 14 cases (13.08%) had chronic bacterial airway colonization, and 8 children (57.14%) had a chronic lung infection with *Pseudomonas aeruginosa*, 3 children (21.43%) were colonized with *Staphylococcus aureus*, 2 patients (14.29%) with *Haemophillus influenzae*. **Conclusions**: Maintaining an optimal immune status in children with asthma or cystic fibrosis can lead to a slower progression of both conditions, delay the deterioration of respiratory function status, and increase the quality of life associated with harmonious mental and physical development. **Keywords**: CYSTIC FIBROSIS, CHRONIC RESPIRATORY DISTRESS, IMMUNE DEFICIENCY.

Studies in the literature indicate that there is a link between chronic respiratory disease, immune deficiency and associated infections, so we looked at the clinical, anamnestic, biological and paraclinical features of pediatric patients diagnosed with asthma or cystic fibrosis and associated with frequent respiratory infections. The relationship between infection and the immune system is certain, and the infection has a negative impact on the functionality of the respiratory system in children with chronic pathology. This forms a vicious circle consisting of the immune deficiency, chronic inflammation and airway remodeling, which accompany cystic fibrosis and asthma, and recurrent respiratory infections that amplify the existing inflammatory process.

MATERIAL AND METHODS

The study group included 107 children with chronic respiratory pathology, diagnosed and followed in the IInd Pediatric Clinic of the "Sf. Maria" Emergency Clinical Hospital for Children from Iasi. The inclusion criterion was represented by the positive diagnosis of asthma or cystic fibrosis, subsequently associated with immune deficiency or hyper IgE, observed by the values of immunoglobulins IgA, IgG and IgM, respectively IgE.

RESULTS

Of the 107 patients enrolled in the study, 66 (61.68%) were boys and 41 (38.32%) were girls. Therefore, an inhomogeneous distribution is observed in the studied group, with a predominance of males.

Of the 107 patients admitted to the study, 94 were diagnosed with asthma and 13 with cystic fibrosis; the tendency to affect the males was observed in both pathologies (fig. 1).

Most hospitalizations are noted in children aged between 3 and 10 years, respectively 66 patients (61.68%); in the age group 0-1 years, no patients were included, in the range between 1 and 3 years there were 17 patients (15.88%), and between 10 and 18 years there were 24 patients (22.44%) (fig. 2).

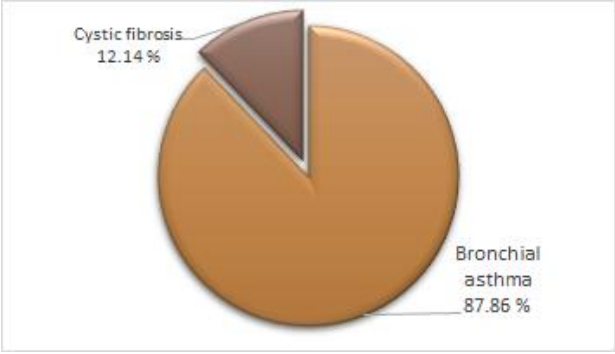

Fig. 1. Chronic pathologies distribution

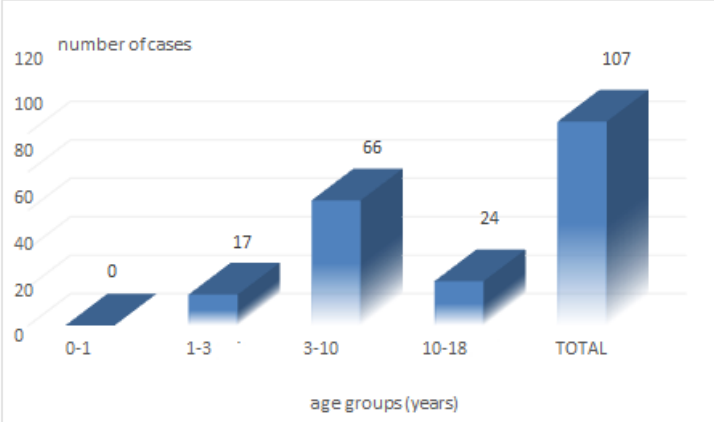

Fig. 2. Age distribution for patients with chronic respiratory pathology

At the time of presentation of the patients at the hospital, 68 (63.55%) of the patients were admitted showing signs and symptoms specific for respiratory infections. For clinical and biological evaluation, 34 (31.78%) patients presented with respiratory impairment, and 5 (4.67%) for gastrointestinal infections.

Pathologies associated with chronic respiratory damage

Out of a total of 107 patients, 79 of them had associated pathologies that can be considered as factors favoring recurrent infections in children with chronic respiratory distress.

In our study, of the 107 children analyzed, 28 (26.17%) did not associate other pathologies. Atopic manifestations were the most common associated pathologies, affecting 36 children (33.64%), and allergic rhinitis was diagnosed in most patients. Cardiovascular malformations followed in 11 patients (10.28%), obesity in 10 children (9.35%), as well as malnutrition, gastroesophageal reflux disease affected 6 children (5.61%) 5 children (4.67%) were associated with malabsorption syndrome, and one child (0.93%) was diagnosed with G6PD deficiency.

Immune deficiency

Following the results on the humoral immune system, out of a total of 107 children analyzed, a number of 70 patients (65.42%) had IgA deficiency. Of these, 30 children (42.86%) had selective Ig A deficiency, 31 (44.29%) also have Ig G deficiency, 6 (8.57%) had hyper Ig E, and 3 (4, 29%) also associated class M immunoglobulin deficiency.

Out of 13 children diagnosed with cystic fibrosis, 8 children (61.54%) had Ig A deficiency, and 5 children (38.46%) had Ig A concentration within normal limits. Of the 94 children diagnosed with asthma, 62 (65.96%) were found to have Ig A values below the normal limit, and 32 (34.04%) had values above 90 mg / dl.

Out of the group of 107 children studied, 57 (53.27%) had Ig G values lower than 800 mg / dl, and 50 children (46.73%) had normal values; 16 children (28.07%) had selective Ig G deficiency, 31 of patients (54.39%) also had Ig A deficiency, 8 children (14.04%) also had hyper Ig E, and 2 children (3.51%) also associated Ig M class deficiency. Of the 13 children diagnosed with cystic fibrosis, 9 of them (69.23%) had low Ig G levels, and 4 children (30.77%) had optimal immunoglobulin concentrations.

Immunoglobulins M represent approximately 8-10% of the total serum antibodies, with a concentration between 60-280 mg / dl. Of the 107 children examined, 11 (10.28%) had low Ig M concentrations, and in 96 children (89.72%) the Ig M concentration was within normal limits. In children with cystic fibrosis, there were 4 cases (30.77%) with Ig M deficiency, and 9 children (69.23%) had normal serum Ig M concentrations. Among the 94 patients diagnosed with asthma, 7 of them (7.45%) had Ig M deficiency, and 87 (92.55%) had a normal M immunoglobulin concentration.

Immunoglobulins E are the type of antibodies present in the lowest amounts in normal serum, constituting approximately 0.004% of the total serum antibodies. Out of a total of 107 patients analyzed, 32 of them (29.91%) had an increase in the concentration of Ig E, and 75 children

(70.09%) had an antibody concentration within normal limits. Among the 32 children with increased immunoglobulin E, 6 children had Ig A deficiency, 8 patients (54.39%) had Ig G deficiency, 2 children (14.04%) had decreased Ig M, and 16 children shows the selective increase of Ig E. Out of the 94 asthmatic children, 28 (29.79%) had elevated concentrations of immunoglobulins E, and 66 children (70.21%) had immunoglobulins E within normal limits; in the group of 13 children diagnosed with cystic fibrosis, 4 of them (30.77%) had a higher concentration of E immunoglobulins, and in 9 children (69.23%), the E immunoglobulins were within normal limits.

Chronic airway colonization

Of the 107 children studied, 14 of them (13.08%) had chronic bacterial airway colonization. In our study, 8 children (57.14%) had a chronic lung infection with *Pseudomonas aeruginosa*, 3 children (21.43%) were colonized with *Staphylococcus aureus*, 2 patients (14.29%) presented *Haemophillus influenzae* chronic infection and in only 1 child (7.14%) the presence of *Burkholderia cepacia* was highlighted (fig. 3).

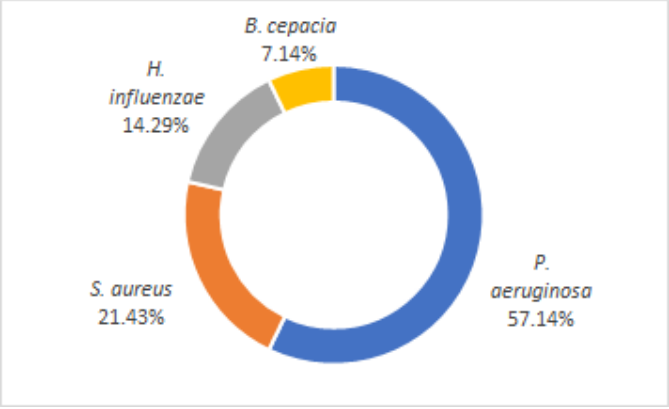

Fig 3. Chronic bacterial colonization in selected patients

DISCUSSION

A number of studies in the literature have shown that there is a higher prevalence of respiratory pathology in males compared to females, the male being also considered a risk factor in a large number of chronic respiratory diseases with childhood onset. Although they have higher lung volumes than girls, most boys have lower values of spirometry testing; other studies show that, with age, in male patients, the increase in airway resistance is much more pronounced than in females (1, 2).

Seemingly healthy male children have a higher Ig E concentration and a higher rate of positive skin tests than girls of the same age. These two hypotheses, in association with narrower airways and increased reactivity, place male children at a higher risk of developing asthma (3). A study conducted in 2013 at the Children's Hospital of Colorado showed that exacerbations caused by asthma were more common in boys than in girls, with values of 1:93 for females and

1:61 for males (4).

Cystic fibrosis, by decreasing the ciliary clearance and the interaction with the defense mechanisms of the respiratory tract, determines the difficult elimination of microorganisms, thus favoring the appearance of infections. Asthmatic children have a four times higher risk of developing pneumococcal diseases, the infections being all the more frequent as the asthma is less controlled, and the corticosteroid administration is in high doses (5, 6).

In our study, 79 of them had associated pathologies that can be considered as factors favoring recurrent infections in children with chronic respiratory distress. Atopic diseases are the most common pathology associated with asthma, and in the case of cystic fibrosis, malabsorption, malnutrition and damage to the cardiovascular system. Allergic rhinitis, atopic dermatitis and urticaria are among the group of atopic diseases, along with allergic asthma. Asthma has been observed more frequently in children who associate with obesity and are much more difficult to control. Studies in the literature state that approximately 40% of children with allergic rhinitis have or will develop asthma. Most often, rhinitis is also associated with allergic conjunctivitis, and atopic eczema frequently precedes the onset of allergic rhinitis (7).

More than 85% of children diagnosed with cystic fibrosis were also associated with protein or fat malabsorption syndromes, which, over time, caused malnutrition. The latter is associated with an acquired immunodeficiency syndrome, decreased secretory Ig A, as well as a degradation of the nonspecific local defense system, such as loss of mucosal integrity and lymphokine overproduction (8).

In children with Ig A deficiency, along with recurrent respiratory infections, autoimmune and atopic disorders often have a much higher incidence compared to those who do not have this immune system impairment. Studies to date have shown that asthma is much more common and has more severe symptoms in children with immunoglobulin A class deficiency. IgA inhibits the attachment of allergens in the environment, which causes the development of asthma. (4, 6).

Chronic airway colonization is a common problem in asthmatic or cystic fibrosis patients, although there is a higher prevalence among children with cystic fibrosis.

A study of a group of 42 patients with cystic fibrosis found isolates of *Candida spp*, and in over 70% of cases, the isolates were positive for *Aspergillus terreus* identified as the most common species of *Aspergillus*, followed by *A. fumigatus*. Bacterial and fungal co-colonization was found in 16.6% of patients and more than half of the samples were positive for *Aspergillus* and *Candida* (9).

Widespread use of immunosuppressive drugs has broadened the spectrum and increased the incidence of human diseases due to common arthroconidia fungi in the environment and ascomycetes that occasionally lead to opportunistic infections in people. A child with cystic fibrosis with pulmonary exacerbation and *Arthrographis kalrae* isolated from bronchoalveolar lavage fluid has been reported (10).

In a study conducted in the AREST CF cohort conducted in Australia, the authors found that children with CF have pulmonary infiltration of CD66b, and granulocytes and elevated levels of MIP-1α, MIG, MCP-1, IL-8 and IL-6. Inflammatory cells

collected by bronchial lavage expressed higher levels of activation and migration markers compared to systemic markers (11).

CONCLUSIONS

Therefore, immune dysfunction is a very important risk factor among these patients, contributing substantially to the morbidity and mortality associated with the underlying pathologies. Studies to date support the significant contribution of immune deficiency in chronic pathologies. In the case of cystic fibrosis, the immune deficiency can have multiple causes: damage to the components of the immune system by mutation or an innate defect, but most often occurs secondary to malnutrition or inflammatory status caused by chronic lung infection. Paradoxically, in this group of patients, the fight of the immune system against chronic lung infection contributes substantially to the progression of lung disease. In asthmatic patients, respiratory infections, common in normal children, can be particularly serious; they also have a significant negative impact on the quality of life and especially on the pathologies they can develop as adults (chronic obstructive pulmonary disease, which is known to be a debilitating condition, associated with multiple pathological implications and huge costs).

In conclusion, focusing on maintaining an optimal immune status among children with asthma or cystic fibrosis can lead to a slower progression of both conditions, delay the deterioration of respiratory function status, and increase the quality of life associated with harmonious mental and physical development.

CONFLICT OF INTEREST AND FUNDING

The author declares that there is no conflict of interest, and she received no specific funding regarding this scientific research.

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NEWS

REDUCTION/ELIMINATION OF BLOOD EOSINOPHILS IN SEVERE ASTHMA: SHOULD THERE BE A SAFETY CONSIDERATION?

Eosinophils play a central role in the inflammation of asthma and are the target of new biologic treatments for patients with severe asthma. Biologics targeting the IL-5 pathway have been shown to reduce asthma exacerbations, improve lung function, reduce oral corticosteroid use, and improve quality of life, accompanied by reduced or even eliminated blood eosinophils. Eosinophils have been associated with host protection and tumor growth, raising potential concerns about the consequences of these long-term therapies that reduce or eliminate them. Areas covered In this review, we explore the current safety profile of biologics regarding the impact they may have on blood eosinophils, trying to answer the question about any safety consideration. There is no doubt that the emergence of specific biologic therapy represents a revolution in severe asthma treatment as the clinical benefit has been clarified through large randomized controlled trials and real-life studies as well. Furthermore, targeting eosinophils through biologic compounds of the IL-5 pathway has shown impressive clinical results irrespective of reduction or elimination of blood eosinophils. However, is it safe to minimize a naturally existing cell? Theoretically speaking no, but practically the answer seems to be yes. Omalizumab, although not a specific antieosinophilic compound has proved to be safe after more than 15 years of use in everyday clinical practice with no safety reports regarding eosinophils. Mepolizumab has already gained a more than 5 years' experience and Reslizumab and Benralizumab have data of up to 3 years exposure with acceptable safety regardless of the degree of blood eosinophils decrease or elimination. Two plausible explanations seem to prevail. First, reduction or elimination of blood eosinophils does not mean vanishing eosinophils from the whole body as they are still present in other sites such as tissue airways or bone marrow. Second, as different types of eosinophils may exist, in different kind of condition such as steady state or inflammatory, it is not clear, yet which type of eosinophils is the real target of the IL-5 pathway biologics. If eosinophilic inflammation still constitutes a treatable trait for severe asthma in the future, it is likely to phenotype eosinophils along with the patients. Despite the different impact of the several biologics on eosinophils depletion, existing evidence does not support an increased infection risk, whereas the risk to benefit ratio, favors the use of IL-5 pathway biologics for severe asthma. Although long term studies are needed, the published evidence up to now supports a convincing anti-eosinophil and anti-IL-5 pathway blockade safety profile (KonKatsoulis K, Kipourou M, Loukides S. Reduction/elimination of blood eosinophils in severe asthma: should there be a safety consideration?, *Expert opinion on biological therapy* 2022; 22(3): 377-384 / doi.org/10.1080/14712598.2021.1960977).

SYSTEMIC COMPLICATIONS OF CHRONIC ALCOHOL CONSUMPTION. CASE-CONTROL STUDY CORPSES *VERSUS* SURVIVING PATIENTS

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SYSTEMIC COMPLICATIONS OF CHRONIC ALCOHOL CONSUMPTION. CASE-CONTROL STUDY CORPSES *VERSUS* SURVIVING PATIENTS (Abstract) **Objective:** The aim of this prospective study is to investigate the forensic cases of the Institute of Legal Medicine (ILM) Iasi compared to a group of living patients, based on chronic alcohol consumption in the past. **Material and methods:** The study group consisted of 120 subjects, of which 60 cadavers from ILM Iasi, alleged victims of chronic ethanol consumption, and 60 patients admitted to the Cardiology Clinic I of the "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, in order to perform a case-control study. **Results**: The studied cases are dominated by male patients over 60 years of age, from rural areas. A cut off value of ethyl glucuronide (EtG) greater than 500 ng/ml proves to be a good predictor in the determinism of liver cirrhosis and dilated cardiomyopathy. **Conclusions**: Elevated levels of EtG have been shown to be a good predictor of liver cirrhosis and dilated cardiomyopathy. **Keywords:** CHRONIC ALCHOOL CONSUMPTION, LIVER, CARDIOVASCULAR, DISEASE.

In forensic medicine, a number of methods are used to determine markers of chronic ethanol consumption in biological products taken from corpses. Alcohol abuse, due to the medical consequences it generates, is an important public health problem (1-3). Studying the protocols in identifying markers of chronic alcohol consumption, in laboratories in Romania and other countries, has shown that alcohol directly affects the most important organs and tissues, leading to systemic dysfunctions, which can have severe health consequences, ranging from decreased quality of life to death (2, 4-7). Chronic alcohol users may experience significant functional and morphological changes (8-13). Overall, all tissues and organs are vulnerable to alcohol

abuse, but almost all medical consequences are only partially reversible with abstinence (14-19).

MATERIAL AND METHODS

The study group consisted of chronic alcohol consumers, divided into two groups, as follows:

- Group I - 60 chronic alcohol consumers, deceased at home; corpses examined in the Institute of Legal Medicine (ILM) Iasi;

- Group II - 60 patients hospitalized in the Cardiology Clinic I of the "Sf. Spiridon" County Clinical Emergency Hospital Iasi.

The present prospective research was conducted between April and August 2021. It was performed with the approvals of the Ethical Commission of "Grigore T. Popa" University of Medicine and Pharmacy, Iasi (Notification no. 9421 / 12th June 2020) and the Ethical Commission of "Sf. Spiridon" County Clinical Emergency Hospital from Iasi (Notification no. 29 / 8th April 2021). A written informed consent from each patient was obtained and enrolment in the research was voluntary. All steps were taken to protect the privacy of the research subjects (patients and corpses) and the confidentiality of their personal information.

In order to highlight the systemic organ changes, tissues taken from the liver, stomach, colon, small intestine, pancreas and heart were microscopically analyzed. Biological samples were taken from the studied cases, at a maximum of 4 hours after death, to determine the level of ethylglucuronide (EtG) by gas-chromatographic method.

The liver tissue samples taken were immunohistochemically analyzed.

In living patients, clinical and laboratory data related to chronic alcohol consumption were selected from the medical records, and the level of EtG in blood or urine samples was determined by the gas-chromatographic method.

The data were centralized in an *SPSS 18.0* database and processed with the statistical functions for which they are suitable, at the significance threshold of 95%.

The ANOVA test was used to calculate descriptive statistical indicators: minimum, maximum, average, median, standard deviation, standard error of the mean, variance.

The Skewness test ($-2 < p < 2$) validates the normality of the series of values.

In calculating the significant difference between the two means, the t-Student test considers the measurement of variability and the weight of observations for the series of values with normal distributions.

Chi square is a nonparametric test that compares 2 or more frequency distributions from the same population; it is applied when expected events are excluded.

RESULTS

Both study groups were dominated by patients over 60 years of age (53.3% *vs.*. 66.7%; p=0.135); male sex (76.7% *vs.*. 83.3%; p=0.360); coming from rural areas (51.7% *vs.*. 66.7%; p=0.094) (tab. I).

By plotting the receiver operating characteristic (ROC) curve, it is proved that age (AUC=0.649; CI 95%: 0.543-0.754) and the environment of origin (AUC=0.603; CI 95%: 0.496-0.711) are good predictors in the determinism of lethality (tab. II, fig. 1).

TABLE I

Comparative demographic data by study group

Demographic characteristics	Group I (n=60)	Group II (n=60)	Statistical test	p
Average age ± SD, years (min-max)	59.52 ± 14.39 (18-81)	64.97 ± 9.42 (43-82)	t-Student test	0.056
≥ 60 years, n(%) < 60 years, n(%)	32 (53.3%) 28 (46.7%)	40 (66.7%) 20 (33.3%)	Chi^2 test	0.135
Males, n(%) Females, n(%)	46 (76.7%) 14 (23.3%)	50 (83.3%) 10 (16.7%)	Chi^2 test	0.360
Rural area, n(%) Urban area, n(%)	31 (51.7%) 29 (48.3%)	40 (66.7%) 20 (33.3%)	Chi^2 test	0.094

TABLE II

ROC curve. Age, sex and environment of origin as death predictors

Demographic data	AUC	Standard error	p	CI95%
Age	0.649	0.054	**0.009**	0.543-0.754
Sex	0.468	0.056	0.576	0.358-0.578
Environment	0.603	0.055	0.060	0.496-0.711

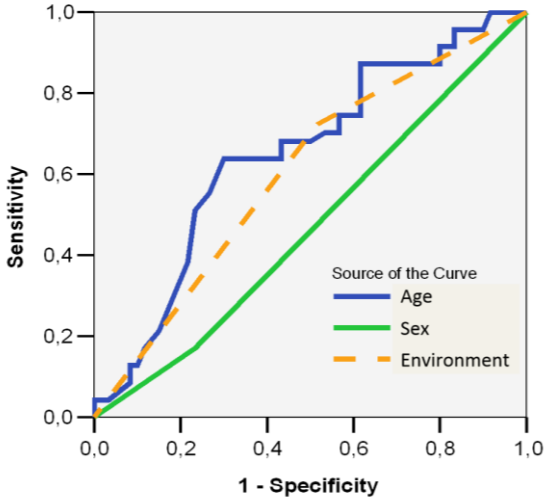

Fig. 1. ROC curve. Demographic data - predictors of death

Digestive complications

In patients who died, excessive accumulation of fat inside the liver cells was highlighted microscopically, which hinders the functioning of the liver (fig. 2).

Also, in the microscopically studied cases, an inflammation of the liver was identified, accompanied by damage of the liver cells (fig. 3).

Alcoholic cirrhosis is the most severe complication of alcoholic liver disease (ALD) (20, 21). In cirrhosis, normal liver cells are replaced by dysfunctional fibrous cells (fig. 4).

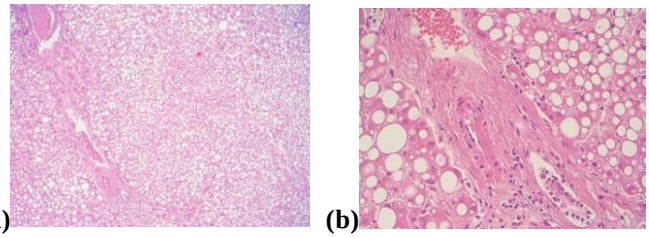

Fig. 2. Steatotic liver (ILM Iasi collection). **(a)** liver with lesions of diffuse micro- and macrovesicular steatosis, HE stain, magnification 10x; **(b)** hepatocytes with micro- and macrovesicular steatosis, reduced mononuclear inflammatory infiltrate in the portobiliary space, HE stain, magnification 40x

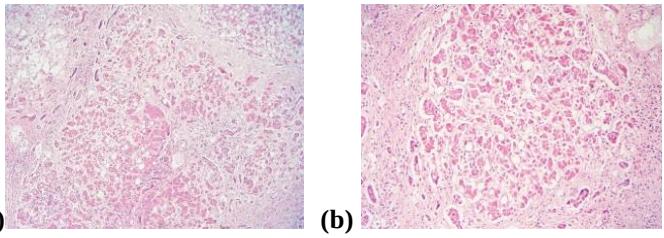

Fig. 3. Liver with alcoholic hepatitis (ILM Iasi collection). **(a)** liver with numerous bile neocanaliculi, inflammatory mononuclear infiltrate, HE stain, magnification 20x; **(b)** micronodule with cu hepatocytes presenting numerous bile neocanaliculi, inflammatory mononuclear infiltrate, HE stain, magnification 20x

Fig. 4. Liver with alcoholic cirrhosis (ILM Iasi collection). **(a)** liver cirrhosis with abundant fibrous tissue, Masson's trichrome stain, magnification 20x; **(b)** liver cirrhosis with micronodules separated by large fibro-inflammatory septa, Masson's trichrome stain, magnification 10x; **(c)** liver cirrhosis with micronodules separated by large fibro-inflammatory septa, Masson's trichrome stain, magnification 20x

Starting from the classical histopathological study (22, 23), it was necessary to continue it with immunohistochemical methods. The following ***immunohistochemical changes*** (IHC) in cirrhosis were found in the study (figs. 5-7):

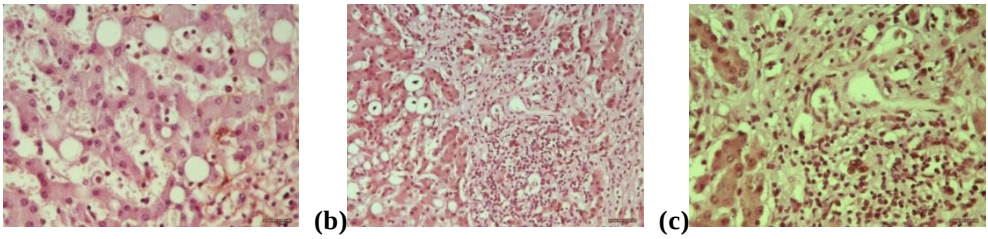

Fig. 5. IHC changes in cirrhosis (ILM Iasi collection). **(a)** 289-2 Ki67, x400. Ki has values below 1% **(b)** 289-2 p53, x200. p 53 it is intensely positive in 10% of the nuclei of bile duct canaliculi and only in 4% of the liver cell nuclei; **(c)** 289-2 p53 x400. Similar image, but with a larger magnification

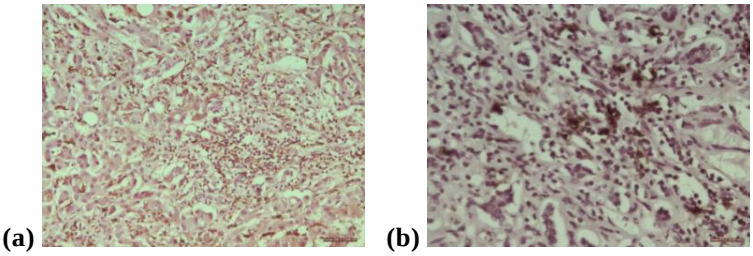

Fig. 6. IHC changes in cirrhosis (ILM Iasi collection). **(a)** 289-2 CD68, x200. CD68 is intensely positive in the macrophages in the inflammatory infiltrate in the portal spaces and in the Kupfer cells in the remaining liver parenchyma; **(b)** 372-2 CD20, x400. CD20 is intensely positive in the rare B lymphocytes in the inflammatory infiltrate in portal spaces

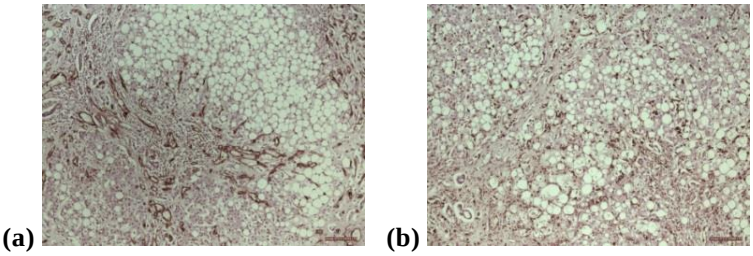

Fig. 7. IHC changes in cirrhosis (ILM Iasi collection). **(a)** 372-2, CD34, x100. CD34 is intensely positive in the endothelial cells of the numerous newly formed capillaries in the greatly enlarged portal spaces; **(b)** 372-2, CD68, x100. CD68 is intensely positive in the macrophages in the inflammatory infiltrate in the portal spaces and in the Kupfer cells in the remaining liver parenchyma

Exposure of the gastric mucosa to ethanol causes pathological changes (fig. 8), such as the inflammatory process, hemorrhagic erosions, and even acute ulcers. Lesions of the gastric mucosa, accompanied by a significant decrease in gastric blood flow and increased reactive oxygen species, imply a role of xanthine oxidase in ethanol-induced gastric hemorrhagic erosions (20).

Alcohol consumption is the main cause of the development of chronic pancreatitis and the second etiology of acute pancreatitis. The mortality of acute pancreatitis depends on its necrotic hemorrhagic character and the initial inflammatory response, while pain, malnutrition and diabetes are the main problems in the management of chronic pancreatitis. From the ILM case series, a histological presentation of a necrotic-hemorrhagic pancreatitis lesion was chosen as an example (fig. 9).

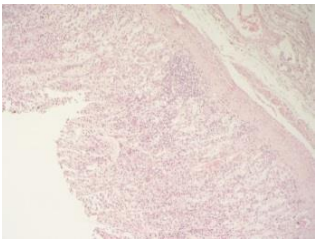

Fig. 8. Gastric mucosa greatly thickened by the presence of an abundant mononuclear inflammatory infiltrate, HE stain, magnification 10x, (ILM Iasi collection)

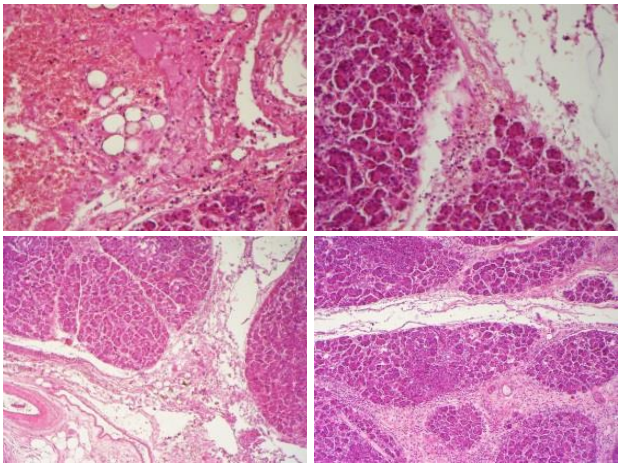

Fig. 9. Microscopic images for necrotic-hemorrhagic pancreatitis, HE stain, magnification 40x, (ILM Iasi collection)

In the studied case series, a significantly higher average level of EtG was found in the corpses in which it has been identified hepatic steatosis (3244 *vs.* 484 ng/ml; p=0.012), alcoholic hepatitis (4,179 *vs.* 520 ng/ml; p=0.001), liver cirrhosis (1,184 *vs.* 817 ng/ml; p=0.05), and chronic gastritis (1063 *vs.* 461 ng / ml; p = 0.049), compared to the level recorded in the living patients (tab. III).

By plotting the ROC curve, a cut off value of EtG of 514 ng/ml proves to be a good predictor in the determinism of liver cirrhosis (AUC=0.635), with a sensitivity of 64.3% and a specificity of 68.9% (tab. IV, fig. 10).

TABLE III

Average level of EtG related to the gastrointestinal complications

Complication type	Group I	Group II	t-Student test
Hepatic steatosis	3418 ± 2103	332 ± 161	**0.004**
Alcoholic hepatitis	6118 ± 3890	328 ± 109	**0.001**
Liver cirrhosis	1710 ± 871	582 ± 464	**0.035**
Chronic gastritis	858 ± 654	307 ± 216	0.319
Chronic pancreatitis	397 ± 388	575 ± 238	0.706

TABLE IV

ROC curve. EtG values as digestive complications predictor

Complication type	Cut off (ng/ml)	AUC	Standard error	p	CI95%
Hepatic steatosis	317	0.574	0.049	0.126	0.477-0.671
Alcoholic hepatitis	334	0.574	0.051	0.157	0.475-0.673
Liver cirrhosis	514	0.635	0.057	**0.027**	0.523-0.746
Chronic gastritis	334	0.513	0.082	0.879	0.352-0.674
Chronic pancreatitis	314	0.539	0.086	0.695	0.370-0.708

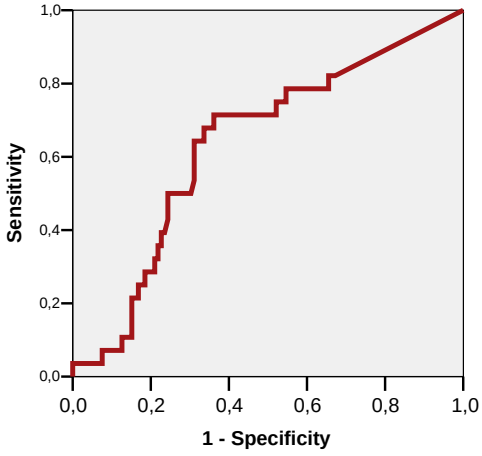

Fig. 10. ROC curve. EtG in the blood - good predictor of liver cirrhosis

Cardiovascular complications

The cardiac response to alcohol reflects the individual's physiology, rate of consumption, and amount of alcohol ingested. The observed postmortem histological changes in the ILM subjects are detailed in

the followings (fig. 11).

Histological evidence of myocardial injury with myocyte death is observed in the intramyocardial fibrous tissue (fig. 12).

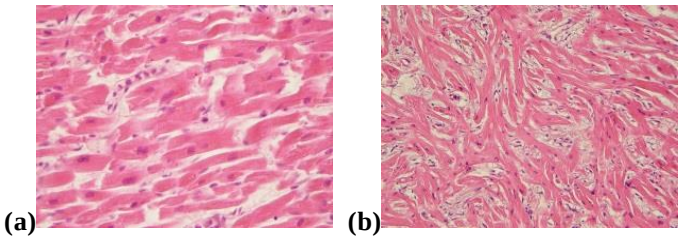

Fig. 11. Dilated cardiomyopathy (ILM Iasi collection). **(a)** myocardial fibers with a wavy, ischemic appearance, HE stain, magnification 40x; **(b)** heart with myocardial fibers with disorganized architecture, separated by fibrous tissue, HE stain, magnification 40x

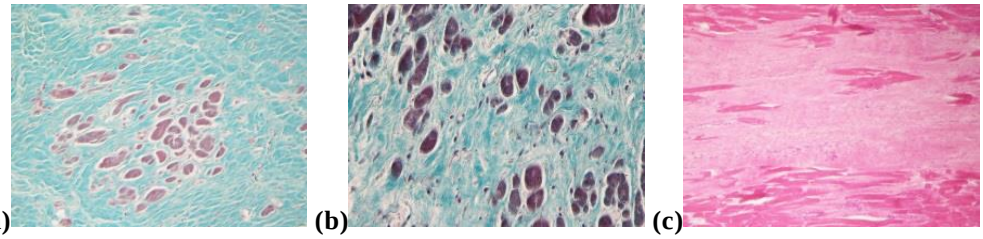

Fig. 12. Myocardial fibrosis (ILM Iasi collection). **(a)** heart with cytological architecture modified by the presence of abundant fibrous tissue, highlighted by green staining, and atrophic myocardial fibers, Masson's trichrome stain, magnification 30x; **(b)** abundant intramyocardial fibrous tissue highlighted by green staining, atrophic myocardial fibers, Masson's trichrome stain, magnification 40x; **(c)** heart with large areas of fibrosis, HE stain, magnification 30x

From the LMI cases it has been selected images of the heart with areas of recent acute myocardial infarction (fig. 13).

In the studied groups, a significantly higher average level of EtG was found in the corpses with chronic heart failure (3361 *vs.* 341 ng/ml; p=0.001) and dilated cardiomyopathy (1178 *vs.* 403 ng/ml; p=0.012), compared to the level recorded in living patients (tab. V).

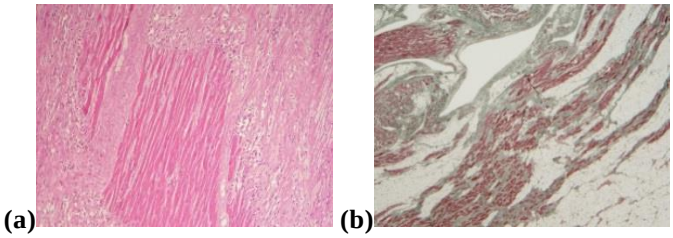

Fig. 13. Acute myocardial infarction (ILM Iasi collection). **(a)** heart with areas of recent acute myocardial infarction, anucleate myocardial fibers, eosinophils, HE stain, magnification 20x; **(b)** heart with cytoarchitecture modified by the presence of abundant fibrous and adipose tissue, atrophic myocardial fibers, Masson's trichrome stain, magnification 20x

TABLE V

Average level of EtG related to the cardiovascular complications

Complication type	Group I	Group II	t-Student test
Dilated cardiomyopathy	1178 ± 372	403 ± 372	**0.012**
Chronic heart failure	3361 ± 1593	342 ± 381	**0.001**
Acute myocardial infarction	462 ± 310	313 ± 266	0.474

By plotting the ROC curve, a cut off value of EtG greater than 500 ng/ml proves to be a good predictor in the determinism of dilated cardiomyopathy (AUC=0.655; CI95%: 0.540-0.770; p=0.026), with a sensitivity of 66% and a specificity of 60% (tab. VI, fig. 14).

TABLE VI

ROC curve. EtG values as cardiovascular complications predictor

Complication type	Cut off (ng/ml)	AUC	Standard error	p	CI95%
Dilated cardiomyopathy	504	0.655	0.059	**0.026**	0.540-0.770
Chronic heart failure	225	0.357	0.071	0.152	0.219-0.496
Acute myocardial infarction	137	0.572	0.061	0.293	0.453-0.691

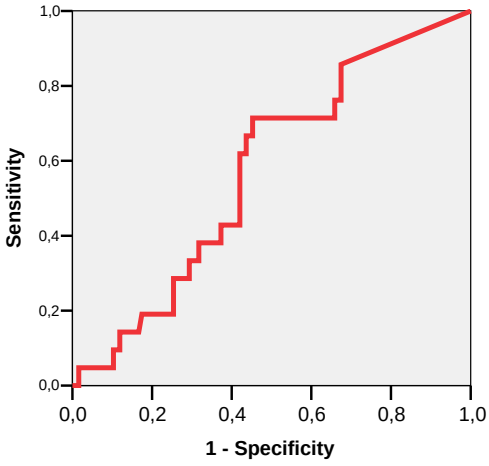

Fig. 14. ROC curve. EtG in the blood - good predictor of dilated cardiomyopathy

DISCUSSION

Alcohol is a major risk factor for many chronic diseases and one of the leading causes of "predictable" morbidity and mortality worldwide. It is estimated that alcohol triggers 2.5-3.3 million deaths in the world each year, accounting for almost 6% of all global deaths (24-26).

A World Health Organization (WHO) report on the relationship between alcohol and health has shown that alcohol abuse is responsible for at least 60 major types of acute or chronic diseases.

The progression of the alcoholic liver disease (ALD) is complex and depends not only on the amount and pace of alcohol consumption, but also on a variety of genetic and environmental factors. In general, men who ingest more than 100 g of ethanol every day, for more than 5 years, are at the highest risk of developing alcoholic hepatitis; however, women may also develop alcoholic hepatitis after ingesting small amounts of ethanol for shorter periods of time (11, 27).

Some studies estimate that about two billion people worldwide are consuming alcoholic beverages in excess (12).

The liver cell translates alcohol consumption as a "preferred fuel". Regardless of whether the consumption of alcohol, in quantity, is reduced or increased, it disrupts gluconeogenesis and leads to the shunting of the metabolic transformation of certain amounts of carbohydrates into fats. In the presence of constant levels of alcohol in the blood (80 mg/dl), liver damage begins with steatotic transformation of the liver, followed by alcoholic hepatitis and perivenular fibrosis, and in the last phase leading to liver cirrhosis (28).

Depending on the frequency of the number of cases, the present study focused on three major liver diseases with thanatogenerative potential: hepatic steatosis (15), alcoholic hepatitis (16, 29-31) and liver cirrhosis (32).

Up to 35% of heavy alcohol users develop chronic, moderate or severe hepatitis. The damage is reversible over time if alcohol intake is stopped. In the severe, acute form, the disease can set in suddenly, after ingesting a large amount of alcohol in a short time and can cause immediate complications leading to death (6).

Between 10 and 20% of heavy drinkers develop cirrhosis, usually after 10 or more years of consumption. Fibrosis is the first stage of scar tissue formation; however, when fibrosis accumulates and significantly occupies the organ, cirrhosis develops (33).

For a long time, hepatic steatosis has been considered a relatively harmless side effect of alcohol consumption, but recent studies have shown that people with alcohol-induced steatosis are vulnerable to developing alcoholic steatohepatitis, liver fibrosis, cirrhosis and even liver carcinoma (14, 15, 34, 35). However, hepatic steatosis is fully reversible if alcohol consumption is completely discontinued (36).

Alcoholic hepatitis is a chronic inflammatory disease, associated with high morbidity and mortality, in the context of chronic or excessive alcohol consumption (29). It causes chronic liver failure, jaundice, coagulation disorders, marked asthenia, ethanolic encephalopathy, etc. Several studies have shown that the prognosis of alcoholic hepatitis is poor if the patient continues to consume alcohol, with death occurring in 20-50% of cases, in approximately 28 days after the clinical onset of the disease (16, 30, 31). Chronic liver inflammation, associated with hepatocyte necrosis, disrupts local homeostasis, and induces a regenerative, anarchic process, characterized by the production of an extracellular matrix (ECM), and especially fibrillar collagen, with the onset of liver fibrosis. Liver fibrosis induced by excessive alcohol consumption is progressive and eventually leads to cirrhosis, which in turn, causes more than 1 million deaths

worldwide each year (37). Moreover, liver cirrhosis can become complicated with ascites that would be refractory to diuretic medication in approximately 10% of patients (38). Often these patients have also renal pathology that progresses to end-stage renal disease (ESRD), necessitating renal replacement therapy. Multiple pathogenetic factors participate in refractory ascites and important morphological changes of the peritoneal membrane also occur in the patients with long term dialysis (39, 40).

The cellular and molecular mechanisms of ALD have become well known in recent years. In the first stage, alcohol induces hepatic steatosis due to the reduction of fatty acids, the activation of lipogenic enzymes, and the appearance of reactive oxygen species (ROS) and proinflammatory cytokines. All this leads to the onset of a local inflammatory process, where neutrophils are predominant. It has been suggested that the large number of neutrophils is triggered by the activation of hepatocyte proteases, and by the appearance and growth of ROS.

Proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), IL-6, and IL-8 are activated and secreted by Kupffer cells and other inflammatory cells (neutrophils, macrophages), which accumulated in Kiernan spaces, following chronic alcohol consumption (29). The presence of a local inflammatory process, even at a low level, can lead to liver fibrosis, a more severe stage of excessive alcohol consumption. Hepatic fibrosis is the result of qualitative and quantitative changes in the ECM. With its onset, the normal architecture of the liver is altered, leading to liver failure. Left untreated, fibrosis can progress to cirrhosis, followed most commonly by severe liver failure and death (32, 41, 42). The process of hepatic fibrillogenesis interferes with the cells present in the liver. Thus, hepatic stellate cells (Ito cells), under the influence of cytokines and chemokines, synthesized by inflammatory cells, undergo a trans differentiation into myofibroblasts. Proliferating myofibroblasts are one of the main sources of ECM molecular excess (type I and III collagen), as well as other proteins in the structure of pathological fibrous tissues (43-45). Kupffer cells are also involved in the process of liver fibrogenesis. They synthesize a multitude of powerful cytokines, through which they activate the liver stellate cells and transdifferentiate them into myofibroblasts (46).

Our study showed that in individuals with chronic or excessive alcohol consumption, there is intense Kupffer cell activity and trans differentiation of liver stellate cells into myofibroblasts.

Based on the chronic alcohol abuse cases analyzed in forensic pathology, pathological anatomy, and forensic toxicology laboratories, we obtained data proving that the amplitude of the histopathology changes in the heart are associated with a significantly higher level of EtG in the blood. Regarding myocardial lesions, we identified most of them in cases with dilated cardiomyopathy, and they were present in both myocytes and the myocardial intercellular conjunctival matrix. We believe that all myocardial injuries and changes are the result of alcohol abuse.

According to some studies, the cardiovascular system is the second most affected by the global toxicity of ethanol, after the liver and the gastrointestinal tract (47, 48). Alcohol abuse can cause systemic atherosclerosis (49), high blood pressure and

cardiac arrhythmias (50). Moreover, in a very high percentage, it leads to progressive damage of the myocardium, also known as "alcohol-dilated heart disease" (51-56).

Chronic alcohol consumption is one of the leading causes of dilated cardiomyopathy, characterized by dilated left ventricle and reduced systolic / diastolic function, which eventually progresses to congestive heart failure. Chronic exposure to alcohol can exacerbate heart failure of several etiologies due to volume overload. The cardiac response to alcohol reflects the individual's physiology and depends on the rate of consumption and the ingested amount. People with left ventricular dysfunction show predominantly the inhibiting effects of the alcohol, which leads to a decrease in ejection fraction (57). Our histopathology findings in the left ventricular myocardium of the subjects with dilated cardiomyopathy can also explain the macroscopic aspect of the heart at necropsy, and provide a morphological substrate for the clinical symptoms the patients complained of, before death.

Myocardial damage, in the case of alcohol abuse, is caused by both the alcohol and the resulting metabolites, especially acetaldehyde, which decrease the structural proteins and reduce the myocyte contractility (58, 59). The lesions produced in myocytes by alcohol are extremely diverse, targeting cell membrane, receptors, mitochondria, ribosomes, or cytoskeleton. This is happening because ethanol has a highly reactive molecule, which is small in size and has a high diffusion capacity (60, 61). Ethanol increases the permeability of the cell membrane, including intracellular organelles, disrupts the activity of ion channels (Ca^{2+}, Na^{+}/K^{+}) together with the cellular signaling mechanisms, and activates apoptosis (62, 63). Other studies have shown that alcohol disrupts the synthesis of ribosomal proteins, contributing to the depletion of non-contractile proteins in the sarcoplasm (61). The excessive consumption causes the mitochondria to enlarge, leading to the occurrence of megamitochondria, the degeneration of the inner membrane folds, and even the fragmentation of these folds (64). Moreover, the mitochondria will produce ROS that can have either adaptive or harmful effects. Elevated ROS levels can eventually lead to apoptosis by promoting the oxidation of lipids, proteins and deoxyribonucleic acid (DNA); a phenomenon that is described in alcoholic dilated cardiomyopathy (65-67).

Our study confirms the observations of other authors who claim that chronic alcohol abuse reduces the structural synthesis of proteins, with sarcomere disorganization, focal dissolution of myofibrils, cell vacuolation and myocytolysis (68). As illustrated above, many studies show that changes in the intercellular conjunctival matrix are present in almost all cases of dilated cardiomyopathy, caused by excessive alcohol consumption (69). The most obvious is myocardial fibrosis, which results from a process of tissue regeneration after the apoptosis or necrosis of myocytes. This myocardial regeneration is inefficient since the fibrosis process is being more extensive, leading to left ventricular dysfunction and heart failure (23, 70, 71).

As medical and endoscopic therapeutic procedures progress, surgical interventions have become increasingly rare, but still remain indispensable for some patients suffering of the complications of chronic alcohol abuse. Alcohol abuse, both alone and in connection with diabetes mellitus,

increases the risk for many cancers (72). Moreover, ethanol consumption by diabetes patients has worsening effect for the efficacy of antidiabetic drugs (73), so novel promising therapies are studied for treatment of diabetes and alcohol dependence involving nanodrug delivery systems (74, 75). Alcohol abstinence is important for patients who receive biologic medication for immune-mediated diseases (76) due to the interaction between alcohol and the proinflammatory cytokines.

The multidisciplinary approach towards patients who consume alcohol is the key to the success of care (8), and a modern approach of alcohol addiction should also consider the degree of satisfaction a patient has with his medication (77), during the abstinence period.

CONCLUSIONS

This prospective case-control study was performed on two groups of chronic alcohol users, 60 deceased patients that had their corpses examined in the Institute of Legal Medicine Iasi (Group I), and 60 living patients, hospitalized in the Cardiology Clinic I of the "Sf. Spiridon" County Clinical Emergency Hospital Iasi (Group II), which were monitored according to the established protocol, in order to highlight the risk factors that may cause lethality.

The dosage of ethylglucuronide (EtG) in blood or urine samples taken from corpses is the main marker for the diagnosis of chronic ethanol consumption, also endorsed by the investigation data and the necropsy diagnosis. All this information can lead to establish a certain diagnosis of chronic ethanol poisoning and help elucidate some inconclusive situations.

In the cases where corpses belonged to individuals suspected of chronic alcohol consumption that we analyzed in pathological anatomy and forensic toxicology laboratories, important histopathological changes of the vital organs were identified, which are correlated with a significantly increased level of EtG. Elevated levels of EtG are a good predictor of liver cirrhosis and dilated cardiomyopathy.

CONFLICT OF INTERESTS AND FUNDING

Authors declare that there is no conflict of interests, and they received no funding for this study.

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NEWS

EFFICACY OF SUBCUTANEOUS CLOSED SUCTION DRAIN IN REDUCTION OF POSTOPERATIVE SURGICAL SITE INFECTION

Surgical site infections (SSIs) are the infections of wound after an invasive operative approach. It remains to be a major morbidity for patients undergoing surgeries although there have been tremendous improvements in the surgical techniques. Different interventions to suppress the selective serotonin reuptake inhibitors have been proposed. Many of them have been routinely used by surgeons like minimizing shaving, hand washing, and preoperative antibiotics and these are well accepted. Drains are used in major abdominal surgeries, hernia repairs, breast surgeries reducing collections in closed areas. Hematoma, serous fluid and dead space in surgical incision wounds raise the risk of infection as they serve as the platform for microbial growth. Studies have proved that the usage of subcutaneous drains has lowered the chances of infection. According to this study the comorbid patients, emergency cases and colorectal surgeries had a higher incidence of developing SSI. The most common organism isolated was *E. coli* followed by *S. aureus*. The assessment of these variables therefore helps in providing a prophylactic treatment to reduce the mortality in the high-risk patients. (Harish R, Kazi FN, Sharma J. Efficacy of Subcutaneous Closed Suction Drain in Reduction of Postoperative Surgical Site Infection. *Surgery Journal (New York, N.Y.)*, 2021; 7(4): e275-e280).

MEANING IN LIFE: CONFESSIONS OF SUICIDAL PATIENTS AND THE NEED FOR A LOGOTHERAPY-BASED NURSING

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MEANING IN LIFE: CONFESSIONS OF SUICIDAL PATIENTS AND THE NEED FOR A LOGOTHERAPY-BASED NURSING (Abstract): A strong meaning in life (MiL) was described as a protective factor against the suicide. **Material and methods:** Accordingly, in this study we conducted a series of interviews with 31 hospitalized suicidal patients (mean age of 55 ±13.5) and 31 control patients (mean age of 58.7 ± 9.7) to evaluate their current MiL and potential means to recover it. The hospitalized patients have been known to the investigator for an average of 4.8 years (median 2.9 years). **Results:** Psychiatric patients have been deeply affected by the ruined family relationships and harmful psychosocial experiences marked by betrayals, deceptions, abuse, threats, insults, contempt, deceit etc. Accordingly, 71% (CI: 0.137-0.432) of suicidal patients and 19 % (CI: 0.637-0.908) of control patients have lost their MiL. Prayer and living their faith in God would represent motivational values to reach a MiL or constitute a MiL by themselves for 84 % (CI: 0.674-0.929) of the patients. It was also observed that the willingness to help other sufferers, the high need to love and to be loved, and to mean something to family and society represented a shared feature of suicidal patients and valuable sources for their MiL. **Conclusions:** a program of supportive logotherapy of suicidal patients and their inclusion in activities of helping other sufferers would represent a beneficial means both to relieve the burden of disease and to help them finding a MiL or to recover it. **Keywords:** MEANING IN LIFE, SUICIDE, NURSING INTERVENTIONS, LOGOTHERAPY, EMOTIONAL DISTRESS, RELIGIOSITY,

"About a third of my cases are suffering from no clinically definable neurosis, but from the senselessness and emptiness of their lives" (1). This quote from Carl Jung, which remains central to modern psychiatry, implicitly incorporates the depressive and suicidal cases whose weight in the global psychiatric casuistry is significant. Accordingly, the WHO has projected depression as the primary cause of global disease burden by 2030 (2), while near 800,000 persons die by suicide every year (3). Given the predicted significant extents and burden of these psychiatric diseases, the therapeutic nursing interventions require further refinements and customizations to deal with the growing mental health problems associated with existential psychological issues of the psychiatric patients affected by loneliness, failure and

emptiness, and who often feel devoid of any MiL. Analysis and understanding of MiL-related beliefs of depressive suicidal patients may improve the mental health outcomes (4).

Life value based therapeutic interventions for suicide prevention emerged from the assumption that people suffering from suicidal ideation have lost any desire to live (5). Different reports on the suicide phenomenon showed that MiL is a key protective factor against suicide attempts, leading to a positive attitude towards life (5-10). Accordingly, suicide-vulnerable people need consistent supportive actions in finding a new meaning in their life or to recover a lost one in order to improve their mental state.

MiL encompasses many complex definitions, but in a simplistic way, for a person with suicidal behavior, MiL might be linked to the following related questions: "Do I still preserve any value, significance, importance or, in short, any meaning for somebody or something in this world? Do I still matter for someone? How useful do I still feel myself for my family, friends or larger society? Have I lost any interest from people on me? How much self-esteem do I still have?". The responses are invariably "no" or "not too much."

From a clinical point of view, studies have underscored in a unitary way the protective impact of MiL on the suicidal ideation, on suicide attempts and completed suicides. The correlations between the search for a MiL and those related to the suicidal state were less consistent, but they were oriented towards a non-protective impact (6). Some studies examining the relationship between MiL and psychopathology have shown that low levels of MiL are associated with mental disorders, addiction disorders, depression, hopelessness and suicide (11).

Viktor Frankl has shown that meaninglessness can lead to depression, addiction and aggression, calling them neurotic triad or existential neurosis (12,13). To address this existential suffering, he developed Logotherapy for his patients, helping them find meaning in their lives and making them aware of the hidden meaning of existence (13). But the best therapeutic approach, according to Frankl, is to ask patients about the meaning of their life (13,14). Consequently, we built the discussions around this particular approach, while the key question about patients' own MiL was addressed directly, albeit carefully and compassionately, in the middle of the conversations. This critical question was placed at the center of the discussion, so as to balance the history of the patient's past and present life experiences with present and potential future actions that could help to better cope with suicide.

Therefore, the objective of this paper was to initiate a dialogue with suicidal patients in order to evaluate their mental state and beliefs related to their MiL. Another objective was to evaluate the potential induced by a friendly supportive discussion, grounded on the mutual confidence built during previous hospitalizations, to get critical confessions about their existential issues associated with beneficial relieving mental states, assuming a cathartic potential of the confessional act.

MATERIAL AND METHODS

Study population and data collection. As a basis for discussions, we have created eleven guiding questions which were addressed to a group of psychiatric Caucasian patients. The patients were recruited from those with multiple hospitalizations in our psychiatric hospital. The target group in-

cluded 31 patients with suicidal behavior while the control group consisted of 31 patients with psychiatric diagnoses from which depression, suicidal behavior, dementia and oligophrenia were excluded. The control patients were diagnosed with schizophrenia, bipolar disorder, organic personality disorder, anxiety disorder, alcohol withdrawal syndrome, sleep disorder. At the time of the conversations, the patients were hospitalized in the same hospital, have been known to the investigator for an average of 4.8 years (median 2.9 years), and were randomly included in the study. No personal or identification data were collected. Participation in this study was exclusively conducted on a voluntary basis and no kind of recompense or favor was offered to patients. The data were collected following a series of individual discussions, initiated only when the patients felt they were prepared for them. From each discussion, we have selected the key confession related to the target question. The confessions were slightly processed, especially grammatically.

Ethical approval. The patients were informed about the subject of the discussions and offered their informed consent. The institutional ethics committee approved the study.

Descriptive statistic. To examine the differences between the two groups, the *Pearson Chi square* test was used. Means and standard deviations were calculated for quantitative variables. The confidence intervals, CI, for proportions were calculated through *Microsoft Excel* 2007. Significance was set at standard $p<0.05$.

RESULTS AND DISCUSSION

Characteristics of the patients are included in first table. The two groups were identically structured, with a women-to-men ratio of about 1.4:1. There was no significant difference with respect to the mean age. The control group included more patients living in rural areas, but the difference is statistically non-significant (p=0.309 CI=0.38-0.71). 80 % of the suicidal patients were diagnosed with suicide attempts.

TABLE I.

Characteristics of the psychiatric patients

Defining characteristics	Suicidal patients		Control patients		Statistical significance	Confidence interval (CI)
	N	%	N	%	p*	95%
Sex						
Women	18	58	*18*	58	1.00	0.46-0.69
Men	13	42	*13*	42		0.3-0.54
Place of residence						
Urban	17	55	*13*	42	0.309	0.36-0.61
Rural	14	45	*18*	58		0.39-0.64
Suicide attempts						
Women	12	39	0	0	1.00	0.11-0.31
Men	13	42	0	0		0.13-0.33
Age (M±SD)	**55 ±13.5**		**58.7±9.7**			
Women	53.5 ± 14.1		57.7 ± 11.3		0.942	
Men	57.1 ± 12.8		60.4 ± 6.5			
* by Chi square test for 5% level of significance						

Table II displays the questions addressed to the two groups of patients. The conversations started with two questions about the importance of the hospital community and the family on their well-being. The following four questions probed their particular psychical state, with an emphasis on their meaning in life, while the next three questions focused on the willingness to help their family and other suffering people as an indicator of their remaining ability to feel useful and, therefore, to have a good reason to continue living. The last two questions sought to ascertain whether religion, prayers, religious activities, and a relationship with God could form a supportive way for these patients. The latter questions were based on an internal survey that showed that more than 90 % of the psychiatric patients are religious (data not published).

The beneficial effects induced by the relationships with other patients and with the medical staff were equally acknowledged by the hospitalized patients. However, the protective role of the family was obvious only for the control group ($p < 0.05$), whereas 35 % of the suicidal patients denied such a role.

A highly significant difference ($p<<0.05$) was noted when asking for their current pleasure to live compared to that felt 5-10 years before. None of suicidal patients responded positively.

The unfulfilled dreams of their life have almost equally affected both groups. It appears that life did not offer those patients compensatory alternatives and opportunities to balance their unfulfilled dreams in order to preserve pleasure, the will and the reason to live at normal levels, which contributes to the failure of their current life. Moreover, by finding themselves now under the roof of a psychiatric institution, often felt as a social stigma, the frustration feeling regarding their unfulfilled life-spanning wishes and dreams was heightened even more.

TABLE II.

Questions addressed to the two groups of patients

QUESTIONS	Responses							
	Suicidal patients			Control patients			Statistical significance	Confidence interval (CI)
	Yes	*No*	*I don't know*	*Yes*	*No*	*I don't know*	*p**	*95 % CI*
1.Do the community of patients in the hospital, the doctors, and medical staff in general, help you feel better?	**29**	2	-	**30**	1	0	0.554	0.86-0.98
2. Does the family help you feel better?	**20**	11	-	**27**	3	1	0.047	0.64-0.85
3. Do you feel that you have the same pleasure to live as 5-10 years ago?	**0**	31	-	**15**	16	-	0.0005	0.15-0.36
4. Have you been very much affected because you could not fulfill certain desires/dreams in your life?	**26**	5	-	**24**	7	-	0.520	0.69-0.88

QUESTIONS	Responses							
	Suicidal patients			Control patients			Statistical significance	Confidence interval (CI)
	Yes	*No*	*I don't know*	*Yes*	*No*	*I don't know*	*p**	*95 % CI*
5. Have you been highly affected / disappointed by what (negatively) happened in your life?	**29**	2	-	**23**	8	-	0.038	0.73-0.91
6. Do you still have any meaning in life?	**8**	22	1	**25**	6	-	0.0001	0.41-0.65
7. Do you think you are or can be useful to your family?	**18**	12	1	**28**	2	1	0.009	0.62-0.83
8. Do you think you are still useful to the others?	**13**	18	-	**31**	-	-	0.0002	0.59-0.81
9. If you had the opportunity to be useful to other people who have different sufferings would you accept to help them?	**28**	2	1	**31**	-	-	0.438	0.87-0.98
10. Do you think an activity in the church dedicated to helping the unfortunate people would help you?	**26**	4	1	**29**	2	-	0.514	0.78-0.94
11. Do you believe that prayer, knowing God and living your faith can give you a meaning in your life?	**26**	5	-	**31**	-	-	0.086	0.82-0.97
* by Chi square test for 5% level of significance								

If the fourth question probed the influence of what the patients dreamt happening in their life, the next question investigated the effect of (negative) events happened in their life. It appeared that negative life experiences affected more patients from the target group (90% *vs.* 74%; p=0.038; CI=0.37-0.31). Considering the medical diagnoses, this is an expected result. It certifies the deeper life sufferings of suicidal patients - generally very sensitive people - who allegedly felt helpless and mentally unprepared to overcome a series of unexpected bad events in their life.

The key question in the discussions concerned the current perception of their MiL. The results showed a significant difference between the groups ($p < 0.05$; CI = 0.41-0.65), most patients from the target group losing any meaning in life (70 %). All of the latter were marked by suicide attempts (tab. I). However, it is worth mentioning that about 20 % from the control group also confessed that they lost their MiL. To recall, they were not diagnosed with depression or suicidal behavior. Hence, since the loss of their MiL is both a specific marker and a trigger for a suicidal behavior, more attention needs to be paid to those patients with undeclared or non-quantifiable signs of suicide behavior. Therefore, questioning psychiatric patients about their MiL may substantially shape the therapeutic approach for a series of cases. Likewise, as Frankl suggested, asking patients about the meaning of their life

would be the best treatment (13, 14).

A significant fraction of the target patients, i.e., 39%, no longer considered themselves useful for their family or were unsure of this (3%). This contrasts sharply ($p = 0.009$; CI = 0.62-0.83) with the control group for which over 90% of the patients believed they were helpful to their families. Almost the same result was obtained when the interest was changed from the family to "other people" (question 8).

Focusing on the target group, it is interesting to note that, while MiL was found as maintained for 26% of patients (question 6), the percentage unexpectedly doubled when their usefulness for the family (question 7) was assessed (58%; $p=0.034$; CI: 0.41-0.74). However, when usefulness was redirected from the family to "other people", the statistically significant difference, with regard to MiL, was canceled (42%; $p=0.352$; CI=0.26-0.59). The results show that patients will feel more stabilized if the family takes into account their support/contribution and acknowledges their meaning/importance in the family life. This underscores the fundamental role of the family in maintaining a MiL for the suicidal patients.

A highly positive attitude was noted when the discussions were directed towards helping other suffering people (question 9). The majority of patients (90 % of the target group) stated their willingness to help these people, as a comprehensive confirmation that suffering deeply sensitizes and unites patients. This is perhaps the reason hospitalization has a healing effect by itself. Accordingly, it can be assumed that, in addition to medication, psychotherapy, ergotherapy or spiritual and religious support, the compassionate confessions exchanged between patients might form a valuable therapeutic means to stabilize their mental state.

Following a survey conducted in our hospital, the majority of patients declared themselves religious (15). Accordingly, the patients stated that engaging in a church activity dedicated to helping suffering people will also help them (question 10), without a statistically significant difference between groups. However, the percentage of those rejecting such a possibility increased slightly for both groups with about 6% as compared to that observed for question 9. The difference is statistically insignificant but might express a tendency to avoid typical restrictive rules imposed by organized social actions, even when conducted under a religious umbrella.

Finally, we also found a dialogue about the possibility that patients' intrinsic religiosity could be considered a means of recovery or finding their MiL to be opportune (question 11). The results showed that prayer, knowing of God and living their faith can afford patients meaning in their life, without a statistically significant difference between the two groups ($p=0.086$; CI=0.82-0.97). However, 16% of the suicidal patients were pessimistic about the help they could receive from God.

Given that 71% of the target patients stated that they no longer had a MiL (question 6), it is remarkable that over 80% of them (question 11) believe that a relationship with God could offer them a MiL. Therefore, the intrinsic religiosity of the suicidal patients should be taken into account a lot more in order to reverse their negative mental state.

Patients' confessions

As Table 3 shows, the patients in each group expressed divergent ideas, opinions, feelings, and desires. The miscellaneous

confessions are most likely the result of their different levels of suffering shaped by type of personality, intellectual condition, self-esteem, life experience, psychological profile, religiosity level, and relationships they used to have with their family, friends, and community. In Table III, the very short responses, e.g., yes/no, were not considered for analysis.

In their confessions, the role of the family as a source of equilibrium and improvement of one's mental state appeared to be crucial for all patients. The lack of the family's emotional support was perceived as a burden difficult to tackle. However, the family itself is being seen as a cause of their difficulties. Patients appeared very affected when the family did not understand their illness and need for communication. They clearly miss the love of the family and suffer from the lack of recognition of their meaning in the family life.

The mental state of patients appeared to degrade significantly quite recently. Or, much likely, they had already been on an accelerating tendency of degrading mental state over the past 5-10 years of suffering. Different causes such as tragic family events, social trauma, betrayals of family members, isolation, lack of shared love, or problems in sexual life quickly degraded their psychological condition. In general, the target patients showed a more depressed condition compared to the control group.

TABLE III

The most relevant confessions during the conversations with psychiatric patients

1. Do the community of patients in the hospital, the doctors, the medical staff in general, help you feel better?	
Suicidal patients	*Control patients*
° The hospital allows me to be isolated from the world, and the personnel respect my desire for minimal interaction. ° In the hospital I can socialize better. I have shared my sufferings with others. I would share my experience every time and I would feel better. ° The hospital gives me the opportunity to talk about topics I know well, to offer advice to and help those who request it. ° Apart from you and the treatment, nobody talks to me. Every time I was hospitalized you stopped to talk to me and asked about me and my illness. Discussions with you made me always feel good. ° The doctors are very important to me because they know more medical information and healing methods than we do.	° Here we can find help, a good word, rest, relaxation, treatment, appeasement, good advice, and good food. ° I like to talk about myself to others, about how I survived cancer, a distressing experience which changed me into an evolved person in many regards. The other patients listen to my life story, and I feel better. ° I felt protected when I came here. ° Here, I get rid of the hard time I usually have at home with my family.
2. Does the family help you feel better?	
Suicidal patients	*Control patients*
° I would like my wife to speak calmly to me, a good word, not to speak to me with contempt, but to be understood, helped. ° My family should believe I am sick, should understand my illness. I miss the love of my family. ° I would like my family to communicate with me through "deeds" and good words, gently, calmly. They have to take me	° I need the love of my family. In the family one is loved and protected. All my life I felt the need for that. ° I need a good word, communication between us. The members of my family do not talk to me. I suffer from this. ° I want to be useful to my family. I cook, wash, and iron the clothes. ° I feel lonely. My family doesn't support or encourage

easier, not to answer back to me; otherwise, I get angry and slam things. ° I want my husband to accept me as I am. ° I want my husband to come to church with me to learn together how to make peace in our lives. ° I don't have to tell anyone what my suffering is. Nothing can help me. ° My wife loved me more in the past. Now, she doesn't love me anymore. I think she has a lover. ° I was exploited by persons close to me. I would have been beaten if I didn't do what they said. So, I don't believe in anyone. ° They don't do anything for me. So, I don't want to do anything for anyone. I want to be useful only to myself. I have 6 years of treatment, and no one can do anything to cure me. Not I.	me. I need their empathy. ° I need my family to understand my situation, to acknowledge my need to know and feel that I am still important to them. ° They simply can't help me. I represent a complicated situation. ° What should I do to be happy? What should I do?! My family can't help me be happy.
3. Do you feel that you have the same pleasure to live as 5-10 years ago?	
Suicidal patients	*Control patients*
° Since my husband fell ill in his lungs, there is no life between two people - sexual life, I mean. There is no love. Because of him my head hurts. You are a woman, and you can understand me, don't you? ° I don't want anything from life anymore. I have no pleasure. Before, I crocheted, watched TV, read. Now, I'm bored of life, with many problems. Many concerns have gathered in my soul. ° I feel helpless. I think I'm useless. I enjoy nothing anymore! I have no expectations, and I feel the need to retire to my carapace. I have no plans for the future. ° I would have liked to be loved, but I wasn't. I would have liked to offer love, but no one wanted my love. This is my real drama. ° I live for myself. I don't want to live anymore. Next time when I decide ° to do something about dying, I'll be more careful not to escape. ° I feel the fatigue and the bad thoughts that press my soul. I am too tired to hang up. ° No, I'm sick now and I'm no longer useful to my family. My wife would like me to work harder, to bring more money. But I can't. You have no value in front of them (i.e., family members, N/A). It can be seen from their words and attitude. ° I don't feel. I don't have that feeling anymore. I do not feel anything. ° After four deaths in my family, I no longer have the pleasure for life I had before. I had nightmares after nightmares. ° Definitely not! I was fooled, betrayed by the family: my mother and my sister. What could be worse in the world than	° I am thinking that I'm alone at home. I am thinking of loneliness. When holidays come, it doesn't seem to be so! Love was lost, everything was lost! I can't even do the fir tree anymore. ° Before, I was more expansive, more joking. I had bigger joys. Now... a lot has happened! I have problems with money. I don't have enough to live on decently. And is my boy that I raised up still on my side? Then, my mother is old. What will I do if she dies? ° Yes, ten times more pleasure! I get my energy from meeting people who have dreams, plans. I have a dream book in which I always tick other and other accomplishments. ° Yes, I have a great love for the grandchildren, and I try to be active, hardworking. I feel excellent, but my physical power diminished, unfortunately. ° I feel joy when I get up in the morning. I want to live my life! ° What could I do to be happy? The treatment does not help me to be happy. It just calms me down. But I'm not happy in my soul. Why has everything changed for the worse?

the mother's betrayal? And even of your only sister? Can you explain this to me, dear lady?! Because I can't understand. You can't even imagine the extent of my suffering. I am still bleeding.	
4. Have you been very much affected because you could not fulfill certain dreams/desires in your life?	
Suicidal patients	*Control patients*
° I wanted to have a job, but my husband did not allow me. I would have done any kind of job just to get out of the house. I sacrificed myself for him and for the children. But he beat me. I did not live a loving life with my husband. ° I wanted my mother and my brothers to love me, but neither my mother nor my brothers received me in their soul. ° I dreamt to be a doctor, but I came out a simple, worthless person, a nurse, not a doctor. ° I dreamed of having a beautiful family. But my wife started drinking a lot of alcohol. Years and years. I wanted to die! Let the bombs, floods come over me only to die. ° I always wanted to be a doctor and to be in the Spanish class, but, unfortunately, the parents chose for me instead; then, they abandoned me and did not understand me.	° I wanted my children to become good-hearted people, with important professional positions, but unfortunately this did not happen. I am disappointed. ° I feel sorry I don't have a child. This is my dream. A child would change my mood and would give me a meaning in life. ° I regret that I did not have a driving license, a hunting rifle and a car. They would have afforded me freedom and confidence. ° I regret that I was not an intellectual. I feel inferior. ° I've made myself a living. I worked at the bus-station, have a small business, cars, family. All my dreams are fulfilled. However, I feel an anxiety in my soul. I can't find peace. ° All my life I wanted to live in solitude, but unfortunately it was not possible. There is no stillness in the monasteries, either. The hospital respects in part my need for loneliness.
5. Have you been highly affected / disappointed by what (negatively) happened in your life?	
Suicidal patients	*Control patients*
° I am very affected. I couldn't trust anyone. Nobody did anything but steal from you, deceive you, pretend to be your friend when actually he was a wolf dressed in a sheep's skin! ° Yes. My wife didn't appreciate those few achievements of mine. She reproached me for my weaknesses, but she didn't see my strengths. ° I had both joys and troubles. I'm not disappointed. ° When I remember that once everything was fine and suddenly everything was reversed! I stopped the treatment and different psychic states began to appear one after another. I treat the agitation, the insomnia appears, I treat the insomnia, and confusions appear. This brain doesn't help me. I can't judge.	° Yes, I didn't have a good relationship with my mother-in-law. I hoped she would be the mother I didn't have, but I was wrong. She didn't love me. I have never known what a mother's love is. Just imagined how it would be. I think she would have understood my suffering. ° Yes, my husband's death traumatized me. It was like the whole world collapsed. All our dreams vanished like a steam. Life is so ephemeral, like a smoke. What does man live and strive for? What is the meaning of life? ° No, I trusted God. No doctor gave me any hope that my boy would live after a very serious road accident in 2011. But I prayed God from all my heart to save him and he recovered. ° No, this is destiny! We must carry it further.
6. Do you still have any meaning in life?	
Suicidal patients	*Control patients*
° No one loves me. What can you do if no one wants you? I am a more withdrawn person while the world is more outgoing. I'm arguing with my wife. She would want a different life, but she doesn't understand my condition, my helplessness. ° No. I was fishing before. It was quiet, it was beautiful. I was happy, content. On the bank of the lake or of the river I was	° I live for my mother to afford her a happy old age and for me not to drink anymore and to solve all my problems. ° The children and grandchildren thank me for having me. I live for them. ° My goal in my remaining life is to get to the people

forgetting about my problems. The water, the fresh air, the sun, the crickets, the birds, all seemed to take the suffering off my shoulders. I was another man after coming back from fishing. Now, my wife doesn't understand me. I'm not fishing anymore. I have to stay with her almost all the time with her endless complains. ° Cold nephews, cold daughter-in-law. I mean nothing in their life or too little. I was good as long as I could help them. I feel isolated and down. Now, I'm sick. I feel a bit better in the hospital than at home, you know? But here there is a lot of suffering. Why is there so much suffering? ° I have reached an age. It's enough. There is nothing like it once was. For my family I do not have much importance anymore. My friends and many dear colleagues died. It's time to leave. I no longer feel joy. Even the food has no taste anymore. ° It's over with my goals in life for a few years. I have finished. There is nothing more to say. ° I want to go to heaven. Not to be tormented anymore! I feel like I can't keep fighting. I feel like something is calling me.	who need me. ° I pray God to give me days to repent for my sins. I have done some wrong things in my life, but I believe God will receive my repentance. I try to correct what I did wrong and help those in need as I can. God is good. I feel this in my heart. All you need is to be sincere in your prayer. God is the goal of my life. ° Yes. I want first of all to do good deeds to the people in order to receive the same. I read once that you are happier when you give than when you receive. It's true. I am content when I am useful to someone. ° Yes. I live for my children. ° I have lost my purpose in life and my faith since my boy died. I don't want to talk about that anymore.
7. Do you think you are or can be useful to your family?	
Suicidal patients	*Control patients*
° To stop drinking. I'm honest. This is how I could help my family. I know, but I can't abstain. I'm not bad, I'm not violent, but I have this problem. I regret. But I had troubles in my life, and I had to drink to forget. ° I think if I were more indifferent… I mean, not to be affected by any minor problem and imperfections of my family. I am a perfectionist, and this led to contradictory discussions with my family. ° Yes, by offering the warmth of my soul to those around me. ° Partially. I feel repulsion. I am closed, non-communicative. ° To work, to have a stable income would be very useful for my family. When you have no work, you get into depression. Family disputes start from lack of money. And I have lived through enough quarrels. I'm tired of everything. ° Yes, I am very useful to the family. I take care of the grand-children, the house. ° Yes, by keeping my calm more and being more open to my family's new ideas and proposal. ° I cannot be useful to the family by any means. I can no longer help anyone. I can't help myself anymore.	° To be positive, to have a sense of humor always created a good atmosphere in the family. I managed to overcome many difficult situations through humor. So does the family. I saw the full half of the glass. From any miserable situation you can learn something good. Don't you agree? Sometimes it is also a lesson you receive from God for your spiritual growth. ° I help them by showing them that I am strong, to raise their morale. Do you know how it is? If I, the one they consider to be sick, show them that I am strong and not afraid of difficulties, then they, who are healthy, must be stronger than me. This is my trick. But they don't know that I'm helping them. I have saved them many times… ° To be respectful towards everyone. That's how I think I help them. ° Yes, by being fair and not troubling them. ° I offer them my love; my good advice and I take care of them. I like to give. ° I would help them if I consumed less alcohol. That's I am here. At least, I am trying.
8. Do you think you are still useful to the others?	
Suicidal patients	*Control patients*
° Yes, I live so I can help others. There are many people more distressed than me. ° I do not think, and I do not want to be useful to others. Kindness costs you dearly. I have learned my lesson! I do not	° Yes. I can offer my knowledge to others. ° Yes. I was glad as much as I have protested against the government. Politicians make laws for their own good. I protested against them to help the poor people.

live for myself, but only so ... only for my tomcat. God does not want to take me. ° No. I think I'm a chore for everyone. I have no power. I can't help them. I'm a big burden. To reach the mercy of the children is very difficult. ° If I were to live, I would help them. ° Very useful. I am a merciful and faithful man. I put myself in their situation. Jesus tells us that what you want people to do onto you, you should do the same onto them. Unfortunately, I was selfish sometimes. But now I'm not anymore. My illnesses helped me understand life differently. I want to help people selflessly. ° No need for me. Everyone is doing well without me. ° I can't, my dear. I have no physical power anymore. I am too old. I am disoriented, confused, and forgetful. ° I cannot. I am too burdened with worries and affected by my husband's behavior; he beats me, he is wicked. ° I don't feel like I have to help anyone.	° Yes, even if I'm not healthy, I can help with a sweet word, some advice. ° Yes, I can help with moral and physical support ° Yes. I have to be. Otherwise, I'm afraid I'm going to hell.
9. If you had the opportunity to be useful to other people who have different sufferings would you accept to help them?	
Suicidal patients	*Control patients*
° Sure. A lot. It would bring me joy. I would always give. Helping and giving is like a medicine to me. ° I'd love to help! Socializing would help me, too. ° It would be useful to me. I feel gratitude in my soul. From my nature, I feel compassion towards those in suffering. ° I would like to help all those who have difficulties in their lives, and I think it would help me a lot. ° Yes. I have given alms with my priest. ° In no case will I help anyone. I helped. I was naive. ° I'm not rich, but I would like to share what I have with others. ° Anytime with a clean heart. Others helped me, too. ° With the greatest pleasure. I felt good, useful. Other people also helped me. My wife doesn't understand me. She wants money.	° Yes, it brings me joy of soul. ° That's why I live. Through everything I do, I share with others what I have accumulated. ° I calm down when I do good things for other people in need. ° I really like a lot to give. ° People helped me when I was in big trouble. It was a time when I had nothing to eat. I lived a humble life. I know what poverty, indigence mean. But people were really good to me. There are still good people in the world. They were merciful to me. Whenever needed, I want to help those who are now like I was once. ° I help old people, orphans, animals. I feel useful. Do you know that animals also have a soul? They feel that I help, and I love them. ° Yes. I have remorse because I can't help as often as I would like.
10. Do you think an activity in the church dedicated to helping the unfortunate people would help you?	
Suicidal patients	*Control patients*
° Yes. It is good to help the sick people also through the Church. When I wanted to commit suicide, I thought of God. I'm afraid of Him. A brother-in-law of mine saved me from drowning. In those moments, God gave me the power to choose life. ° Yes, but the younger Father (priest) of our church disappointed me. He told me: "As you can go to other churches to attend the religious services, you can also come to my	° Yes. When I was in great distress, I wanted to meet people to help me, and the Lord listened to my prayers. I was diagnosed with cancer, and I prayed the Lord to give me days to live to help my children. I prayed Him to do what He thinks is best for me. And so, it was. Many people need God's help. ° We are so many sick and unhappy people. I would participate, but I have nothing to give. God have mercy

church." But I feel bad, and I cannot stay in our church because there are not enough seats. I would go to stay for up to an hour, but I have nowhere to sit. For this reason, it would be hard for me to go with him to help others. But I would go with the older Father. ° Yes. I would help because God helped me, too. I was seriously injured in 2006. I was in the 4th grade coma, and I could have been dead. However, God gave me life to live, but then I did only nonsense. Helping other sufferers would wash my sins. ° Yes. I, as a (psalm) reader in the Church, along with my Father have visited and helped many unfortunate people. Their suffering made us sad, but we felt great peace of soul sharing their troubles and helping them as best as we could. We have seen many tears on their cheeks. ° No. If I want to do good things for other people, I do them alone.	and help everyone who helped me! ° I never participated. But I think it would be great for those in the parish who are sick and have no one else. I would go with other believers to help. I probably would feel good to help them as I can. ° Yes. Sick people understand best the poor fellows. And through the Church it is even better to help one another. Sometimes, God is all we have left.
11. Do you believe that prayer, knowing God and living your faith can give you meaning in your life?	
Suicidal patients	*Control patients*
° Yes. Through prayer and in the Church, I feel free and spiritually fulfilled. I feel as I would be unchained. ° Prayer helped me a lot. I would not have resisted if I had no faith against all the troubles I had. ° It is a very great spiritual joy. I wish to go to the Metropolitan Cathedral and to spend a full day in the Church service. I pray the Lord to give me health, peace, joy. ° No. I insulted God. Anyway, I'll go to hell. ° No. I don't believe in the Church anymore. I want to sell my soul to the devil.	° I'm religious. Even though I have some medical problems, these are because of my sins, and I do not accuse God. On the contrary, I am grateful. Prayer and faith are everything to me. ° I believe that people of faith are everywhere. I help everyone who comes my way. I confess my faith through good deeds that bring me spiritual fulfillment. ° I go out on the road praying. I pray at home. God is with me, bringing peace and safety in my soul. ° Faith keeps me alive. If I experience joy, I feel I have to share it. ° Faith has given me a good meaning in life. The discussions about God cheer my soul. I feel joy in my heart. ° Without God, we don't exist. I have great faith in God. Without the Church we would have no purpose and without prayer we could not live. ° Yes. I have a great faith in God. Although I had myocardial infarction, I had the power to heal myself with the Lord's power. I said: "God, if you exist, as I know you exist, help me when I go to the cardiac control that there will be no sign left." When I went to the hospital, there was no sign of illness anymore. I think you don't really believe me ... but this is it.

Unfulfilled wishes represented a source of inhibitions and humiliations for some patients. They suffered from the freedom limitation, failure in appreciating their sacrifice by their own family, deteriorated family prestige by an alcoholic spouse, frustration and shame generated by not assuming their current professional status,

abuses and lack of protection, or clearly-expressed lack of MiL.

The majority of patients, especially suicidal ones, experienced a series of severe life traumas, mostly occurred in their families, which have continuously influenced their perception towards the people they interact or live with. The deterioration of confidence in people, the loss of any hope of healing, and the inability to overcome some unexpected cold family relationships have constituted long-term triggers for their present mental condition. However, in the control group, some patients found strong support in God or seemed to be mentally stronger in facing the critical events that occurred in their lives.

Patients have expressed some universally-valid motivations for living: to love and be loved, to mean something to their family and/or society, to help and enjoy children and grandchildren, to be useful to the needy people, to live for God, or simply to enjoy life for itself. Degradation of these motivations, corroborated with a mental illness, especially in a geriatric context, can quickly lead to the loss of MiL with consequent suicidal tendencies. In addition, especially for the suicidal patients, narrowing the mentally comforting activities, such as fishing for a particular case evaluated here, due to the inability of the family to understand the psychiatric disease, may be critical in triggering a suicide. Hence, the family should be specifically trained and alerted to the specific needs of these very sensitive patients.

Despite their fragile mental condition, most patients felt that they could be useful to their family, being aware of both their qualities and weaknesses in backing it. Part of the control group revealed an unexpectedly good supportive behavior, expressing their high capability to change the family's mood in difficult situations.

When the focus was shifted from family to other people, things were slightly changed for the target group of patients. It was observed that suicidal patients were less willing to help others outside their family, even if the difference was statistically insignificant ($p=0.286$; CI=0.38-0.62). The reason might include subjective and objective factors such as the absence of affective ties with foreign people, reluctance or even fear of the unknown, physical inability to move to other people, high burden with marriage problems, social disappointments, or feelings of worthlessness.

When the question regarding helping the others included a narrowing feature such as *suffering*, their reaction changed significantly. Suffering by itself immediately sensitized them, showing a high capacity to empathize for majority of suicidal patients. They feel compassion towards people found in conditions similar to theirs, seeing themselves as beneficiaries of these charitable acts rather than as benefactors. Such charitable activities are confessed as bringing them mental peace and satisfaction. The opportunity to help the suffering people has awakened in them the most noble and uplifting human feelings. Overall, involving in helping activities may afford a strong MiL to these patients.

Prayer, the Church and God have proven to be a means of restoring mental balance and releasing internal stresses, affording a good disposition, but also a motivational basis for helping unfortunate people in the case of 84% suicidal patients. God and the Church turned out to be a source of joy and MiL, affording sometimes a strong belief in the divine intervention for healing

the sick. However, some of the suicidal patients rejected God and the Church, though not for atheistic reasons, but as an aversion gained probably from their failures and life problems that were not resolved by divine intervention when they probably prayed for it.

Patients' willingness to participate in volunteer activities coordinated by the Church dedicated to suffering people (related to the last question) were generally grounded on distressing personal experience in which religiosity has played an important supporting role. As a result, participating in such activities was perceived as a moral and religious duty in response to the help they received from God during their traumatic experiences.

The results of the analysis match the theoretical and other empirical findings related to (a) psychosocial characteristics associated with suicide such as loneliness and social isolation (16) which can strongly impact the need for love, (b) family supportive relationships/activities which were also found as very important among patients with and without a non-psychiatric illness (17), (c) religious practices whose positive function is often acknowledged by the general literature, but little described in specific reports focusing on the relationship between health and wellness, and prayer practices (17), (d) prosocial actions (i.e., "acting in ways that benefit others" (18)) which were theorized as MiL sources (19, 20) and empirically validated by Van Tongeren *et al.* (18).

Although the interpersonal theory of suicide does not consider MiL as a significant factor in preventing suicide (22), according to the theory of meaning, the search for MiL make aware people to appreciate the value of their lives, with positive direct outcomes consequences (16, 21, 22). The latter theory was confirmed also by this work, at least in defining patients' current health condition and finding and orienting their future actions in order to improve the mental state.

CONCLUSIONS

In this study, a number of confessions of suicidal patients, mainly related to their MiL, were collected and analyzed. Patients' need to talk about their depressive emotional states, often marked by a painful life experience and unfulfilled wishes and dreams, was perceived as equally intense for both groups. It was revealed that the main reason for living was essentially linked to receiving and giving love, and the need to represent a meaning for someone, especially for family and suffering people. They were especially affected by the corrupted relationships with their own family, as well as by profound negative life experiences in which they felt betrayed, cheated, abused, threatened, despised, or wrongly guided and left alone. As a result, the majority of the suicidal patients lost their meaning in life.

Patients acknowledged the high potential of helping activities to improve their meaning in life by backing other suffering people individually or collectively via Church's community. In addition, they confessed that prayer and living the faith in God would represent mediating motivational values and beliefs to reach a MiL or constitute a MiL by themselves.

Finally, we can state that by recovering the patients from broken family relationships, restoring their love feelings, improving communication and intimate relationships with the spouse, regaining social esteem and trust in people, getting them

involved in volunteering actions to help other suffering people, as well as applying a personalized religious support, a great improve of their mental condition could be possible in order to recover a MiL or to find a new one. Moreover, friendly, compassionate, and empathetic logotherapy of the suicidal patients conducted by the medical staff, performed on a regular basis, might work as a functional integrated platform of medical and social guidance, psychological and spiritual/religious support, and place of relieving confessions.

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NEWS

ARE THE CURRENT SEIZURE SEMIOLOGY CLASSIFICATION AND DESCRIPTIVE DENOMINATIONS WIDELY IMPLEMENTED?

The current way of classifying epilepsy patients is based on a 3-layer approach for the purpose of syndromic refining. In the last 4 years, this has proved to be a limited subdivisional approach failing to take account of the volatility of two aspects, the seizure onset and the temporal relationship of the interconnected structures. A new study published in 2021 in *Epilepsia* proposes an updated model of the neural mechanisms of seizures, framing epilepsy as a partially-defined network in which the clinical expression is secondary to dynamic rhythmic interactions. The correlation of these factors was studied using information coming in the narrative and recorded seizures, the former being prone to inaccuracies. One methodological challenge resides in the inter-observer variability. From the first visit at an epileptologist, each patient can be included in one of the proposed subdivisions, but a caveat for future classification systems would be to properly integrate individualized semiological features (McGonigal A, Bartolomei F, Chauvel P. *On seizure semiology, Epilepsia*. 2021; 62(9): 2019-2035 / doi:10.1111/epi.16994).

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EDITORIAL

HEALTHCARE-ASSOCIATED INFECTION

Healthcare-associated infection (HAI), also known as nosocomial infection or intrahospital infection, is one of the major problems of contemporary healthcare worldwide. This notion has evolved over time (1).

The negative consequences regarding the state of health, as well as the high costs resulting from additional care, require the implementation of feasible strategies, with objectives oriented towards the selection of measures to prevent, limit and control HAIs (2).

The etymology of the word "nosocomial" (hospital) comes from the Greek language, the word nosokomeion (νοσοκομειων), where (nosos means disease, and komeo - to care) (3).

Joseph Felsen, a physician in New York, was the first to use the term "hospital epidemiologist" to describe an expert in "investigating outbreaks of infectious disease occurring or spreading within an institution." In a 1939 presentation to the American Public Health Association, Felsen called for the necessity of an epidemiologist in every hospital as part of a comprehensive program to prevent infectious diarrhea (3).

In 1962, New York City's public health authorities created a local hospital epidemiology program (2).

Since 1970s, there has been an increase in the prevalence of HAIs due to the excessive use of broad-spectrum antibiotics and cephalosporins, under the pressure of which multi-resistant microbial strains have been selected (for example, methicillin-resistant *Staphylococcus aureus* - MRSA, extended-spectrum beta-lactamase – BLSE, vancomycin-resistant *enterococcus* - VRE) (5).

The increased prevalence of HAI has been associated with:

- the invasiveness of diagnostic and therapeutic procedures,
- the care of a high number of immunosuppressed patients or patients with diseases,
- the high incidence of infectious pathology of viral or fungal etiology,
- the emergence of emerging infections,
- the neglect of the control system by the failure of the medical staff to observe the standard precautions,
- failure to adapt to HAI surveillance system (6).

The most important clinical distributions are urinary tract infections, surgical wound infections, respiratory tract infections, skin infections, bacteremia.

The infectious disease specialist is facing an increasingly acute problem related to:

a) antibiotic resistance;

b) the presence of multidrug-resistant bacteria in what is another "community infection". This is due to the population mix, the development of the home care sector, patients with multiple comorbidities who have an increased average life span, with survival in the conditions of intensive care and with frequent access to health services;

c) the complicated introduction on the market of a new antibiotic that can take decades

d) the presence of opportunistic germs in immunosuppressed patients, which have become etiological factors of serious sepsis,

bronchopneumonia.

From the experience of the last years, in current practice, we encounter germs that we can call "classics", that have acquired resistance to multiple antibiotics, especially Escherichia coli and *Klebsiella spp.,* which are producing ESBL. Taking this into account, we observed that the cases of resistance to the reserved antibiotics, like carbapenems, are increasingly frequent. Another germ that was panresitent is *Aspergillus fumigatus* that is encountered more often in immunocompromised patients especially HAI in intensive care units (ICU).

In our area, the number of cases of fungal sepsis caused by *Candida spp*. has shown an important increase and there have been a rise in the number of reports for *Candida krusei* and even cases of *Mucor mycosis.* These patients require long hospitalization, antibiotics and antifungals of the latest generation, placement in ICU, support of vital functions, implantation of devices that expose the patient to lingering infections on implanted material and microfilm. All this leads, not only to an increase in the costs of hospitalization, but as well to a decrease in the quality of life of the patients and their relatives. More rigorous control of infections by raising the awareness of patients and family doctors regarding the rigorous use of antibiotics, as well as the persistent and continuous implication of the hospital epidemiologist and the structure he leads, could limit the expand of MDR and panresitent bacteria by checking sanitation measures and correct identification, and reporting of HAI. This would contribute to stopping the phenomena of resistance. In the future, we expect that along with MRSA and BGN-ESBL, we will encounter fungous, as well as atypical mycobacteria, which will lead to a new mutation that will have expensive repercussions regarding the first-line antibiotic-antimycotic-tuberculostatic therapy. All of this will maintain at high levels the consumption of antibiotics and antimycotics of the last generation, with the perpetuation of a vicious circle in terms of multidrug resistance (7, 8).

The prophylactic administration of antibiotics is justified only in special situations, for example in surgical interventions involving the intestinal tract or the repair of traumatic injuries, where the surgical site is contaminated with pathogens (7-9).

The main categories of microorganisms involved in the etiology of HAIs are bacteria (*Clostridioides difficile, Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumanii, Klebsiella pneumoniae, Escherichia coli),* followed by viruses, fungous, and parasites (5). Compared to the general population, hospitalized patients and immuno-compromised patients (with neoplasia, diabetes, hemodialysis), are much more susceptible to infections. Many patients have a fragile integumentary barrier (surgical or accidental injuries), which is the main way for germs to enter. The lack of intact skin and mucosal integrity provides easy access to infectious agents (10).

Over the past three decades, CDC data have shown that although overall rates of HAI have decreased, antimicrobial resistance of the pathogens involved, and severity of healthcare-associated infections have increased. Measures to prevent and control infections require a detailed knowledge of the epidemiology of the infection (11).

In conclusion, HAIs remain a tangible contemporary reality in all hospital structures. Patients hospitalized in intensive care units, hematology, and oncology departments, those with immunosuppression, premature, dystrophic, catheterized patients, polytransfused patients, or those with long

hospitalization, are the most exposed to nosocomial risk (12, 13).

The problem of HAI is widely recognized and almost all hospitals now have infection prevention and control programs. Hospitals must have programs that include the prevention and surveillance of HAIs, both in patients and in all medical staff. Therefore, each hospital must develop and implement its own procedures and protocols (9).

Due to technical achievements, including in the medical field, the emergence of new molecules, with a life-saving effect in the context of oncological and non-oncological patients, life expectancy in certain categories of patients, especially immunosuppressed ones, has increased. Infections associated with medical care with atypical mycobacteria and fungous are more common among them.

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EDITORIAL

THE CHALLENGES OF USING DIRECT ORAL ANTICOAGULANTS IN PATIENTS WITH CHRONIC KIDNEY DISEASE

Direct oral anticoagulants (DOAC) are undeniably a vital pillar of antithrombotic therapy. Dabigatran is a direct thrombin inhibitor that acts by preventing the conversion of fibrinogen to fibrin. Apixaban, betrixaban, edoxaban and rivaroxaban are selective factor Xa inhibitors. Since the first market launch of a DOAC 15 years ago, the number of indications has greatly expanded, based on their superior efficacy and safety in many clinical scenarios compared to classical vitamin K antagonists (VKAs). The indications include prevention of stroke and systemic embolism in patients with atrial fibrillation (AF); prevention of major cardiovascular events in patients with chronic coronary artery disease or peripheral artery disease; treatment of deep vein thrombosis and pulmonary embolism; prevention of recurrent venous thromboembolism (VTE); and prevention of VTE after total knee or hip replacement, or in hospitalized acutely ill medical patients (1). DOACs have several advantages over VKA, such as rapid onset and offset of action, limited food and drug interactions and a wider therapeutic window. They have a stable and predictable effect that allows the administration of fixed doses and does not require monitoring of the anticoagulant effect.

There is a solid bidirectional link between cardiovascular and kidney disease. Not only do they share the same risk factors, such as age, hypertension, dyslipidemia, diabetes, and obesity, but these two diseases are connected by pathophysiological links that allow them to increase each other's incidence and rate of progression in a downward spiral ending in premature heart failure, end-stage renal disease (ESRD) and death.

Individuals with chronic kidney disease (CKD) have an increased risk of thrombotic events, both arterial and venous, with up to half of patients with stage 4-5 CKD being affected during their lifetime. The prothrombotic state is the consequence of complex imbalances affecting the vascular endothelium, platelets and plasma factors of coagulation and fibrinolysis. Toxins related to renal disease decrease the expression and limit the activation of endothelial NO synthase, thereby reducing NO bioavailability and NO-mediated endothelial vasodilation (2). The endothelium becomes activated and expresses adhesion molecules, including the von Willebrand factor, which is responsible for the adhesion of platelets to the endothelium as the first step in generating thrombosis. The functional changes occurring in platelets transform their phenotype into a preactivated one (3). At the level of the damaged vessel wall, the tissue factor is exposed and initiates coagulation and thrombin generation. CKD patients have increased levels of factors V and VIII and reduced levels of antithrombin III, proteins C and S, which favor thrombosis (4). Impaired fibrinolysis is another important factor. The most important change is the increase in fibrinogen and PAI-1 levels. Moreover, fibrin clots

have a dense structure and are less permeable to the action of fibrinolytic enzymes, therefore more difficult to lyse.

CKD patients have a cluster of traditional cardiovascular risk factors, accelerated atherosclerosis and a double prevalence of atrial fibrillation compared to the general population (5). In addition, factors deriving from the presence of renal disease, such as volume overload, albuminuria, abnormal calcium-phosphate metabolism and anemia, also contribute to very high cardiovascular risk. Thus, patients with CKD not only have an increased risk of acute coronary syndromes (ACS) and peripheral arterial disease of the lower limbs but also of ischemic stroke. The greater the decline in renal function and the greater the albuminuria are, the more severe the acute arterial vascular events and the worse the prognosis. The risk of stroke is 5-30 times higher in CKD patients compared to the general population, especially in those on dialysis, and fatal in almost 90% of cases. CKD doubles the mortality rate among ACS patients and ranks third after cardiogenic shock and congestive heart failure as a predictor of mortality (6). A moderate and severe decrease in renal function causes a 2.5 times and 5.5 times increase in the risk of VTE, respectively, compared to normal renal function (7). In addition, the risk of VTE recurrence is 5.3 times higher in CKD patients compared to individuals with normal renal function (8). In dialysis patients - higher in those with hemodialysis than with peritoneal dialysis - the major risk is to develop pulmonary embolism, a risk that is double that of the population without CKD (9).

Patients with CKD not only have an increased risk of thrombosis, but also an increased risk of bleeding. Platelet hypo reactivity is the main contributor. Uremic toxins interfere with platelet-vessel wall interaction, platelet adhesion, aggregation and release of alpha granules, leading to a hemorrhagic phenotype (3). The risk of bleeding increases gradually as kidney function declines. Patients with ESRD have a threefold increased risk of bleeding compared to those without CKD. One in seven patients with stage 5 CKD will experience a major bleed within 3 years of starting dialysis. Of all bleeding sites, hemorrhagic stroke and upper gastrointestinal bleeding are particularly common (10).

As expected, the risk of bleeding increases with the addition of antithrombotic drugs, either antiplatelet or anticoagulant or combination therapy as needed in patients with AF and ACS treated by percutaneous coronary intervention (PCI). Moreover, the difficulty of antithrombotic therapy also lies in the fact that patients with advanced disease, stages 4-5 CKD, have generally been excluded from large, randomized trials or have been underrepresented. When choosing a DOAC for a patient with renal failure, a number of aspects must be considered, such as the severity of renal function decline, the type of DOAC, and the indication (AF or VTE; prevention or therapy).

All DOACs have a renal route of elimination, but there is great variability among them: dabigatran (80%), edoxaban (50%), rivaroxaban (35%), apixaban (27%) and betrixaban (7%). Thus, assessment of renal function is mandatory before initiation of any DOAC and periodically throughout treatment. Moderate or severe renal impairment may contraindicate the use of DOAC or require dose reduction in patients with AF. Dabigatran requires dose reduction at CrCl 30-49ml/min and is contraindicated at CrCl less than 30 ml/min. CrCl is

an essential reference point for establishing the indication for dose reduction in the remaining DOACs. Caution is advised when using low doses of apixaban, edoxaban and rivaroxaban in patients with CrCl 15-29ml/min. In a pooled analysis of patients with AF and stages 3-4 CKD of the pivotal trials of DOAC, all-cause mortality and the rate of all stroke and systemic embolism were slightly reduced by DOAC compared with warfarin (11). In addition, patients receiving a DOAC had less major bleeding than patients treated with VKA, particularly those with stage 4 CKD. Compared with warfarin, patients receiving a DOAC had a trend toward a reduction of intracranial hemorrhage, but slightly more gastrointestinal bleeding. To summarize, DOACs are preferred over VKAs in stages 1-4 CKD, except for dabigatran, which cannot be used in stage 4 CKD (12).

To date, all DOACs have been contraindicated at CrCl less than 15 ml/min (13). The 2020 ESC guidelines for the diagnosis and management of atrial fibrillation state that all DOACs are contraindicated in AF patients on dialysis. However, off-label use has been reported. Increased risk of bleeding was found in patients receiving dabigatran and rivaroxaban (14), but not apixaban (15). Furthermore, dose reduction was considered unnecessary because the composite outcome, including stroke/systemic embolism, major bleeding, and death, was more favorable with a dose of 5 mg bid compared with a lower dose of 2.5 mg bid, especially in terms of stroke prevention (16). New data has recently become available. The RENAL-AF trial published its results in December 2022 and reported that among patients with AF and ESRD on hemodialysis, apixaban treatment had similar rates of bleeding and stroke as warfarin (17). Of note, 71% of patients in this study received apixaban 5 mg bid. Although enrollment challenges led to the premature termination of the study and there was inadequate power to draw any conclusion, one finding should be highlighted, namely, that patients on hemodialysis experienced clinically relevant bleeding approximately ten times more frequently than thrombotic events (stroke cerebrovascular or systemic embolism).

A large meta-analysis showed that DOACs have similar efficacy and better safety than warfarin in preventing VTE recurrence across the spectrum of CKD severity (18). Although rates of intracranial bleeding were similar, DOAC users had fewer clinically relevant major and non-major bleeds than patients receiving VKAs. A systematic review focusing on the prevention of recurrent VTE in CKD patients reinforces these findings (19). Of note, one study showed better efficacy of dabigatran compared to warfarin in elderly patients with moderate renal impairment. DOACs and warfarin similarly reduced the risk of recurrent VTE and had a similar overall risk of bleeding. However, the risk of overall major bleeding from DOACs increased with decreasing renal function, except with apixaban. Additionally, in dialysis patients, apixaban had less overall bleeding compared to warfarin. Based on pharmacokinetic and pharmacodynamics studies, apixaban and rivaroxaban were recently approved by the Food and Drug Administration (FDA) for use in ESRD patients. Furthermore, in patients with VTE and ESRD, a retrospective analysis showed that apixaban has similar efficacy and better safety than warfarin. The incidence of major bleeding, including intracranial and gastrointestinal bleeding, was significantly lower in DOAC users (20).

Another element that differentiates DOACs is the binding to plasma proteins and implicitly the possibility that the drug is dialyzable. Protein binding is strongest for rivaroxaban (92-95%) which is not dialyzable, followed by apixaban (87%), betrixaban (60%), edoxaban (55%) which are partially dialyzable and finally dabigatran (35%), which is the most dialyzable.

A debate is related to the best method of evaluating the renal function, in order to adjust the dose or establish the contraindication for DOAC administration. In patients receiving apixaban, the parameter that defines the renal function is the value of serum creatinine. In patients receiving dabigatran, edoxaban or rivaroxaban, creatinine clearance is used to estimate renal function. The FDA, the highest authority providing guidance to the pharmaceutical industry, recommends that dose adjustment in patients with renal impairment be based on CrCl estimates and explicitly states that the Cockcroft-Gault formula should be used. Therefore, the pivotal trials with DOACs estimated renal function according to this recommendation (21-24). Because the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formulas estimate renal function more accurately than the Cockcroft-Gault formula and are widely implemented in current practice, dose mismatches may occur. It was recently shown that approximately 40% of AF patients would have required DOAC dose adjustment if either the MDRD or CKD-EPI formulas had been used instead of the Cockcroft-Gault formula (25). The consequences of this reclassification would have been 5 times increase in the risk of thrombotic events and a numerical increase in major bleeding events. This situation must be known and recognized as very important. Until the academic community and the pharmaceutical industry provide guidance for dose adjustments based on MDRD or CKD-EPI-estimated renal function, DOAC dose adjustments should continue to be made on the basis of renal function estimated by the formula used in the pivotal trials to ensure reproducibility in terms of efficacy and safety of DOAC use.

In the absence of CKD, renal function should be assessed at least annually, and for patients over 75-80 years of age, at least once every 6 months. When CKD is present (CrCl $\leq$ 60 mL/min), the interval between assessments is calculated according to the formula: the number of months = ClCr/10. In addition, renal function will be assessed whenever there is an acute event that may suddenly alter renal function.

We can conclude that the evidence of efficiency and safety of DOAC accumulated in recent years represents a strong ally in the reliable management of anticoagulant therapy in this fragile category of patients, which combines at the same time an increased thrombotic risk, but also a hemorrhagic one. There is increasing evidence that DOACs can be used across the spectrum of CKD.

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OBESITY IN WOMEN TREATED FOR BREAST CANCER - CONTROVERSIAL OBSERVATIONS ASSOCIATED WITH MENOPAUSAL STATUS AT DIAGNOSIS

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OBESITY IN WOMEN TREATED FOR BREAST CANCER - CONTROVERSIAL OBSERVATIONS ASSOCIATED WITH MENOPAUSAL STATUS AT DIAGNOSIS (Abstract): Breast cancer is a polymorphic disease, obesity being an important risk factor. The aim of the study is to assess different clinical, imagistic, biological determinants of fat mass and of gonadotropic axis in breast cancer treated patients according to their menopausal status at diagnosis. **Material and methods**: Seventy-eight breast cancer female patients were recruited cross-sectionally. All women were at menopause (physiologic or therapeutically induced). Fat mass was assessed by dual-energy X-ray absorptiometry, and immunoassays were used to determine serum hormones levels. **Results**: Post-menopausal diagnosed women showed statistically significant higher BMI (t=-3.159, p=0.002), abdominal (t=-2.305, p=0.036) and hip circumference (t=-2.683, p=0.009), but not hip/abdominal ratio (t=-0.647, p=0.520); also, they had significantly higher body fat mass (either total, subtotal or trunk fat; $p<0.05$). No statistically significant differences were observed in fasting glucose (t=-0.800, p=0.426), cholesterol (t=-0.566, p=0.573) nor triglycerides (t=0.969, p=0.336). Insulin levels, however, were significantly increased in post-menopausal group (t=-2.420, p=0.018), and subsequently HOMA-IR index assessing insulin-resistance was significantly increased (t=-2.305, p=0.024). Regarding sex hormones, no statistically significant differences were observed in circulating estrogens (estradiol and estrone) nor androgens (total testosterone and calculated free androgen index). However, for both pre-menopause and post-menopause groups, no statistically significant differences were observed in sex hormones according to weight status (obese *vs* non-obese). **Conclusions**: There is significant difference between weight status and risk of breast cancer regarding menopausal status, many reports showing that obesity is a risk factor in post-menopause patients and a protective factor in pre-menopause women, underlying the polymorphism of breast cancer disease. **Keywords**: BREAST CANCER, OBESITY, MENOPAUSE.

Obesity is a disease characterized by excess amount and/or unhealthy distribution of body fat (1). According to the World Health Organization (WHO), excess

weight in adults is characterized by a body mass index (BMI, calculated as weight measured in kilograms divided by height measured in meters squared) ≥ 25.0 kg/m^2, including obesity with a BMI ≥ 30.0 kg/m^2 and overweight status with a BMI between 25 - 29.9 kg/m^2 (1). Excess weight is thus divided into two categories: overweight and obesity.

Compared to people with normal weight, overweight or obese patients have an increased risk of developing multiple diseases, the most common being diabetes, cardiovascular diseases, and cancer (at least 13 types of cancer are being associated with obesity including breast cancer, colorectal cancer, renal cancer, liver cancer, ovarian cancer, multiple myeloma, meningioma) (2-8).

Breast cancer is by far the most common neoplasm among women, with approximately 2.26 million new cases diagnosed in 2020 (24.5% of cancer cases). It is also the most common cause of cancer death in women (15.5%, 684,996 deaths), ranking fifth place globally after the cause of death by cancer (following lung, colorectal, liver, and gastric cancers) (9). Of these, 23.7% of breast cancer cases and 20.7% of deaths were reported in Europe, although Europe has only 10% of the world's female population. In Central and Eastern European regions, 158,708 new cases and 51,988 deaths from breast cancer were reported, representing 29.9% of newly diagnosed cases but 36.3% of deaths caused by this type of tumor (9).

In Romania, 12,085 new cases of breast cancer and 3,918 deaths were reported in 2020, accounting for 2.27% of new cases and 2.4% of deaths attributed to breast cancer in Europe, and 8.5% of newly diagnosed cases but 7.6% of reported deaths for the central and eastern European region (9). A study (10) that analyzed differences in breast cancer mortality in Romania compared to the European Union (EU) over a period of 10 years (from entering EU, 2007-2016) showed a greater increase in the number of deaths caused by breast cancer in Romania, a trend of increasing mortality (opposite to that in the EU) and a reduction in life expectancy that gradually increased from 0.45 years in 2007 to 0.48 years in 2016 (Romania already having one of the lowest life expectancies in the EU, at 76.8 years, 5.4 years lower than the European average) (10).

WHO European Regional Report on Obesity in 2022 estimated that only in Europe, obesity is directly responsible for at least 200,000 new cases of cancer annually and this value is projected to increase in the coming decades (6). In 2012, globally, 33% of breast cancer cases (in postmenopausal women) were attributed to excess weight and obesity totaling 110,000 cases of which 28,000 could have been prevented if average BMI values in general population remained constant since 1982 (11).

Breast cancer is a polymorphic disease, and the current investigation has the objective to assess the differences of different clinical, imagistic, biological determinants of fat mass and of gonadotropic axis in breast cancer treated patients according to their menopausal status at diagnosis of the disease.

MATERIAL AND METHODS

Seventy-eight breast cancer female patients were recruited cross-sectionally. All women were at menopause, either physiologic or therapeutically induced (in function of when the cancer was diagnosed: pre-menopause or post-menopause). Fat

mass was assessed using dual-energy X-ray absorptiometry (DEXA); data recorded included total fat mass, subtotal fat mass (without head region) and trunk fat mass measured in grams (g) or as percentage (%) of total body mass. Immunoassays were used to determine serum hormone levels of insulin, FSH (follicle stimulating hormone), estradiol, estrone, total testosterone. Fasting glucose, total cholesterol and triglycerides were also measured. Insulin-resistance was calculated by HOMA-IR index, and the free testosterone was assessed by determining FAI (free androgen index).

All statistical analyses were performed using *SPSS Version 24.0* software (IBM Corp., Armonk, NY, USA). Associations between continuous variables were analyzed using Spearman correlation coefficients. The level of statistical significance was established at $p<0.05$.

Ethical approval was obtained from the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy Iasi (no. 1642/25.01.2017). The research protocol was approved by the institutional ethics committees of "Sf. Spiridon" County Clinical Emergency Hospital Iasi (no. 78/21.12.2016) and "Grigore T. Popa" University of Medicine and Pharmacy Iasi.

RESULTS

Seventy-eight female patients evaluated at the Endocrinology Department for metabolic or endocrine disease associated to breast cancer treatment were included in the current analysis. Patients had a mean age of 53.3 ± 11.6 years (age between 35-78), among which 45 were diagnosed with breast cancer before menopause, and 33 patients after the menopause. However, all patients were at menopause (physiological or induced) at the moment of assessment.

The group of women diagnosed pre-menopause had a mean age at assessment of 47.5 ± 9.5 years old, being diagnosed at a mean age of 41.6 ± 7.8 years, with mean years of disease of 5.9 ± 5.6 years. They had a mean duration of fertile years (between menarche and induced menopause) of 28.8 ± 7.7.

Women diagnosed after menopause had a mean age at assessment of 61.2 ± 9.3 years old, being diagnosed at a mean age of 57.9 ± 9.3 years, with mean years of disease of 3.4 ± 3.6 years. They had a mean duration of fertile years (between menarche and physiological menopause) of 33.6 ± 4.6.

Morphometric parameters analysis showed that patients did not have a significant difference between height ($t = -0.436$, $p = 0.664$), however the weight was higher in post-menopausal diagnosed patients ($t = -2.8$, $p = 0.006$), determining subsequent variations in BMI ($t = -3.159$, $p= 0.002$). According to the WHO classification of weight status by BMI, in the group of women diagnosed pre-menopause: 57.8% had normal weight (n=26), 28.9% were overweight (n=13) and 13.3% obese (n=6). Post-menopausal group comprised of 36.4% patients with normal weight (n=12), 27.2% overweight (n=9) and 36.4% with obesity (n=12) (fig. 1).

Abdominal circumference ($t = -2.305$, $p = 0.036$) and, also, hip circumference ($t = -2.683$, $p = 0.009$) varied significantly in the two groups, however not the hip/abdominal ratio ($t = -0.647$, $p = 0.520$). The same thing was valid also for fat mass, either total, subtotal or trunk fat mass ($p<0.05$).

Regarding biological parameters, no statistically significant levels were observed in fasting glucose ($t = -0.800$, $p = 0.426$), total cholesterol ($t = -0.566$, $p = 0.573$) and triglycerides ($t = 0.969$, $p = 0.336$) levels between the groups.

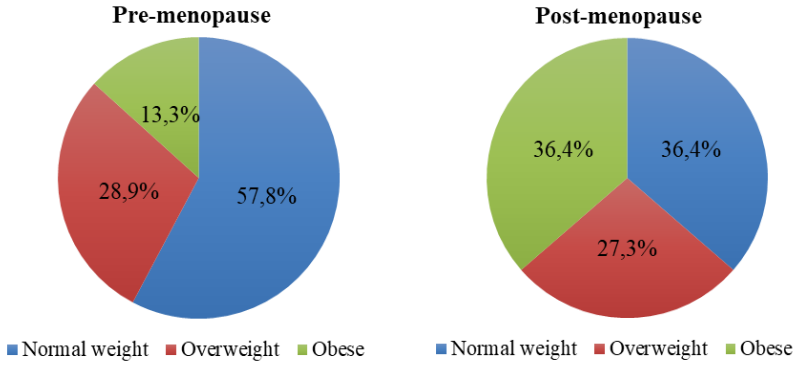

Fig. 1. Distribution of breast cancer cases in pre-menopause (right) *vs.* post-menopause (left) patients according to weight status

However, when analyzed, insulin levels were significantly different (t = -2.420, p = 0.018) in the two groups, pre-menopausal diagnosed women had a lower mean insulin level (9 ± 5.9 µUI/mL), than post-menopausal diagnosed cases (14 ± 11.8 µUI/mL), and subsequently the HOMA-IR assessing insulin-resistance (normal insulin-sensitivity when HOMA-IR below 2) was significantly different (t = -2.305, p = 0.024): in the pre-menopausal group HOMA-IR was lower (mean of 1.8) than in post-menopausal group (mean of 3.2).

Regarding ovarian function, even if all patients were at menopause at the moment of assessment, a difference was observed in FSH levels, as pre-menopausal diagnosed women had a lower mean FSH levels (33.1 mUI/mL), than post-menopausal diagnosed cases (53.4 mUI/mL), statistically significant (t = -2.899, p = 0.005). Estradiol levels measured in these groups showed slightly elevated values for what is normally considered in menopause (normally below 30 pg/mL): pre-menopausal diagnosed group had mean estradiol levels of 53.38 pg/mL, and post-menopausal group of 50.6 pg/mL, however not statistically significant different (t = 0.233, p = 0.817). Similarly, estrone levels also varied between the groups, but not reaching the statistical significance of $p<0.05$ (t = 1.870, p = 0.065): pre-menopausal group had a slightly higher mean value (84.9 ± 39.9 pg/mL) that the post-menopausal group (70.3 ± 25.3 pg/mL). Testosterone levels assessed by total testosterone (t = -2.899, p = 0.005) or as calculated free androgen index (t = -2.899, p = 0.005), were not significantly different between the two groups.

However, for both pre-menopause and post-menopause groups, when checked for differences in sex hormones according to weight status (obese *vs.* non-obese) no statistically significant differences were observed.

DISCUSSION

The major confirmation was that post-menopausal diagnosed women had significant higher fat mass indicators (BMI, abdominal and hip circumference, and quantity of body fat mass in all regions, assessed by DEXA). Even if the number of subjects included in the current analysis was small,

the data is concordat with global results.

Worldwide, the incidence of obesity has almost tripled since 1975 reaching pandemic levels (12). In Romania, available epidemiological data from 2007-2008 (13, 14) estimated a prevalence of overweight of 30-36% of the adult population and obesity of 20-25% (13, 14), with similar values reported in 2015 (15). WHO European Regional Report on Obesity 2022 placed Romania among countries with over 55% of the adult population being overweight and where more than 20% of the population is obese (6). Excess weight has a higher prevalence in men compared to women. In Romania excess weight has a lower prevalence of 51.1% in women compared to 64.3% in men (average prevalence in both sexes of 57.7%); on the other hand, obesity has a higher prevalence in women, of 23.4%, compared to 21.6% in men (average prevalence of obesity in both sexes of 22.5% in Romania, assessed by BMI) (6)We reported the differences between weight and gonadotropic axis in a cross-sectional analysis of female patients treated with breast cancer.

The association between excess weight (overweight/obesity) and breast cancer risk was reported to vary depending on menopausal status, with multiple studies showing a negative correlation between obesity and this risk in premenopausal women (16-18). Van den Brandt *et al.* (19) conducted an analysis of 7 prospective studies totaling a cohort of 337,829 women, of whom 4,385 were diagnosed with invasive breast cancer, finding that in pre-menopause women with a BMI over 31 kg/m^2 have a relative risk (RR) of 0.54 [95% confidence interval (CI 95%): 0.34-0.85] of developing breast cancer compared to those with a BMI below 21 kg/m2 (19). In 2001 Bergström *et al.* (20) conducted another meta-analysis including 9 studies also demonstrating an inverse correlation between obesity and increased BMI regarding breast cancer risk in premenopausal women (RR: 0.98; CI 95%: 0.97-0.99) (20). Renehan *et al.* (19) presented similar results in a meta-analysis consisting of 34 studies (totaling over 2.5 million women) that investigated breast cancer risk in relation to BMI (21). In total breast cancer was reported in 7,930 premenopausal women and 23,909 postmenopausal women. Breast cancer risk was reduced by approximately 8% per 5 kg/m^2 increase in BMI in premenopausal women (RR: 0.92; CI 95%: 0.88-0.97; p=0.001); on the other hand, in postmenopausal women breast cancer risk was increased (RR 1.12; CI 95%: 1.08-1.16; p<0.0001) (21).

The largest and most recent study investigating the association between obesity and breast cancer was conducted by the Premenopausal Breast Cancer Collaborative Group in 2018 (22). This multicenter study included 758,592 premenopausal women aged 18-54 years. They were followed for a median period of 9.3 years, during which 13,082 cases were diagnosed with breast cancer. The analysis showed that underweight women have a 4.2 times higher risk of developing breast cancer than obese women under the age of 24 and a 1.9 to 2.5 times higher risk in women over this age. Taking these results into account, the authors determined an estimated reduction of 12% to 23% in premenopausal breast cancer risk with a 5 kg/m^2 increase in BMI depending on age. Breast cancer positive for estrogen (ER) and/or progesterone receptors (PR) was associated with BMI in all age groups while hormone receptor-negative breast cancer was associated with BMI only in women aged 18-24

years. In this younger age group, the strength of the association between ER and/or PR positive breast cancer and BMI (HR: 0.75; CI 95%: 0.70-0.81) was much stronger than that for hormone receptor-negative breast cancer (HR: 0.85; CI 95%, 0.76-0.95) (22). The lack of association of triple-negative breast cancer with BMI in women over 25 years was contrary to previous reports (23). The authors considered that the discrepancy possibly reflects differences in study design (case-control versus prospective study), the former being more susceptible to potential selection bias (22).

Unlike women of reproductive age, several large studies and meta-analyses have demonstrated a direct and consistent association between obesity and breast cancer risk in postmenopausal women. The Million Women Study (24) followed 1.2 million women in the UK (including 45,037 cases with breast cancer) aged 50-64 years for a median period of 5.4 years. The study identified a risk about 30% higher for breast cancer in obese women compared to non-obese women (RR: 1.29; CI 95%: 1.22-1.36) in the postmenopausal period (24). These findings are comparable to those of Renehan *et al.* (21), previously described (breast cancer risk in postmenopausal women was positively associated with each 5 kg/m^2 increase in their BMI: RR: 1.12; $p < 0.001$) (21).

Local and systemic changes are hypothesized to support the relationship between obesity and breast cancer, including increased circulating levels of insulin and glucose as well as adipose tissue-derived estrogens, adipokines and inflammatory mediators (25).

Subsequent to increased fat mass, insulin levels and HOMA-IR (insulin-resistance) were significantly increased in post-menopause diagnosed women, and not pre-menopause breast cancer cases. No changes were observed with fasting glucose, total cholesterol, or triglycerides between the groups.

Also, no statistically significant differences were observed between circulating sex hormones levels in the two groups. All cases were at menopause and the only difference was in FSH levels; even if it was increased (considered inhibiting for the ovarian function), it was still lower in patients diagnosed with breast cancer in pre-menopause than in women with breast cancer after menopause, possibly in accordance with the number of years since the menopause occurred.

Given the impact of obesity on the development and progression of breast neoplasm, the recommendation is for overweight women to avoid weight gain during treatment and for obese women to lose weight immediately after treatment (26, 27). In the meta-analysis conducted by Chan *et al*. (28), which analyzed 23,182 deaths from 213,075 breast cancer survivors confirmed that the current indication for breast cancer patients is to remain as much as possible within the normal weight range (28).

CONCLUSIONS

Obesity and breast cancer are often associated; however, there is a significant difference between weight status and the risk of breast cancer regarding menopausal status, many reports showing that obesity is a risk factor in post-menopause patients and a protective factor for breast cancer in pre-menopause women. Our result show that post-menopause breast cancer patients present increased fat mass parameters,

insulin levels and insulin-resistance, compared to women diagnosed in premenopause, underlying the polymorphism of breast cancer disease.

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CONFLICT OF INTEREST AND FUNDING

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URTICARIA IN CHILDREN - A DISEASE IN DISGUISE

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URTICARIA IN CHILDREN-A DISEASE IN DISGUISE (Abstract): Urticaria is considered a multifactorial condition, initializing or triggering chronic conditions with dermal or/and hypodermic localization, more common in children and young people. **Material and methods:** A number of 87 children were considered in a retrospective study aiming the diagnostic of acute and chronic urticaria, evolutive particularities and immunological aspects of the allergic condition. Descriptive analysis and correlation with biological parameters of the immunological status (total IgE, allergen-specific Ig E IgA, IgM, IgG) were performed. **Results:** The age distribution showed that most children aged 10-15, with a balanced gender distribution (59.57% male *vs.* 40.43% female patients). Immunological profile associated immune deficiency (IgA and IgG, a minority of patients presenting deficiency of IgM). Elevation of IgE values were found in 9% of cases. Low serum levels of Ig G, A, and M associated clinical features such as upper respiratory infection, gastroenteritis, and otitis media. Four patients with multiple immunoglobulin deficiency and 5 cases with single immunoglobulin deficiency were noticed, including a complete IgA deficiency. Arthritic impairment was observed in 10% of cases. **Conclusions:** Various immune deficiencies were found in children with acute and chronic urticaria. Prospective research is needed to clarify the real role of triggers in the pathogenesis of urticaria and their relative prevalence, in order to apply, with greater accuracy, prevention methods in children at risk. **Keywords**: URTICARIA, CHILDREN, IMMUNO-GLOBULINS.

Allergic diseases can be considered systemic disorders, regarding the polymorphism of the general causes, the associated symptoms and the simultaneous damage to several organs and systems (1). From food allergies with digestive impact to sensitivity to aeroallergens with the atopic cascade towards bronchial asthma, this pathology has aroused the interest of specialists from all fields (2). Nowadays, urticaria is considered to be, in fact, a multifactorial condition, initializing or triggering chronic conditions with dermal or/and hypodermic localization, more common in children and young people. Particularly, less common causes of urticaria, other than allergic agents, aroused specific interest in identifying and interpreting this symptom along with infectious, autoimmune and idiopathic conditions in pediatric patients (3).

The constant concern of allergologists to study the pathogenesis of the disease, the

evolution of clinical manifestations, the biological pattern, but also the evolutionary complications of urticaria confirm the importance of the early implementation of treatment methods (4). Pathogenic and symptomatic medication can momentarily improve the clinical status, but long-term surveillance of these cases is essential, in the event of silent complications that may appear (autoimmune diseases, infection, chronic evolution) (5).

In the present study, we tried to capture the clinical and evolutionary characteristics of children urticaria, a category of patients in which studies of prevalence and etiology are still poor from an informational point of view. For this reason, specialists can take into consideration the ethics of research on pediatric subjects, as well as the reluctance of parents or relatives to be co-opted in clinical trials, and last but not least, the impossibility of exhaustively extrapolating the results obtained in studies conducted on adult patients.

MATERIAL AND METHODS

Study group

A number of 87 children were considered in a retrospective study aiming the diagnostic of acute and chronic urticaria, evolutive particularities and immunological aspects of the allergic condition, other than an allergic-induced trigger. Patients were hospitalized in the Allergology Clinic of the "Sf. Maria" Emergency Clinical Hospital for Children Iasi in a 2-year interval of time. In order to systematize the ideas derived from the study of the material represented by the documentation of the evolution of cases, authors aimed the following objectives:

1. Analysis by observation and correlation of some reference parameters for a representative study group, in which the diagnosis was accurately established and the provision of clinical and biological surveillance in dynamics.

2. Descriptive analysis of the study group for the main defining essential epidemiological parameters: distribution by age groups, gender, distribution according to the type of the disease, distribution according to the associated pathology with acute respiratory disease, also the distribution according to evolutionary complications in the patients included in the present study.

3. Correlation with biological parameters of the immunological status, performing blood sampling to measure total IgE, allergen-specific Ig E IgA, IgM, IgG.

RESULTS

Descriptive analysis

Most of the children were admitted in acute phase of the urticaria, almost 88% of the total of patients, the remaining percentage being assigned to chronic cases of urticaria (fig. 1).

It is rather difficult to assess the chronic status of urticaria, regarding the timing of the rash (appearance, evolution pathway, aspect, associated signs and symptoms), but also determining the minimum six weeks interval of time that is necessary for defining chronic urticaria. In some cases, we can also discuss the possibility of a second allergen involvement, with a new rash appearing, or even multiple triggers (allergens, medicines, stress etc.).

The age distribution showed that most cases were in 10-15 years old children, on behalf of chronic urticaria (fig. 2). Hormonal changes within the adolescence and the possibility of triggering autoimmune diseases, or even the similarity with other pathological conditions are the facts that can enhance allergy status in this age group.

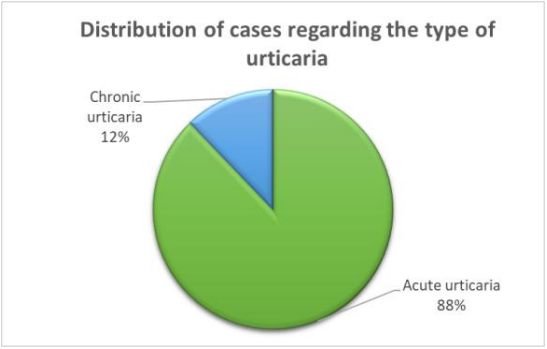

Fig.1. Distribution of cases regarding the type of the urticaria

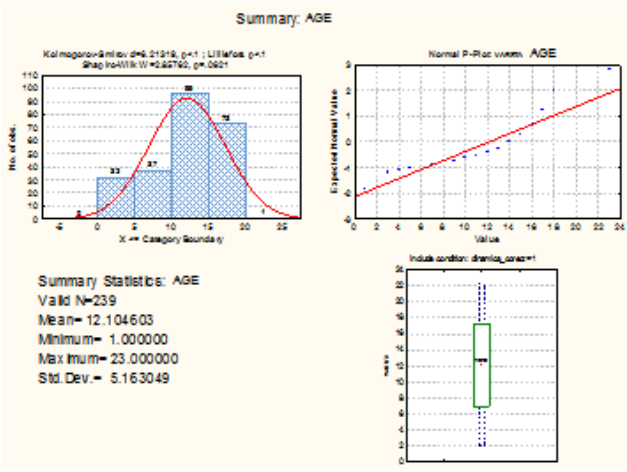

Fig. 2. Histogram of age for the patients with acute and chronic urticaria

Gender distribution was rather balanced, with a slight deviation in favor of male patients (59.57% versus 40.43% female patients). This situation can be explained by the fact that males are more prone in developing, enhancing and transmitting the atopy (in a genetic pathway related to Y chromosome).

Immunological findings

Immunological profile of the patients associated also immune deficiency (Ig A and Ig G, a minority of patients presenting deficiency of Ig M). Elevation of Ig E values were only found in 9% of the cases, leading to the possibility of involving non-atopic mechanisms in the pathogeny of urticaria (fig. 3).

Low serum levels of immunoglobulins G, A, and M were also associating outstanding clinical features of each group - upper respiratory infection, gastroenteritis, and otitis media. In 9 cases, immunoglobulin E levels were above the normal range for age for the laboratory. Four examples of multiple immunoglobulin deficiency and 5 examples of single immunoglobulin deficiency were noted, including a complete IgA deficiency.

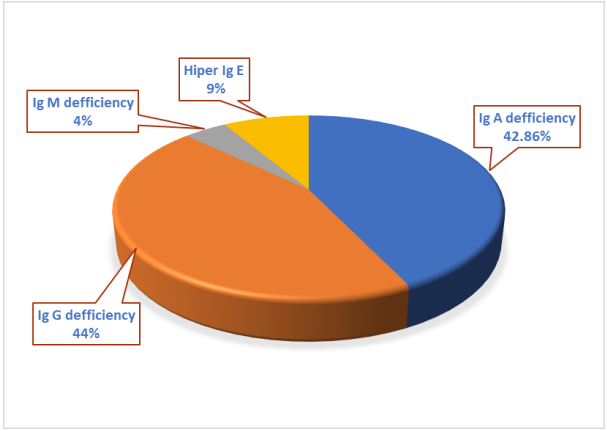

Fig. 3. Immunological findings in patients with acute and chronic urticaria

Atopic and non-atopic associated conditions were determined in the study group, most of cases presenting other allergic manifestations (allergic rhinitis, bronchial asthma, cow s milk allergy, histamine intolerance). Arthritic impairment in selected cases was also observed in a minority of cases (fig. 4). The discussion regarding the obesity versus malnutrition in allergic patients leads to considering food allergy, gastroesophageal reflux and/or autoimmune inflammatory bowel disease to be ruled out in differential diagnostic. Adipokine involvement in determinism of chronic urticaria should be further assessed also in obese pediatric patients.

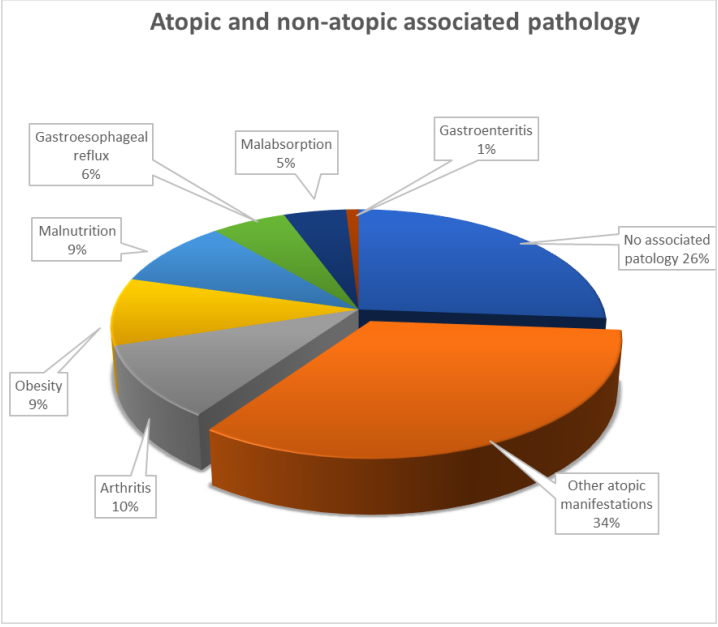

Fig. 4. Associated pathologies in children with urticaria

DISCUSSION

Many bacterial infections have been associated with urticaria manifestation, such as those due to *Helicobacter pylori*, *Streptococcus*, *Staphylococcus*, *Mycoplasma pneumoniae*, *Salmonella*, *Brucella*, *Mycobacterium leprae*, *Borrelia*, *Chlamydia pneumoniae*, and *Yersinia enterocolitica*. In some cases, the skin manifestations, falsely considered as urticaria, the presence of the microorganism containing toxins, or the complement activation mediated by circulating immune complexes were, in fact, the trigger factors for the skin rash (6).

This is the case of a young male patient hospitalized with rash and fever, associating cough and alteration of the general status, in which case a bronchial lavage culture revealed the presence of *Mycoplasma pneumoniae* (fig. 5).

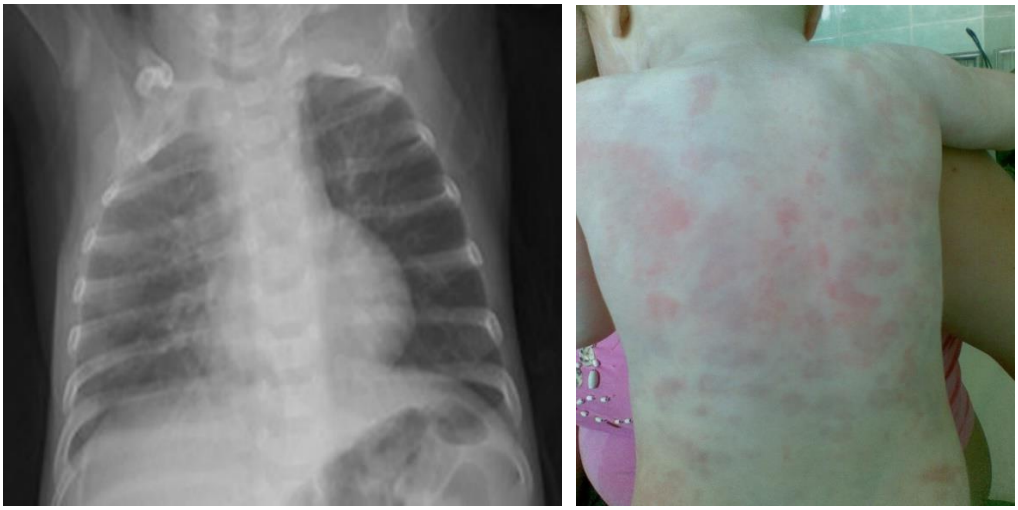

Fig. 5. Male patient, 18 months old, with *Mycoplasma pneumoniae* infection and urticaria

Urticaria may appear also as prodroming various infectious diseases, as seen in one of the patients (female patient, 8 years old) included in the study group (fig. 6).

In this case, the presumed urticarial manifestation was, in fact, a pre-icteric complication of a viral hepatitis, being pathogenically linked to the deposition of circulating immune complexes at the level of the dermis and being frequently associated from a clinical point of view with arthritis and headaches (Caroli's triad). However, an obvious causal relationship between the occurrence of urticaria and chronic viral hepatitis infection has not been objectified. In the case of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, eruptions are cited as extrahepatic manifestations, but these are probably more related to the underlying vasculitis than to actual urticaria (7). Many patients with urticaria and angioedema were diagnosed with chronic infection with HBV, the markers of hepatitis infection being detected by chance or following subjective accusations that required testing (8). Unlike them, anti-HCV antibodies are less frequent among those diagnosed with urticaria and/or angioedema, according to existing studies in the specialized literature, so we

cannot speak of a significant association between the two conditions. If routine testing for HCV in patients predisposed to eruptions or angioedema does not appear to be effective, viral markers for chronic HCV infection should be sought in these patients.

Fig. 6. Female patient, 8 years old, with chronic urticaria and hepatitis C

Urticarial vasculitis was not identified in the absence of typical clinical features. Food allergy was encountered in a small number of patients and clinical significance was found in the subgroup of patients with positive history of allergy to related foods. Also, intestinal parasitic infestation was found in 5% of children with chronic urticaria, but the clinical correlation could not be established. Several studies demonstrated that 21% of patients with chronic urticaria had no laboratory abnormality. Chronic autoimmune urticarial was identified in more than one-third of children with chronic urticaria, without protracted clinical course. However, a long-term follow-up would be important to identify any association of CAU and other autoimmune diseases (9).

Chronic urticaria-like lesions may also appear in systemic lupus erythematosus (SLE), cases in which the manifestation of chronic urticaria is diverse and often characterized by flares and remissions, and there may be an overlap between clinical features of chronic urticaria and SLE. Alt-

hough patients with acute or chronic urticaria usually do not exhibit LE-specific skin lesions and do not meet the diagnostic criteria for SLE, urticaria may represent the first complaint in these patients before the more classical form of SLE manifests. Urticaria and SLE may have similar cutaneous manifestations, and both are related to immunopathological conditions; however, few studies have examined the effects on SLE of a history of urticaria.

Abnormally low immunoglobulin levels were found in 6 immediate family members (representing 2 of the studied families), and there were also frequent positive family histories of allergy, repeated infection, and collagen disease within the second and third generation relatives. These findings suggest that abnormal immunologic function with resultant repeated bacterial infection may be causally related to the development of urticaria in certain individuals, and that chronic urticaria may be the presenting clinical manifestation of an immunologic-deficiency state.

CONCLUSIONS

Urticaria is one of the most common skin diseases, and pediatric patients may present with a wide spectrum of symptoms, including sudden development of pruritic wheals or angioedema. Chronic urticaria has a significant impact on quality of life due to the constant sensation of itching, regular recurrence, and unknown etiology. Chronic urticaria is diagnosed when disease has been continuously or intermittently present for at least 6 weeks. The chronic and acute forms of urticaria differ in etiology, pathophysiology, and underlying mechanism, being hard to assess mostly in early age.

Previous studies have reported that chronic idiopathic urticaria is associated with infection, pseudo-allergy to a food, autoimmunity or other condition. However, there is little information on the relationship of chronic urticaria developing with non-allergic triggers in children. The association with infectious disease, such as viral and bacterial determined rash, was previously assessed in young children, whereas pneumonia, pharyngitis, gastroenteritis and other diagnostic status created challenges in investigating and treating the patients.

Although only a weak association with urticaria of unclear pathogenesis exists, clinicians should consider these bacterial agents, autoimmune factors or pseudo-allergens in the workup of the patients with urticaria. Prospective studies and well-structured research are obviously needed to better clarify the real role of non-allergic triggers in the pathogenesis of urticaria and their relative prevalence.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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THE ENIGMATIC PROFILE: UNRAVELING CLINICAL AND BIOLOGICAL FEATURES IN COVID-19 PATIENTS

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THE ENIGMATIC PROFILE: UNRAVELING CLINICAL AND BIOLOGICAL FEATURES IN COVID-19 PATIENTS (Abstract): The outbreak of COVID-19, caused by the novel coronavirus SARS-CoV-2, has emerged as an unparalleled global health crisis, affecting millions of lives worldwide. This respiratory illness exhibits a wide spectrum of clinical presentations, ranging from mild flu-like symptoms to severe acute respiratory distress syndrome (ARDS), leading to high morbidity and mortality rates, especially among vulnerable populations. In this abstract, we provide a concise overview of the clinical and biological characteristics observed in COVID-19 patients. **The aim** of this study was to evaluate patients with COVID-19 presenting with mild to moderate clinical and paraclinical features and their subsequent outcomes. **Materials and methods:** The study group included 200 patients diagnosed with confirmed COVID-19, exhibiting mild to moderate symptoms, who were admitted for treatment and health monitoring. **Results:** The estimated risk of SARS-CoV-2 infection was over 5 times higher in asymptomatic afebrile patients (RR=5.82; 95% CI: 0.88-38.65; p=0.004). Among asymptomatic patients, C-reactive protein (CRP) levels ranged from 0.58 to 39.88 mg/dL, while in symptomatic patients, the range varied from 0.20 to 200.17 mg/dL. The mean level of CRP was significantly higher in symptomatic patients (13.67 *vs.* 5.73 mg/dL; p=0.001). Regarding imaging examinations, 38.6% of subjects did not show pathological aspects. The time to viral clearance ranged from 9 to 41 days in asymptomatic patients and from 2 to 38 days in symptomatic patients, with a similar mean duration in both groups (18.71 *vs.* 18.79 days; p=0.936). **Conclusions:** The estimated risk of SARS-CoV-2 infection was over 5 times higher in asymptomatic afebrile patients, and among laboratory markers, the inflammatory marker CRP was associated with a more severe COVID-19 outcome. Our study has several limitations that need to be acknowledged, including the small number of patients and the short follow-up period. **Keywords:** COVID-19, SARS-CoV-2, CLINICAL CHARACTERISTICS, BIOLOGICAL FEATURES, PANDEMIC, RESPIRATORY ILLNESS.

COVID-19, caused by the coronavirus SARS-CoV-2, has emerged as one of the most devastating pandemics in modern history, profoundly impacting the lives and health of the global population. This respiratory disease was first identified in December

2019 in the city of Wuhan, Hubei Province, China, and quickly evolved to spread worldwide, becoming a major public health threat. The onset of the COVID-19 pandemic was characterized by a rapid spread of new cases, accompanied by increasing concerns among scientific communities, governments, and international health organizations. The SARS-CoV-2 virus has demonstrated an exceptional capacity for human-to-human transmission through respiratory droplets and direct contact, fueling its rapid dissemination across diverse social and geographic settings (1).

The stage of the COVID-19 pandemic continues to be dynamic and to evolve constantly. Despite substantial efforts to control the virus spread and develop vaccines and treatments, the adaptability of SARS-CoV-2 and the emergence of new strains have posed significant challenges for effective pandemic management.

In this article, we aim to analyze the progress of the COVID-19 pandemic, highlighting essential aspects of epidemiology, clinical and biological characteristics, as well as efforts to fight the disease and enhance medical responses. Understanding the evolution of the pandemic and the factors contributing to the success or failure of control measures is crucial for developing future strategies and improving global capacity for potential future pandemic threats.

Thus, this article seeks to provide a comprehensive overview of the COVID-19 pandemic evolution, with the purpose of making relevant contributions to the current management of the situation and identifying potential directions for action in the near future.

MATERIALS AND METHODS

We conducted a retrospective study that included 200 patients with clinically and radiologically diagnosed COVID-19, admitted to the Medical Clinic V of C.F. Iasi Clinical Hospital, Iasi, Romania, between 1st April and 29th June, 2020. The inclusion criteria were patients with confirmed diagnosis of COVID-19 who signed the informed consent. Patients with severe COVID-19 we excluded. All patients signed informed consent for study inclusion, and there were no age-related restrictions.

The study group was divided into two subgroups based on the presence of symptoms: I - 67 asymptomatic COVID-19 patients (33.5%), II - 133 symptomatic COVID-19 patients (66.5%). Complete clinical examination (medical history + objective clinical examination) was performed on admitted subjects; blood samples were collected for serological and biochemical tests, and imaging investigations (chest X-ray, computer tomography examination) were performed.

For statistical analysis, SPSS version 18.0 was used. Tests such as ANOVA were used to analyze the dispersion of the dependent variable, both intra and intergroup; the coefficient of variation (CV%) highlighted the percentage deviation between two means, providing results on the homogeneity of the value series. To calculate the significant difference between two or more groups, depending on the distribution of the value series, at a significance level of 95%, the following tests were applied for quantitative variables: the t-Student test - a parametric test that compares mean values recorded in 2 groups with normal distributions, and the F-test (ANOVA) used when comparing 3 or more groups with normal distributions. The chi-square test (c2) is a non-parametric test that compares 2 or more frequency distributions from the same population, applied when the

expected events are excluded. Sometimes, when a frequency in the calculation formula is small, Yates correction is applied to obtain a higher estimate of the difference. The Kruskall-Wallis correlation compares ordinal variables from 3 or more groups, while the Pearson correlation coefficient (r) represents the correlation between two variables from the same group (direct/indirect correlation). The ROC curve establishes the sensitivity/specificity balance of one parameter in relation to another.

RESULTS

Gender distribution showed a higher proportion of female patients, with a ratio of 2:1. Age ranged from a few days to 86 years, with a mean age of 44.34 years ± 15.88, and a median of 45 years. The majority of cases were found in the age group of 40-49 years (36.5%), both in females and males (40.6% *vs.* 28.4%; p=0.113). Regarding gender, it was observed that 38.6% of males and 44.4% of females were asymptomatic (p=0.452) (fig. 1).

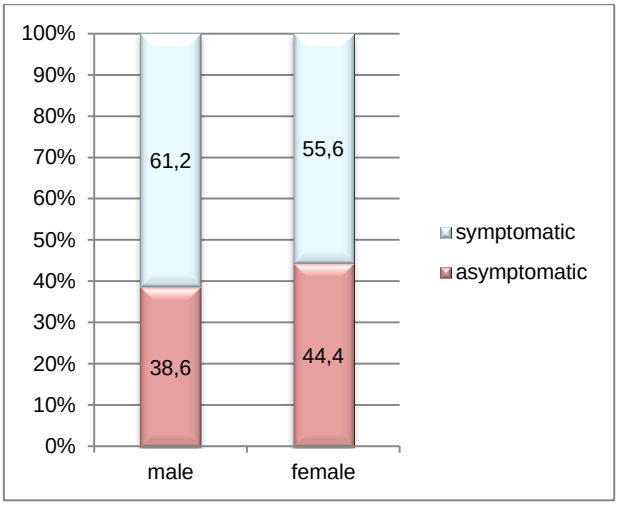

Fig. 1. Distribution of asymptomatic cases by gender

The proportion of asymptomatic cases by age groups revealed the following aspects (p=0.002) (fig. 2):

- In the age group under 10 years, 63.6% of children were asymptomatic.
- All children in the 10-19 age group were asymptomatic.
- Among adults, the highest proportion of asymptomatic cases was recorded in the 20-29 years group (58.3%) and 40-49 years group (49.3%).
- 77.8% of cases were asymptomatic in the 70-79 years age group.
- All elderly individuals in the over 80 years age group were symptomatic.

Upon admission, the estimated risk of SARS-CoV-2 infection was over 5 times higher in asymptomatic afebrile patients (RR=5.82; 95% CI: 0.88-38.65; p=0.004). Interestingly, if an afebrile patient presents with a cough, this symptom seemingly acts as a protective factor (RR=0.12; 95% CI: 0.02-0.84; p=0.001). Among symptomatic

patients, the presence of cough (RR=1.75; 95% CI: 1.47-2.09; p=0.001), anosmia (RR=1.80; 95% CI: 1.59-2.05; p=0.001), dyspnea (RR=1.78; 95% CI: 1.57-2.02; p=0.009), headache (RR=1.84; 95% CI: 1.59-2.06; p=0.001), and abdominal or chest pain (RR=1.77; 95% CI: 1.57-2.01; p=0.018) was associated with an approximately 2-fold higher risk of SARS-CoV-2 infection (tab. I).

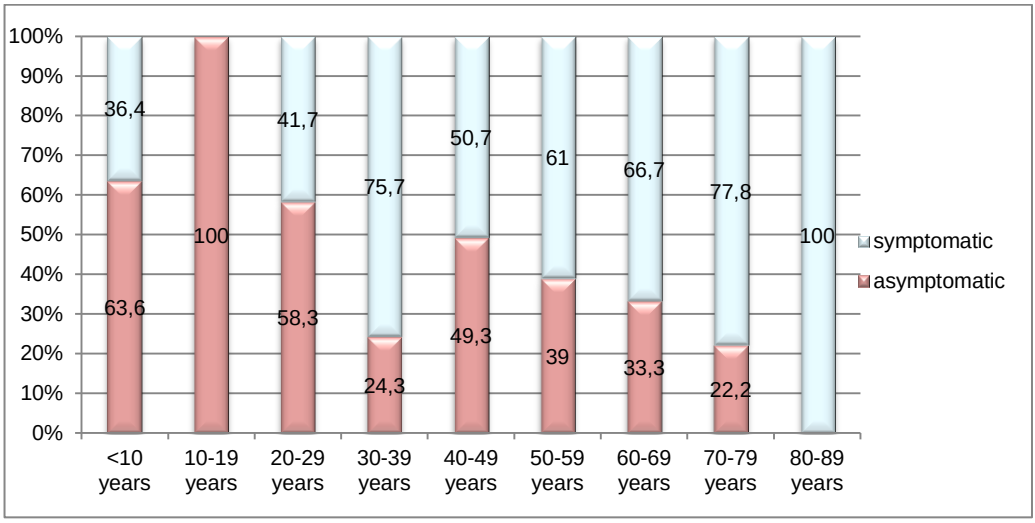

Fig. 2. Distribution of asymptomatic cases by age groups

TABLE I.

Estimated Risk of SARS-CoV-2 in Asymptomatic/Symptomatic Patients

Symptoms	Asymptomatic (n=85)	Symptomatic (n=115)	p-value
Afebrile	84 (98.8%)	103 (89.6%)	0.004
Cough	1 (1.2%)	17 (14.8%)	0.001
Anosmia	0 (0.0%)	9 (7.8%)	0.001
Dyspnea	0 (0.0%)	6 (5.2%)	0.009
Fatigue	1 (1.2%)	6 (5.2%)	0.101
Headache	0 (0.0%)	10 (8.7%)	0.001
Nausea	1 (1.2%)	2 (1.7%)	0.743
Pain	0 (0.0%)	5 (4.3%)	0.018

Note: RR - Relative Risk, IC95% - 95% Confidence Interval

Among comorbidities, in asymptomatic patients, only myocardial infarction induces an estimated risk of COVID-19 that is approximately 2 times higher (RR=1.74; 95% CI: 10.35-8.64; p=0.364) (tab. II).

Regarding biological parameters, in asymptomatic patients, CRP ranged from 0.58 to 39.88 mg/dL, while in symptomatic patients, the range was 0.20-200.17 mg/dL. The mean level was significantly higher in symptomatic patients (13.67 *vs.* 5.73 mg/dL; p=0.001).

TABLE II.
Estimated Risk of COVID-19 in Patients with Personal Pathological History (Comorbidities)

Comorbidities	Asymptomatic (n=85)	Symptomatic (n=115)	p-value
Type 2 Diabetes	5 (5.9%)	7 (6.1%)	0.952
Hypertension	15 (17.6%)	22 (19.1%)	0.789
Asthma	1 (1.2%)	1 (0.9%)	0.830
Chronic Hepatitis	1 (1.2%)	7 (6.1%)	0.059
Obesity	4 (4.7%)	7 (6.1%)	0.670
COPD	0 (0.0%)	2 (1.7%)	0.135
Thyroid Disorder	3 (3.5%)	4 (3.5%)	0.984
Autoimmune Disease	0 (0.0%)	2 (1.7%)	0.135
Myocardial Infarction	2 (2.4%)	1 (0.9%)	0.396

Note: RR - Relative Risk, IC95% - 95% Confidence Interval.

The mean level of white blood cell count did not differ significantly based on asymptomatic versus symptomatic status (6.45 *vs.* 6.61 thousand/mL^3; p=0.731) (tab. III).

The mean values of blood glucose (121.47 *vs.* 107.83 mg/dL; p=0.136), Alanine Aminotransferase-ALAT (49.98 *vs.* 54.40 U/L; p=0.445), and Aspartate Aminotransferase-ASAT (36.92 *vs.* 35.18 U/L; p=0.675) did not differ significantly between the analyzed groups (tab. III).

TABLE III.
Biological Parameters

Parameter	Asymptomatic	Symptomatic	-Student Tes
CRP, mg/dL	5.73±1.05 (0.58-39.88)	13.67±3.72 (0.20-200.17)	**0.001**
White Blood Cells (thousand/mL^3)	6.45±1.71 (3.14-12.05)	6.61±2.85 (2.62-25.86)	0.731
Blood Glucose, mg/dL	121.47±51.93 (79-316)	107.83±36.90 (60-310)	0.136
ALAT, U/L	48.98±32.86 (15-160)	54.40±39.96 (10-219)	0.445
ASAT, U/L	36.92±27.35 (16-181)	35.18±15.54 (10-86)	0.675

CRP-Protein C-reactive, ALAT-Alanine Aminotransferase, ASAT-Aspartate Aminotransferase

For imaging diagnosis, computed tomography (CT) scan was performed in 42.4% of asymptomatic patients and in 41.2% of symptomatic patients (p=0.874) (fig. 3).

Out of the total radiological investigation, 38.6% had a normal appearance (tab. IV).

The treatment for patients with COVID-19 was as follows: among asymptomatic patients, 37.6% received lopinavir/ritonavir and 15.3% received hydroxychloroquine, while symptomatic patients were treated with lopinavir/ritonavir in 57.4% of cases (p=0.006) and with hydroxychloroquine in 27.8% of cases (p=0.033) (fig. 4).

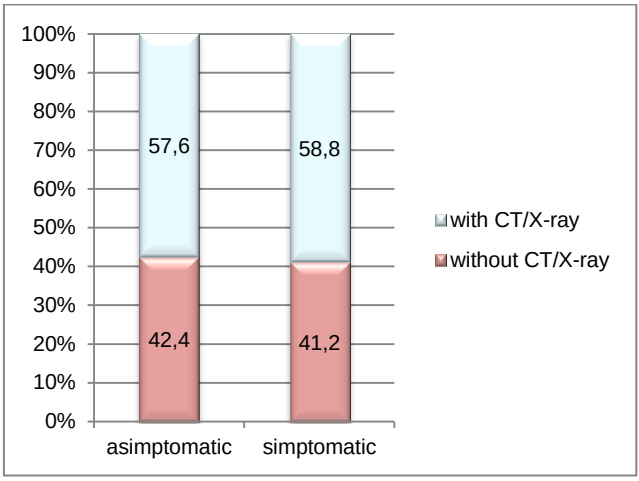

Fig. 3. Distribution of asymptomatic cases with imaging diagnosis

TABLE IV.
CT/X-ray

	n	%
Normal aspect	32	38.6%
Ground- glass opacity	23	27.7%
Multifocal nodular areas	11	13.3%
Large opacity	9	10.8%
Moderate opacity	3	3.6%
Small areas of alveolar condensation	4	4.8%
Pulmonary lesions	1	1.2%

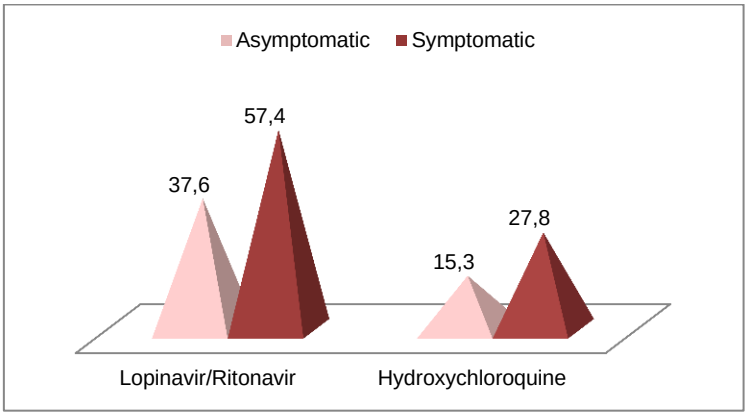

Fig. 4. Distribution of symptomatic/asymptomatic COVID-19 cases based on the received treatment

The number of days until negativity varied from 9 to 41 days in asymptomatic patients and from 2 to 38 days in symptomatic patients, with a similar average level in both study groups (18.71 *vs.* 18.79 days; p=0.936).

DISCUSSION

Identification of patients with COVID-19 is challenging, and risk monitoring strategies for disease progression are limited. Previous studies have reported that SARS-CoV-2 has been isolated from asymptomatic individuals, and a significant percentage of the infections have been transmitted by asymptomatic patients (2, 3, 4). Thus, the high proportion of asymptomatic subjects in our study (1/3 asymptomatic subjects, 38.6% of men, and 44.4% of women) supports the importance of social distancing for controlling the pandemic. In this study, approximately 1/2 of children under 20 years old were asymptomatic. Among adults, the highest proportion of asymptomatic cases was recorded in the 20-29 years and 40-49 years age groups (49.3%), and 2/3 of individuals over 70 years were asymptomatic.

In the current research, the most prevalent symptom, accounting for approximately 15% of cases, was cough, followed by anosmia and headache. A study including 13 subjects with mild COVID-19 reported nasal congestion (62%) as the main symptom (5). Interestingly, hyposmia was quite commonly encountered in subjects with mild COVID-19. Although hyposmia is caused by post-viral olfactory dysfunction (6), further studies are needed to determine the specificity of this symptom in COVID-19. Moreover, future pathophysiological studies are necessary to evaluate whether this unique symptom is associated with nasal congestion, olfactory nerve dysfunction due to viral infection, or other comorbidities. In our study, anosmia was present in 7.8% of symptomatic patients and represented one of the main subjective complaints declared by the patients.

Previous studies show that 50% of COVID-19 patients develop dyspnea after approximately 8 days after the onset of symptoms (7). Dyspnea was present in 5.2% of the current research, involving five subjects.

Previous studies suggest that advanced age, male gender, and the presence of comorbidities may represent risk factors for disease progression and a reserved prognosis (8). In another recent epidemiological study conducted in China, it was reported that patients with pre-existing comorbidities accounted for 23.2% of mild cases and 37.6% of severe cases (9). Other observational studies evaluating the characteristics of COVID-19 patients have shown similar results (1, 10), indicating that the presence of comorbidities is associated with disease severity. In our study, it was demonstrated that myocardial infarction induces an estimated risk of SARS-CoV-2 infection that is approximately 2 times higher. In the present study, 17.6% of patients had a personal history of hypertension.

As reported in one study, some biomarkers differ significantly among patients based on the severity of the disease (9). Another study indicates that the percentage of leukocytes predicts the outcome of individuals with COVID-19 (10). In our study, leukocytosis was more commonly found in symptomatic patients.

It is also reported that elevated D-dimer concentration, lymphopenia, and an in-

creased neutrophil-to-lymphocyte ratio are frequently encountered in patients with severe COVID-19 compared to those with mild forms of the disease (11). Lymphopenia, which is a common feature of symptomatic COVID-19 (12), may be a direct consequence of SARS-CoV-2 infection or mediated by inflammatory cytokines.

Similarly, in our study, although the mean levels of CRP exceeded the reference value in both analyzed groups, it was more severe in symptomatic COVID-19 patients.

Other studies (13, 14) report that pathological lung changes range from ground-glass opacities to consolidation during the course of the disease, and the appearance of consolidative features was more frequent at 1-3 weeks after the onset of the first symptom.

The occurrence of pulmonary consolidative features is associated with a deterioration of the patient general condition. Various types of pulmonary changes were observed in both symptomatic and asymptomatic subjects. Previous reports on imaging in COVID-19 also mention changes in asymptomatic individuals (15). In the present study, approximately 40% of the subjects underwent imaging explorations, but in one-third of them, the results were normal.

One-third of asymptomatic patients were treated with lopinavir/ritonavir, and approximately 15% with hydroxychloroquine. The number of days until negative conversion was around 19±7 days.

CONCLUSIONS

Our article provides valuable insights into the multifaceted nature of the pandemic. It sheds light on the significant proportion of asymptomatic cases, highlighting the need for social distancing measures to control transmission. Age and pre-existing health conditions were identified as risk factors for severe outcomes, emphasizing the importance of protecting vulnerable populations.

Symptoms of COVID-19 varied, with cough, anosmia, and headaches being some of the common manifestations. The understanding of anosmia, even in mild cases, sparked further interest for future research.

Laboratory tests, such as CRP levels and leucocyte counts, proved essential in predicting disease progression and guiding medical interventions. Additionally, imaging played a critical role in monitoring lung changes and the extent of lung involvement. Treatment strategies, were explored to manage asymptomatic cases, but ongoing research is needed to assess their effectiveness fully.

Throughout this challenging journey, the importance of vaccination emerged as a beacon of hope, offering a way to build immunity and control the spread of the virus. The pandemic has taught humanity valuable lessons about global collaboration, scientific advancements, and the significance of adhering to public health measures. It serves as a reminder that the fight against COVID-19 is ongoing, but with continued resilience and unity, the world can overcome this unprecedented crisis.

As we move forward, it is imperative to maintain a vigilant approach, embrace scientific evidence, and foster international cooperation to safeguard public health and ensure a healthier and safer future for all.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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COVID-19 NEUROLOGICAL MANIFESTATIONS - THE EXPERIENCE OF A TERTIARY HOSPITAL FROM NORTHEASTERN ROMANIA

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COVID-19 NEUROLOGICAL MANIFESTATIONS - THE EXPERIENCE OF A TERTIARY HOSPITAL FROM NORTHEASTERN ROMANIA (Abstract): Well-known for its respiratory tropism, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can further affect both the central and peripheral nervous system causing a substantial source of morbidity and mortality. This study aimed to evaluate the neurological manifestations in SARS-CoV2-infected hospitalized patients and to evaluate the short-term outcomes based on clinical features, neurologic comorbidities, and laboratory. **Materials and methods**: We retrospectively analyzed medical records of patients with SARS-CoV-2 infection admitted to the "Sf. Parascheva" Clinical Hospital of Infectious Diseases Iasi between May 2020 and December 2022. Clinical features, frequency of neurological manifestations, and correlation with risk factors, pandemic waves, and the severity of the disease were evaluated. **Results**: We identified 160 patients hospitalized during the investigated period, the most frequent neurologic manifestations at the time of admission were fatigability (85% cases), headache (51.25% cases), myalgia (41.25% cases), vertigo (26.88% cases), confusion (21.25% cases) and less frequent anosmia (16.88% cases), ageusia (15% cases), paresthesia (3.13% cases). In all phases fatigability was predominant, being in over 80% of cases one of the symptoms at the moment of hospitalization, followed by cephalalgia and myalgia. Anosmia (25%) and ageusia (27%) were found most in phase two compared to all phases. In the second phase, the variant of concern was delta, causing more critical cases than all the other mentioned periods. Previous neurologic comorbidities such as dementia and stroke were correlated with a higher mortality risk. **Conclusions**: Neurological symptoms were a relatively frequent part of the clinical picture of SARS-CoV2 infection, and their incidence varied in different pandemic waves. Fatigability was the most constant finding. Previous neurologic comorbidities such as dementia and stroke were correlated with a higher mortality risk. **Keywords**: NEUROLOGICAL, CORONAVIRUS, SARS-COV-2, FATIGUE.

The coronavirus disease 2019 (COVID-19) was the most challenging public health issue in decades and was caused by the severe acute respiratory syndrome corona-

virus 2 (SARS-CoV-2). Known for its respiratory tropism, the virus can further affect both central and peripheral nervous systems causing a substantial source of morbidity and mortality.

The central nervous system manifestations are headache, encephalitis, and encephalopathy; the peripheral nervous system involvement can be expressed as anosmia and ageusia and skeletal muscle manifestations, such as myalgia and myasthenia (1). The underlying mechanisms of neurologic complications in patients with COVID-19 are di-verse and multifactorial. One of them is represented by systemic dysfunction; patients with severe illness admitted for respiratory failure can develop encephalopathy or other neurological manifestations due to hypoxemia and metabolic imbalances. The immune dysfunction induced by the virus due to a proinflammatory state and autoimmunity also plays an important role in the pathogenesis of acute neurological symptoms. Patients with COVID-19 can develop signs of severe systemic inflammation consistent with a cytokine release syndrome-like presentation shown as elevated inflammatory markers (D-dimer and ferritin) and proinflammatory cytokines (IL-6 and TNF-alpha) (2). High levels of circulating proinflammatory cytokines can cause confusion and alteration of consciousness (3). SARS-CoV-2 enters the cells using a membrane-bound protein called angiotensin-converting enzyme 2 (ACE-2). ACE-2 has a wide distribution in multiple organs including the nose, lungs, kidneys, liver, blood vessels, immune system, and the brain; its physiological effect is to regulate blood pressure and to facilitate the conversion of angiotensin II to angiotensin 1-7 (4, 5). When SARS-CoV-2 binds to ACE2, the levels of angiotensin II rise causing damage to the endothelial cells therefore promoting vasoconstriction, kidney failure, heart disease, apoptosis, and oxidative processes that accelerate aging and boost brain degeneration (6,7). Direct viral invasion of the nervous system is another possible culprit mechanism (8).

Multiple variants of SARS-CoV-2 have emerged during the pandemic, including Alpha, Gamma, Zeta, Delta, Lambda, and Omicron, each with its subtypes presenting different patterns of transmission and immunology and different levels of severity. With the evolution of the virus and the increasing immune protection of the population (following vaccination or natural infection), the clinical picture of COVID-19 is also changing, with the frequency and intensity of some symptoms (including neurologic ones) being different in the present. COVID-19 effects on the body, including the neurological impact vary due to viral strain, host, and environmental conditions.

We conducted a retrospective study to determine the incidence of neurological symptoms during an acute COVID-19 episode and the way they varied during the waves of the COVID-19 pandemic and to identify how different neuro-psychiatric disorders may influence the short-term outcome of COVID-19 infection in hospitalized patients from our geographical region.

MATERIALS AND METHODS

Retrospective study of patients diagnosed with SARS-CoV-2 infection, admitted at the “Sf. Parascheva” Hospital of Infectious Diseases Iasi between May 2020 and December 2022. The diagnosis was confirmed by real-time reverse transcrip-

tion-polymerase chain reaction (RT-PCR) assay from nasopharyngeal swab samples.

The study period was stratified based on the RT PCR SARS-CoV-2 positive test month to identify temporal trends in cases. Population data were divided into four phases (fig. 1):

- Phase 1: May 2020-February 2021 (first three waves)
- Phase 2: August-December 2021 (fourth wave)
- Phase 3: January-May 2022 (fifth wave)
- Phase 4: June 2022- December 2022 (sixth wave)

The variants of concern in the studied periods are as follows: in phase one the dominant strain was Alpha (lineage: B.1.1.7, Q), followed by Delta (lineage: B.1.617.2, AY.4.2) in phase two and Omicron (lineage: B.1.1.529, BA) during phase three and four.

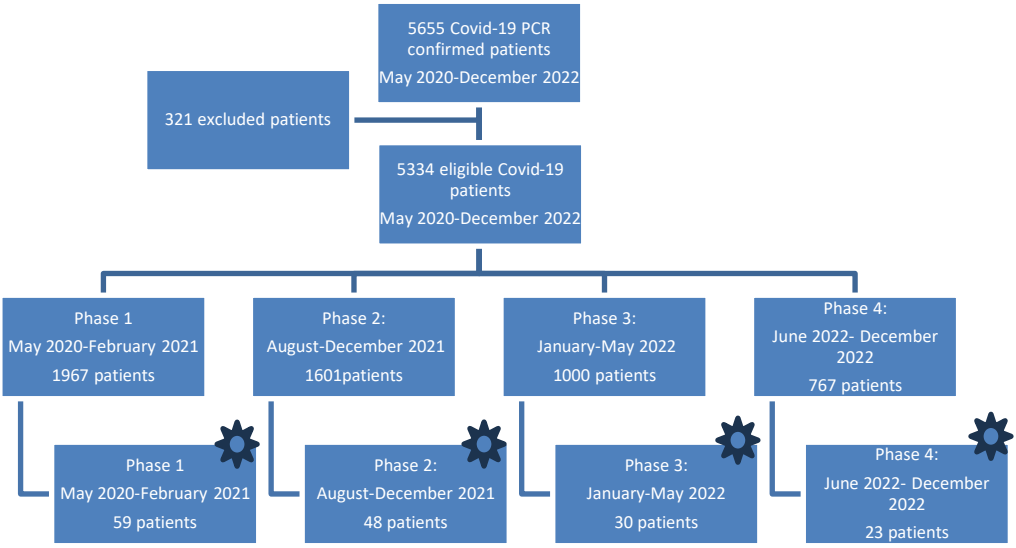

Fig. 1. Study population flow-chart. -3% sample extraction following simple randomization

All hospitalized COVID-19 patients were included in an anonymous database. Patients in a comatose state at admission who required immediate admission to the intensive care unit, the ones with acute psychiatric conditions, and those who were unable to offer data regarding their symptoms and history were excluded. 3% of admitted patients in each one of the studied phases were selected using simple randomization through a randomization software (Power Analysis & Sample Size - PASS© 2023).

A total of 160 cases were obtained.

The neurological symptoms considered were represented by: fatigue, headache, anosmia, ageusia, vertigo, paresthesia, confusion, and myalgia.

We reviewed electronic files, collecting paraclinical and clinical data including demographic data, medical history, the onset and the severity of symptoms at admission, and the outcome.

The severity of the illness was graded according to National Institute of Health

US COVID-19 guidelines as follows: mild illness: various signs and symptoms of COVID-19, but without shortness of breath, dyspnea, or abnormal chest imaging; moderate illness: evidence of lower respiratory disease during clinical assessment or imaging and oxygen saturation measured by pulse oximetry (SpO2) ≥94% on room air; severe illness: individuals who have SpO2 <94% on room air at sea level, PaO2/FiO2 <300 mm Hg, a respiratory rate >30 breaths/min, or lung infiltrates >50%; critical illness: individuals who have respiratory failure, septic shock, and multiple organ dysfunction.

Encephalopathy was defined as a disturbance in the level of awareness and attention, leading to an alteration in cognitive function, not primarily due to structural organic brain disease.

We monitored the predictors of short-term outcomes of the patients dividing them into four separate groups. The first two groups were divided by the severity of the illness including critical patients who needed ICU care due to acute respiratory failure and multiple organ dysfunction and patients who maintained a moderate/severe form of illness without needing ICU support. The two other groups were divided by the lethality rate, one included the patients who survived, and the other one the ones who died due to COVID-19 infection.

Statistical analysis was performed using XLSTAT software version 2022. Descriptive data are presented as absolute values, percentages, and means. Differences between groups were tested for statistical significance using unpaired T-Student and χ square tests. $p < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

There were 83 women and 77 men, aged 27-94 (median: 67, interquartile range (IQR) 56-75). The interval from the onset of symptoms and hospital admission was 6 days median, with an interquartile range IQR 4-7. Other patient characteristics are summarized in first table.

TABLE I.
Patient characteristics

	Critical N=28		Moderate and severe N=132		Survivors N=145		Deceased N=15	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Age at COVID-19 onset	71	66.2-77	66	55.2-75	67	56-75	71	67-78
Days since symptoms onset	7	4.2-7	6	3-8	6	3-6	7	4-7
Hospitalization days	14.5	6.7-22.2	12	9-16	12	9-16	12	9-16

None of the selected patients presented a mild form of disease. From the total number of patients, 48.12% had a moderate illness, 34.37% had a severe illness and 17.5% had a critical form of the infection. Phase 2 contained the highest proportion of severe and critical cases, the difference from the other phases being significant (p=0.004). (tab. II).

In the group of patients who were stable with moderate/severe forms of illness and improved by the time of discharge the me-

dian age was 66 years and IQR 55.25-75, more women were included (54.55%) from urban areas; the most frequent associated comorbidities were cardiovascular diseases (68.18%) such as hypertension, cardiomyopathies and heart failure.

TABLE II.
Number of cases and illness severity in different phases

	Phase 1		Phase 2		Phase 3		Phase 4	
No cases	59		48		30		23	
	n	%	n	%	n	%	n	%
moderate	36	46.7	11	14.2	16	20.7	14	18.1
severe	17	30.9	23	41.8	9	16.3	6	10.9
critical	6	21.4	14	50	5	17.8	3	10.7

In the group of critical patients, the median age was 71 years and IQR was 66.2-77, the patients being older in this group; 60.71% were male, 96.43% had comorbidities, over 60% had associated cardiovascular disease, 28.57% had previous neurological illnesses such as stroke (including intracerebral hemorrhage, ischemic stroke and transient ischemic attack), dementia, neuromuscular disorder, motor neuron disease, spinal cord disorder, the last three ones being less frequent. In this group, the hospitalization period was longer compared to the other ones, with a median of 14.5 days and IQR 6.75-22.25.

The most frequent neurologic manifestations were fatigability (85% cases), headache (51.25% cases), myalgia (41.25% cases), vertigo (26.88% cases), confusion (21.25% cases) and less frequent anosmia (16.88% cases), ageusia (15% cases), paresthesia (3.13% cases) (fig. 2)

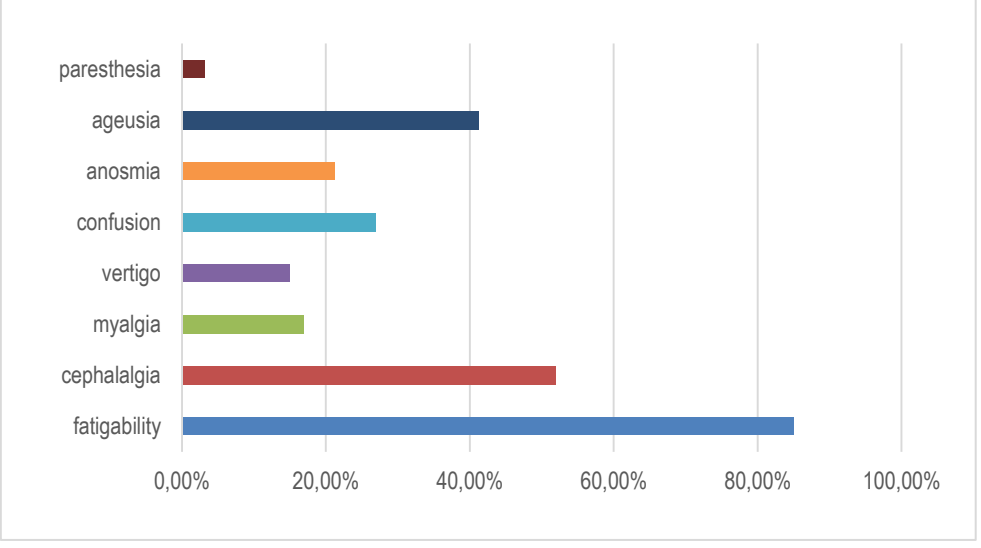

Fig. 1. Neurological symptoms

In all phases, fatigability was predominant, being in over 80% of cases one of the symptoms at the moment of hospitalization, followed by cephalalgia and myalgia. Anosmia (25%) and ageusia (27%) were found most in phase 2 compared to all phases.

TABLE III.
Frequency of neurological manifestation in different phases of the pandemic

	Phase 1	Phase 2	Phase 3	Phase 4	p
	(n=59)	(n=48)	(n=30)	(n=23)	
	%	%	%	%	
Fatigability	81.36%	83.33%	93.33%	86.96%	0.49
Cephalalgia	42.37%	43.75%	76.67%	60.87%	**0.009**
Anosmia	11.86%	25.00%	13.33%	17.39%	0.31
Ageusia	8.47%	27.08%	10.00%	13.04%	**0.04**
Vertigo	15.25%	31.25%	30.00%	43.48%	**0.048**
Confusion	23.73%	20.83%	23.33%	13.04%	0.74
Myalgia	32.20%	52.08%	50.00%	30.43%	**0.09**
Paresthesia	0.00%	4.17%	0.00%	13.04%	**0.01**

The temporal trend in the described periods showed that fatigue was constant in all phases, headache was more frequent during phases 3 and 4, ageusia during phase 2, vertigo in phase 4, myalgia in phases 2 and 3, and paresthesia in phase 4 (tab. III).

During the hospitalization, 12 patients (7.5%) developed encephalopathy. The incidence has sub sequentially decreased during the studied periods, with a peak in the first two phases (9/12 patients). The median age and hospitalization duration were significantly higher in this group (73 *vs.* 67 years, p=0.04 and 16 *vs.* 12 days, p=0.03). Nine of the 12 encephalopathy patients had a lumbar puncture performed, but none showed an inflammatory reaction in the cerebrospinal fluid (CSF). A borderline elevation of the CSF albumin was detected in 3 patients. No other specific investigations for the diagnosis of a possible acute inflammatory demyelinating polyneuropathy were performed.

In all, 15 patients died in the hospital. Compared to those discharged alive, patients who died were older (the median age at COVID-19 onset 71 years, IQR 67-78), predominantly male (73.3%) from urban areas, with more comorbidities (cardiovascular disease including hypertension, diabetes type 2, obesity. 46.76% had previously neurological diseases such as stroke (p=0.0023) and dementia (p=<0.001), both being correlated with death. Almost all deceased people were admitted to the ICU (80%), and all of the ICU patients required mechanical ventilation (tab. IV). Between the alive and dead groups, there's no difference between the hospitalization days.

The days from the onset of COVID-19 symptoms to hospital admission are similar

in all groups, with a median of 7 in the critical and dead groups and a median of 6 in the stable/improved outcome and alive groups.

In what concerns the laboratory parameters used to regularly monitor the patients, we found a significant statistical correlation ($p < 0.05$) between anemia (hemoglobin <11.7 g/dL in females and <13.2 g/dL in males) p=0.005, neutrophilia (>8*10³/μL) p<0.0001, lymphocytopenia (lymphocytes < 1*10³/μL) p<0.0001, thrombocytopenia (<150,000/mm³) p<0.0001, high levels of D-dimer (>0.5mg/L FEU) p<0.0001, high levels of ferritin (>400ng/mL) p<0.0001 and both worse outcome and mortality risk. From this group 64.29% had anemia, 85.71% had lymphocytopenia, 53.57% had neutrophilia, 53.57% had thrombocytopenia, 67.86% had high levels of D-dimer and 60.71% had high levels of ferritin. The significant statistical correlation persists in the deceased group, between anemia (73.33%) p=0.00132, neutrophilia (66.67%) p<0.0001, lymphocytopenia (86.67%) p<0.0001, thrombocytopenia (53.33%) p<0.0001, high levels of D-dimer (93.33%) p<0.0001, high levels of ferritin (66.67%) p<0.0001 and death.

The C reactive protein levels were high in all groups, but in the deceased patient's group, it was high in 100% of the cases.

TABLE IV.

Patient's characteristics and outcome

	Critical		Moderate/ severe		P value	Alive		Dead		P value
	N=28		N=132			N=145		N=15		
	N	%	N	%		N	%	N	%	
Gender										
Female	11	39.29%	72	54.55%	0.06193	80	55.17%	4	26.67%	0.0722
Male	17	60.71%	60	45.45%		65	44.83%	11	73.33%	
Provenience										
Urban area	16	57.14%	83	62.88%	0.56708	88	60.69%	11	73.33%	0.33906
Rural area	12	42.86%	49	37.12%		57	39.31%	4	26.67%	
Comorbidities										
Any comorbidity	27	96.43%	114	86.36%	0.11606	126	86.90%	14	93.33%	0.47605
Cardiovascular	17	60.71%	90	68.18%	0.43325	100	68.97%	7	46.67%	0.09496
Diabetes type 2	7	25.00%	38	28.79%	0.55547	43	29.66%	2	13.33%	0.17158
Obesity	9	32.14%	37	28.03%	0.58176	45	31.03%	3	20.00%	0.37062
Pulmonary	7	25.00%	20	15.15%	0.17006	23	15.86%	4	26.67%	0.245
Oncologic	2	7.14%	12	9.09%	0.59854	14	9.66%	0	0.00%	0.19131
Other	2	7.14%	25	18.94%	0.16264	26	17.93%	2	13.33%	0.65626

	Critical		Moderate/ severe		P value	Alive		Dead		P value
	N=28		N=132			N=145		N=15		
	N	%	N	%		N	%	N	%	
Laboratory *Variables*										
Anemia	18	64.29%	46	34.85%	**0.00521**	50	34.48%	11	73.33%	**0.00132**
Leukocytosis	13	46.43%	17	12.88%	0.23962	22	15.17%	5	33.33%	0.06903
Leukopenia	8	28.57%	20	15.15%	0.08572	23	15.86%	4	26.67%	0.29989
Neutrophilia	15	53.57%	18	13.64%	**<0.0001**	23	15.86%	10	66.67%	**<0.0001**
Lymphocytopenia	24	85.71%	67	50.76%	**<0.0001**	78	53.79%	13	86.67%	**<0.0001**
Thrombocytosis	9	32.14%	37	28.03%	0.56666	40	27.59%	4	26.67%	0.93428
Thrombocytopenia	15	53.57%	25	18.94%	**<0.0001**	32	22.07%	8	53.33%	**<0.0001**
High D-dimer	19	67.86%	45	34.09%	**<0.0001**	51	35.17%	14	93.33%	**<0.0001**
High ferritin	17	60.71%	38	28.79%	**<0.0001**	45	31.03%	10	66.67%	**<0.0001**
High CRP	27	96.43%	125	94.70%	0.63204	137	94.48%	15	100.00%	0.23696

All variables with a $p < 0.05$ on the univariate categorical analysis were included in a multivariate logistic regression, which demonstrated that in patients with neurological symptoms at the time of hospitalization for SARS-CoV-2 infection, history of stroke ($p=0.0023$, odds ratio (OR) 0.22, 95% confidence interval (95% CI) 0.1-0.4) and dementia ($p < 0.001$, OR 0.092, 95% CI 0.04-0.21) as comorbidities were significant predictors for an increased risk of mortality independent of the other factors (tab. V).

TABLE V.

Neurological comorbidities and outcomes

	Critical		Moderate/ severe		P value	Alive		Dead		P value
	N=28		N=132			N=145		N=15		
	N	%	N	%		N	%	N	%	
Neurologic comorbidities	8	28.57%	22	16.67%	0.153	24	16.55%	7	46.67%	
Stroke	4	14.29%	10	7.58%	0.226	11	7.59%	4	26.67%	**0.002**
Dementia	2	7.14%	2	1.52%	0.084	3	2.07%	2	13.33%	**<0.001**
Neuromuscular disorder	1	3.57%	0	0.00%	1	0	0.00%	1	6.67%	1
Motor neuron disease	2	7.14%	2	1.52%	**0.044**	3	2.07%	1	6.67%	0.264
Spinal cord disorder	1	3.57%	1	0.76%	**0.154**	1	0.69%	1	6.67%	0.441
Parkinson disease	0	0.00%	1	0.76%	0.237	1	0.69%	0	0.00%	0.237
Others	2	7.14%	7	5.30%	0.641	8	5.52%	1	6.67%	0.803

Concerning laboratory investigations, independent predictors for an increased risk of mortality were: anemia (p=0.001, OR 5.2, 95% CI 1.58-17.25), neutrophilia (p<0.0001, OR 10.61, 95% CI 3.31-33.91), lymphocytopenia (p<0.0001, OR 5.58, 95% CI 1.21-25.6), thrombocytopenia (p<0.0001, OR 4.03, 95% CI 1.36-11.97), high D-dimer levels (p<0.0001, OR 25.8, 95% CI 3.29-201.8) and high ferritin levels (p<0.0001, OR 4.44, 95% CI 1.43-13.7) (tab. VI).

TABLE VI.
Significant predictors for increased mortality

Predictors	P value	Odds ratio (OR)	95% confidence interval (95% CI)
Stroke	0.0023	4.43	1.20-16.23
Dementia	<0.001	7.28	1.11-47.59
Anemia	0.00132	5.22	1.58-17.25
Neutrophilia	<0.0001	10.61	3.31-33.91
Lymphocytopenia	<0.0001	5.58	1.21-25.6
Thrombocytopenia	<0.0001	4.03	1.36-11.97
High D-dimer	<0.0001	25.80	3.29-201.8
High ferritin	<0.0001	4.44	1.43-13.7

DISCUSSION

This study aimed to identify the incidence of neurological symptoms during the acute COVID-19 infection and the way they varied in different periods of the pandemic, in hospitalized patients from northeastern Romania. It was found that the most common neurological symptom is fatigue followed by headaches. The temporal trend in the described periods showed that fatigue was constant in all phases, headache was more frequent during phases 3 and 4, ageusia during phase 2, vertigo in phase 4, myalgia in phases 2 and 3, and paresthesia in phase 4. To mention, the study was conducted in a tertiary care hospital, in the infectious diseases department. The patients who needed acute neurological care were directed to another hospital. A study from India (9) compared the first two waves of the pandemic in the matter of neurological manifestations, identifying headaches and fatigability as the most frequent symptoms in both waves, similar to our findings.

The neurological symptoms of COVID-19 infection are known to be more common in patients with severe illness (10), and none of our patients who showed neurological symptoms during hospitalization had a mild form of disease.

Every virus can mutate, more often with no significant effect on its pathogenic properties, however, during the major pandemic waves, some mutations result in new strains with higher transmission rates, like the SARS-CoV-2 Delta strain, which has been associated with higher viral loads, enhanced transmissibility, and higher hospitalization rates (11). A study conducted during a period of co-circulation of Omicron and Delta concluded that the Omicron variant appears to cause significantly less severe disease compared to Delta (12). In our study, the most critical cases of SARS-CoV-2 infection were found during the period when Delta was the main variant of concern.

Systemic reviews of the literature identified headache, fatigability, and dizziness/myalgia as the most constant neurological symptoms in COVID-19 patients (13-16). These symptoms are not highly specific to COVID-19 and don't presuppose necessarily an infection to underlying neurological structures, but they could also occur via indirect mechanisms of neuropathogenicity (dehydration, fever, hypoxia, or respiratory distress) as well influenced by the proinflammatory state (16). The neurotropism of the SARS-CoV-2 virus has been debated during the pandemic, in 2020 Moriguchi *et al.* and Poyiadji *et al.* described the first case of encephalitis with the detection of SARS-CoV-2 RNA in cerebrospinal fluid (CSF), but in other encephalitis case reports published, viral RNA was not detected in CSF or complementary investigations were not performed (17). An autopsy study from 2022, of 20 brains of decedents with acute COVID-19 infection, hasn't detected viral RNA or viral proteins in the CSF or brains, the acute changes found in the study have mostly a vascular origin with acute hypoxia, infarcts, and hemorrhages and inflammatory reactions (18).

Headache has been described as a frequent non-specific neurological symptom in a series of studies (19). It is experienced by 12-70% of patients with COVID-19 (20). In our study, 50% of the patients complained of this symptom. Different types of headaches have been reported such as tension-type headaches, headaches resulting from systemic viral infections that cause chronic cough or hypoxia, and migraine-type headache exacerbation (21).

Fatigability was the most common finding in our study, 85% of the patients complained about different grades of fatigability, and most of them described it as having "never felt so tired", but it is one of the most difficult symptoms to quantify because of its subjective nature, therefore is not always considered as an important finding. The pathology behind this symptom is diverse and cannot be explained by just one mechanism. Some promising factors include inflammation, mitochondrial dysfunction, sleep alterations, autonomic nervous system abnormalities, and poor nutritional status (22). This is one of the most persistent symptoms in long-covid, causing persistent tiredness, and effort-independent, which reduces the quality of life.

Taste and smell disorders were the most common, sudden neurologic symptoms of COVID-19 at the beginning of the pandemic, and are thus considered a useful early diagnostic marker (23). Anosmia and ageusia are more frequent in patients with mild illness who usually don't require hospitalization (24), therefore the lower incidence in our study: 16% for anosmia and 15% for ageusia. In one study, the incidence was over 80% (25), but in a meta-analysis with more than 27000 subjects included, anosmia affected 48% of patients (26). These symptoms are rarely the only COVID-19 manifestations, are more common in younger people and females reported it more often (27). The prognosis of smell and taste disorders associated with COVID-19 is generally favorable and, in most cases, it resolves spontaneously (28).

Besides the common neurological symptoms, major and devastating SARS-CoV-2 virus-associated CNS complications can occur such as encephalopathy and encephalitis (27). Encephalopathy is a major neurological manifestation, with a multifactorial origin, including systemic disease and in-

flammation, coagulopathy, direct neuroinvasion by the virus, endothelytis, and possibly post-infectious auto-immune mechanisms (30). Additionally, other risk factors associated with intensive care units should be taken into account as sedation and analgesia doses, disruption of sleep/wake cycles, and infectious complications (31). Previous studies showed an increase in mortality and morbidity associated with encephalopathy, as well as an increase in the hospital length of stay, this finding being consistent with our data (29, 32).

We stratified the patients into two groups according to the outcome of the infection. In all, 15 patients died in the hospital. Compared to those discharged alive, patients with increased mortality were older, predominantly male from urban areas, with more comorbidities such as cardiovascular disease, including hypertension, and diabetes type 2 obesity. 46.76% had previously neurological diseases such as stroke and dementia, both being correlated with death. Almost all deceased people were admitted to the ICU, and all of the ICU patients required mechanical ventilation. In two other studies (33, 34) stroke and dementia were associated with increased disease severity.

Concerning laboratory investigations, independent predictors in our study for an increased risk of mortality were anemia, lymphocytopenia, thrombocytopenia, high D-dimer levels, and high ferritin levels. Azhideh (35) also noticed lower lymphocyte and platelet counts in patients with severe central nervous system (CNS) involvement, while laboratory findings were not helpful in patients with peripheric nervous system involvement or individuals with non-severe CNS involvement. Similar findings were reported by Trofin *et al*. (36).

Given the global impact of COVID-19 and the emerging recognition of its neurological effects, this research adds to the body of knowledge required for comprehensive disease understanding and management.

It is now recognized that the clinical picture of COVID-19 has changed since the start of the pandemic, the most important drivers for these changes being the different virus variants involved and the immune response gained by a partially vaccinated or naturally immunized population (37, 38). Some authors also recognize some regional differences in the reported symptoms of COVID-19, due possibly to cultural or genetic differences (39,40). Our study, with its regional focus, provides specific insights that could be relevant for understanding the disease's manifestation in particular demographic and geographical settings.

Our study has several limitations, first of all, by its retrospective nature and by the small sample of analyzed patients, even though they were selected from various periods, matching with different waves of the pandemic and different variants of the virus. Most of the symptoms were self-reported and due to the retrospective nature of our study, we were unable to use more objective tools to quantify them. At this time, we were able to include only short-term outcomes at discharge. Long-term follow-up is needed to assess the outcomes more efficiently and to identify the persistent symptoms and the impact of neurological symptoms due to COVID-19 on daily life including possible sequelae.

CONCLUSIONS

Neurological symptoms were a relatively frequent part of the clinical picture of SARS-CoV2 infection, and their incidence

varied in different pandemic waves. Fatigability was the most constant finding. Previous neurologic comorbidities such as dementia and stroke were correlated with a higher mortality risk. Other independent predictors for an increased risk of mortality are anemia, lymphocytopenia, thrombocytopenia, high levels of D-dimer, and high levels of ferritin.

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CHRONIC VENOUS DISEASE AND PSORIASIS - A RANDOM ASSOCIATION?

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CHRONIC VENOUS DISEASE AND PSORIASIS- JUST A RANDOM ASSOCIATION? (Abstract): **Aims:** Chronic venous disease and psoriasis are two common pathologies in dermatological practice. The aim of this study is to determine the prevalence of venous disease among the psoriatic population and to identify risk factors that lead to their association**.** **Materials and methods**: This study included 375 patients diagnosed with psoriasis vulgaris, hospitalized in "Sf. Spiridon" County Clinical Emergency Hospital's Dermatology-Venereology Clinic, between 01.01.2019 and 31.05.2021. **Results**: The prevalence of chronic venous disease in the study group was 37.3%, reaching the highest values in the age group of 36-60 years (women: 40.7%, men: 48.6%, $P = 0.040$). Among the common risk factors, obesity had a prevalence of 19.2% and smoking 24.3% ($P = <0.001$). Among common comorbidities, hypertension had a prevalence of 47.1% and diabetes mellitus 13.6% ($P = <0.001$). **Conclusions**: In the future, more studies are required, in order to fully elucidate the mechanisms by which venous disease can be associated with psoriasis. **Keywords**: PSORIASIS, CHRONIC VENOUS DISEASE, OBESITY, SMOKING.

INTRODUCTION

Psoriasis is a chronic inflammatory disease, with a global prevalence of 2-4% and a mild evolution in 2/3 of cases (1). The etiopathogenesis of psoriasis is not fully elucidated. Clinical manifestations of psoriasis vulgaris include the appearance of erythematous-scaly plaques, well delimited, with the surface covered by white, fine, easily removable scales (2, 3). Other clinical types are guttate, pustular, erythrodermic and inverse psoriasis. It usually affects young people, between the ages of 20 and 40 years (1). Internal or external risk factors cause immunologic and metabolic dysregulations, that can trigger the disease in genetically predisposed individuals (4, 5). The immune system responds with T cells hyperactivation and T-helper 1 cells upregulation

(6, 7).

Chronic venous disease (CVD) is a common vascular pathology, produced by disturbances in the return of blood in the deep, superficial and perforating venous systems. Increased venous pressure occurs most often by weakening of the vascular wall and secondary to valvular insufficiency. Venous hypertension causes extravasation of water and erythrocytes in the tissues, leading to skin lesions (8). Clinical manifestations of CVD include discomfort and pruritus of lower limbs and suggestive signs include the appearance of varicose veins, leg edema, purple lesions or lipodermatosclerosis, as well as ulcers (9).

Common risk factors involved in the etiopathogenesis of psoriasis and chronic venous disease are obesity and smoking. Obesity is a risk factor in the development of venous valvular insufficiency in CVD due to the mechanical component and the systemic proinflammatory status produced by adipose tissue, accentuated by sedentary lifestyle and impaired pumping function of the leg muscles (10). Obesity can also lead to psoriasis (11). Hypertension is considered a comorbidity of psoriasis but there are studies in which hypertensive patients have associated an increased incidence of psoriasis (12). Also, high blood pressure can be a factor in the onset or worsening of venous disease (13).

We performed a cross-sectional study, including 375 patients, aiming to determine the prevalence of CVD among the psoriasis population and to identify risk factors that lead to their association.

MATERIALS AND METHODS

We included in this study all patients diagnosed with psoriasis vulgaris (L40.0) between 01.01.2019 and 31.05.2021, resulting 375 patients, hospitalized in "Sf. Spiridon" County Clinical Emergency Hospital's Dermatology-Venereology Clinic. The characteristics of the patients admitted in the study were: age, sex, environment, primary diagnosis and secondary diagnoses, PASI and DLQI severity scores. All patients included in the study signed their consent to the processing of personal data.

The assessment of the severity of psoriasis was performed by the doctors, using the PASI score (Psoriasis Area and Severity Index) and the impact on the quality of life was quantified using the DLQI score (Dermatology Life Quality Index). The assessment of the severity of chronic venous disease was performed using the CEAP (Clinical Etiology Anatomy Pathophysiology) classification, as follows: C0-without signs of venous disease; C1-intradermal telangiectasias or subdermal reticular veins (diameter between 1-3mm); C2- varicose veins (diameter over 3 mm); C3-edema in the ankle or calf; C4-venous eczema, lipodermatosclerosis or white atrophy; C5-healed venous ulcer; C6- active venous stasis ulcer (14).

In determining the obesity class, we used the Body Mass Index (BMI), as follows: grade I-BMI obesity with values between 30 and 34.99; grade II-BMI obesity with values between 35 and 39.99 and grade III-BMI or morbid obesity with values of 40 or more (15).

The statistical analysis of the patients' data was performed using the SPSS program version 20.0 and the calculation of the chi-square test, as follows: p <0.05, the statistical value is moderately significant (S, 95% c); p <0.01, the statistical value is very significant (S, 99% confidence); p <0.001, the statistical value is highly significant (HS, 99.9% confidence); p> 0.05, the statistical value is not significant (NS).

RESULTS

The prevalence of chronic venous disease among the psoriatic patients was 37.3%, representing 140 patients out of a total of 375 patients included in the study.

From first table, we can observe that the prevalence of CVD in the psoriatic population has the highest values in the age category 36-60 years, both in females and males, with values of 40.7% and 48.6%, followed by the age category with patients over 60 years, with a percentage of 35.9% for females and 38.0% for males. The lowest prevalence was in the age category 18-35 years, with a value of 23.4% in women and 13.5% in men (P = 0.040).

In the group of psoriatic patients who also associated the diagnosis of CVD, there was a slight predominance of males (55%) (P = 0.055) and the mean age was 60.06 ± 12.35 years (P = 0.027). Regarding the local environment, 55.7% of patients belong to urban areas (P = 0.033) and 44.3% come from rural areas (P = 0.041).

From second table, we can observe that after the classification according to the clinical stage of the chronic venous disease, most of the patients were classified in CEAP C3 (32.1%), followed by C4 (28.6%) and C2 (26.4 %) (P = <0.001). Stages C5 and C6 had a lower representation, of 6.4% (P = 0.047).

After classifying the patients according to the PASI score, most of the patients included in our study fall into the moderate-severe type of the disease (61.4%), followed by the moderate type (20.7%). The severe (11.4%) and mild (6.4%) types had lower percentages. Regarding other forms of psoriasis associated with CVD, we noticed that the pustular type had the highest prevalence, being diagnosed in 3.6% of patients, followed by guttate and inverted types, with a prevalence of 2.9% and 2.1%, (P = <0.001).

TABLE I.

Chronic venous disease prevalence by age and gender

		Gender				
		Female		Male		
		N	N %	N	N %	p- value
Age	18-35	39	23.4%	28	13.5%	0.040
	36-60	68	40.7%	101	48.6%	
	over 60	60	35.9%	79	38.0%	

TABLE II
Characteristics and risk factors of patients diagnosed with chronic venous disease and psoriasis vulgaris

Parameter	N (%)	P-value	Parameter	N (%)	P-value
Gender			Environment		
• female	63 (45%)	0.041	• Rural	62 (44.3%)	0.041
• male	77 (55%)	0.055	• Urban	78 (55.7%)	0.033
Age	60,06±12,35	0.027	Severity of psoriasis		
CEAP C categories			Moderate-to-severe	86 (61.4%)	
• C2	37 (26.4%)	<0.001	Moderate	29 (20.7%)	
• C3	45 (32.1%)	<0.001	Severe	16 (11.4%)	
• C4	40 (28.6%)	<0.001	Mild	9 (6.4%)	
• C5	9 (6.4%)	0.047	PASI <10	123 (87.9%)	0.043
• C6	9 (6.4%)	0.047	PASI 10-20	12 (8.6%)	0.068
Obesity	27(19.2%)	<0.001	PASI >20	5 (3.6%)	0.055
• grade I	7 (5%)	0.012	DLQI <10	117 (83.6%)	0.001
• grade II	16 (11.4%)	0.009	DLQI 10-20	19 (13.6%)	0.033
• grade III	4 (2.9%)	0.029	DLQI >20	5 (3.6%)	0.074
Other types of psoriasis			Hypertension	66 (47.1%)	<0.001
• Guttate	4 (2.9%)	<0.001	Diabetes mellitus	19 (13.6%)	<0.001
• Inverse	3 (2.1%)	<0.001	Smoking	34 (24.3%)	<0.001
• Pustular	5 (3.6%)	<0.001			

From first figure we can observe that the moderate-severe forms of Psoriasis are mainly associated with CEAP C3 (19.3%) and C4 (17.1%), the moderate type is associated with class C3 (8.6%) and C2 (7.1%) predominantly. The severe type is associated especially with the C4 class (5.0%) and the mild type is especially associated with the C2 class (2.9%), (P = 0.099).

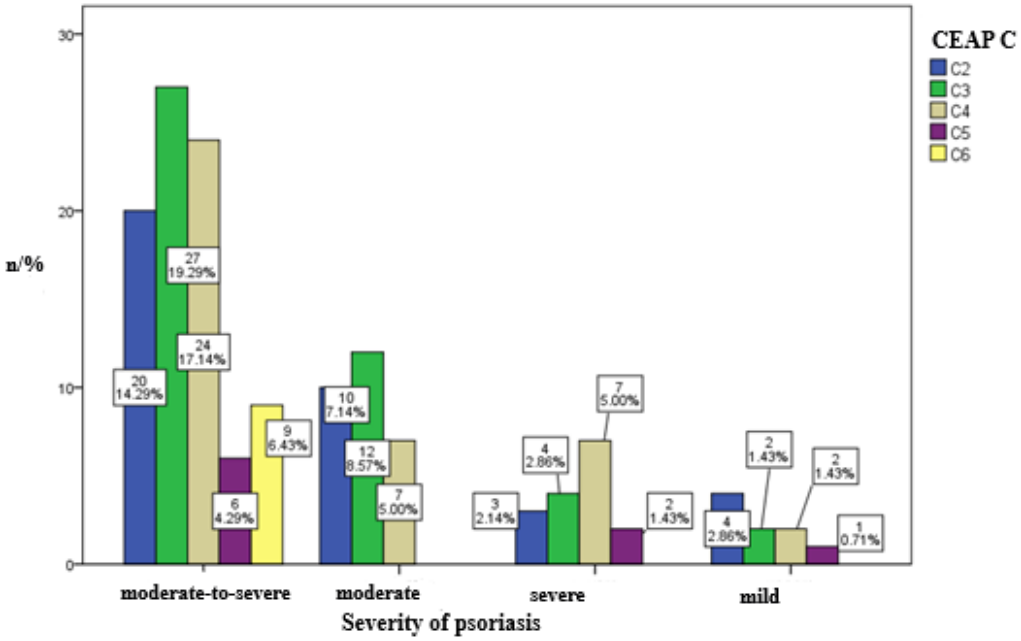

Fig. 1. Distribution of the severity forms of CVD on the severity of Psoriasis.

DISCUSSION

Compared to the experience of other medical centers, we can consider that in our study, the prevalence of chronic venous disease among the psoriatic population is high. A study conducted at the Sibiu Dermatology Clinic, during 2010-2013, on a cohort of 270 patients diagnosed with psoriasis, CVD prevalence was 22.22% (16). In another study conducted in Tunis, published in 2019, evaluating a cohort of 163 patients diagnosed with Psoriasis, the prevalence of CVD was 1% (17) There are, however, few studies published in the literature on this association. The highest prevalence of venous disease was in the age group 36-60 years, followed by the age group over 60 years. Women have a higher prevalence compared to men in the age group 18-35 years, and males have a higher prevalence in the age groups 36-60 years and over 60 years. Most patients included in the study have mild to moderate forms of venous disease. Psoriatic lesions cause the patient to consult a dermatologist, thus facilitating the early diagnosis and treatment of venous disease.

There are common risk factors involved in the etiopathogenesis of psoriasis and chronic venous disease (CVD) (1, 18). Excess body weight can trigger or maintain psoriasis or venous disease. Adipose tissue acts as an accessory endocrine organ, synthesizing over 700 peptides with a proinflammatory or metabolic role, called adipokines. Leptin, an adipokine involved in regulating appetite but also in perpetuating systemic inflammatory syndrome, has elevated serum values in psoriatic

patients (19). Other molecules involved in the onset of psoriasis are Tumor Necrosis Factor α (TNFα), Interleukin-6 and Interleukin-1 (1, 10). Obesity is also a risk factor for CVD. Recent studies show that elevated body mass index values are associated with more severe forms of venous disease (10, 13). In vivo, obesity is associated with decreased venous tonus in animal studies (11). In our study, we found a high prevalence of obesity, 19.2% of patients associated a BMI> 30 kg/m^2 (statistical value is highly significant, P = <0.001). Vlajinac *et al.* conducted a study on a group of 1116 patients diagnosed with CVD, and 15.8% had associated obesity (20). In a study of a group of psoriatic patients, Jacobi *et al.* found a prevalence of 27.6% in obesity (21). We can consider that in this study this association is also verified.

Smoking has been demonstrated as a risk factor for both psoriasis and chronic venous disease (18, 22). A meta-analysis study showed that among smokers or ex-smokers there is an increased prevalence of psoriasis. Smoking is responsible for the development of a chronic proinflammatory status and is especially associated with pustular forms of psoriasis (18). The number of packs/years is directly proportional to the severity of psoriasis. For CVD, smoking is a risk factor in young patients (<35 years) and especially in males (23). Gourgou *et al.* demonstrated that the appearance of varicose veins is closely related to the number of cigarettes consumed per day (24). In this study, 24.3% of patients are smokers or ex-smokers. Adışen *et al.* conducted a study on 563 patients diagnosed with plaque psoriasis between 2007 and 2013, and the prevalence of smoking in this population was 50.1% (25). Regarding the association of smoking with CVD, Gourgou *et al.* conducted a study on 1806 patients with venous disease and the prevalence of smoking in this group was 37% (24).

Cardiovascular comorbidities are associated with both psoriasis and venous disease. Psoriatic patients, especially those with severe forms, have an increased prevalence of hypertension (26). On the other hand, hypertension is a risk factor involved in the onset of psoriatic disease, as demonstrated in the study performed by Kim *et al*. in 2018, in which hypertensive patients had an increased incidence of psoriatic disease (12). Criqui *et al*. found that elevated blood pressure, both systolic and diastolic, is associated with chronic venous disease (22). A study conducted in Catalonia on a population of psoriatic patients, concluded that the most common comorbidities of psoriasis are hypertension and type II Diabetes, with prevalences of 34.4% and 14.7% (27). In our study, the prevalence of hypertension was high, with a value of 47.1%, and diabetes mellitus had a prevalence of 13.6%. Psoriasis is a risk factor for the onset of type II diabetes, regardless of age or disease severity (18). Diabetes mellitus may be considered a comorbidity in the patient with CVD. The vascular pathological changes induced by chronic hyperglycemia are represented by endothelial dysfunction, reduced peripheral perfusion and local angiogenesis (13).

CONCLUSIONS

Both psoriasis and chronic venous disease are common pathologies in dermatological medical practice, both of which have a negative impact on the patient's quality of life. In this study, the prevalence of CVD among psoriatic patients was significantly higher than the worldwide published results. There are, however, very few studies that have included this association.

In this population, there is a statistically significant high connection with hypertension, diabetes, obesity and smoking. Considering the results of our study, we consider that it is necessary to deepen this topic in future studies.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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CHALLENGES OF IMPLEMENTATION OF A FAST-TRACKED PRE-HOSPITAL POCUS ROUTINE

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CHALLENGES OF IMPLEMENTATION OF A FAST-TRACKED PRE-HOSPITAL POCUS ROUTINE (Abstract): The COVID-19 pandemic has changed the way of care for our patients and for our staff, many departments have compared the pandemic period to a 4 yearlong mass casualty situation managing scarce resources with insufficient manpower in a constantly shifting environment. A major bottleneck for healthcare systems were imagistic studies and the constant need for it, a foundation of modern medical practice. With the aim to improve care and facilitate better decision making we introduced a portable ultrasound device to the pre-hospital team, after providing Point of Care Ultrasound workshops to practicing physicians. **Materials and methods:** We performed a prospective observational study over 6 months, during the height of the COVID pandemic, using the extended Focused Assessment Sonography in Trauma (eFAST) protocol and the Shock Index (SI) to understand patient status; voluntary sampling was done by emergency physicians. The final outcomes were patient numbers, severity and type of cases and usage trends. **Results:** the study registered 34 patients: 41% traumas, 35% cardiac arrests, 18% shock, 6% acute respiratory distress. **Conclusions:** The overall increase and necessity of POCUS use during the pandemic or other mass casualty incidents does not equate to competency or familiarity of use despite workshops, instruments and willingness. Adapting and improving implementation of any new techniques requires a mature and professional team willing to collaborate, establish transparent feedback loops and implementation programs catered to the local needs. In this article we discuss the benefits and flaws we identified from a direct approach. **Keywords:** EMERGENCY, FAST-TRACKED PRE-HOSPITAL, POCUS, ROUTINE.

Imagistic input is a staple of modern medical investigation, diagnosis and treatment but with high convenience comes a high vulnerability in critical times such as mass casualty situations or pandemics. One of the first evocative, and dramatic, experiences of modern casualty management was during the 1988 earthquake in Armenia that had over 150000 victims and over 25,000 deaths, imagistic triage was accomplished

with 30 ultrasound scanners and one computer tomography machine (1). Romania has a symbiotic relationship with radiology while providing patient care (2) but with an ever-increasing reliance on advanced imagistic techniques to evaluate patients with critical illness, fatigue was evident in the radiology team and a way to quickly assess but also triage the need for future imagistic investigations needed to be adopted. In late 2020 following one year of pandemic experience, we added a portable ultrasound device to the pre-hospital phase, the GE Handheld VSCAN Dual Probe (General Electric Company, New York, USA), to help physicians provide better patient care and help with decision-making under difficult conditions due to pandemic fatigue and limitations imposed by personal protective equipment (PPE).

MATERIALS AND METHODS

Study Aim: Study how a fast-tracked implementation of ultrasound workshops influences emergency physician use of Point of Care Ultrasound in pre-hospital. We provide emergency physician pre-hospital teams with specific training and an ultrasound machine to improve critical patient diagnosis and management before arrival at the hospital.

Study Setting. A prospective observational study with voluntary sampling submitted by emergency physicians that are performing pre-hospital shifts on the Mobile Intensive Care ambulance (MIC) during the COVID-19 pandemic. The study was done one year after the introduction of the ultrasound machine to the ambulance.

The submitting physicians had previously participated in at least one Point of Care Ultrasound Workshop that was done with support from the European Society of Emergency Medicine (EuSEM). The workshops were structured over a two-day teaching period with multiple working stations focused on participant scanning experience and scan time. Workshop covered ultrasound basics, extended Focused Assessment Sonography in Trauma (eFAST), aorta lung and cardiac views.

The MIC ambulance is sent to cases in various environments following initial calls to 112 that meet the requirements for potential life-threatening situation, in support to lower ranked ambulances that require assistance or to facilitate intra-hospital transfer of critical patients. The environment can present different levels of accessibility difficulty, and at times can be at considerable distances from the closest hospital due to local geographical features and hospital placement making field decisions (stay and play approach) even more important.

The study took place in the county of Iasi, Romania, between January and June 2022, with ethics commission approval number 147/31.01.2022 from the "Grigore T. Popa" University of Medicine and Pharmacy Iasi and the "Sf. Spiridon" County Clinical Emergency Hospital from Iasi ethics commission board – The ethics approval was a part of a larger study.

Study Design

Participating physicians were instructed to complete a standard report form detailing patient demographics, cardiovascular parameters, causes for examination, ultrasound findings, diagnosis, elapsed time from arrival of crew till hospital transport and difficulties encountered. The encouraged scanning protocol was eFAST but pinpoint observations were also openly accepted.

Data Collection

The reporting physician gathered all the information required and submitted it

anonymously.

Data Analysis

Shock Index was calculated after data submission to quantify hemodynamic status. The cases were divided in 4 groups: shock, acute respiratory distress, cardiac arrest and trauma depending on presenting motive, positive findings were noted, elapsed time from crew presentation till hospital arrival, and final diagnosis.

Descriptive data analysis was performed.

RESULTS

The starting point for the study was 2019, when POCUS was not performed in our Emergency Department by emergency physicians, due to ease of access and close collaboration with the radiology department for diagnostic ultrasonography and advanced imaging (2).

The total number of participating physicians was 23 and the total number of cases submitted was 34. Emergency physicians preferred to use ultrasound with the following types of cases: 6 with shock, 2 with acute respiratory depression, 14 with trauma and 12 with cardiac arrests. The age varied between 18 and 81 years with an average of 57 and a ratio of female to male of 2.1:1. Shock Index was above 0.75 in 14 cases out of 22.

TABLE I
Categories of patients' groups

Category	Patients	Shock Index > 0.75	Causes/initial examination	US Findings
Overall	34	14		19 Positive findings
Shock	6	4	2 Undifferentiated, 2 Septic, 4 Cardiac,	4 Hypokinesia, 2 RV Strain
Trauma	14	8	12 Road Traffic Accidents, 2 Falls from Height	6 Free abdominal fluid, 2 Pneumothorax
Cardiac Arrest	12	N/A	8 Asystole, 2 PEA, 2 VT/VF	3 Mechanical activity, 2 RV strain
Acute Respiratory Distress	2	2	N/A	N/A

The **shock group** had a total of 6 patients out of which, 2 had an undifferentiated shock, 0 presented as septic, 4 had a cardiogenic shock and none presented with obstructive shock. A Shock Index of >0.75 was identified in 4 patients. Ultrasound findings reported were positive in 4 patients with hypokinesia and 2 with right ventricle (RV) strain.

The **group** with **acute respiratory distress** numbered 2 patients both with a SI > 0.75 but with no significant findings on ultrasound scans.

The trauma group numbered 14 patients, 12 were due to road traffic accidents and 2 fell from height (more than two meters). From the 14 patients with trauma, 8 patients had a SI >0.75. eFAST scans were done and 6 patients were found positive for abdominal free fluid and 2 for pneumothorax.

The cardiac arrest group numbered 12 patients that had as initial rhythm as fol-

low, 8 with asystole, 2 with pulseless electrical activity and 2 with Ventricular Fibrillation or Ventricular Tachycardia. On ultrasound scan during CPR 3 patients were found to have mechanical activity 2 of which presented Right Ventricle strain.

The following difficulties while performing ultrasound in pre-hospital were reported by operators: 7 cases were difficult to exam/obtain images due to body status, 2 were very agitated, 1 due to extremely low ambient temperature and 1 due to patient lodged foreign body and the way he/she was immobilized in a patient from road traffic crash.

The following data was excluded from the data presented due to inconsistencies in submissions: The time elapsed at the scene and the time of arrival at the hospital. Accuracy of findings was not possible as follow-up was not completed due to multiple factors described in the discussion subheading.

DISCUSSION

The COVID pandemic challenged the way we practice medicine and the way we implement new techniques and habits into daily practice due to a constant struggle to reduce risk of infection for both patients and staff with several rushed decisions having to be made to cope with new patient influx and adjust to current working realities.

A lot of suspected COVID patients were investigated at the start of the pandemic by computer tomography (CT); drastically increasing the rate of non-contrasts scans (4). Although a shift from early CT assessment was done, usage remained high due to an increased risk of COVID patient population complications such as pulmonary embolism or co-infections. CT was also recommended to patients with an initial negative RT-PCR but a high suspicion of severe infection especially in environments with limited test in (5, 6, 7). Alongside the influx of COVID infections, non-COVID medical and surgical patients had diagnostic, management and treatment requirements that needed radiology input. Overall, this contributed to a tremendous stress on the radiology department and increased waiting times in some already standstill emergency departments.

The high workload was taking a toll the medical personnel and already in 2020 reports were coming out stating that 46% of radiologists were burned out compared to the average of 42% in all other specialties due to workload, complexity of cases, constant change of schedule and longer time between scans due to decontamination measures (8, 9).

As the pandemic progressed, technology started to step in and relieve pressure from repetitive tasks. Deep learning models were implemented to help analyze chest x-rays and chest CT obtaining a sensitivity of 92.3% and specificity of 85.1% for CT at the start of the pandemic progressing to a sensitivity of 97.6% and specificity of 78.6% with chest x-rays, but this was not available everywhere (10, 11, 12). Lung ultrasound such as the BLUE protocol (13) is seen as a viable way of imaging patients with accuracy rates comparable to CT or X-rays (14-17) but with obvious limitations in the form of required user experience.

The decision to initiate advanced imaging requests is directed by physicians, and with a high workload, decision fatigue was high. The choice to develop POCUS protocol use within our department was appropriate, especially since steps to develop a POCUS program was underway prior to the pandemic. The goal was to relieve pressure

from the radiology department by optimizing patient evaluation and provide the means of getting the required simple, straightforward answers needed in most cases. The goal of POCUS has always been that of answering simple but important questions at the right time to provide clarity to the attending physician (18).

The workshops we provided are supported by experienced POCUS providers from the European Union Society of Emergency Medicine (EUSEM) and started two years prior to ultrasound introduction to the ambulance crew. They were geared to provide participants with the maximum scanning time and patient experience within the two days of the workshop, covering different topics and learning goals. Initial enthusiasm was high for the first workshop in the first half of 2019, before the pandemic, and during the second one, that took place in 2021 in the middle of the pandemic. The introduction of an ultrasound machine to the ambulance was also meet with great enthusiasm and seen as a breath of fresh air in a very difficult environment due to epidemic restrictions, high patient load and personal protective equipment (PPE).

The study was started one year after the introduction of ultrasound to the pre-hospital, to have a better idea of trends and usage. The initial enthusiasm did not carry well to practice, at least when translated into submitted cases for the study. The meager 34 properly submitted cases did not meet surveyor expectations but the reasoning behind it provided valuable information on local learning requirements and characteristics.

The lack of cases submitted was partly explained by 1) the fact that POCUS was not considered to be of use in all encountered cases and 2) when faced with critical cases that would have benefited from POCUS but required fast actions, most doctors preferred to fall back on ingrained behavior from Advanced Life Support or Advanced Trauma Life Support protocols without utilizing POCUS due to feelings of inadequacy from lack of experience with ultrasound.

Participants also declared that ultrasound was used a lot more than the submitted cases but due to long mission times, downtimes due to decontamination or back-to-back missions, information was omitted and not submitted to not pollute results.

The main request from most of the participants was more workshops or even implementation of POCUS curricula for residents, where even established physicians could participate. This led us to further implement an expansion of POCUS teaching intro actual clinical practice to stress its usability in critical situations. These aspects have been studied and detailed in another study, performed and published by the authors (18). Without guidance and experience sharing the risk of inconsistent results or wrongful use is high and this in turn makes it dangerous for the operator to trust in the gained skills but also for them to practice in a wrong or inefficient manner.

Experience gathered during this period, can easily be applied to future mass casualty situation or other pandemics that may or may not come, solidifying the importance of adaptability and agility.

Documentation is a normal part of daily medical life, especially in pre-hospital where every step, procedure, medication and detail need to be noted to ensure safety of all parties. A severe disruption in effective documentation was noted during the heights of the pandemic, due to a multitude

of factors such as: personal protective equipment, sterilization protocols for ambulances and equipment, translating into long down-times and sometimes inability to maintain correct medical documentation. (19) An important limitation of our study was the lack of integration of data from the ultrasound machine to safe localized data storage and thus a heavier reliance on human scribing and submitting.

An implementation of the lessons learned in this study with the addition of easier to use and automate reporting mechanisms would provide a better understanding of POCUS usage and changes required to improve teaching and study protocols for future research.

CONCLUSIONS

Implementing correct and confident POCUS use in the daily practice of acute high paced departments cannot be achieved by providing ultrasound workshops. These will provide respite in resource strained environments but in the case of patients that require fast decisions, seldom will care providers, with just workshops experience, use these skills effectively in their evaluation and care, falling back to ingrained behavior or risk misusing said skills. The lessons learned from adapting to difficult conditions such as those created by the pandemic are that we need to cultivate flexibility and adaptability, both of which are characteristics POCUS. Creating the prerequisites for a POCUS training curriculum would provide better current care and better resistance in the event of future mass casualty situations.

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LUNG DAMAGE IN RHEUMATOID ARTHRITIS - A PROSPECTIVE ANALYSIS

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LUNG DAMAGE IN RHEUMATOID ARTHRITIS - A PROSPECTIVE ANALYSIS (Abstract): The objective of this prospective study was to make correlations between demographic and rheumatoid arthritis (RA)-related characteristics and the type and severity of the interstitial lung involvement (ILD), being able to quantify a potential "gold standard" in predicting the development of these extra-articular manifestations (EAMs) that worsen the disease and increase the morbidity. **Materials and methods**: 60 RA patients were included in the analysis over a period of 2 years and the following data were centralized: demographic characteristics, the presence of lung damage quantified by pulmonary high-resolution computed tomography (HRCT), the presence of specific RA antibodies (anticitrullinated peptide antibodies - ACPAs), duration and activity of RA, pulmonary function measured by diffusing capacity for carbon monoxide (DLCO). Using different statistical tests, we analyzed the correlations between these parameters, trying to highlight the possible risk factors involved in the appearance of pulmonary manifestations in RA. **Results:** We included in our prospective study 60 patients, mostly women (n=46; 76.7%) with a mean age of 63.02±10 years old and with a mean age of disease onset of 51.89 years (95% CI: 48.86 to 54.93). Out of the 60 cases, data on smoking status was available for 34 participants, 82.4% being non-smokers, while 17.6% having an active smoker status. Regarding pulmonary function tests, 60% of the patients showed a decreased DLCO. More than half of the subjects (63.18%) had increased values of ACPAs. The results obtained were promising, demonstrating a direct relationship between both the DLCO decrease and the presence of specific ACPA antibodies, as well as between the appearance of lung-specific changes on HRCT and the increased titer of ACPAs. Also, the decrease in lung function was correlated with the radiological manifestations highlighted on HRCT. **Conclusions:** The presented study supports the close relationship between AR and ILD, pointing out the need for a careful pulmonary evaluation in these patients. The more active the disease, the higher the risk of developing lung damage. An early diagnosis and an adequate treatment can improve the prognosis of these patients. **Keywords**: RHEUMATOID ARTHRITIS, INTERSTITIAL LUNG DISEASE, DIFFUSING CAPACITY FOR CARBON MONOXIDE, ANTICITRULLINATED PEPTIDE ANTIBODIES.

INTRODUCTION

RA is a systemic autoimmune condition which has a vast panel of EAMs, interstitial lung disease being one of the most frequent and problematic EAMs due to an unfavorable prognosis and a delayed diagnosis. RA-

ILD is a multifaced disease, in which the ACPAs play a key role (1, 2). Being first described in 1948 by Ellman and Ball (3), ILD is much more common nowadays. The prevalence of ILD in RA patients may vary between 5-67% (4, 5). The women are more predisposed to the disease (6). The disease does not respect a certain pattern, any segment of the lungs compartment can be involved. The etiology of RA-ILD is yet not fully elucidated. The presence of ACPAs can precede the disease onset with many years (7-11).

The main steps in the diagnosis of RA-ILD are represented by an impairment of pulmonary function (mainly a decrease value of DLCO), a specific lung pattern highlighted by HRCT, and an active disease characterized by an inflammatory status and a high disease activity determined by the Disease Activity Score in 28 joints-C-reactive protein (CRP)(DAS28-CRP). The later the onset of the disease is, the more preserved the prognosis can be (12-14). HRCT scan represents the main tool in the diagnosis of ILD. There are two most frequent and specific patterns in RA-ILD: usual interstitial pneumonia (UIP) in 40-60% patients, named "temporal heterogeneous pattern" and non-usual interstitial pneumonia (NSIP) which can appear in 10-30% patients, named "spatial homogeneous pattern". UIP illustrates a symbiosis of lung fibrosis areas combined with healthy pulmonary tissue having a distribution in subpleural sites and being characterized by the "honeycombing" aspect. In contrast, NSIP has a bilateral distribution, usually in the inferior lobes, known as "ground-glass opacities" and bronchiectasis (15-18).

MATERIAL AND METHODS

RA-ILD patients (n=60) attending Rheumatology department of Clinical Rehabilitation Hospital, Iasi, Romania from august 2022 to august 2024 were enrolled in this prospective study. The patients were diagnosed using 1987 ACR or 2010 ACR/EULAR classification criteria. The inclusion criteria were a positive diagnosis of RA according to the classification criteria, a signed informed consent, and age more than 18 years old. The exclusion criteria were age under 18 years old, the presence of an Overlap syndrome, active infections, neoplasms, severe comorbidities (hepatic, cardiac, or renal involvement), and non-adherence to treatment.

The objective of our study was to highlight the correlations between different parameters of RA and the type and severity of the lung involvement. In the patient's medical records, the following data were included: demographic characteristics, the presence of lung damage quantified by HRCT, the presence of specific RA antibodies (ACPAs), duration and activity of RA, pulmonary functionality measured by DLCO. There was no restriction on the use of any conventional synthetic disease-modifying antirheumatic drugs (csDMARD) or biological therapy.

After collecting these data and using different statistical tests, we analyzed the correlations between them in order to highlight some possible risk factors for the development of pulmonary manifestations in RA.

The disease activity was measured using the DAS28-CRP score. Recent studies highlighted that CRP is more specific for RA-ILD, than erythrocyte sedimentation rate (ESR). The seropositivity, especially the presence of ACPAs was evaluated using Alegria device by an enzyme-linked immunosorbent assay (ELISA), a robust and sensitive technique. High titers of ACPA were considered those over three times

the normal value (N:0-20 U/mL). The functional lung tests were accomplished in accordance with the standards in force. We took into consideration the value of DLCO, and we included only the percent of predicted values of DLCO. All patients underwent a pulmonary HRCT. Patients who had traction bronchiectasis, reticular abnormalities, a basal distribution, "honeycombing" aspect or "ground glass opacification" were included, having a characteristic aspect for RA-ILD.

Statistical analysis

The data analysis was conducted using *SPSS version 26.0* statistical software. Descriptive statistics were presented as means (M), standard deviations (SD), and percentages (%). For continuous variables with a normal distribution, the mean (M) and standard deviation (SD) were used, while non-normally distributed variables were described using the median and interquartile range (IQR). Group comparisons were performed using chi-square tests (χ^2) for categorical variables, with Fisher's exact test applied when expected frequencies were below 5. For continuous variables, independent samples t-tests or Mann-Whitney U tests were utilized, depending on the data distribution. Kendall's tau-b correlation coefficient was used to analyze associations between continuous variables, such as DLCO, Age, and ACPA. All tests were two-tailed, with statistical significance set at $p < 0.05$, except where noted (e.g., marginal significance at $p < 0.10$). Abbreviations used include M (Mean), SD (Standard Deviation), IQR (Interquartile Range), CI (Confidence Interval), t (t-test statistic), χ^2 (Chi-Square statistic), p (p-value), N (Sample Size), and OR (Odds Ratio).

RESULTS

60 RA-ILD patients met the criteria for the inclusion in the study. The median age of the patients was 64.5 years. The demographic and RA-related data are presented in the first table.

TABLE I.

Demographic and RA-related characteristics of patients.

Characteristics	N=60	%
Gender		
Male	14	23.3%
Female	46	76.7%
Median age	64.5±10 years	
Smoking history		
Yes	6	10.0%
No	28	46.7%
Seropositivity		
Yes	49	81.7%
No	11	18.3
Disease activity (DAS28-CRP)		
Low	29	48.3%
Moderate	21	35.0%
High	10	16.7%
HRCT		
Specific pattern	43	71.7%
No specific pattern	17	28.3%
DLCO		
Normal values	24	40.0%
Decreased values	36	60.0%

Of the 60 cases, 23.3% were male and 76.7% female. The average age of males was 62.93 years (95% CI for Mean ranges from 56.50 to 69.36) and the average age of females was 63.02 years (95% CI for Mean means, the true mean age of females in the population is likely between 59.93 and 66.11 years). The age distribution for both males and females is symmetrical, with similar means and medians.

Data on smoker status were available only for 34 subjects. Among those with valid data, 82.4% reported being non-smokers, while 17.6% reported being active smokers. Non-smokers had a mean age of 65.04 years with a 95% CI ranging from 60.67 to 69.40 years and a median of 66.5

years. In contrast, smokers had a lower mean age of 61.33 years (95% CI: 54.67 to 67.99) and a median of 62 years.

In the studied group, 81.7% of cases were seropositive. Out of the 60 patients, 23.3% had ACPA levels below the normal value of 20. 6.7% had ACPA levels 4-5 times higher than normal range (80-100), while 16.7% had ACPA values exceeding 80-100. A significant 18.3% of individuals had ACPA levels at or exceeding 1000 (tab. II).

TABLE II.

The ACPAs values in seropositive patients.

ACPA	Frequency	%	Valid %	Cumulative %
Below normal (**<20**)	14	23.3%	35.0%	35.0%
4-5 times higher than normal (**80-100**)	4	6.7%	10.0%	45.0%
Over 80-100	10	16.7%	25.0%	70.0%
Max ACPA levels (**1,000** and above)	11	18.3 %	27.5%	97.5%
Missing data	21	35%	N/A	100.0%
Total	60	100.0%	100.0%	

For seronegative individuals, the mean age was 62.18 years (95% CI: 55.95 to 68.42), with a median of 65 years. For seropositive individuals, the mean age was slightly higher reaching to 63.18 years (95% CI: 60.08 to 66.28), with a median of 64 years. Overall, seropositive individuals exhibited higher age variability with a more balanced distribution, while seronegative individuals had a more concentrated age range with a noticeable skew towards younger ages.

Out of the total 60 patients, nearly half (48.3%) had a low Disease Activity Score (DAS28-CRP), 35% had a moderate disease activity, and 16.7% were categorized as having high disease activity. For the low disease activity group, the mean age was 64.10 years (95% CI: 59.68 to 68.53), with a median of 65 years. For the moderate disease activity group, the mean age was slightly lower at 63.86 years (95% CI: 59.69 to 68.03), with a median of 65 years. For the high disease activity group, the mean age was decreased at 58.00 years (95% CI: 51.65 to 64.35), with a median of 62 years. Overall, as disease activity increases, the mean age tends to decrease, and the variability in ages becomes slightly more constrained (fig. 1).

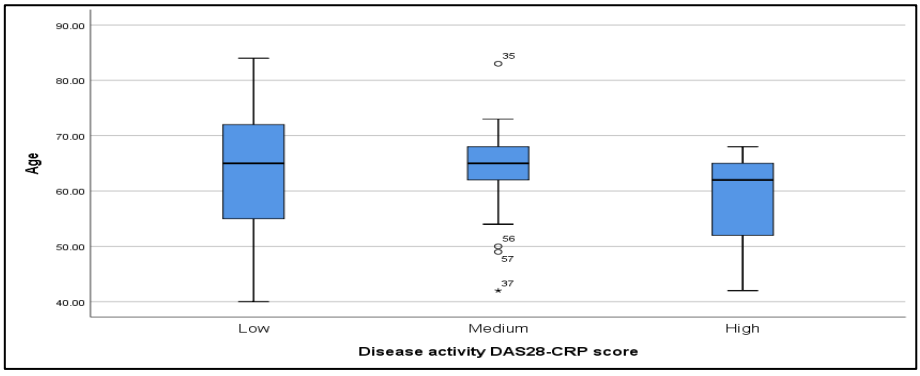

Fig. 1. The mean age regarding disease activity.

Out of the 60 subjects, the majority (71.7%) showed a specific pattern on HRCT, while 28.3% did not exhibit any specific pattern. For individuals who did not have a specific pattern on HRCT scan, the mean age was 60.24 years (95% CI: 54.57 to 65.90), with a median of 63 years. In contrast, individuals with a specific pattern on HRCT scan had a higher mean age of 64.09 years (95% CI: 60.96 to 67.23), with a median of 65 years.

Regarding functional lung tests, 60% cases exhibited a decrease in DLCO, while 40% had normal DLCO values. For individuals having a normal DLCO, the mean age was 59.79 years (95% CI: 55.57 to 64.01), with a median of 62 years. For individuals with a decreased DLCO, the mean age was 65.14 years (95% CI: 61.63 to 68.65), with a median of 67 years. Among the 36 cases having a decreased DLCO, 83.3% were seropositive, and 16.7% were seronegative. As a percentage of the total sample, 50% of the participants had both a decreased DLCO and were seropositive, while 10% had a decreased DLCO and were seronegative. Most individuals having a decreased DLCO were seropositive, suggesting a strong association between seropositivity and a decreased lung function.

The mean age of disease onset was 51.85 years (95% CI: 49.15 to 54.55), with a median of 52 years. For males, the mean age of disease onset was 51.71 years (95% CI: 45.02 to 58.41), with a median of 50.5 years. For females, the mean age of disease onset was 51.89 years (95% CI: 48.86 to 54.93), with a median of 52 years. For seronegative patients, the mean age of disease onset was 49.91 years (95% CI: 45.12 to 54.70), with a median of 52 years. For seropositive patients, the mean age of disease onset was slightly higher 52.29 years (95% CI: 49.10 to 55.47), with a median of 52 years. Overall, while the median ages were similar, seropositive individuals showed more diversity in the age of disease onset. For patients having a low disease activity, the mean age of onset was 53.03 years (95% CI: 48.51 to 57.56), with a median of 54 years. For individuals with a moderate disease activity, the mean age of onset was 50.48 years (95% CI: 46.09 to 54.86), with a median of 52 years. For the cases having a high disease activity, the mean age of onset was 51.30 years (95% CI: 45.74 to 56.86), with a median of 51.5 years. Overall, as disease activity increased, the age of disease onset showed less variability.

The crosstabulation analysis between DLCO and ACPAs presence showed that among the 24 patients having a normal DLCO, 5 patients (45.45%) were seronegative, and 19 patients (38.78%) were seropositive. In contrast, among 36 patients having a decreased DLCO, 6 patients (54.55%) were seronegative, while 30 patients (61.22%) were seropositive. The overall distribution indicates that a higher proportion of patients with a decreased DLCO were seropositive compared to those having a normal DLCO. This suggests a potential association between seropositivity and DLCO decline (tab. III).

TABLE III.

The correlation between DLCO and the presence of ACPAs.

DLCO	Seronegativity	Seropositivity	Total
Normal value	5 (45.45%)	19 (38.78%)	24
Decreased value	6 (54.55%)	30 (61.22%)	36
Total	11	49	60

However, The Chi-Square test assessing the relationship between DLCO (Normal *vs.* Decrease) and Seropositivity and Seronegativity yielded non-significant results. The Pearson Chi-Square value was 0.167, with a p-value of 0.683, suggesting no association between DLCO and seropositivity / serone-gativity. The Continuity Correction, applied due to small-expected counts, showed a p-value of 0.946, reinforcing the lack of significance. The Likelihood Ratio produced a p-value of 0.684, consistent with the Pearson test, while Fisher's Exact Test (appropriate for small samples) showed no significant association with a p-value of 0.741.

Analyzing the link between HRCT findings and the presence of ACPAs, we had a total sample of 60 patients; 71.7% had a specific pattern identified on HRCT scans, while 28.3% had no specific HRCT pattern. When looking at the distribution of seropozitivity, the majority of patients with specific HRCT pattern (73.5%) were seropositive, compared to only 26.5% who were seronegative. Among those without specific pattern on HRCT, 76.5% were seropositive and 23.5% were seronegative. Overall, the results suggest that patients having a specific pattern on HRCT are more likely to be seropositive. In the Chi-Square test for the association between HRCT findings and seropositivity/seronegativity, the Pearson Chi-Square value was χ^2 (1, N = 60) = 0.428, with a p-value of 0.513, indicating no significant association between the two variables ($p > 0.05$). The Continuity Correction for continuity in small samples also yielded a non-significant result ($p = 0.777$), confirming that the relationship is not statistically significant. Additionally, the Likelihood Ratio and Fisher's Exact Test provided p-values of 0.521 and 0.712, respectively, both of which support the absence of a significant association (tab. IV).

TABLE IV.
The correlation between HRCT and the presence of ACPAs.

HRCT	Seronegativity	Seropositivity	Total
No specific pattern	4 (23.5%)	13 (26.5%)	17 (28.3%)
Specific pattern	7 (41.2%)	36 (73.5%)	43 (71.7%)
Total	11	49	60

The crosstabulation between HRCT pattern and DLCO showed that among the 17 patients with no specific pattern on HRCT, 10 (58.82%) had a normal DLCO, while 7 (41.18%) showed a decrease in DLCO. On the other hand, among the 43 patients with a specific pattern on HRCT, 14 (32.56%) had a normal DLCO, while a higher proportion of 29 (67.44%) had a decrease in DLCO. Overall, in the total sample of 60 patients, 24 (40%) had normal DLCO, and 36 (60%) experienced a decreased DLCO. This distribution suggests that patients having a specific pattern on HRCT tend to have a higher likelihood of a decreased DLCO compared to those with no specific modifications. For a significance level of 10% ($\alpha = 0.10$), the Pearson Chi-Square test showed a Chi-Square value of 3.502 with 1 degree of freedom and a p-value of 0.061. Since this p-value is slightly above the conventional 5% threshold but below 10%, the result suggests a marginally significant association between the variables. The Likelihood Ratio test,

with a value of 3.460 and a p-value of 0.063, similarly suggested a marginal association, just missing the 5% cutoff but significant at 10%. The Fisher's Exact Test (p = 0.082) and Linear-by-Linear Association (p = 0.063) tests both indicate a significant result at the 10% level, reinforcing the idea of a weak association between the variables (tab. V).

TABLE V.
The correlation between HRCT pattern and DLCO.

HRCT	Normal DLCO	Decreased DLCO	Total
No specific pattern	10 (58.82%)	7 (41.18%)	17
Specific pattern	14 (32.56%)	29 (67.44%)	43
Total	24 (40.0%)	36 (60.0%)	60 (100.0%)

DISCUSSION

In the last decades, RA has won its leadership among systemic inflammatory autoimmune diseases, the prevalence of pulmonary involvement increasing. Nowadays, modern medicine has the necessary tools for an early and correct diagnosis (3, 6). ILD is diagnosed more frequently in RA patients presenting risk factors such as: an active smoking status, male gender, advanced age and high ACPA titer (6, 19).

EAM in RA represents a factor of disease severity, lung damage proving to be more and more frequent and increasing the risk of death up to 3 times (20-22). Although the new therapeutic strategies are promising, the mortality rate is still high (23). Even though the patients are mostly asymptomatic, at least 27-63% will have low values of lung function tests, especially DLCO decrease (6, 24). Regarding the changes highlighted by HRCT scan, symptomatic patients present in 90% of cases an ILD pattern, but even asymptomatic ones can have non-specific changes in a high percentage of up to 68% (25). Moreover, regardless of the stage of RA activity, the changes observed on HRCT can anticipate the decrease of pulmonary functionality (26). A decreased DLCO has been correlated with a progressive RA-ILD disease, being the most sensitive independent predictor of this association (6, 27).

The objective of our study was to highlight the correlations between different parameters of RA and the type and severity of the lung involvement. The positivity of ACPA seems to be the most important risk factor for the development of pulmonary damage in the analyzed group of patients. The increased values of ACPA were correlated with the decrease of DLCO as well as with the appearance of specific pulmonary interstitial changes highlighted by HRCT. In addition, interstitial lung disease was associated with functional impairment quantified by DLCO, the low values of this parameter being closely related to the occurrence of ILD on HRCT.

In a multicenter cross-sectional study carried out over a period of 2 years, Mena-Vázquez *et al.* (28) found a certain predisposition for ILD in RA patients with a history of smoking (p=0.013) and with ACPA positivity (p=0.004). Also, in the group with RA and lung damage, the ACPA titer and DLCO decrease below 70% were correlated with the occurrence of articular erosions (p<0.001). Moreover, the authors described the characteristic aspects

highlighted on HRCT as follows: UIP pattern in 66% of cases and NSIP pattern in approximately 32% of subjects (28-31).

Our results are in accordance with the data from the literature and support the close relationship between RA and ILD. The patients included in the analysis were mostly women (76.7%), with an increased level of ACPA, with specific lung changes on HRCT (71.7%) and with a decrease in lung function described by a decrease in DLCO (60%). A distinctive fact is represented by the non-smoker status, which was more frequently present in our cohort.

Supporting the same idea, Zhang *et al*. (32) evidenced a decreased DLCO in 92% of patients with RA-ILD. Kim *et al*, whose study took place over a period of 7 years (2001 - 2008), pointed out the close connection between ILD and RA and described an equality between the 2 distinctive patterns UIP and NSIP (33). Another study that focused on the HRCT aspect highlighted that the most frequent findings were UIP aspect (37.22%) and also NSIP aspect (25.94%) (34). The study included 266 RA-ILD subjects, mostly women (n=162; 60.90%) with a mean age of disease onset at 64.80 ± 10.71 years old. The percentage of smokers was higher among males (75.92%) (34).

Other data that confirm the results obtained from our study, were published by Samy (35). He analyzed 160 cases with RA, of which 85% were women, with a low DLCO, with high ACPA titer and with specific HRCT changes of interstitial damage. The published data reinforce the link between all these parameters and the appearance of RA-ILD (35). Moreover, Paulin *et al*. showed, in a multicenter cross-sectional study on 83 subjects (mostly women - 83%), with a mean age of 46.49 years, the association between a decreased DLCO, an increased ACPA level and pulmonary impairment (36). Last but not least, Oak *et al* (37) and Bessa *et al.* (38), through their studies, support this close link between ACPA high level - low DLCO - HRCT changes, which can be considered an axis in the development of RA-ILD.

Our study has some limitations due to the small number of enrolled subjects. However, we highlighted important aspects regarding RA-ILD. Some of our results did not have statistical value but can open new perspectives on the horizon of knowledge. The presence of ACPAs in high titers correlated with the decreased levels of DLCO and the presence of specific patters on HRCT scan, may be considered significant predictors of pulmonary impairment in RA.

CONCLUSIONS

This prospective study highlighted that seropositivity tends to be more prevalent among patients who have a decreased DLCO and a specific lung pattern on HRCT, thereby indicating a potential lung impairment. The positivity of ACPA seems to be the most decisive risk factor in the development of RA-ILD disease. The clinical and paraclinical evaluation of these patients is particularly important and the presence of EAM should not be neglected. The association between RA and ILD is a certainty that worsens the evolution of the disease and increases mortality. Therefore, an early diagnosis and an appropriate treatment, which requires a multidisciplinary team, becomes a priority in everyday medical practice.

CONFLICTS OF INTEREST AND FUNDING

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INSULIN PUMP THERAPY REDUCES THE SEASONAL HBA1C DRIFT IN CHILDREN WITH TYPE 1 DIABETES

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INSULIN PUMP THERAPY REDUCES THE SEASONAL HBA1C DRIFT IN CHILDREN WITH TYPE 1 DIABETES (Abstract): Glycated hemoglobin A1c (HbA1c) levels are known to fluctuate seasonally in children with Type 1 Diabetes (T1D), typically decreasing during warmer months and increasing during colder months. Given that continuous subcutaneous insulin infusion (CSII) may lower HbA1c by approximately 0.3% (3 mmol/mol) relative to multiple daily insulin injections (MDI), the well-documented seasonal drift might offset the immediate benefits of initiating CSII in warm seasons. This study examines how CSII and MDI each modulate the expected rise in HbA1c from warmer to colder weather. **Materials and methods**: A total of 504 T1D patients under 18 years old attending the pediatric diabetes department of a major university hospital were evaluated. Exclusion criteria included disease duration under 1 year, lack of continuous glucose monitoring (CGM) access, fewer than 6 months on CSII, or incomplete data. Propensity score matching was used to yield two groups (CSII versus MDI), matched by age, HbA1c, body mass index, and calendar month (n=65 in each group). Follow-up visits occurred at 180±90 days after baseline. **Results:** Follow-up time was comparable in both groups (174±35 days for CSII *vs.* 169±38 days for MDI, p=0.44). Children in the CSII group demonstrated no significant change in HbA1c between baseline and follow-up. Conversely, the MDI group exhibited a statistically significant increase in HbA1c at follow-up (7.58±1.0%; p=0.01 *vs.* baseline). Among adolescents, the same pattern emerged, indicating a stable HbA1c level in the CSII group and a marked rise in MDI users (p=0.01). **Conclusions:** Children receiving MDI experienced the expected seasonal HbA1c increase, whereas CSII users did not demonstrate significant changes. These findings highlight the potential buffering role of CSII on seasonal HbA1c drift, even when follow-up conditions and baseline characteristics are comparable. **Keywords:** PEDIATRIC DIABETES, INSULIN PUMP, HBA1C SEASONALITY.

INTRODUCTION

Diabetes mellitus continues to be a significant public health problem, although diabetes-associated mortality rates seem to be declining in recent years (1). Despite the increasing recent recognition of time in

range importance, glycated hemoglobin A1c (HbA1c) is still the pillar of glycemic control evaluation in type 1 diabetes mellitus (T1D) in children. In many temperate regions worldwide, HbA1c levels exhibit seasonal variations, with lower values observed in the second and third quarters compared to the rest of the year (2-8). In addition, HbA1c variability tends to be greater in countries that experience a more prominent contrast in temperatures between winter and summer (5,9).

As costs and access improve, continuous subcutaneous insulin infusion (CSII) using an insulin pump may become the primary treatment option for children with T1D. Compared with multiple daily insulin (MDI) treatment, its advantage on HbA1c of approximative 0.3% (3 mmol/mol) is strikingly similar to the average differences in HbA1c induced by seasonality (2,10). Consequently, initiating CSII in summer might not translate into an immediate HbA1c benefit simply because of the known summer-winter HbA1c inherent drift. CSII appears to ameliorate HbA1c seasonality, but a direct comparison with MDI on this aspect is still missing (7).

Researchers should carefully consider these aspects when using descriptive data from real-world registries. Therefore, we tried to contribute data to the general knowledge about the impact of CSII and MDI on the HbA1c expected increase from warm to cold seasons. The primary outcome of the current study was to assess short-term (six months) changes in HbA1c starting from the second and third trimester of the year in T1D children treated with either CSII or MDI. Secondary outcomes included changes in the total daily insulin dose, the prandial-to-total insulin ratio, and the units of insulin per kilogram of body weight.

MATERIALS AND METHODS

Study population. This retrospective observational cohort study compared the short-term metabolic control in type 1 diabetes children on either CSII therapy or MDI. All patients attending the pediatric diabetes department of a major university hospital from 2019 to 2024 were eligible for this study (n=504). This major pediatric diabetes center serves a large urban area and its surroundings within a 300 Km radius. As part of the larger SWEET Project (www.sweet-project.org), the local pediatric diabetes registry contained information on anthropometrics, biochemistry, and therapy, including data before 2019. As continuous glucose monitoring (CGM) was known to impact metabolic control significantly, patients were required to have continuous CGM access to be eligible for this study. Other exclusion criteria at baseline were age 18 or more, diabetes duration under one year, and time since CSII initiation less than 180 days.

The study has been conducted in accordance with all the relevant national regulations and institutional policies and with the tenets of the Helsinki Declaration, and the local Ethics Committee has approved it. Informed consent was obtained from all legal guardians of individuals included in this study.

Baseline. The baseline was defined as the first available recording, in which the age was less than 18 years, the diabetes duration was 365 days or more, CGM was used, and the date of visit was in the second or third trimester of the year. Propen-

sity score matching was used to control differences in age, diabetes duration, HbA1c, BMI and calendar month at baseline in a 1: 1 group ratio. The final cohort retained 130 subjects, split into a CSII (n=65) and an MDI group (n=65) based on the baseline insulin delivery method (see fig. 1). The MDI group was formed using the remaining patient pool after constructing the CSII group. The treatment option at baseline had to remain unchanged at the follow-up visit.

Follow-up visits occurring at 180 ± 90 days after baseline were eligible for this study. Where multiple follow-up visits were available, only the one closest to 180 days was retained in the primary analysis. Patients without at least one follow-up visit were excluded (n=52) (fig. 1).

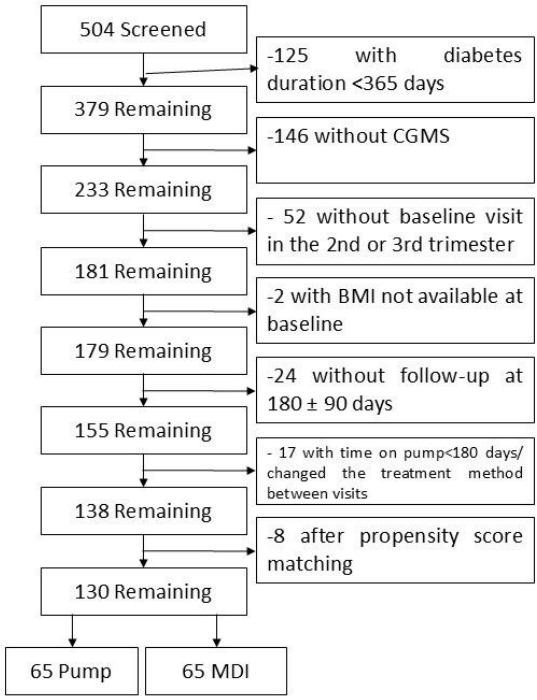

Fig. 1. Flowchart of patient attrition in the study. (CGMS: continuous glucose monitoring system, BMI: body mass index, MDI: multiple daily injections)

Statistical analysis was performed using *SPSS version 29.0.1.0*. A 2-sided p-value less than 0.05 was considered statistically significant. Propensity score matching used the multivariable logistic regression model. Only patients with baseline and follow-up data were retained when individual variables were analyzed. Primary outcome variables were available in all study subjects. The primary outcome was supplementary checked in a sensitivity analysis, using all available follow-up visits at

180 ± 90 days after the baseline. This analysis was performed to check the reliability of the main results.

The primary outcome was further explored in the adolescents' group, defined as age at baseline between 10 and 17 years (n=79). Preadolescents could not be investigated due to the limited number of patients in this age category.

Secondary outcomes were investigated in a subgroup of patients (n=53 CSII and n=58 MDI) due to missing information in some patients. The number of sick days over the last three months was available in a limited patient sample (n=27) containing a mixture of CSII and MDI patients. Due to the limited number of cases, the Wilcoxon matched-pairs signed-ranks test was used in this subset of data.

RESULTS

Primary outcome. The final cohort retained 130 subjects, respecting all the inclusion and none of the exclusion criteria. Mean age was 10.7 ± 3.5 years, with a diabetes duration of 4.5 ± 2.8 years. In the CSII group, the baseline mean time since pump initiation was 858 ± 491 days (range 193-2327 days). Propensity score matching was responsible for the similar results at baseline for age and HbA1c among pump users (n=65, mean age 10.7±3.7 years, HbA1c 7.28 ± 0.9%) and MDI users (n=65, mean age 10.7±3.4 years, HbA1c 7.35 ± 1%; all p>0.05 *vs.* CSII), (tab. I).

HbA1c at baseline was also similar between the two groups in adolescents (7.23 ± 0.9 CSII *vs.* 7.32 ± 1 MDI).

The mean follow-up was similar in the CSII (174 ± 35 days) and the MDI group (169 ± 38 days, p=0.44). HbA1c at the follow-up visit was similar to baseline in the CSII group. However, the MDI group experienced a statistically significant rise in HbA1c at the follow-up visit (7.58 ± 1%, p= 0.01 *vs.* baseline) (tab I). The supplementary sensitivity analysis using all available follow-up visits occurring 180 ± 90 days after the baseline did not change these results.

Adolescent restricted analysis showed the same pattern: no significant changes from baseline in the CSII group (7.23 ± 0.9% *vs.* 7.4 ± 0.8 %, p=0.93), but a significant rise in the MDI users (7.32 ±1.0% *vs.* 7.63 ± 1.1%, p=0.01).

Secondary outcomes. The total daily insulin dose at follow-up increased significantly in both CSII (+4.5U, 95%CI 1.7-7.3, p=0.02) and MDI group (+3.5U, 95%CI 1.4-5.5, p<0.001), see Tab I. During the follow-up, the prandial to basal ratio remained relatively unchanged (p>0.05). MDI group had a significantly higher prandial to basal ratio as compared with pump users at baseline (p<0.01), but not at the follow-up visit (p=0.173). During the follow-up, the CSII group showed a significant increase in insulin per body weight (p=0.02), accompanied by significant increases in BMI (p=0.006), weight (+2.2Kg, 95%CI 1.5-2.8, p<0.001), and height (+2.4 cm, 95%CI 1.9-2.9, p<0.001). The MDI group experienced an increase in insulin per body weight (p=0.041), weight (+2.1 Kg, 95%CI 1.5-2.7, p<0.001) and height (+3.2 cm, 95%CI 2.4-4, p<0.001), but not BMI (p=0.131).

There was a significant rise in the number of sick days from baseline (1.2, 95%CI 0.2-2.4) to follow-up visit (7.1, 95%CI 3.7-11.3, p=0.005). Data could not be further detailed by the treatment group.

TABLE I.

Baseline and follow-up characteristics of children with type 1 diabetes

	Insulin pump		Multiple daily injections	
	Baseline	**Follow-up**	**Baseline**	**Follow-up**
Primary outcome				
N	65	65	65	65
Age (years)	10.7 ± 3.7	-	10.7 ± 3.4	-
Diabetes duration (years)	5.7 ± 2.8	-	3.3 ± 2.3	-
HbA1c (%)	7.28 ± 0.9	7.38 ± 0.8^{NS}	7.35 ± 1	7.58 ± 1*
Secondary outcomes				
N	53	53	58	58
Age (years)	10.8 ± 3.8	-	10.6 ± 3.5	-
Diabetes duration (years)	5.6 ± 2.8	-	3.3 ± 2.2	-
Total insulin (U)	34.6 ± 19.9	39.2 ± 22.5*	32.7 ± 17	36.2 ± 17.9**
P: B ratio	1.23 ± 0.62	1.39 ± 0.96^{NS}	1.65 ± 0.68	1.61 ± 0.65^{NS}
IBW (U/Kg)	0.77 ± 0.24	0.83 ± 0.23*	0.8 ± 0.19	0.84 ± 0.2*
BMI (Kg/m^2)	19.1 ± 3.5	19.5 ± 3.8**	18.6 ± 3.8	18.8 ± 3.9^{NS}

^{NS} not significant; * p<0.05; ** p<0.01 *vs.* baseline; P: B ratio - prandial to basal insulin ratio; IBW - insulin per body weight; BMI - body mass index

DISCUSSION

This observational study investigated the hypothesis that CSII (n=65), MDI (n=65), or both could change the natural rise of HbA1c from warm to cold seasons in children with T1D and uncovered three major findings. First, children on MDI treatment experienced the expected rise in HbA1c during follow-up. Second, the CSII group did not show a significant rise in HbA1c despite similar follow-up conditions. And third, BMI showed different dynamics in CSII compared with the MDI group.

Seasonal variation in HbA1c is an important concept, especially in older children with T1D from world regions with a high temperature contrast between winter and summer (5, 11, 12). Primary contributors to higher HbA1c in cold seasons might be increased susceptibility to viral infections, winter celebrations related to excessive eating, school stress, and decreased physical activity (3, 11, 13, 14). Parallel variation in blood pressure, lipid profile and insulin sensitivity might also contribute to some seasonal variations in HbA1c (12, 15, 16).

Moreover, some children have an acute recovery of insulin secretion a few days after insulin initiation following T1D onset. Insulin requirements may drop significantly, and sometimes the patients can live insulin-free for a limited amount of time. This partial or total remission may last for a couple of weeks or months but rarely exceeds one year in children. HbA1c levels and variability are exceptionally low during this period but sharply rise following its end. The current study enforced at least one year of diabetes duration at baseline, but most children had significantly more than that. The total number of sick days over the previous three months was used to proxy

for viral infection incidence. Although results could not be detailed by treatment group, this important parameter did rise significantly in the limited sample of patients investigated. No data was available for physical activity, but the authors' observation is that summer is associated with increased leisure-related physical activity compared with late autumn and winter.

Cold stress-related rise in mean glucose levels, asserted by HbA1c, might also be explained by increased glucagon, epinephrine, cortisol, growth hormone, and thyroid hormones plasma concentrations as a natural adaptation to the body's demand for heat production (17-19). Besides increased levels, resistance to glucocorticoid action was described as an adaptation to a cold environment (19). Increased counter-regulatory hormone concentrations translate into a parallel increase in insulin resistance during the cold seasons and, ultimately, in HbA1c variability (19).

The results from a retrospective observational study by Tanaka *et al*. indicate that insulin pump treatment may significantly reduce seasonal variation in HbA1c levels. The researchers examined the seasonal variation of HbA1c levels and how the COVID-19 pandemic and related restrictions affected this variation in a cohort of 52 adults with T1D. The study found that individuals using CSII and sensor-augmented pumps experienced the least seasonal variation in HbA1c levels. In contrast, greater variability was observed in groups using MDI without CGMS or with intermittently scanned continuous glucose monitoring. Notably, variability in HbA1c levels was present in 2019 but diminished in 2021 during the COVID-19 restrictions (20).

In an observational study on 454 T1D children, 231 on CSII and 223 on MDI, Fendler *et al.* found a significantly lower HbA1c variability in the CSII group compared with the MDI group (21). Although similar to results from the current study, they used a different approach to assess HbA1c variability, i.e., the mean standard deviations of the mean HbA1c during the mean three years follow-up. Also, they declared that only acute hospitalizations were considered during the follow-up, disregarding the scheduled hospital admissions. Adding the age imbalance at baseline, with the MDI group being around two years older than children on CSII, results might express the expected higher HbA1c variability in older T1D children when admitted to a hospital for an acute presentation. The current study used propensity score matching to control for age and other possible imbalances between groups. Results can also be interpreted for situations outside acute presentations in the hospital.

As strength points, this study used propensity score matching to control for important confounding variables. Besides enforced similar age, HbA1c, BMI and calendar month at baseline, the protocol excluded patients with no access to CGM, recognizing the importance of this confounding variable on HbA1c. The influence of partial remission phase was minimized by enforcing at least one-year duration of diabetes at baseline. HbA1c variability due to MDI to CSII switch was accounted for by including only patients with at least 180 days since insulin pump initiation.

As weak points, this study was performed in a single center, with a limited selection base (N=504). The number of cases retained in the final groups was also limited (n=65 each). Moreover, it did not

evaluate gender impact on HbA1c dynamic in studied groups. Although many girls (especially adolescents) seem to have slightly higher HbA1c levels than boys starting right from T1D onset, no gender differences were previously reported in studies investigating HbA1c variability (3, 11, 22). Information on physical activity and incidence of viral infections was not available. These parameters are part of the possible explanations of HbA1c variability. The number of sick days in a limited sample of patients from both investigated groups only offered limited compensation for this shortage. Although there is no obvious indicator that physical activity and viral infections differed substantially between the CSII and MDI groups, no direct systematic measurements were available.

CONCLUSIONS

In conclusion, children receiving MDI experienced the anticipated seasonal HbA1c increase, while CSII users exhibited no significant changes. These findings emphasize the potential buffering effect of CSII on seasonal HbA1c drift, even when follow-up conditions and baseline characteristics are similar. Pediatric endocrinologists and researchers should recognize the seasonal variability in HbA1c and the potential impact of CSII on modifying this phenomenon when used alongside CGM.

CONFLICT OF INTEREST AND FUNDING

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CHALLENGES IN MANAGING DIVERSE GENDER ROLES AMONG ROMANIAN MEDICAL PROFESSIONALS

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CHALLENGES IN MANAGING DIVERSE GENDER ROLES AMONG ROMANIAN MEDICAL PROFESSIONALS (Abstract): This original research examines the perceptions and attitudes of Romanian medical professionals towards gender nonconformity, focusing on stereotypes and their implications for healthcare delivery. The **aim** is to identify cultural and psychological barriers that influence healthcare delivery to gender diverse individuals and to propose educational strategies for improving inclusivity and non-discrimination. **Materials and methods:** Through a qualitative, phenomenological approach, the research explores stereotypes and conformity to assigned gender roles among a sample of medical students that were evaluated through interviews focusing on personal and professional views shaped by cultural, familial and societal influences. The study combined systematic literature reviews from international databases with a transversal study involving 50 medical students aged 20-52 years. Participants' views on traditional axioms and other popular gender stereotypes were critically evaluated to identify variations across age, gender, marital status and cultural background. **Results:** Results showed that gender role conformity is prevalent, particularly among married and religious participants, underscoring the influence of traditional patriarchal norms on societal integration. The findings emphasize the need for specialized education to foster acceptance and tolerance toward gender diversity. Addressing cultural stereotypes and providing adequate training are critical for developing ethical medical practices that ensure equitable care for all patients. The findings highlight the urgent need for specialized education to dismantle stereotypes, improve professional competence and foster ethical medical practices. **Conclusions:** This research concludes that recognizing and addressing gender role biases is pivotal in reducing stigma and ensuring equitable care for nonconforming individuals within the Romanian healthcare system. **Keywords**: HEALTHCARE ETHICS, MEDICAL PROFESSIONALS, GENDER DIVERSITY, INCLUSIVE EDUCATION, GENDER STEREOTYPES.

INTRODUCTION

In Western modern societies, the visibility of gender incongruence is mostly reflected in the phenomena of nonconforming gender roles. Sometimes such persons face challenges in accessing medical care. This can be a problem of limited competence among medical professionals or of

stigma rooted in traditional intolerance. Therefore, medical proper education in gender care is important for medical professionals (1).

In Romania, gender healthcare is still in its infancy. We need education for social acceptance and integration. The issue of the relationship between those persons and healthcare providers is much debated in the international literature (2).

This research explores the stereotypical perception of gender-assigned traits among medical professionals, which can provide information about their attitude towards nonconforming gender patients who need care. We believe that our medical staff has limited knowledge about the subject. We wanted to see whether they understand and accept gender nonconformity, because this might be the first step to adjust their medical attitude towards nonconforming gender people. We tend to address the psychological barriers of medical stuff, because they need to ensure ethical and adequate medical care for all. Gender nonconforming roles remain unfamiliar and challenging concepts for many (1, 3, 4, 5). Worldwide contemporary evolutions seem to preserve and even amplify the phenomenon of gender nonconformity and there will probably be many people in need for general and specific medical care. Therefore, recognizing acceptance and respecting the diversity of gender experiences and expressions should be the trigger point to empathetic psycho-behavioral responses, supporting professional competence (6). We believed that the gender roles assumed by medical professionals inevitably shape their attitudes toward individuals with diverse gender identities.

Gender role is conditioned by "cultural and historical traditions, ethnic particularities, stereotypes and norms related to sexuality" (7). Nowadays, changes in gender role affirmation, fully recognized in many modern "progressive" societies, influence social, economic, and political life. But, nevertheless, there still are persistent stereotypes, which perpetuate inequalities and discrimination. Gender roles shape self-image and societal integration, varying across cultures and are influenced by stereotypes that simplify complex ideas and resist change (8, 9, 10).

As cultural constructs based on sexual differentiation, they are seen as "natural" or "appropriate" (10, 11). These stereotypes, perpetuated through education, media, and family traditions, reinforce distinctions between sexes (4). Historically, religion and cultural norms have cemented these differences, assigning women subordinate roles. Patriarchal views have been used to justify discriminatory practices in Romanian culture, too (12, 13). Rejection of nonconforming gender identities is strongly linked to religious fundamentalism, often cited as a root cause of homophobia and aggression (14).

Authors believe that traditional gender role stereotypes limit personal development. Joseph Pleck's "fragmentation" of gender roles highlighted their adaptability, reducing conflicts and enhancing psychological well-being (12). Researchers recommend that specialized gender diversity education should be introduced in the professional training of medical staff (1, 5).

Professional training and continuing medical education often lack explicit information on the specific needs of gender diverse individuals, resulting in limited understanding of specialized care (1, 15).

International studies highlight that nursing, a predominantly female role (5, 11, 16, 17, 18), has women significantly outnumbering men, as reported by the World Health Organization (19).

This stereotype has historically exclud-

ed men from nursing (4, 19, 20, 21, 22, 23). While professional value in healthcare is often influenced by gender, feminine traits are sometimes seen as less experienced, while masculinity is associated with leadership. However, Kirchmeyer's study shows that femininity can predict professional dedication, fostering innovation, patient focus, and effective teamwork, complementing leadership qualities linked to masculinity (18).

As international databases show, cultural norms that have historically tied caregiving roles to women are reflected in our study too (mostly women participants). A 2021 analysis (24) shows that women dominate healthcare executive roles but are less likely to achieve higher professional or academic ranks (25, 26). Men are often perceived as better leaders (27), while women in medical professions frequently experience higher rates of burnout (28). This is about women's efforts to balance professional responsibilities with traditional roles as mothers and homemakers (29). Women are approached differently than men in medical training, both by educators and patients, as well as by patients' families (30, 31).

Medical students are evaluated differently based on gender stereotypes, with male achievements often labelled as "leadership" while women's efforts are categorized as "team focus" and "caring ability (32)." Research on women in healthcare shows they prioritize collaboration, consensus, and work-life balance, often juggling family and professional roles. Men, conversely, emphasize career advancement and recognition (33, 34, 35).

As society increasingly embraces gender role diversity, it is essential for medical staff to recognize and address both stereotypical and nonconforming roles that individuals embody in their lives. Acknowledgment of the fact is important to psychological well-being and societal balance (23). Also, international literature highlights the importance of psychological traits and gender role identity assumption for career development. Gender role conformity, shaped by "social axioms" affects medical workers. These stereotypes of rigid traditional roles can lead to resistance and intolerance, especially as gender diversity becomes more visible, so educating medical staff to manage culturally influenced stereotypes is crucial for fostering acceptance and delivering inclusive care.

Gender roles evolve with changing societal norms, challenging the belief that men and women were inherently created for specific roles. Diversifying gender roles improves psycho-emotional balance, benefiting individuals, including those in the medical field (35).

Traditionally, women managed caregiving and households, while men provided financially. Although roles are now more flexible, gender inequality persists. In Romania, conformity to religious values shapes coping mechanisms and identity but often resists gender diversity. Addressing gender stereotypes among healthcare workers is key to improving care for nonconforming individuals and guiding trainees toward ethical, inclusive practices.

The hypothesis of the research was that the stereotypical views held by medical professionals about gender-assigned roles shape their expectations and attitudes towards nonconforming gender patients.

MATERIALS AND METHODS

First, we performed a review of international literature from PubMed and ScienceDirect databases using specific search terms like "gender role", "transgender healthcare" and "gender nonconformity." Systematic reviews published between

2020 and 2023 in English that were open access were included. To refine results, combinations of terms were used, followed by sorting abstracts and analysing full texts. A limitation identified was the scarcity of studies linking the proposed concepts, as most focus on individual topics.

We performed a qualitative transversal study which utilized a convenience sample of 50 third-year medical students. Voluntary participation ensured informed consent, while anonymity and confidentiality were maintained. The phenomenological approach explored stereotypes and social axioms on gender roles ("The man is the head of the family"/ "The man must ensure family safety"/ "The woman looks after the household and the children"/ "Women should obey their husband/partner"). Written responses and oral in-depth interviews allowed participants to express authentic perspectives. Open-ended questions addressed conformity to gender roles and correlations with daily life, covering professional, familial, and societal dimensions. The free text interview method was selected so that participants could provide responses in their own words, allowing for rich, detailed and authentic data.

Secondary analysis compared psychological traits identified in participants with those documented in established Romanian studies. Insights were gathered on how medical education and cultural exposure affect tolerance and receptiveness to gender diversity. The open-ended questions were formulated on the basis of the general stereotypes assumed by the respondents and aimed at assessing the presence of the qualitative phenomenon of conformity to the assigned gender role, as a starting point for accepting the diversity of nonconforming gender roles, which we are currently facing mostly in Western culture. The free expression of the conceptions was correlated with the evaluation of their concordance with concrete aspects of the practitioner`s life and work (declared and/or observed by the researcher): living environment, marital status, children, declared practice of traditional religious rituals, general level of medical education, involvement in the debate on the proposed gender role nonconformity topics, level of information about them, interest shown in the proposed topics, frequent reference to dogmas, refusal to debate the proposed topics, circumstantial answers, indicative level of knowledge of the general duties of their profession.

Data were analyzed using Colaizzi's method, identifying key themes, formulating meanings, and verifying results with participants for accuracy. This qualitative phenomenological interpretation emphasized lived experiences and perceptions, shedding light on the intersection of gender roles and healthcare practice. The design of the study was to utilize interpretative phenomenology, in order to explore the lived experiences and perceptions of medical students regarding gender nonconforming individuals. Ultimately, the findings highlighted cultural influences, conceptual conformity to traditional gender roles, and the need for inclusive education to address diversity in healthcare.

RESULTS

The study group consisted of 50 medical students, predominantly women (45 females, 5 males) aged between 20-52 years old. Most participants were aged 20-29, many already engaged in healthcare roles or training, with complex life experiences. Seven women over 40 years of age stood out for their broader perspective on healthcare, often pursuing higher education for professional advancement. Out of the 50 students, 33 were employed in various medical fields, including ICU, surgery,

ambulance services and oncology, while 15 were high school graduates in basic training. The employed students demonstrated confidence and firm beliefs but had limited knowledge on gender nonconformity topics. Resistance to discussing gender roles was noted among older professionals. Male participants displayed moderate interest and restraint on these topics.

Most students (46 people) were urban-based. Marital status revealed 24 married women, 9 single, 7 divorced, and 4 in consensual relationships, with gender nonconforming traits observed but often unrecognized by respondents. Of the women, 28 had children, with 5 pregnant during the study.

Regarding confessional affiliation, all participants identified as Christian, with the majority being Orthodox. Qualitative analysis showed familiarity with patriarchal concepts like "the man is the head of the family", with 23 participants agreeing in principle, including 20 women and 3 men. Traditional views were juxtaposed with evolving roles, such as professional aspirations, partially challenging traditional gender norms. The study highlights that 75% of married female participants (15 out of 20) supported patriarchal views, despite having independent careers. Younger women (aged 20-39) showed a mix of traditional and modern influences, with cultural and religious upbringing shaping perceptions. Of 5 male participants, 3 upheld patriarchal views regardless of marital status. 8 women, aged 20-39, and 2 men categorically opposed patriarchal axioms, while 11 respondents showed ambivalence, acknowledging a need for family structure without assigning dominance to men. Agreement on stereotypes like "the man must support his family" was prevalent among 35 women and 3 men, mostly aged 20-39, during career-building years.

Stereotypes about women's roles in household and child care were evident in 29 women and 3 men, reinforcing traditional norms while reflecting partial change in role perceptions. Among 13 exceptions, 11 women aged 20-29 and 2 aged 30-49 deviated from stereotypical gender axioms, reflecting modern trends toward diversifying family roles. Notably, 5 young women rejected these axioms, while 3 women remained neutral.

DISCUSSION

Analysis identified agreement with two social axioms: "Women should obey their partner/husband", supported by 15 women (mostly aged 20-29) and 2 men, influenced by spiritual and cultural education. However, 30 women and 2 men disagreed with the obedience aspect while acknowledging respect for traditional family roles. Male participants displayed ambiguous interpretations, revealing discomfort with nonconforming gender roles in healthcare. Positive discrimination in favor of traditional male roles was accepted by 15 women, while 30 rejected such biases.

Further, two broader axioms were analyzed. For the statement: "A woman ought to get married" we noticed: 35 women and 4 men agreed, thus in accordance with the stated social axiomatic concept, as follows: 16 women in the 20-29 age group, 12 women in the 30-39 age group, and all women in the 40-50 and over age groups, respectively all men except one in the 20-29 age group, who did not express an opinion. So, 39 people out of the total of 50 in the researched group agreed with the stated social axiom, and out of the total of 45 women, 35 said "yes". Out of the 16 women agreeing with the axiom aged 20-29, 9 were married, 1 in consensual relationship, 2 divorced and 4 singles. Of the 12 women in the 30-39 age groups agreeing with the

axiom, 9 were married and 2 divorced, and in the 40-49, 50 and over age groups, 2 were married, 3 divorced. So, the marital status 'married', present in 21 respondents out of 35, is a predictor of gender role conformity in the study group. Disagreeing with the traditional axiom were 10 women, 9 of them aged 20-29 and 1 in the 30-39 age group, mostly single.

The interpretation of the axiomatic concept: 'It is better to stay in an unsuccessful marriage than not to marry at all', provided the following results: Agreeing were 21 women and no men, as follows: 10 women in the 20-29 age group, 8 in the 30-39 age group, and 3 in the 40-50 and over age groups, 16 married. All of them, either have children, or were pregnant at the time of the study. Disagreeing were 22 women, of whom 8 were married and 3 had children, and all 5 men. Two were undecided or avoided the answer. Approximately the same extent female students related to the traditional concept of marriage as a necessity regardless of the quality of the relationship, a deeply stereotypical cultural gender-role aspect. The status of "married" almost equally influenced the critical attitude towards the "unsuccessful" nature of marriage, which for women who disagree with the concept, represents a nonconforming feature of their gender role. All 5 men expressed disagreement with this social axiom, which is applicable not only to women but to men as well, a nonconforming aspect of the male gender role as well.

Furthermore, 21 women and 3 men who had children stated that they take care of their children's education and share household chores. These, doesn't confirm the discussed stereotype.

The strongest acceptance of cultural gender stereotypes occurred for "the man has to ensure family security" "the woman takes care of the household and the children" and "a woman ought to get married." These align with traditional societal norms, showing high conformity among medical students. Notably, 34 out of 50 participants agreed with "The man is the head of the family," further reflecting paternalistic conceptions.

The lowest acceptance rates were for "Women should obey their partner/husband" and "It is better to stay in an unsuccessful marriage than not to marry at all," representing modern, non-discriminatory perspectives. The contradiction between declared traditionalism and low acceptance of subservience highlights participants' mixed understanding and awareness of these stereotypes.

The in-depth interviews revealed correlations between gender role perceptions and participants` daily family and professional life. They included: married women balancing healthcare careers, household duties, childcare, and advanced studies, as well as single/divorced mothers, women in consensual relationships, and women pursuing medical specialization while working non-medical jobs. Nonconforming traits observed included explicit or partial disagreement with traditional axioms, career dedication reducing household responsibilities, single-parenting, female breadwinners, male nurses, men sharing household chores and childcare, and divorcees. These findings illustrate deviations from traditional gender roles.

CONCLUSIONS

Gender roles and their diversification are increasingly relevant in Romanian society but remain poorly understood among medical professionals, risking intolerance and discrimination. This study emphasized that adherence to traditional roles is mostly linked to divinatory practices, and the majority of married participants supported

patriarchal views despite pursuing education and careers. Younger participants displayed a mix of traditional and modern influences, shaped by cultural and religious factors and only a minority categorically opposed patriarchal beliefs. Many participants were unaware they practiced diverse gender roles and only recently began exploring this subject. Specialized education is critical to address gender role ideologies, prevent discrimination, and promote acceptance in professional and societal contexts, ensuring a respectful and adequate conduct in medical care.

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EXPLORING SUICIDAL MOTIVATION IN PATIENTS WITH DEPRESSION. A COMPREHENSIVE ANALYSIS OF THE PSYCHOLOGICAL, SOCIAL, AND BIOLOGICAL FACTORS INVOLVED

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EXPLORING SUICIDAL MOTIVATION IN PATIENTS WITH DEPRESSION. A COMPREHENSIVE ANALYSIS OF THE PSYCHOLOGICAL, SOCIAL, AND BIOLOGICAL FACTORS INVOLVED (Abstract): Depression is a prevalent and severe mental disorder affecting millions globally, significantly increasing the risk of suicide, which is the second leading cause of death among young individuals. **Aim:** This study examines the complex interplay between depression, anxiety, personality disorders, and suicidal behavior, highlighting the multifaceted nature of these relationships. **Materials and methods:** Utilizing a sample of 40 patients diagnosed with depressive episodes, both in isolation and as part of major depressive disorders or bipolar disorders, the research investigates various psychological, social, and biological factors contributing to suicidal ideation. The findings underscore that not all individuals with depression exhibit suicidal thoughts, yet specific risk factors including neurochemical imbalances, low self-esteem, and social isolation are prevalent. A primary focus of the research is the declared suicidal motivation of each patient in relation to the identified factors, offering insights into the motivational drivers behind suicidal ideation. Through qualitative analysis of individual cases, the research emphasizes the significance of familial and social dynamics, personality traits, and early-life traumas. **Results** indicate a critical need for integrated therapeutic approaches that address these complex interrelations to effectively reduce suicidal risk. Furthermore, the study advocates for enhanced access to psychotherapeutic services and the implementation of preventive measures tailored to the unique needs of individuals struggling with depression and associated disorders. Conclusions: the findings highlight the fundamental role of social, familial, and professional contexts in shaping suicidal motivation, alongside the significant impact of accentuated personality traits that precipitate suicidal ideation and behavior, emphasizing the need for comprehensive, individualized interventions that address both psychosocial and psychopathological dimensions. **Keywords**: DEPRESSION, SUICIDE, SUICIDAL IDEATION, ANXIETY, PERSONALITY DISORDERS, MENTAL HEALTH, PSYCHOPATHOLOGY, QUALITATIVE ANALYSIS, MOTIVATIONAL DRIVERS.

INTRODUCTION

Depression is one of the most common and severe mental disorders, affecting over 264 million people globally (1). Beyond impairing quality of life, it is a significant risk factor for suicide, the second leading

cause of death among young people. The relationship between depression and suicide is complex: while not all depressed individuals develop suicidal thoughts, various biological, psychological, social, and cultural risk factors increase vulnerability (2). Neurochemical imbalances involving serotonin and dopamine have been associated with depression, impulsivity, and suicidal behavior (3). Life experiences such as trauma, abuse, and social isolation, along with low self-esteem and negative self-perception, further intensify depressive symptoms (4-5). Social determinants, including socio-economic status, stigma, and lack of access to healthcare, exacerbate risk by limiting treatment-seeking (6).

Suicide accounts for about 700,000 deaths annually, and depression is one of its strongest predictors (7). Recent evidence shows rising suicide rates among adolescents and young adults (8), with particularly high risks in LGBTQ+ and marginalized groups (9). Nearly 60% of individuals who die by suicide suffer from mood disorders (10). Theoretical frameworks help explain these associations: cognitive theories emphasize distorted thinking and hopelessness (11); the Interpersonal Theory of Suicide highlights perceived burdensomeness and social disconnection (12); and the Diathesis-Stress Model underscores the interaction between genetic vulnerabilities and environmental stressors (13).

Comorbidity further complicates the picture. Anxiety disorders contribute to suicide risk by intensifying fear, stress reactivity, and difficulty coping with adverse events (14). Their frequent co-occurrence with depression amplifies vulnerability (15). Personality disorders, especially borderline and Cluster C types, increase risks through emotional instability, impulsivity, and problematic interpersonal relationships (16-17). Depression, anxiety, and personality disorders often interact cyclically, reinforcing each other and heightening suicidal behavior (18). This overlap necessitates integrated approaches that address multiple dimensions simultaneously.

Psychiatric patients are particularly vulnerable. Mental illness is associated with reduced life expectancy, often 15-20 years shorter than the general population, partly due to elevated suicide risk (19). Around 44% of suicide victims have a history of psychiatric hospitalization (20), with a 5% risk of self-harm during inpatient stays far higher than in the general population (21). Overall, affective disorders increase suicide risk 150-fold compared to individuals never hospitalized, while even outpatients face six to eight times higher risks than the general population (22-23). Thus, psychiatric care settings play a crucial role in prevention (24).

Demographic and socio-economic variables also influence suicide. In Western countries, higher risk is linked to male gender, advanced age, education, and employment status, with motivations including fears of financial loss, job insecurity, stigma, and illness progression (25). Stable predictors of suicide during hospitalization include depressive symptoms, affective or schizophrenia spectrum disorders, and prior self-harm (1). Additional risk factors include family history of suicide, hopelessness, guilt, and prolonged antidepressant use (27). However, suicide risk remains difficult to quantify due to fluctuating symptoms and impulsivity. Current prevention relies on better communication between clinicians, patients, and caregivers, along with comprehensive thera-

peutic interventions that address biological, psychological, and social dimensions of mental health.

OBJECTIVES

The aim of this study is to explore the peculiarities of suicidal ideation among hospitalized patients and those treated in outpatient psychiatric services. A comprehensive overview was developed to examine the correlation between suicidal risk and psychological, social, and biological characteristics, including clinical and syndromic features. The analysis focused on relative risk, trends, and socio-demographic and clinical factors in patients with non-organic depression. Demographic data and suicidal risk were compared across groups to identify specific vulnerabilities.

The main objective was to highlight pathophysiological and clinical perspectives that may support the development of practical preventive tools, while also examining each patient's declared suicidal motivation within the broader bio-psycho-social context. Beyond statistics, this study aims to humanize suicide in depression by describing psychopathological situations united by the desire to end life and the absence of reasons to continue. While the literature extensively addresses risk factors and predispositions, motivational aspects remain understudied despite their central role in suicidal behavior.

MATERIALS AND METHODS

This research presents 40 cases of patients with depressive episodes, either isolated or within recurrent major depressive disorder or bipolar affective disorder, with or without psychotic symptoms, all monitored between January 2022 and January 2024. Patients were almost equally distributed by gender and evaluated through psychiatric interviews, anamnesis, and psychological assessments, including Hamilton scales for depression and anxiety and a scale for accentuated personality traits, routinely applied at the Socola Psychiatric Institute in Iași. Data collected included personal and family history, socio-demographic conditions, and intrafamilial relationships, with results recorded in observation sheets. Both hospitalized and outpatient cases were analyzed, focusing on psychopathological indicators, suicidal risk, and declared motivations. For consistency, only patients with severe depressive or bipolar episodes, with or without psychotic symptoms and accompanied by suicidal ideation or attempt, were included.

Case structuring included the following components:

a) History of illness - reasons for hospitalization or psychiatric referral.

b) Hereditary background - psychiatric disorders and aberrant behaviors in relatives, with special focus on suicidal attempts in close family members.

c) Family background - relationships with family of origin and current family, communication patterns, and family situation at the time of the study.

d) Educational and religious background - behaviors related to education, confession, and relation to the divine.

e) Personal pathological history - somatic or psychiatric comorbidities, chronic pain, therapeutic adherence, and prior psychotropic treatment.

f) Living and working conditions - socio-professional status and psychosocial events acting as potential triggers of depressive episodes.

g) Psychiatric examination - psycho-

pathological elements (cognitive, volitional, affective functions), addictive behaviors, and declared suicidal motivation.

h) Psychological examination - scores from Hamilton scales (depression, anxiety) and the PA questionnaire (accentuated personality traits).

i) Positive diagnosis - established according to ICD-10 (International Classification of Disorders 10th edition), including type of suicidal behavior and secondary psychiatric diagnoses.

j) Therapeutic conduct - summarized treatment principles, medication classes, and combinations.

Ethical considerations: Informed consent was included in the standard agreement for hospitalization and therapeutic intervention. All patient data were anonymized.

RESULTS

Within the study group (N=40), patients diagnosed with depression spectrum disorders without associated personality disorders represented 55% (n=22), while those with comorbid personality disorders accounted for 45% (n=18), as illustrated in first figure.

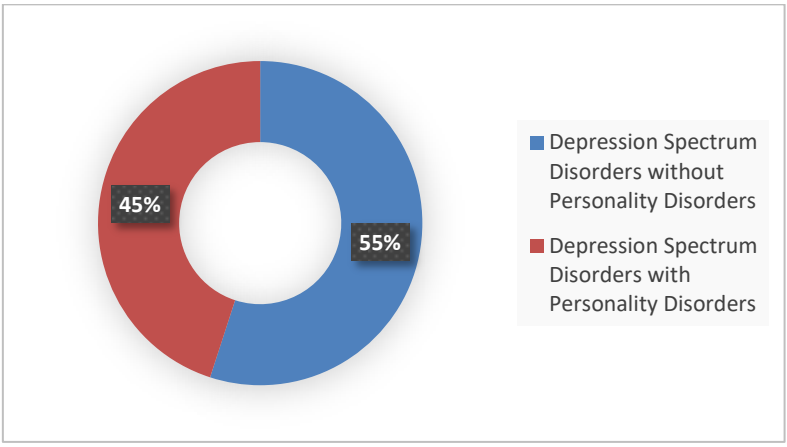

Fig. 1. Distribution of patients with depression spectrum disorders according to the presence of personality disorders.

In our cohort, patients with depression frequently presented with multiple personality disorders associated with an increased risk of suicide, as illustrated in second figure.

Distribution of personality disorders among patients with depression spectrum disorders. Borderline personality disorder was identified in 8 cases, avoidant personality disorder in 4 cases, and obsessive-compulsive personality disorder in 6 cases. These personality traits were variably associated with different forms of depressive episodes: borderline traits were most frequently observed in bipolar affective disorder with and without psychotic symptoms, avoidant traits appeared mainly in severe depressive episodes, while obsessive-compulsive traits were more frequent in major depressive disorder with psychotic symptoms. This distribution highlights the significant role of comorbid personality disorders in shaping suicidal risk in depressive spectrum disorders.

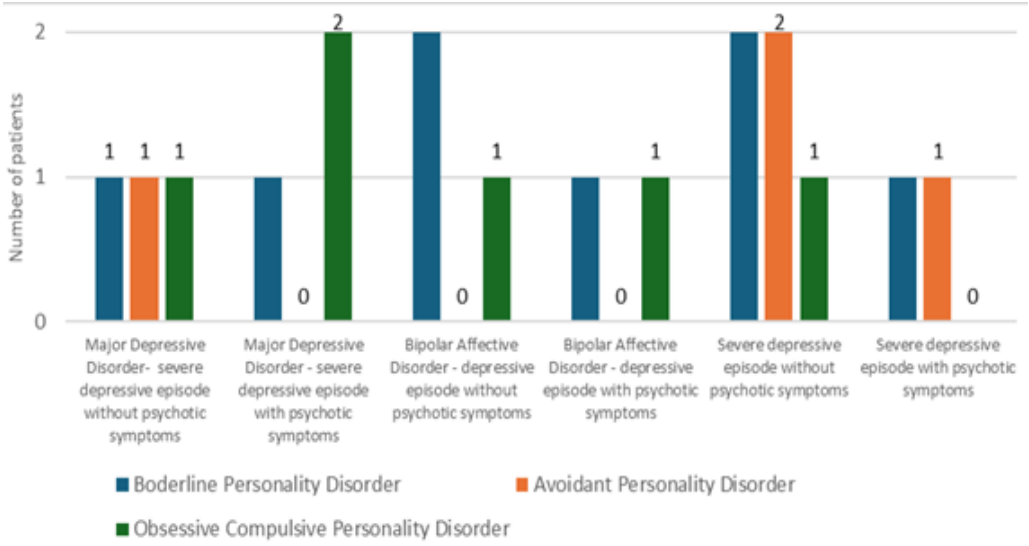

Fig. 2. Depression and Personality Disorders

The distribution of diagnostic categories within the study group (N=40) is shown in Figure 3. The largest subgroup consisted of patients with severe depressive episodes without psychotic symptoms (37.5%, n=15), followed by those with major depressive disorder, severe episode without psychotic symptoms (12.5%, n=5). Patients with major depressive disorder, severe episode with psychotic symptoms, and those with severe depressive episodes with psychotic symptoms each represented 7.5% (n=3). Bipolar disorder cases accounted for smaller proportions, with 5% (n=2) presenting with depressive episodes without psychotic symptoms and 5% (n=2) with depressive episodes with psychotic symptoms.

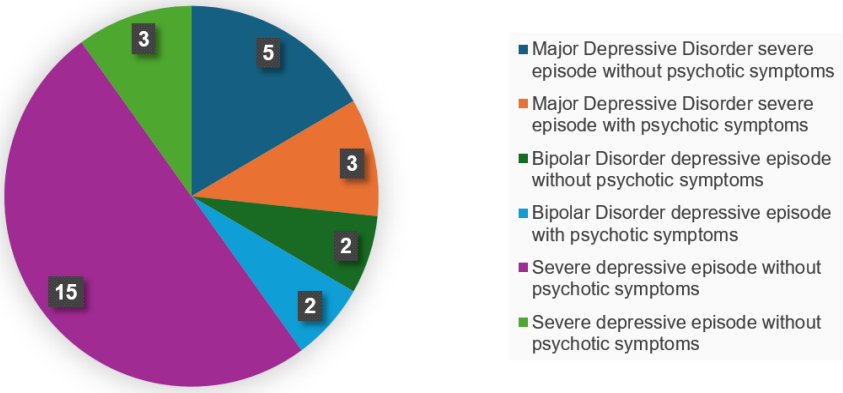

Fig. 3. Distribution of cases by diagnosis

Each case was assigned one primary suicidal motive: anxiety-intolerance (n=12), intense self-blame/guilt (n=14), socio-professional/family insertion problems related to childhood/adolescent trauma (n=18), and other mixed.

In the PD subgroup 10 out of 18 cases were coded with anxiety-intolerance *vs.* 2 out of 22 in the non-PD subgroup. Fisher exact test p = 0.006; Pearson $\chi^2(1) = 10.17$, $p \approx 0.0014$, indicating a statistically significant association. The estimated odds ratio (OR) for anxiety-intolerance in PD *vs.* non-PD = 12.5 (95% CI ≈ 2.23-70.3), showing substantially higher odds of anxiety-intolerance being the principal suicidal motive among patients with PD. the Pearson χ^2 gives consistent evidence of association when approximations are acceptable (fig. 4).

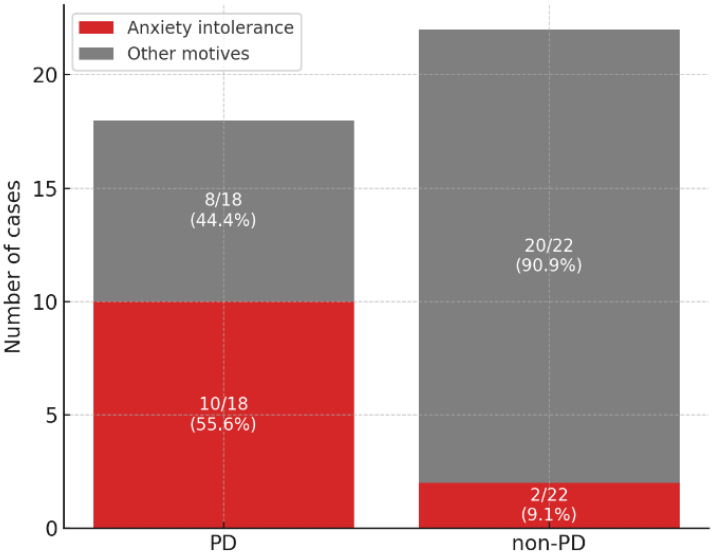

Fig. 4. Suicidal motives by PD status

Also, seven of the eight psychotic patients were coded as having intense self-blame as the primary motive versus 7 out of 32 among non-psychotic patients (psychotic: self-blame 7/other 1; non-psychotic: self-blame 7/other 25).

Fisher exact p < 0.001; Pearson $\chi^2(1) = 12.12$, $p \approx 0.0005$. OR (psychotic *vs.* non-psychotic) = 25.0 with 95% CI ≈ 2.62-237.7, indicating a very large point estimate but wide CI due to the small number of psychotic cases; the strong association is statistically robust but imprecisely estimated (fig. 5).

Socio-family motives were more frequent in the non-PD group: PD: socio-family 4/other 14 (18); non-PD: socio-family 14/other 8 (22). Fisher exact p = 0.002; Pearson χ^2 (1) = 6.86, $p \approx 0.0088$. OR for PD *vs.* non-PD ≈ 0.163 (non-PD *vs.* PD ≈ 6.13), 95% CI ≈ 0.040-0.671, indicating lower odds of socio-family motives in PD patients (or conversely higher odds in non-PD patients), with a CI that excludes unity but is moderately wide. Multiple comparisons: three primary hypothesis tests were adjusted (fig. 6).

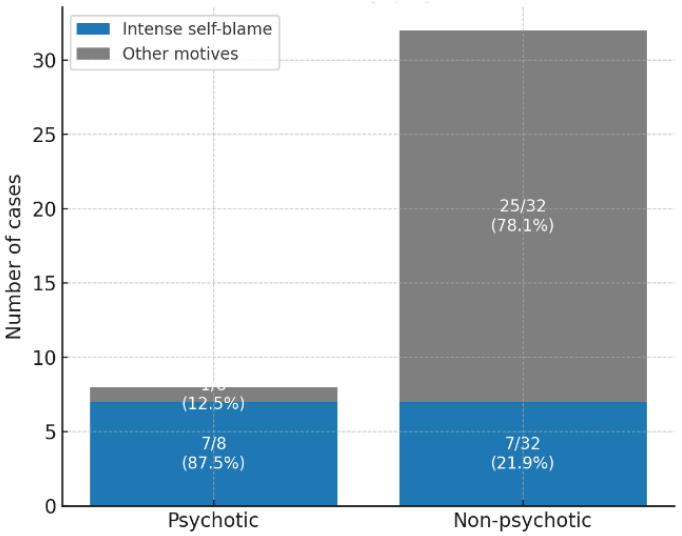

Fig. 5. Suicidal motives by psychotic status

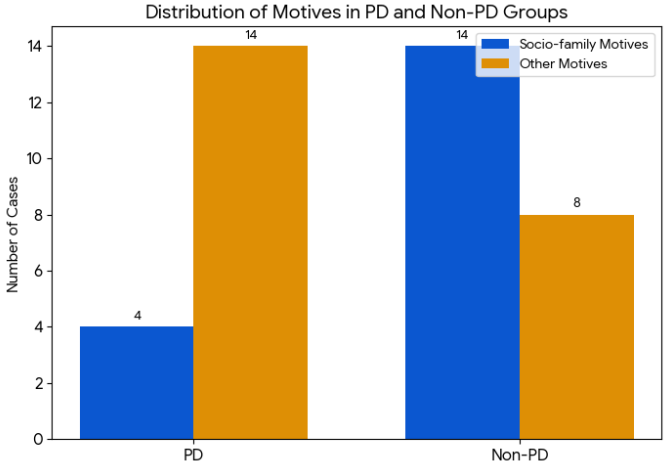

Fig. 6. Socio-family motives in PD and non-PD group

Bonferroni-corrected p-values (m = 3) are ≈ 0.0042 (anxiety-PD), 0.0015 (self-blame-psychotic), and 0.0264 (socio-family-PD) all remain below 0.05 after correction. Benjamin-Hochberg FDR ranks the p-values similarly (adjusted pBH ≈ 0.0015, 0.0021, 0.0088 respectively), so, the findings survive standard FDR control (fig. 7).

Sensitivity analyses (simulated reallocations) illustrate stability limits: moving two anxiety cases from PD to non-PD (PD 8/18 *vs.* non-PD 4/22) increases Fisher p ≈ 0.03 (association weakens and approaches the 0.05 threshold); reducing self-blame in the psychotic group by one case (6/8) yields Fisher p ≈ 0.0015 (association remains strong); moving two socio-family cases from non-PD to PD (non-PD 12/22, PD

6/18) yields Fisher p ≈ 0.015 (association weakens but remains below 0.05). Thus, the largest effect estimates (self-blame with psychosis; anxiety-PD) are robust to small reallocations, whereas the socio-family *vs.* PD association is more sensitive to case reclassification and has wider relative variability (fig. 8).

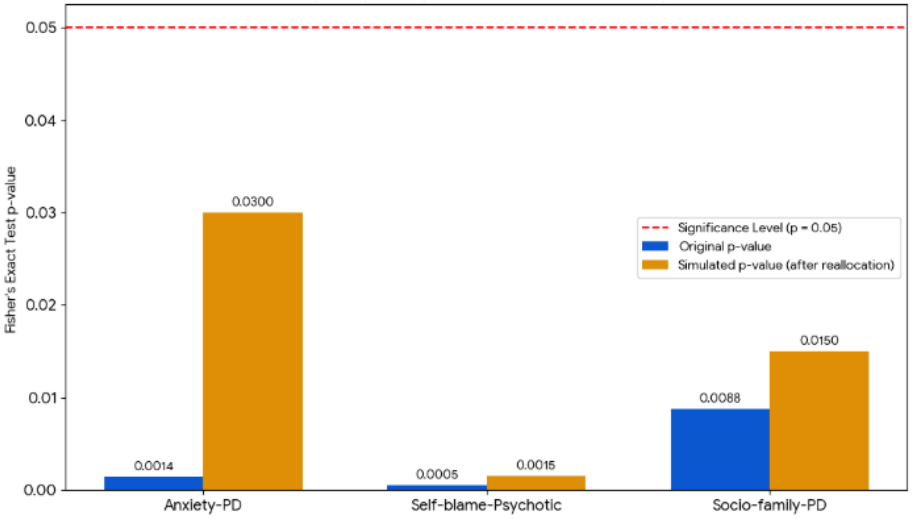

Fig. 8. Sensitivity analyses impact of cases on p-values

Data reported with contingency tables, Fisher and Pearson χ^2 tests, ORs and approximate 95% CIs, multiple comparison corrections and sensitivity checks-show strong, statistically significant associations of PD with anxiety-intolerance as the primary suicidal motive, of psychotic symptoms with intense self-blame, and a higher frequency of socio-professional / family motives in patients without PD; precision is limited for comparisons involving small cell counts (notably the psychotic subgroup).

DISCUSSION

Approximately 4-5% of patients hospitalized in psychiatric units present a suicide risk, which fluctuates over time due to multifactorial influences. The link between psychiatric disorders and suicidal behavior has been widely debated (1, 27), and therapeutic interviews can reveal the mosaic of ideations underlying such behavior. Suicide risk is heightened during clinical decompensation and shortly after hospital discharge, making predisposition to self-harm a frequent reason for hospitalization (2, 3, 27). Although the prevalence of in-hospital suicidal acts is low, some cases evade monitoring, highlighting the protective yet limited role of the hospital environment in restoring psychosocial-emotional balance.

Diagnoses were established according to ICD-10, with descriptive extensions capturing ideation, rumination, self-harming attempts, and clinically significant anxiety. The Hamilton scales for depression (21-items version) and anxiety, alongside the PA questionnaire (Accentuated Personality Traits Questionnaire), assessed accentuated personality traits, including emotional instability, impulsivity, hyperthymia, cyclothymia, dysthymia, anxiety,

and demonstrativeness, to explore correlations with depression and suicidal behavior (2, 4, 27).

Organic pathologies and certain psychiatric comorbidities (Axis I disorder except anxiety spectrum, cognitive deficits, dementia, or active addictive syndromes) were excluded, while cases with mild personality disorders or accentuated traits were included (5-6).

Cases were presented descriptively to explore familial, social, and psychological dimensions, aiming to identify correlations between multiple life domains and suicidal motivation (15, 16). Therapeutic schemes effective in the short- and medium-term, including guideline-approved and off-label medications, were documented (7). Antidepressants were often combined with antipsychotics such as Olanzapine or Quetiapine, providing both symptom control and mood stabilization for depressive or bipolar episodes (12). Medication doses were tailored to each patient's clinical context and semiological characteristics, with minimal effective doses prioritized.

Suicidal ideation emerged variably, influenced by social, familial, or professional integration, early-life traumas, psych genealogical patterns, or psychotic symptoms, but consistently tied to a diminished or absent motivation to live. In some cases, religious values acted as protective factors, while psychotic depression involved delusional or hallucinatory motivations for self-harm. Accentuated personality traits, impulsivity, and emotionality, often combined with relational problems or family history of aggression or suicide, contributed to vulnerability (8, 9). Low or fluctuating self-esteem was a constant factor throughout patients' lives (fig. 9).

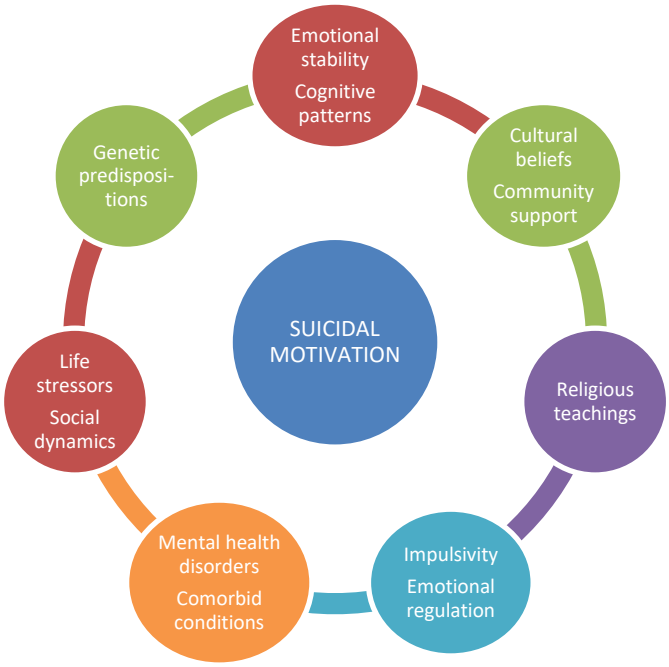

Fig. 9. Factors that Influence Suicidal Motivation

Qualitative analysis indicated that suicidal behavior results from the interaction of socio-familial, educational, psychological, and biological factors, raising questions about neuroplasticity and neuromodulation (10-11). Pharmacological treatment focused on alleviating depressive symptoms and eliminating suicidal ideation, usually combining antidepressants with antipsychotics for both therapeutic and preventive effects (12-14). A significant limitation in suicide management in the country is limited access to psychotherapeutic services, which can provide deeper insights into the individual motivational drivers of suicidal behavior (13).

CONCLUSIONS

In conclusion, the findings highlight the fundamental role of social, familial, and professional contexts in shaping suicidal motivation, alongside the significant impact of accentuated personality traits that precipitate suicidal ideation and behavior. Across individual cases, patterns of psychotic symptomatology further increase suicidal risk, sometimes serving as the primary driver for self-harming actions or persistent planning of such acts. Importantly, whether occurring in isolated depressive episodes or within major depressive or bipolar disorders, suicidal ideation consistently reflects an absence of motivation to live, rooted in early developmental stages. Once the motivation to live diminishes, the drive to suppress one's existence emerges, emphasizing the need for comprehensive, individualized interventions that address both psychosocial and psychopathological dimensions.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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ANALYSIS OF RISK FACTORS FOR NEUROLOGICAL COMPLICATIONS OF SARS-COV-2 INFECTION: FOCUS ON CHRONIC FATIGABILITY

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ANALYSIS OF RISK FACTORS FOR NEUROLOGICAL COMPLICATIONS OF SARS-COV-2 INFECTION: FOCUS ON CHRONIC FATIGABILITY (Abstract): **Aim:** Chronic fatigue syndrome is a long-term neurological manifestation that significantly affects the quality of life of patients. Its etiology is incompletely known, although it can occur after various infections including COVID-19, and the associated risk factors are not fully defined. The aim of the current study was to evaluate neurological manifestations in long-COVID and to identify potential risk factors for these complications. **Materials and methods:** We performed retro - prospective study on patients with history of COVID-19 infections. Patients were assessed up to 3 years after infection. Clinical and neurological investigations, brain imaging, analysis of inflammatory markers, levels of vitamin B_{12}, folic acid and vitamin D were carried out. Neurocognitive status was assessed using a validated semiquantitative scale. **Results:** In 132 eligible patients, 41.67% of cases developed chronic fatigability. Other neurological manifestations associated with Long-COVID were dizziness (29.17% cases), and memory impairment (25% cases). The deterioration of the neurocognitive status 1 year after infection was significantly correlated with the patient's age ($p<0,0001$), serum vitamin D ($p=0.015$), as well as vitamin B12 ($p=0.006$). **Conclusions:** Chronic fatigability is a common neurological complication of COVID-19 infection. Older people, those with low vitamin D and vitamin B12 levels, have higher risk of developing this syndrome. The COVID-19 infected patients need multiple investigations and a multidisciplinary approach to prevent these long-term complications. **Keywords:** RISK FACTORS, NEUROLOGICAL COMPLICATIONS, SARS-COV-2, INFECTION, FATIGABILITY.

INTRODUCTION

More than three years after the COVID-19 pandemic began, accumulating evidence highlights how SARS-CoV-2 infection has impacted patients' quality of life. Initially, it was considered that the new coronavirus had only respiratory tropism; however, later real - world data showed that it produces a wide range of symptoms, including neurological ones. Moreover, the persistent complications following SARS-Cov-2 infection are extremely varied. For this reason, a series of clinical manifestations have been correlated with the infection and classified as the Long–COVID complication.

In fact, several forms of post-COVID-

19 complications have been identified and defined so far and included in this umbrella term of Post COVID-19 conditions (1). Center for Disease Control and Prevention (CDC) defines this complication as Long COVID that "include a wide range of ongoing symptoms and conditions that can last weeks, months, or even years". According to the CDC, the symptoms of Long COVID should be improved within 3 – 6 months (2).

The World Health Organization (WHO) later provided a definition for Post COVID-19 condition, describing it as "the continuation or development of new symptoms three months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least two months and with no other explanation" (3).

Clinical sequelae of COVID-19 include respiratory (lung fibrosis), cardiovascular (thrombosis, myocarditis, arrhythmias), metabolic (diabetes), dermatologic (alopecia), renal, and neurological complications (4). Neurological manifestations of Long COVID include fatigue, "brain fog," sleep disturbances, mood disorders, cognitive dysfunction, anxiety, depression, and stroke (5). Their prevalence is uncertain but may reach 50% worldwide. Reported risk factors described in other studies include female sex, comorbidities, lack of COVID vaccination, obesity (increased) BMI, with women more frequently experiencing headache, stress, concentration difficulties, and fatigue (6).

In this context, we conducted a retro-prospective study to evaluate neurological manifestations of Long COVID and potential risk factors.

MATERIALS AND METHODS

We performed a retro-prospective study which included patients with history of SARS-CoV-2 infections. Patients were hospitalized and evaluated in Sf. Parascheva Clinical Hospital of Infectious Diseases Iasi and Equilibrium Clinic between 2021-2024.

The study was approved by the Ethics Committee of the hospital, the Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy Iasi. The data was collected in agreement to ensure data protection. The informed consent was obtained from the patients or their legal representatives before the inclusion in the study.

Eligible patients met the criteria: written informed consent obtained from the patient or their legal representative, patients aged over 18 years with history of SARS-CoV2 infection, patients evaluated up to 3 years after COVID-19 infection. Patients with history of chronic fatigue syndrome before COVID-19 infection or with incomplete data were excluded.

Data collection. Data collected from medical records included demographics (age, sex), medical and medication history, SARS-CoV-2 infection details (severity, reinfections, treatment, outcome), laboratory findings (complete blood count, biochemistry, inflammatory markers, serum vitamin D, folic acid, vitamin B_{12}), neurological evaluation, and cerebral CT. Neurological status was assessed with the extended SCGI scale, covering cognition, affective state, sleep, fatigue, headache, motor and sensory function, balance and coordination, functional autonomy, and quality of life. Each domain was scored 0–3, with total scores categorized as mild (0–10), moderate (11–20), or severe (21–30). This is an interim analysis of data obtained one year after infection. Therefore, only

data up to this time point have been collected and analyzed.

Statistical analysis. Normally distributed data are presented as means ± standard deviations, and nonnormally distributed data are reported as medians and ranges). The chi-squared test was performed for the single-factor analysis of count data, and the Kruskal-Wallis was performed for the single-factor analysis of non-normally distributed data. Pearson correlation coefficients were used for correlation between different variables. A p-value ≤0.05 was considered statistically significant. Statistical analysis was performed using XLSTAT software version 1.3, 2025 (Lumivero, USA).

RESULTS

Of a total of 183 patients, 132 eligible cases were included in our analysis. Of these, 41.67% cases were males. The mean (St. dev.) age was 49.43 (±18.98) years in females and 57 (±15.51) years in males (fig. 1).

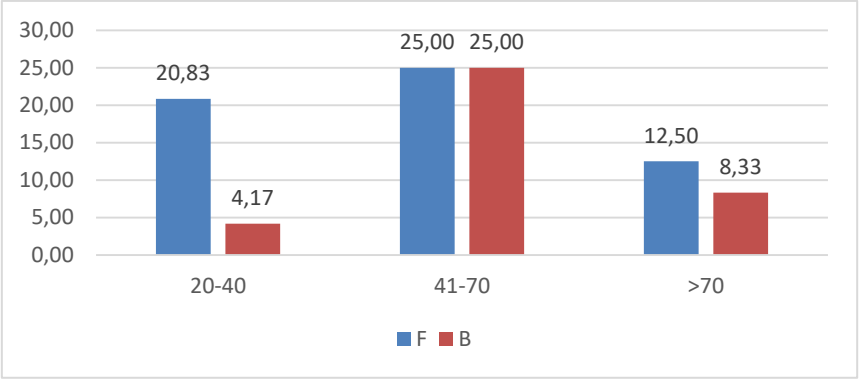

Fig. 1. Demographic data - gender repartition by age group (%)

Almost 67% of cases described associated disorders, most of them with negative prognostic impact associated with COVID-19 infection (hypertension, metabolic disorders, hematological diseases).

The neurological manifestations recorded up to 1 years after SARS-CoV-2 infection in the patients surveyed were varied: paresthesia, nonspecific myalgias or fibromyalgia, headache or migraine, various neuralgias (trigeminal, facial), confusional syndromes or new or aggravated cognitive disorders in patients with previous cognitive impairment (with MMSE score 18-20). However, the most frequent neurological disorder was chronic fatigue syndrome reported in all age groups (41.67% of cases) (fig. 2).

The 55 patients with chronic fatigue syndrome were subsequently analyzed to establish correlations between demographic and biological parameters with the degree of neurocognitive impairment (SCGI Scale) (tab. I, fig. 3).

The data obtained revealed a significant correlation between the severity of cognitive impairment (SCGI score between 27-30) and the patient's age ($p<0,0001$). A weaker, but clinically significant correlation was also obtained for serum vitamin D values ($p=0.015$), as well as for vitamin B_{12} ($p=0.006$). Patients' gender was not correlated with neurological manifestations in our analysis ($p=0.589$).

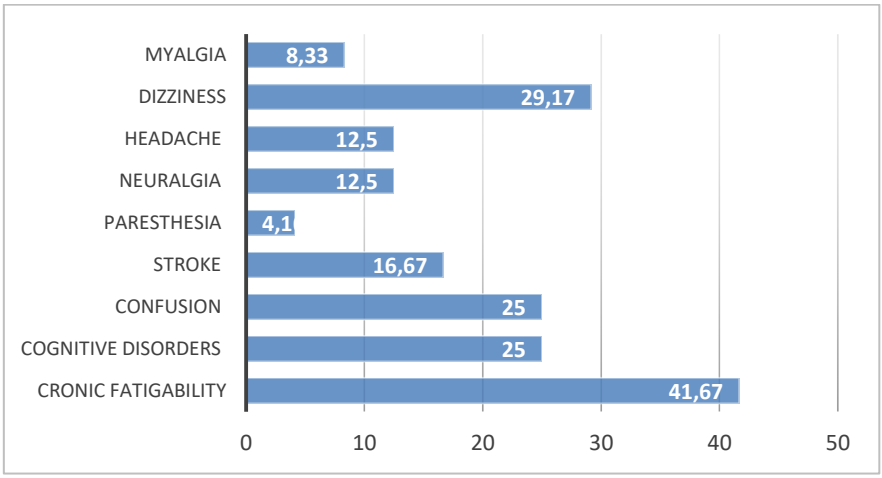

Fig. 2. Neurological manifestations associated with Long - COVID (% cases)

TABLE I.

Correlation between various parameters and SCGI score (Pearson test)

Variable	Pearson's r	p - value	CI95%
Age	-0,683	<0,0001	[-0,803; -0,510]
Gender	-0,182	0,589	[-0,195; 0,333]
IL-6	0,074	0,467	[-0,170; 0,356]
LDH	0,100	0,502	[-0,349; 0,177]
D-dimer	0,019	0,891	[-0,248; 0,283]
Ferritin	0,027	0,844	[-0,240; 0,290]
Vit D level	0,327	0,015	[0,068; 0,545]
Vitamin B12	-0,369	0,006	[-0,578; -0,115]
Folic acid	-0,156	0,254	[-0,405; 0,114]

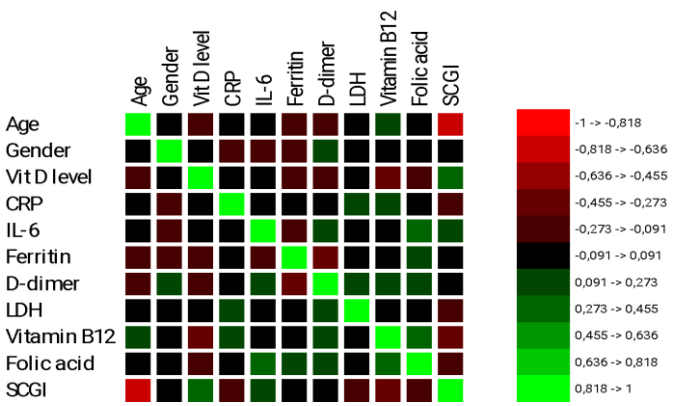

Fig. 3. Correlation map (Pearson test) between SCGI and various parameters

DISCUSSION

The SARS-CoV-2 virus is responsible for a multisystem infection, and neurological manifestations are common in the acute phase of infection. Multiple clinical studies, as well as real-world clinical experience, have shown that symptoms such as headaches, anosmia, ageusia, paresthesia, and myalgias are significant in COVID-19. A retrospective analysis of patients admitted to the "Sf. Parascheva" Infectious Diseases Hospital in Iasi between 2020 and 2022 showed that the most common symptoms were fatigue (85% cases), headache (51.25% cases), myalgia (41.25% cases) (7). To date, various hypotheses have been proposed regarding the pathophysiological mechanisms of neurological disorders in COVID-19, including direct invasion of the central nervous system, disruption of the blood-brain barrier, or dysregulation of the gut–brain axis (5).

On the other hand, the SARS-CoV-2 virus can trigger a "cytokine storm," characterized by the excessive release of pro-inflammatory cytokines (TNF-alpha, IL-6, IL-1, etc.), with significant changes in the immune system that ultimately lead to a systemic hyperinflammation syndrome (8). This mechanism can also induce neuroinflammation, which is responsible for both acute symptoms and long-term neurological changes (5, 8, 17).

Analysis of a cohort of over 150,000 patients in the USA identified the following neurological complications of SARS-CoV-2 infection, classified as Long-COVID syndrome: cerebrovascular diseases, cognitive and memory disorders, peripheral nerve disorders, migraine, extrapyramidal and motor disorders psychiatric disorders, and other complications (dizziness, drowsiness, Guillain–Barré syndrome, encephalitis, transverse myelitis, encephalopathy) (9-11). Our data are in line with extensive studies published by other authors. The most common neurological manifestations were chronic fatigue syndrome (over 40% of cases), followed by cognitive deficit with new onset or worsening of a pre-existing cognitive deficit.

Chronic fatigue syndrome defined as Myalgic Encephalomyelitis is a complex condition, characterized by significant asthenia, reduced exercise capacity, cognitive disorders (including "brain fog"), sleep disorders, myalgia or other forms of pain, vegetative syndrome (12). The etiology is not completely known, but a prevalence of up to 11% has been identified in severe infections (Epstein Barr virus (EBV), Ross River virus, Giardia lamblia, parvovirus B19, and Q fever infections) (13).

In an extensive analysis of 41,802 women in the US, chronic fatigue syndrome was identified in only 102 women. Risk and predisposing factors for Myalgic Encephalomyelitis or severe fatigue were advanced age, higher BMI in adulthood, hormone therapy, alcohol intake (14). In our study, patients' age, as well as serum levels of vitamin D and vitamin B_{12}, were significantly correlated with the occurrence of chronic fatigue.

The protective role of vitamin D in infectious pathology has been intensively studied and is well defined. In addition to its direct involvement in calcium metabolism and the maintenance of bone health, vitamin D exerts numerous effects, including anti-inflammatory, immunomodulato-

ry, and even antibacterial or antiviral properties (15). Increasing amounts of data are available regarding the relationship between vitamin D and cognitive function. Vitamin D receptors have been identified in the nervous system (neurons and glial cells). Vitamin D has been found to impact numerous brain processes, including cell differentiation, neurotrophic factor production and release, neurotransmitter synthesis, intracellular calcium homeostasis, prevention of oxidative damage, neuronal structure and metabolism, and ultimately, cognitive functioning (16). Hypovitaminosis D has been associated with the onset of neurodegenerative diseases, schizophrenia (18). Clinical data support this benefit of vitamin D for the treatment of several neurocognitive disorders, including those associated with chronic fatigability.

The small sample size evaluated is the main limitation of the current analysis. However, this is an interim analysis of patients monitored up to three years and the final conclusions will be disseminated.

CONCLUSIONS

Chronic fatigability is a common neurological complication of COVID-19 infection. Older people, those with low vitamin D and vitamin B_{12} levels, have higher risk of developing this syndrome.

The COVID-19 infected patients need multiple investigations and a multidisciplinary approach to prevent these long-term complications.

CONFLICT OF INTEREST AND FUNDING

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BASELINE PREDICTORS OF ONE-YEAR DISEASE-RELATED MORBIDITY IN PATIENTS WITH NEWLY DIAGNOSED ULCERATIVE COLITIS

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BASELINE PREDICTORS OF ONE-YEAR DISEASE-RELATED MORBIDITY IN PATIENTS WITH NEWLY DIAGNOSED ULCERATIVE COLITIS (Abstract): Early identification of factors predicting disease course in ulcerative colitis (UC) is essential for guiding therapeutic decisions and improving patient outcomes. Recognizing patients at higher risk of unfavorable evolution allows closer monitoring and timely treatment escalation. Clinical severity, disease extent, biochemical abnormalities, and concomitant infections may all influence prognosis. Among these, *Clostridioides difficile* infection (CDI) is increasingly recognized in UC, although its independent impact on disease course remains uncertain. **Aim:** To assess baseline clinical and biochemical severity markers, as well as concomitant CDI at diagnosis, as predictors of disease-related morbidity during the first year following UC diagnosis. **Materials and methods:** We conducted a retrospective study including 125 patients newly diagnosed with UC and followed for at least 12 months. Collected data included demographic characteristics, clinical presentation, biochemical markers, endoscopic findings, and the presence of CDI at diagnosis. Disease-related morbidity was defined as any UC-related hospitalization occurring within the first year after diagnosis, excluding the initial diagnostic admission. Statistical analysis comprised bivariate testing and multivariate logistic regression to identify independent predictors of hospitalization. **Results:** Independent predictors of UC-related hospitalization were Mayo clinical score (OR=1.28; 95% CI 1.00-1.64; p=0.043), pancolitis (OR=3.45; 95% CI 1.35-8.79; p=0.009), and hypoalbuminemia (OR=3.73; 95% CI 1.09-12.71; p=0.035). CDI was present in 20% of patients but did not independently predict hospitalization (OR=1.84; p=0.265). **Conclusions:** Baseline clinical activity, disease extent, and hypoalbuminemia are independent predictors of early UC-related morbidity. These findings underscore the importance of comprehensive initial assessment to support early risk stratification and individualized management strategies. **Keywords:** ULCERATIVE COLITIS, DISEASE-RELATED MORBIDITY, HOSPITALIZATION, BASELINE PREDICTORS.

INTRODUCTION

Ulcerative colitis (UC) is a chronic condition characterized by mucosal inflammation, characterized by a relapsing - remitting course. It typically begins in the rectum and extends proximally throughout the colon in a continuous manner.

During the past decades, the incidence

of inflammatory bowel disease (IBD) has increased, especially in developing countries. Immune system dysfunction and intestinal microbiome imbalance, along with the influence of environmental factors on genetically predisposed individuals, are the main mechanisms involved in the development of ulcerative colitis. Currently, the treatment of ulcerative colitis relies on corticosteroids for acute flares, amino salicylates, conventional immunosuppressants, and targeted biologic or small-molecule agents (1).

Therapeutic goals in IBD have recently evolved into a structured framework of short-term, intermediate and long-term targets, as outlined in the STRIDE-II consensus (2).

Short-term targets include symptomatic relief and normalization of C-reactive protein (CRP) as a general inflammatory marker, intermediate targets focus on decrease of fecal calprotectin, while long-term goals aim for achieving endoscopic healing, improved quality of life, and prevention of disease-related complications, disability and surgery. While not formally defined as treatment target, transmural healing in Crohn's disease and histological healing in UC are recognized as valuable therapeutic milestones (2). The lack of histological inflammation is linked to decreased relapse rates, as well as fewer hospital admissions, surgical interventions, and lower risk of neoplastic complications (3).

Accordingly, current strategies emphasize individualized care and close monitoring to guide therapy optimization. Furthermore, the early identification of factors that predict disease course in ulcerative colitis is essential to guide therapeutic decisions and improve patient outcomes. Timely recognition, ideally at diagnosis, of patients at higher risk of unfavorable evolution allows for more intensive monitoring and appropriate escalation of therapy within the "window of opportunity".

Certain baseline parameters, including clinical and laboratory characteristics, disease phenotype, laboratory findings, and histological grading have been associated with poor prognosis (4). Additionally, superimposed infections such as *Clostridioides difficile* may further complicate the clinical course.

The aim of our study was to identify baseline predictors of unfavorable disease progression, with a specific focus on early relapse during the first year after diagnosis.

MATERIALS AND METHODS

Study design and patients. We conducted a retrospective observational study including 125 consecutive patients newly diagnosed with UC who were followed for at least 12 months after diagnosis.

Patients were eligible for inclusion if they were newly diagnosed with UC based on clinical, endoscopic, and histologic criteria, and had at least 12 months of documented follow-up. Exclusion criteria were: previous total or subtotal colectomy, unconfirmed diagnosis, missing key data regarding *Clostridioides difficile* testing, endoscopic or histologic assessment, or loss to follow-up before completing the 12-months monitoring period.

The primary endpoint of our study was one-year disease-related morbidity, defined as any UC-related hospitalization occurring in the first year after diagnosis, including admissions due to disease flares or UC-related complications. Hospitalizations unrelated to UC were excluded from the analysis.

Data collection. Clinical, demographic, biochemical, endoscopic, and histologic data were collected from electronic medical records at the time of diagnosis. Disease severity and extent were assessed using the Mayo clinical score, Mayo endoscopic sub score, and the Montreal classification. All hospitalizations occurring within the first 12 months following baseline presentation were reviewed for each patient using the institutional electronic medical database.

Statistical analysis. Descriptive statistics were used to summarize baseline characteristics. Between-group comparisons were performed using the Mann-Whitney U test for continuous variables and the Chi-square test for categorical variables. Variables with a p-value <0.1 in univariate analysis were entered into a multivariate logistic regression model to identify independent predictors of UC-related hospitalization. Results were expressed as odds ratios (OR) with corresponding 95% confidence intervals (CI). A two-tailed p-value <0.05 was considered statistically significant. Analyses were performed using IBM *SPSS Statistics*, version 26 (IBM Corp., Armonk, NY, USA).

RESULTS

Patients' characteristics and one-year UC-related morbidity rate

A total of 125 patients with newly diagnosed UC were included in the study. The median age at diagnosis was 39 years (interquartile range [IQR] 26-49), and 54,4% were male. Extensive colitis (E3) was present in 36,5% of patients. The baseline demographic, clinical, and laboratory characteristics of patients are summarized in first table.

During the first year after diagnosis, 40 patients required UC-related hospitalization, predominantly for disease flares, corresponding to a 1-year morbidity rate of 32%.

Predictive factors for one-year UC-related morbidity

The correlations between baseline clinical, biological and endoscopic parameters and the risk of hospitalization were assessed by univariate and multivariate models. Results of the univariate analysis are presented in second table.

TABLE I.

Demographic, clinical and biological characteristics of patients

Parameter	Patients, n Total number=125
Age(years), median (IQR) Range, years	39 (26-49) 18-74
Gender ratio, n (%)	
Female Male	57 (45,6%) 68 (54,4%)
Hemoglobin value, g/dL, median (IQR)	12.9 (11.3-14.2)
White blood cells x10^3/μL, median (IQR)	8.08 (6.6-10.38)
C-reactive protein, mg/dL, median (IQR)	1.13 (0.38-3.86)
Serum albumin, g/dL, median (IQR)	4.12 (3.5-4.54)
Fecal calprotectin μg/g, median (IQR)	742 (323.5-1750)

Parameter	Patients, n Total number=125
Mayo score	
3-5 (mild), n (%) **6-10 moderate), n (%)** **11-12 (severe), n (%)**	37 (29.4%) 84 (66.7%) 4 (3.2%)
Mayo endoscopic sub score	
1 (mild UC), n (%) **2 (moderate UC), n (%)** **3 (severe UC), n (%)**	6 (4.8%) 70 (56%) 49 (39,2%)
Extension	
E1 (proctitis), n (%) **E2 (left-sided UC), n (%)** **E3 (extensive UC), n (%)**	13 (10.3%) 66 (52.4%) 46 (36.5%)
CDI, n (%)	25 (20%)

TABLE II.

Correlation between clinical, biological and endoscopic parameters and hospitalization risk; parameters marked with * were included in multivariate analysis (p<0.1)

Variable	Not hospitalized within 1 year (n=85)	Hospitalized within 1 year (n=40)	p-value
Age, years **Median (IQR)**	37 (26-48)	42 (27-52)	0.268
Gender, n (%)			
Female **Male**	45 (78.9%) 40 (58.8%)	12 (21.2%) 28 (41.2%)	0.016*
Mayo score **Median (IQR)**	6 (4-7)	8 (6-9)	0.002*
EIM at diagnosis, n (%)	6 (85.7%)	1 (14.3%)	0.301
Extension E3, n (%)	22 (47.8%)	24 (52.2%)	<0.001*
CDI at diagnosis, n (%)	12 (48%)	13 (52%)	0.017*
Hemoglobin g/dL **Median (IQR)**	13.3 (11.9-14.2)	12.2 (9.7-13.7)	0.010*
C-reactive protein mg/dL **Median (IQR)**	0.99 (0.25-3.5)	1.62 (0.7-4)	0.026*
White blood cells (x10^3/µL) **Median (IQR)**	7.84 (6.6-10.5)	9.04 (7-11.4)	0.148
Albumin g/dL **Median (IQR)**	4.30 (3.75-4.61)	3.72 (3.20-4.27)	0.001*
Fecal calprotectin µg/g	687 (299-1489)	857 (347-2232)	0.255
MES 3, n (%)	26 (53%)	23 (47%)	0.004*

Patients who were hospitalized within the first year had significantly higher baseline Mayo scores (p=0.002), lower hemoglobin (p=0.010), lower serum albumin (p=0.001), and higher CRP levels (p=0.026). Hospitalization was also more frequent among male patients (p=0.016), those with extensive colitis (E3) (p < 0.001), and patients with *Clostridioides difficile* infection (CDI) at diagnosis (p=0.017).

The Mayo endoscopic sub score was strongly associated with hospitalization risk (*p*=0.004), with more severe endoscopic activity corresponding to increased morbidity rates.

Multivariate logistic regression identified the following independent baseline predictors of one-year hospitalization (tab. III): higher Mayo clinical score (OR=1.28; 95% CI 1.00-1.64; p=0.043), extensive colitis (OR=3.45; 95% CI 1.35-8.79; p=0.009) and hypoalbuminemia (OR=3.73; 95% CI 1.09-12.71; p=0.035).

Although gender, hemoglobin levels and CDI status were associated with hospitalization in univariate analysis, they did not retain independent significance in the multivariate model (p > 0.05). Interestingly, C-reactive protein showed an inverse association with hospitalization risk (OR=0.86; 95% CI 0.77-0.97; p=0.015), likely reflecting the influence of extreme CRP elevations observed in patients with concomitant *C. difficile* infection.

A sub analysis comparing CRP levels between CDI-positive and CDI-negative patients at diagnosis revealed higher inflammatory activity among CDI-positive patients (median 1.74 mg/dL vs 0.98 mg/dL; p=0.055) (tab. IV).

TABLE III.

Independent predictive factors for hospitalization - multivariate analysis

Variable	B	P	Exp (B)	95% C.I for Exp (B)	
				Lower	Upper
Mayo score	0.25	0.043	1.28	1.00	1.64
CRP	-0.14	0.015	0.86	0.77	0.97
Hypoalbuminemia (< 3,5 g/dL)	1.31	0.035	3.73	1.09	12.71
Extension (E3)	1.23	0.009	3.45	1.35	8.79
CDI at diagnosis	-0.27	0.608	0.75	0.26	2.19
Gender	0.91	0.063	2.50	0.95	6.57
Hemoglobin	-0.14	0.287	0.86	0.67	1.12

TABLE IV.

Correlation between CRP levels in CDI positive and negative patients at diagnosis

Variable	CDI positive	CDI negative	p-value
C-reactive protein mg/dL, Median (IQR)	1.74 (0.87-6.11)	0.98 (0.33-3.42)	0.055

DISCUSSION

Demographic and clinical characteristics such as age, gender and disease phenotype at onset have been explored as potential prognostic indicators in UC. The impact of age remains debated. Unlike Crohn's disease, the Montreal classification for UC does not include age at diagnosis as a defining parameter, owing to inconsistent findings across studies. Some reports associate younger age, female sex, and extraintestinal manifestations with higher relapse rates, whereas others link male sex and extensive colonic involvement with an increased risk of colectomy (5-7). In our study no correlation between group age and gender was noted.

Clinical and laboratory markers reflecting high disease activity at diagnosis are key prognostic determinants in UC. Stratifying disease severity is crucial for guiding treatment decisions and anticipating disease progression. A combination of clinical, biological and endoscopic indicators provides valuable prognostic information. Features as persistent fever, frequent bloody stools, elevated inflammatory markers, and low serum albumin levels have been consistently associated with poor response to therapy and higher colectomy rates (4). Among biological markers, CRP remains a widely used and accessible inflammatory biomarker. Elevated CRP at diagnosis is associated with more extended disease and an increased risk of colectomy. Persistently high CRP levels during follow-up are also correlated with worse outcomes, regardless of treatment, highlighting its value as a surrogate for disease burden and prognosis (8).

In our analysis, a higher Mayo score - incorporating both clinical and endoscopic activity, was an independent predictor of UC-related hospitalization during the first year. These findings confirm the Mayo score's prognostic utility beyond activity assessment, suggesting that greater baseline disease severity translates into a higher morbidity burden over time.

Although CRP and fecal calprotectin are established inflammatory markers, they were not independently associated with one-year morbidity in our cohort. This may reflect their limited prognostic value once acute inflammation diminishes after treatment initiation. They primarily indicate inflammatory burden at diagnosis but do not necessarily predict mid-term outcomes in newly diagnosed patients.

Disease extent is another well-recognized prognostic factor. Patients with extensive colitis typically experience a more aggressive disease course, are more likely to require immunosuppressive or biologic therapy, and have lower response rates to amino salicylates (4). In our cohort, extensive colitis emerged as an independent predictor of UC-related hospitalization (OR=3.45; p=0.009), emphasizing the importance of accurately determining disease extent at baseline, ideally by colonoscopy and, when needed, supported by cross-sectional imaging.

Serum albumin is classified as a negative acute phase reactant, and its levels tend to decrease in the presence of inflammation. In UC, hypoalbuminemia results from increased catabolism and protein loss through the inflamed mucosa, while additional factors such as malnutrition and malabsorption further contribute to decreased levels (9). Previous studies have identified hypoalbuminemia as a strong predictor of poor outcomes, including treatment failure, colectomy,

and non-response to thiopurines or anti-TNF therapy (9,10). In line with these findings, our study demonstrated that hypoalbuminemia is associated with an increased risk of hospitalization in the first year after diagnosis (OR=3.73; p=0.035). These results underscore the prognostic value of albumin as a simple, cost-effective marker for early risk stratification in UC.

The impact of CDI in UC remains controversial. CDI occurs more frequently during UC flares and has been linked to worse short- and medium-term outcomes, including increased healthcare costs, longer hospital stays, higher colectomy rates, and more frequent hospital visits (8). However, data are conflicting. Some studies reported increased colectomy and mortality in CDI-positive UC patients (11, 12), while others found no significant long-term impact (13, 14). In our cohort, CDI at diagnosis was associated with higher CRP levels, indicating greater inflammatory activity, but did not independently predict one-year hospitalization. These findings support the hypothesis that CDI may exacerbate inflammation or unmask preexisting mucosal disease without directly worsening long-term prognosis.

Interestingly, 20% of our patients were diagnosed with UC in the context of CDI, suggesting that infection may act as a trigger in genetically or immunologically susceptible individuals.

Several limitations should be acknowledged. The retrospective, single-center design limits causal inference. The relatively small number of CDI-positive patients may reduce statistical power to detect differences in some outcomes. Additionally, although hospitalization was used as an objective marker of morbidity, we did not perform subgroup analyses based on the cause of admissions (e.g., flare vs. complication), which may have limited specificity. Moreover, using hospitalization as a marker of morbidity may overlook important aspects such as health-related quality of life or outpatient care requirements.

CONCLUSIONS

This study identified a higher Mayo clinical score, extensive colitis, and hypoalbuminemia as independent baseline predictors of UC-related hospitalizations within the first year after diagnosis. These findings highlight the importance of a comprehensive initial assessment, integrating clinical, biochemical, and endoscopic parameters to improve early risk stratification and guide timely, individualized treatment decisions.

Clostridioides difficile infection was associated with more pronounced biochemical inflammation, but it did not independently predict one-year morbidity. This suggests that CDI may exacerbate mucosal inflammation or unmask underlying disease rather than directly worsening mid-term outcomes.

From a clinical perspective, patients presenting at diagnosis with extensive colonic involvement, elevated Mayo score, and low albumin levels may benefit from closer surveillance and timely therapeutic escalation.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interests, and they received no specific funding regarding this scientific research.

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THE ETIOPATHOGENETIC PATHWAYS CONNECTING PSORIASIS AND MENTAL HEALTH DISORDERS

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THE ETIOPATHOGENETIC PATHWAYS CONNECTING PSORIASIS AND MENTAL HEALTH DISORDERS (Abstract): Psoriasis is a chronic immune-mediated skin condition that goes beyond the cutaneous symptoms, often affecting patients' mental health and social interactions. Psychiatric comorbidities like depression, anxiety, and sleep disorders are prevalent and can be worsened by stigma and discrimination. The psychology of psoriasis is discussed in this article based on a 2023 study that used a questionnaire to investigate discrimination, treatment expectations, sleep, and perceived psychosocial effects of psoriasis. **Materials and methods**: A questionnaire of 101 questions was given to psoriasis patients via social media (n = 155). The responses were assessed descriptively and using multivariable regression. Correlations between discrimination, sleep disturbances, expectations, and side effects on family life, work, and relationships were the focus of the study. **Results**: Almost half of the participants (43.2%) had experienced discrimination or stigma. Discrimination was strongly related to self-reported negative effects on family life (52.3%), work performance (49.0%), and interpersonal relationships (54.2%), with a moderate effect size (χ^2 = 32.26, $p < 0.001$). Sleep issues were less frequently reported (6.5%) and were not strongly related to discrimination. **Conclusions**: Psoriasis is inextricably linked with mental health, not just a cause of biological inflammation but also social stressors such as stigmatization. The results identify the fact that discrimination plays an underlying part in reducing quality of life, and problems with sleep may be less centrally involved. An integrated medical, psychological, and social treatment approach is needed for overall treatment. **Keywords:** PSORIASIS, PATHOLOGY, MENTAL DISORDERS, HEALTH.

INTRODUCTION

Psoriasis is more than a skin disease; it is an inflammatory condition with strong psychological and social consequences. Although scientific research has mainly targeted immune processes and treatment (1), mounting evidence illustrates the substantial psychosocial burden, such as stigma and discrimination, and their adverse impact on mental health (2, 3). In a previous questionnaire-based study on psoriasis, the necessity of approaching this topic from a new perspective was brought up, linking psoriasis to different comorbidities, interrelationships

and its multifactorial factors was taken more seriously (4). Patients must often contend with a double burden: visible lesions that garner unwanted attention and inner turmoil that is not observable, including shame and a changed self-image (5). Comorbid depression, anxiety, and sleep disorders are prevalent, which puts psoriasis in the realm of mind-body medicine, in which psychological and physical components cannot be separated (6, 7).

Stigma also plays a significant role in increasing emotional vulnerability through social rejection and internalization of negative beliefs that decrease self-worth and hope (2, 3). Discrimination, sleep disturbances, and expectations about treatment and their impact on everyday life and mental health are reviewed here, emphasizing the necessity for comprehensive care addressing physical as well as psychosocial domains. With the pioneering research of Wittkower in the 1940s connecting psoriasis with emotional stress (8), studies have explained complex bidirectional associations among psoriasis and psychiatric illness. Stress disrupts the hypothalamic–pituitary–adrenal axis and the sympathetic-adrenal-medullary system, resulting in chronic systemic inflammation (9).

Improved cytokine secretion by cytokines such as TNF-α and IL-6 plays a role in psoriatic skin lesions and also psychiatric illnesses such as depression, thereby providing a two-way link between inflammation and distress (10, 11). Other psychosocial elements such as stigma, inefficient coping mechanisms, alexithymia, and discrimination could augment the burden of disease regardless of lesion severity (2, 3).

Understanding these entangled biological and psychosocial processes presented in first figure is essential for the creation of multicomponent treatments that address both dermatological symptoms and mental health, along with social functioning.

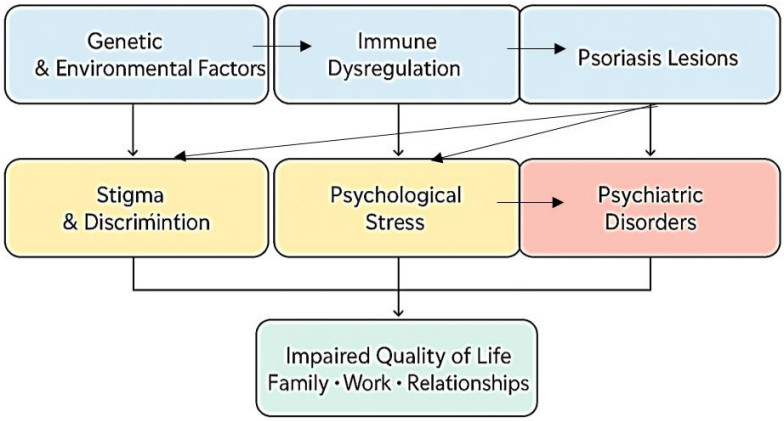

Fig. 1. Etiopathogenetic Pathways Connecting Psoriasis and Mental Health Disorders

MATERIALS AND METHODS

A structured, 101-question survey was developed to collect sociodemographic information, clinical history, and psychosocial results of the patients with psoriasis. The survey was sent in 2023 to a sample of individuals with psoriasis via social media (n = 155). Because the questionnaire was distrib-

uted primarily through psoriasis forums, the sample may be subject to selection bias, potentially overrepresenting individuals who are more engaged in online patient communities. Questions addressed areas like perceived discrimination, insomnia or sleep disruption, expectations from treatment, and family, occupational, and interpersonal relationship impacts of psoriasis.

Descriptive statistics were calculated for all responses. Discrimination and psychosocial variables' cross-tabulations were tested using Pearson's chi-square test and Cramer's V to establish the effect size. Multivariable logistic regression was performed with discrimination (yes/no) as the outcome variable, predictors such as age, sex, insomnia, and expectation categories.

The Declaration of Helsinki was followed in the conduct of the study. Each participant received information about their rights regarding data protection and how their personal data was processed in accordance with Regulation (EU) 2016/679 (GDPR) and any applicable national laws. Each participant provided written informed permission attesting to their knowledge of data protection.

RESULTS

One hundred fifty-five questionnaires were completed and returned. Respondents' average age was 39 years, ranging from 18 to 73 years, 79% of them were female and 21% male.

Discrimination and Stigma. Almost half of the sample (43%) said they had been discriminated against or stigmatized due to their psoriasis, while 57% said they had never been treated this way (fig. 2). Those who had been discriminated against were slightly younger, with an average age of 31.47 years, than those who had not, with an average age of 50.04 years.

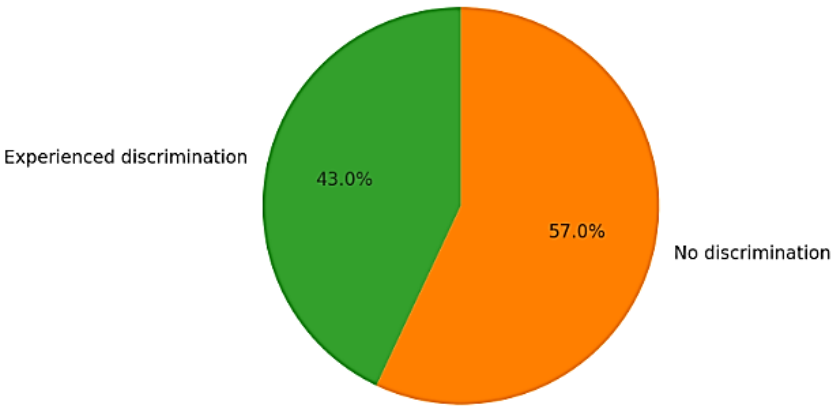

Fig. 2. Experience of discrimination or stigma due to psoriasis

Sleep Disturbances. Only 6.5% reported having insomnia or difficulty sleeping, while the majority (74%) did not have a sleeping problem (fig. 3).

Approximately 19% could not answer or were unsure of this question. No significant association was found between the experiences of discrimination and insomnia.

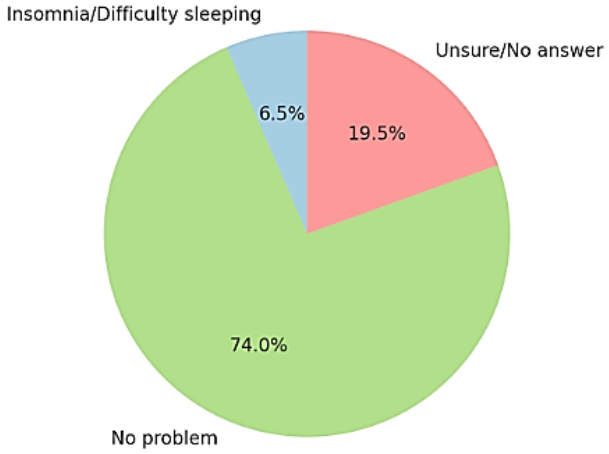

Fig. 3. Reported sleep disturbances among respondents

Expectations about Disease Course. The participants gave diverse responses when questioned about what they hoped for the psoriasis of their future (fig. 4). 42 out of 155 responders stated that they had no hope for the future of their disease. Though 29 participants wished to become totally well, 32 participants wished that they would improve. Five of the small sample predicted that their illness would worsen, and five predicted that any treatment that they received would be only temporarily effective. 29 subjects had not given a clear answer or were not sure how to respond, and 12 subjects had responded differently or more elaborately before.

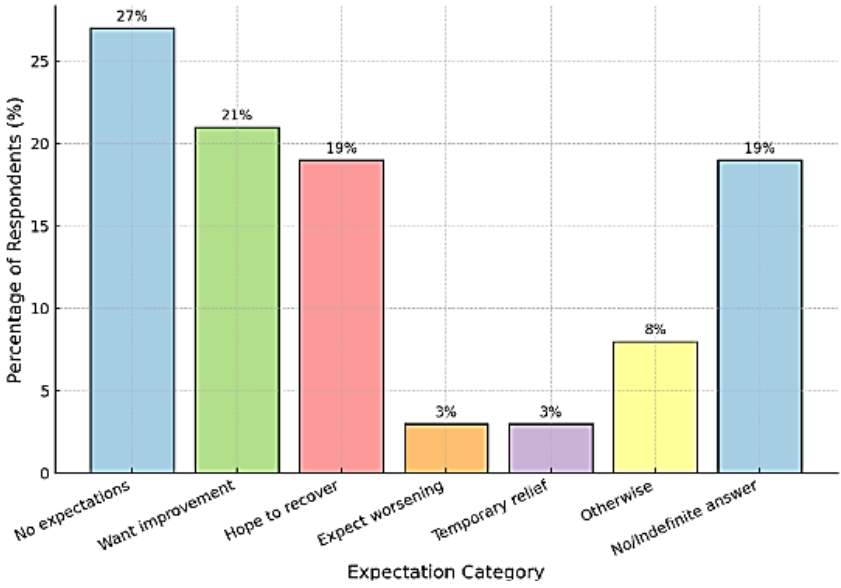

Fig. 4. Expectations about the future course of psoriasis

Impact on Family, Work, and Relationships.

When asked to describe how psoriasis had affected different parts of their lives, participants most often reported negative effects on a variety of important areas (fig. 5). Eighty-one reported that their families were affected negatively, and 76 reported that their performance at work was affected negatively as a result. Similarly, 84 reported that their dating relationships were affected negatively by their psoriasis. A little over 23 of the responses in these categories were either absent or too general to be categorized properly.

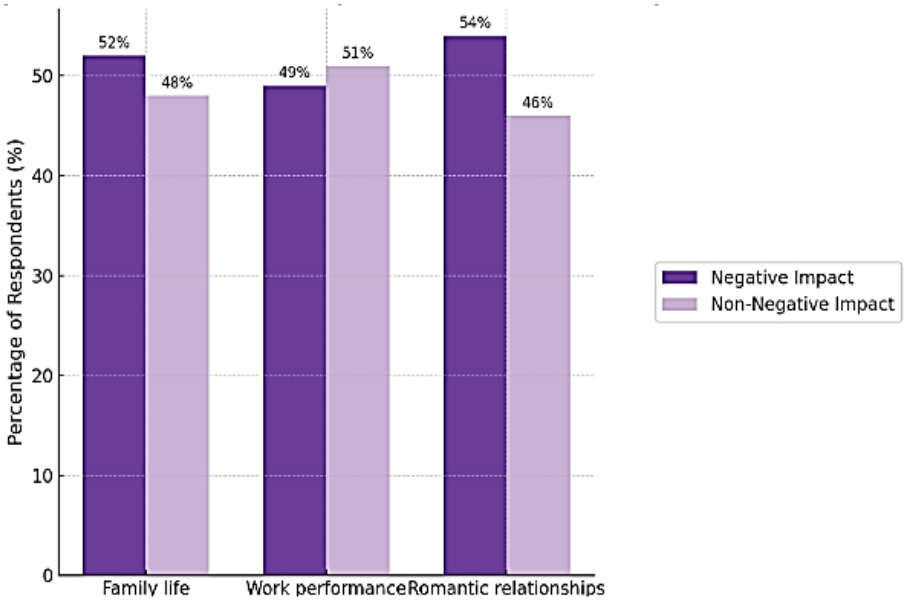

Fig. 5. Impact of psoriasis on family, work performance and romantic relationships

Psychosocial Correlations. There was a statistically significant and moderate relationship between discrimination reports and adverse effects on family, employment, and interpersonal relationships, suggesting that individuals reporting discrimination were more likely to report strain in these domains. Pearson's chi-square tests yielded χ^2 (9) = 32.26, $p < 0.001$, with Cramer's V = 0.388, indicating a moderate effect size. Discrimination was not significantly related to insomnia, though.

DISCUSSION

The study unveils the complex overlap of psoriasis and mental health, where psychosocial issues, led by discrimination, are the root cause of impaired quality of life. The study also has a few limitations. Self-report data limitation is prone to in-built subjectivity and recall bias. Female over-sampling (79%) and relatively low sample size might limit generalizability.

Nevertheless, the results offer significant patient-centered evidence to empha-

size imperatives of the integration of psychosocial issues into models of psoriasis clinical management.

Nearly half of the participants reported being stigmatized or discriminated against, consistent with earlier research placing psoriasis at the center of the most stigmatized dermatological disease (5). In 2021, Zhang *et al*. (12) mentioned that the lack of public understanding of these dermatological diseases would lead to people being stigmatized and treated with disgust and hostility. Also, stigma may lead to social rejection and internalization of negative self-beliefs, increasing emotional vulnerability and psychiatric comorbidity (3). Back in 2020, a similar survey was conducted by Wan *et al*. (13), in which they reported that 106 patients were stigmatized based on their psoriatic lesions. Two-thirds of the patients who completed this survey anticipated being stereotyped as contagious or unattractive, whereas about one-third of them thought that their lesions would elicit disgust from other people upon seeing them.

In contrast to studies with high levels of sleep disturbance among psoriasis patients, as often explained by pruritus, depression, and systemic inflammation (14, 15), the current sample had comparatively low levels of sleep complaint (6.5%), not correlated with a seeming association with insomnia and discrimination. This result may be explained by underreporting, the effect of non-psoriasis-related sleep disorder, or sample-specific characteristics. Lack of correlation indicates that stigma acts directly on psychosocial adjustment by means of relational stress and not by means of biological sleep disturbance. In a 2024 study guided by Guo *et al*. (16), fifteen studies including 1274 patients with psoriasis and 775 controls were analyzed, and they concluded that psoriasis is associated with sleep disturbances, especially among young patients. They also mentioned that patients with psoriasis are more likely to experience insomnia.

Contrary to expected responses, the disease course differentiated subgroups with no expectations from those with expectations of complete remission. These subgroups also showed higher perceived discrimination, although not significantly. Such trends may signal higher psychological vulnerability, whereby reduced hopefulness or excessive optimism increases the distress of social rejection. This aligns with earlier evidence that coping styles, resilience, and perceptions of illness significantly influence the psychological burden of psoriasis (17). After treatment, 72% of patients expected 90%-100% clearance, whereas only 28% expected <90% clearance of psoriasis in a study conducted by Clemmesen *et al.* in 2023 (18). So, it would be possible to suggest that some patients still have hope for clearance despite the odds.

Particularly, discrimination experience was most closely associated with adverse impact on family relationships, work functioning, and interpersonal relationships, indicating that the psychosocial morbidity of psoriasis extends far beyond individual self-esteem to influence central relational and functional areas of daily living. This extends beyond self-esteem to relational and functional domains, supporting Grossschaedl *et al.* (19) study from 2024 on high emotional burden for partners and relatives

The multivariable analysis identified

patterns in discrimination predictors, but none were statistically significant. Males experienced discrimination less frequently than females, and greater age was associated with a lower likelihood, patterns that could reflect gendered social dynamics or cumulative coping resilience over time. There was no prognostic role for insomnia. Notably, individuals who had no expectations about their illness or hopes for a full recovery were more likely to experience discrimination than those who anticipated improvement. This suggests that despondency or false optimism may increase feelings of vulnerability and social rejection.

CONCLUSIONS

Psoriasis has to be visualized as a multidimensional disease well beyond the scope of dermatopathology. Stigma and discrimination are placed in the focus of this article as parts of the forces that influence the psychosocial existence of patients and significantly lower quality of life in family, occupational, and relational areas of life. Systemic inflammation biologically links psoriasis with psychiatric disease, and social stressors raise disease burden and create a vicious circle of distress.

Effective management necessitates a careful, multidisciplinary strategy. Apart from biologic and systemic anti-inflammatory treatment, routine mental health screening, measurement of stigma experience, and availability for psychological and social support treatment are required. Cognitive-behavioral therapy, psychoeducation, and peer support interventions have the ability to empower patients to reconceptualize illness representations and become resilient. Integrative psychodermatology thus promises improved outcomes through management not just of overt cutaneous lesions but also of silent wounds of social discrimination.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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EXTRAINTESTINAL MANIFESTATIONS IN INFLAMMATORY BOWEL DISEASE - RESULTS FROM NORTH-EASTERN ROMANIA

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EXTRAINTESTINAL MANIFESTATIONS IN INFLAMMATORY BOWEL DISEASE - RESULTS FROM NORTH-EASTERN ROMANIA (Abstract): The presence of extraintestinal manifestations (EIM) in inflammatory bowel disease (IBD) is a common and well-known finding. **Material and methods:** Using the national database IBD Prospect, we conducted a prospective case-control study that included 325 patients diagnosed with Crohn's disease (CD) or ulcerative colitis (UC). Between these cases, 81 (24.92%) were classified in the group CD, 242 (74.46%) having UC and 2 cases (0.62%) were classified as having undifferentiated colitis. Among them, 30 patients (9,23%) had EIM including 24 cases of articular manifestations. Regarding joint events, we found 5 cases of arthritis, 10 cases having axial manifestations like sacroiliitis or ankylosing spondylitis and 9 cases of multiple EIM including articular damage. **Results:** In both study arms, articular manifestations occupy the first place into EIM (14/17 CD *vs.* 10/13 UC, 82.35% *vs.* 76.92%, p=0.927). Into the CD group, joint manifestations first correlated with the ileo-colonic form of CD (8/14; 57,14%) followed by the colonic involvement (4/14; 28,57%). Into the UC arm, joint damage was associated with an extended colonic involvement (4/10; 40%) followed by proctitis and left side colitis. Both groups of patients with articular manifestations were associated with a moderate form of IBD activity - 10/14 CD *vs.* 5/10 UC (71.43% *vs.* 50%, p = 0.199). **Conclusions:** Most patients included in this study and having EIM belong to CD phenotype. Articular manifestations occurred at a higher frequency in patients with CD as compared to those diagnosed with UC. The most common articular manifestation is the axial involvement, followed by peripheral arthritis. **Keywords:** INFLAMMATORY BOWEL DISEASE, CROHN'S DISEASE, ULCERATIVE COLITIS, ARTICULAR MANIFESTATIONS, EXTRAINTESTINAL MANIFESTATIONS.

Inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory diseases different from both the pathogenic mechanisms, as well as from clinical manifestations. In addition to inflammatory intestinal damage, CD and UC may have lots of extraintestinal manifestations (EIM). The most common EIM are at the level of musculoskeletal system - axial and peripheral arthritis, followed by skin involvement - erythema nodosum, pyoderma gangrenosum or eye damage - uveitis, iridocyclitis.

EIM can be divided into three catego-

ries: extraintestinal immune- related manifestations of IBD, autoimmune disorders associated to IBD and extraintestinal complications. Extraintestinal immune- mediated manifestations have the same pathogenic mechanism and correlate with the activity of intestinal inflammation. Autoimmune diseases associated with IBD develop independently of IBD activity and are characterized by a high genetic susceptibility. Extraintestinal complications may occur due to: intestinal inflammatory activity, use of medication, anatomical and metabolic changes that accompany IBD, malabsorption etc. (1, 2).

MATERIAL AND METHODS

We performed a prospective case-control study using the national database IBD PROSPECT (Inflammatory Bowel Disease Prospect). All patients signed an informed consent form before being included in the database. In University Center Iasi were included 325 patients with IBD. Among these, 140 (43.08%) were female and 185 (56.92%) male. Most patients were coming from urban areas - 227 cases (69.85%), while in rural areas were recorded only 98 cases (30.15%). The average age was 43 years with a minimum of 17 years and a maximum of 76 years. Among the patients included, 81 (24.92%) were classified in the group of CDs 242 (74.46%) in the UC's group and 2 cases (0.62%) were categorized as undifferentiated colitis. The detailed patient history also included the smoking status. 40 cases (12.30%) of smokers, 122 cases (37.54%) of ex-smokers and 163 cases (50.16%) of never-smoking were recorded.

All patients were examined clinically and paraclinically. The history followed significant personal or family history, the clinical symptoms like: the number of stools / 24 hours, the presence of rectal tenesmus, lower gastrointestinal bleeding, weight loss within 3 months, abdominal pain and asthenia.

Evaluation of the disease phenotype was performed using colonoscopy with biopsy and histopathological examination. CD has been divided, according to the localization of the inflammation, in L1 - ileal, L2 - colonic, L3 - ileo-colonic and depending on the type of disease in B1 - inflammatory, B2 - stenosing and B3 - penetrating disease. Depending on the location of the inflammatory process, UC was divided into E1 - proctitis, E2 - left colitis and E3 - extensive colitis. In both groups of patients, the assessment of severity of the disease flare was quantified by remission, mild, moderate or severe.

The study also analyzed the treatment of these patients. There have been considered the following classes of drugs: 5 ASA (5-aminosalicylic acid), corticosteroids, budesonide, the use of antibiotics, probiotics and immunosuppressive agents such as azathioprine, methotrexate or biological therapy with anti-TNF-α agents such as infliximab or adalimumab.

Regarding the complications related to the disease, they were divided into two categories: intestinal and extra-intestinal manifestations. The intestinal complications included: abscesses, intestinal and perianal fistulas, stenosis, the presence of toxic megacolon, intestinal perforation, lower intestinal bleeding and the development of a malignancy. Extraintestinal manifestations included joint signs like arthritis or sacroiliitis/ankylosing spondylitis (SI/AS), skin manifestations: erythema nodosum, pyoderma gangrenosum, eye manifestations: uveitis / episcleritis, gastrointestinal symptoms: peri cholangitis, primary sclerosing cholangitis or renal manifestations such as

oxalate nephrolithiasis, renal amyloidosis or multiple urinary infections.

RESULTS

In the study group, 30 cases (9.23%) with EIM were highlighted. Of these, we noticed 5 cases (16.66%) of arthritis, 10 cases (33.33%) of SI/AS, erythema nodosum 2 cases (6.66%), pyoderma gangrenosum 1 case (3.33%), 1 case of primary sclerosis cholangitis (3.33%), multiple urinary infections 2 cases (6.66%) and associations between two or more EIM - 9 cases (30%). Because all the cases with multiple EIM also have articular involvement, they were considered as articular manifestations, being in total 24 cases (80%) (tab. I).

In the group of patients who had articular manifestations (n = 24; 80%), 14 cases (58.33%) of axial manifestations (sacroiliitis/ankylosing spondylitis), 9 cases of arthritis (37.5%) and one case (4.16%) of association between peripheral and axial involvement were recorded (fig. 1).

TABLE I.

Extraintestinal manifestations in study group

Extraintestinal manifestation	n (%)
Articular manifestations (n, %)	24 (80%)
Arthritis	5 (16,66%)
SI/AS	10 (33,33%)
Association of EIM	9 (30%)
SI/AS + pyoderma gangrenosum	2 (6,66%)
SI/AS + uveitis/episcleritis	1 (3,33%)
SI/AS + uveitis/episcleritis + oxalate urolithiasis + multiple urinary infections	1 (3,33%)
Arthritis + multiple urinary infections	1 (3,33%)
Arthritis + erythema nodosum	1 (3,33%)
Arthritis + uveitis/episcleritis	2 (6,66%)
Arthritis + SI/AS	1 (3,33%)
Erythema nodosum (n, %)	2 (6,66%)
Pyoderma gangrenosum (n, %)	1 (3,33%)
Primary sclerosing cholangitis (n, %)	1 (3,33%)
Multiple urinary infections (n, %)	2 (6,66%)

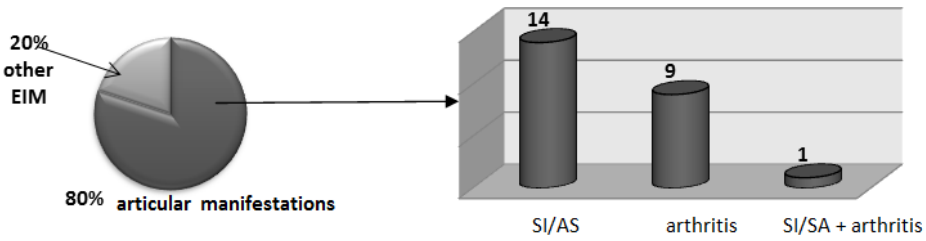

Fig.1. Articular manifestations in patients with IBD

CD and EIM

There were 17 patients diagnosed with CD who also had EIM as follows: 14 cases (82.35%) with articular manifestations, one case (5.88%) having erythema nodosum, 1 case (5.88%) with pyoderma gangrenosum,

1 case (5.88%) with multiple urinary infections. Most patients with CD and EIM were female - 11cases (64.70%) than male - 6 cases (35.30%). 16 patients (94.12%) were from urban areas, only one patient (5.88%) being from rural areas. The analysis of smoking status revealed the following data: 8 cases (47.06%) of active smokers, 3 cases of ex-smokers (17.65%) and non-smoking 6 cases (35.29%).

For patients with CD and EIM, the most frequent localization of the inflammatory process was L3 (ileo-colonic disease) - 9 cases (52.94%), followed by L2 (colonic disease) - 5 cases (29.41%), L1 (ileal disease) - 2 cases (11.76 %) and L4 (upper digestive tract disease) - 1 case (5.88%). Referring to the form of the disease, 13 patients (76.47%) had B1 (inflammatory form) and 4 patients (23.53%) had B2 (stricturing form). Most of cases - 11 (64.7%) - had a moderate intestinal inflammation, while 6 cases (35.30%) had remission or a mild form of BC (fig.2).

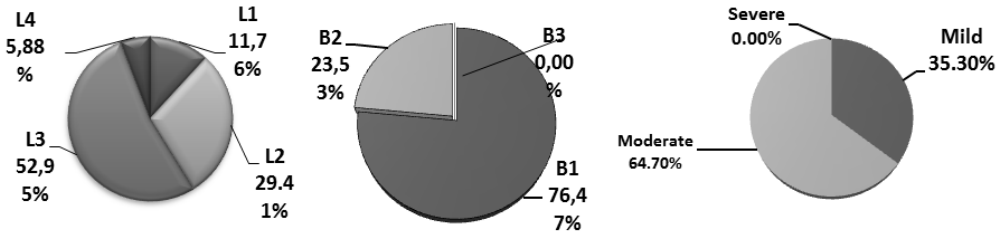

Fig. 2. Association between different forms of CD and EIM

The group of CD patients presented 14 cases (82.35%) of articular manifestations, which are the most common EIM. There were 3 cases (17.65%) of arthritis, 6 cases (35.30%) of SI/AS and 5 cases (29.41%) of association between different EIM including joint symptoms.

Articular manifestations occurred in a higher percentage in patients with L3 localization of CD - 8 cases (57.14%), followed by L2 - 4 cases (28.57%) and L1 - 2 cases (14.28%). The largest number of cases with an inflammatory phenotype (B1) - 11 (78.57%), followed by the stenosing form (B2) - 3 cases (21.43%), was recorded in subjects with associated articular manifestations. Also, moderate inflammation of the gut was associated with joint symptoms - 10 cases (71.43%), followed by the mild form - 4 cases (28.57%) (fig.3).

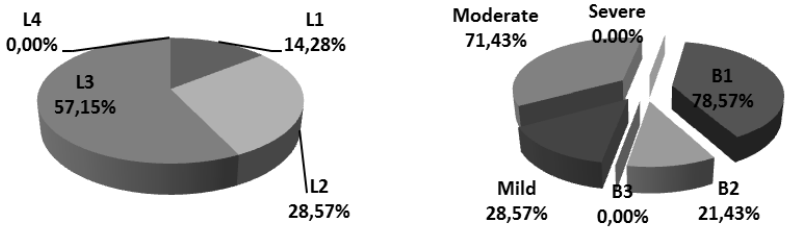

Fig. 3. Association between different forms of CD and articular manifestations

It should also be noted that patients with CD and EIM can develop intestinal complications. There were seven cases (41.17%) of such complications: 4 cases (57.14%) of stenoseses, 1 case (14.28%) of lower digestive hemorrhage, 1 case (14.28%) of fistulas + lower digestive hemorrhage, 1 case (14.28%) of abscesses + fistula. Of the 7 cases of intestinal complications, 6 (85.71%) have developed in patients who also associate articular manifestations.

UC and EIM

The group of patients with IBD include 13 cases of UC and EIM: 10 patients (76.92%) with joint manifestations, 1 patient (7.69%) with primary sclerosing cholangitis, 1 patient (7.69%) with erythema nodosum and 1 case (7.69%) of multiple urinary infections. Of these 13 cases of UC, 8 (61.54%) were women and five (38.46%) men. Most patients came from urban areas - 9 (69.23%), 4 (30.77%) being from the countryside. Analyzing the smoking status, we found 8 patients (61.54%) non-smoking, 5 patients (38.46%) ex-smokers, none active smoker.

The location of the intestinal inflammation in UC correlated differently with EIM. Therefore, left colitis (E2) was found in 6 cases (46.15%) of patients who associate EIM, extended colitis (E3) in 4 cases (30.77%) and proctitis (E1) in 3 cases (23,07%). Regarding the severity of the intestinal inflammation, 6 patients (46.15%) with EIM showed a mild form of the disease, 5 patients (38.46%) a moderate form, only 2 patients (15.38%) having a severe inflammation of the bowel (fig. 4).

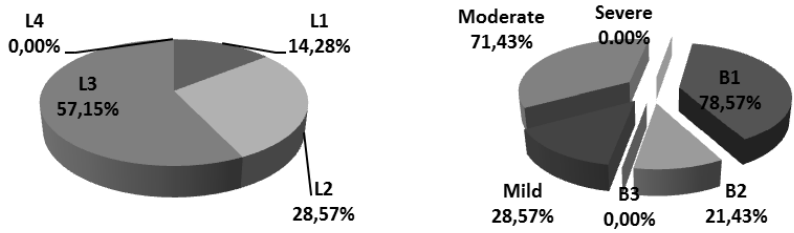

Fig. 4. Association between different forms of UC and EIM

Articular manifestations associated with UC were 10 (76.92%) distributed as follows: 2 cases (20%) of UC and arthritis, 4 cases (40%) of UC and SI/AS, 4 cases (40%) multiple EIM and UC.

According to the location of the inflammation of the bowel, articular manifestations associated with UC patients correlate as follows: 3 cases (30%) with form E1, 3 cases (30%) with form E2 and 4 cases (40%) with E3. The severity of the gut inflammation was divided into: 3 cases (30%) of mild symptoms associated with joint symptoms, 5 cases (50%) of moderate inflammation and 2 cases (20%) of severe bowel symptoms associated with axial or peripheral joint manifestations (fig. 5).

It must be pointed out that 2 patients (15.38%) with UC and EIM also associated intestinal complications such as lower digestive hemorrhage. Both patients had peripheral joint symptoms - arthritis.

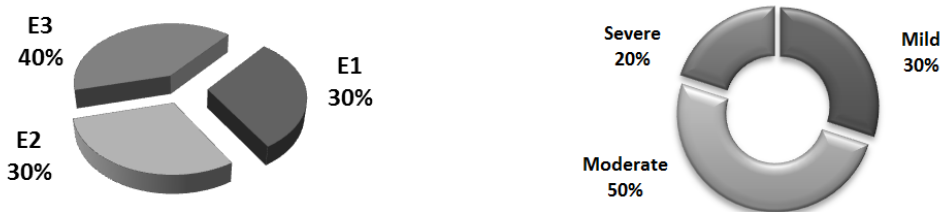

Fig. 5. Association between different forms of UC and articular manifestations

DISCUSSION

The aim of this study was to highlight the presence of EIM in patients with IBD. The overall prevalence of EIM found was 9,23% quite like that reported in the literature ranging from 6 and 47% (3-6). Both in the CD and UC group predominate female cases - 11/17 *vs.* 8/13 (64.70% *vs.* 61.54%, p = 0.838). Most patients come from urban areas - 16/17 CD *vs.* 9/13 UC (94.12% *vs.* 69.23%, p = 0.187). In the group of CD patients, 8/17 cases are active smokers, while in the case with UC patients 8/13 cases are non-smokers (p = 0.015) (statistically significant).

EIM occurred with a higher incidence among patients with CD and among females, this result being supported by other studies (7). In both study arms, articular manifestations have been shown to be the most frequent EIMs (14/17 CD *vs.* 10/13 UC; 82,35% *vs.* 76,92%, p=0,927). Numerous clinical trials have demonstrated that axial and peripheral articular manifestations occur frequently in patients with IBD, being classified as the most common EIMs (8-11).

In the group of patients with CD there is no difference between joint manifestations and other EIMs regarding the association with the location and the phenotype of the disease. Most cases of CD and EIM, including articular manifestations, correlated with form L3 (ileo-colonic) - 9/17 (52.94%), followed by form L2 (colonic) - 5/17 (29.41%). Regarding the phenotype, 11/17 (64,70%) had a moderate form of intestinal inflammation.

As opposed to patients with CD, in the group of cases with UC there is difference between joint manifestations and other EIMs regarding the association with the location and the phenotype of the disease. Most cases of UC and EIM correlated with left-colitis (E2) - 6/13 (46.14%), followed by pancolitis (E3) - 4/13 (30.77%). Regarding articular manifestations, they first correlated with pancolitis, then with left-colitis (4/10 *vs.* 3/10).

In both groups of patients with IBD and EIM, intestinal complications occurred with a higher rate in CD patients compared to those with UC - 7/17 *vs.* 2/13 (41.17% *vs.* 15.38%, p = 0.260). We raised the following hypothesis: a more severe disease characterized by the presence of intestinal complications can be considered as a risk factor for the emergence of EIM, including articular manifestations.

CONCLUSIONS

The results of our study confirmed that UC is more frequently found both in Northeastern Romania and in city of Iasi. Most of the patients enrolled in the study and who presented EIM belong to CD phe-

notype. Joint manifestations have been shown to be the most common EIM in both CD and UC patients. The most common articular manifestations are the axial forms (SI/AS), followed by peripheral arthritis. Joint manifestations occurred with a higher frequency among CD patients compared to those diagnosed with UC.

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SHORT-TERM PREDICTIVE FACTORS FOR THE OUTCOME OF PRETERM INFANTS DEPENDING ON THE METHOD OF RESPIRATORY SUPPORT

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SHORT-TERM PREDICTIVE FACTORS FOR THE OUTCOME OF PRETERM INFANTS DEPENDING ON THE METHOD OF RESPIRATORY SUPPORT (Abstract): Respiratory support issues regarding preterm newborns show increasingly theoretical and clinical interest. **Aim:** To monitor the outcome of preterm infants with respiratory distress receiving different methods of respiratory support. **Materials and methods:** We performed a 5-year observational study (2013-2016) of 750 preterm infants with respiratory distress. The cases included in the study were divided into three groups as follows: group 1 - 476 premature newborns from IMSP IMsC (The Institute for Mother and Child Health) Chisinau (level III maternity hospital); group 2 - 240 premature infants transferred by the AVIASAN aerial service from level I and II to level III maternity hospitals, and group 3 - 34 premature babies from IMSP IMsC Chisinau which received surfactant administered by the LISA (Less Invasive Surfactant Administration) method. Data were analyzed using *SPSS 21.0* software (IBM Statistical Package for the Social Sciences, Chicago, Illinois). **Results:** 42.67% of the cases did not require initial respiratory support and in 36.8% of cases, CPAP (Continuous Positive Airway Pressure) was used. Of the cases which were at first on CPAP, 6.27% were transferred on conventional mechanical ventilation (CMV) and 4.8% on high frequency ventilation (HFV). Of the newborns who initially received CMV, 5.33% were later transferred on CPAP and 4.13% required HFV. Our results showed that mechanical ventilation had been applied particularly in low gestational age newborns - the mean gestational age was significantly lower in these infants ($p < 0.01$). Both in newborns from level III perinatal center and those from level I and II maternity hospitals, gestational age was significantly lower in those who required CMV or HFV. **Conclusions:** Due to the implementation of contemporary methods of respiratory support for preterm infants their survival rate has greatly improved. However, the possibility for oxygen therapy remains limited in low-resource settings. Thus, in the Republic of Moldova, high quality respiratory support remains a valid option only for the level III perinatal center - IMSP IMsC. **Keywords:** PREMATURE NEWBORN, MECHANICAL VENTILATION, CPAP, T-PIECE RESUSCITATOR, RESPIRATORY DISTRESS SYNDROME.

The most frequent acute respiratory disease for very premature infants is Respiratory Distress Syndrome (RDS). Treatment includes respiratory support and surfactant replacement therapy. The recent development of methods for respiratory management of the preterm infant in the delivery room includes limiting the use of excess oxygen during resuscitation and limitation of inspiratory pressure through using a T-piece resuscitator, which can be also used for early non-invasive ventilation and surfactant administration (1).

Some authors have highlighted the need to improve therapeutic strategies in the delivery room for fragile newborns. Jobe and other authors (1-3) mentioned that only few very low birth weight infants need resuscitation, while most of them only require transitional assistance to adapt to extrauterine life. While early CPAP safety and effectiveness are well established (2, 4, 5), the most effective approach to the preterm newborn with respiratory distress is a combination of early administration of surfactant during spontaneous breathing with CPAP support and with limited exposure to positive pressure ventilation.

Although many premature infants can be successfully treated with non-invasive ventilation, intubation should not be overlooked as a variant of respiratory support with long-term benefits. Children with severe RDS can continue to deteriorate despite non-invasive ventilation and there is a potential risk of administering high positive-end-expiratory pressures (PEEP) with very high levels of inspired oxygen. Signs of worsening of respiratory distress in a newborn, such as PEEPs > 7 cm H_2O, oxygen concentrations > 40% or pH < 7.25 are accepted as triggers for increasing respiratory support. Some researchers confirm that by using these criteria, the duration of mechanical respiratory support is reduced (6-9).

The aim of our research is to monitor the outcome of preterm infants receiving different methods of respiratory support and surfactant administration.

MATERIAL AND METHODS

We performed an observational study of 750 preterm newborns admitted between 2013 and 2016 to the Neonatal Intensive Care Unit (NICU) of the Institute for Mother and Child Health (IMSP IMsC) in Chisinau, Republic of Moldova. The cases included in the study were divided into three groups as follows: group 1 - 476 premature newborns from IMSP IMsC Chisinau (level III maternity hospital); group 2 - 240 premature infants transferred by the specialized aerial AVIASAN service, from level I and II to level III hospitals and group 3 - 34 premature infants from IMSP IMsC Chisinau which received surfactant by the LISA method. Infants in group 3 were selected throughout the year 2016, based on the clinical and radiological severity of the RDS and the presence of spontaneous breathing.

The inclusion criteria were respiratory distress, either due to surfactant deficit or congenital pneumonia, and gestational age less than 34 weeks. The exclusion criteria were: congenital malformations, genetic syndromes and hydrops fetalis (immune and non-immune).

To assess the outcome of the infants, we considered the following complications while infants were hospitalized in the NICU: pulmonary hemorrhage, intraventricular hemorrhage, air-leak syndromes, ventilator associated pneumonia, and chronic lung disease.

Data were analyzed using the *SPSS 21.0* software (IBM Statistical Package for the

Social Sciences, Chicago, Illinois). Continuous variables with normal distribution were expressed as mean ± standard deviation, and continuous variables with abnormal distribution were expressed as median (min-max). Categorial variables were expressed as absolute or relative frequencies. Continuous variables were verified by either the Levene test or Kruskal-Wallis test. The statistical significance level (p-value) was considered 0.05. The multivariate analysis used the multinomial logistic regression and the odds-ratio (OR) was calculated for each predictive parameter.

RESULTS

There are significant statistical group differences regarding gestational age (GA): 30.2 weeks in group 1, 30.8 weeks in group 2 and 29.1 weeks in group 3 (p=0.003).

There were 8.61% infants in group 1 and 6.25% infants in group 2 who received positive pressure ventilation in the delivery room. 37.92% neonates in group 2 and 27.94% in group 1 did not require respiratory assistance in the delivery room (Table I). Our results show a significant correlation between the method of initial respiratory support and the level of maternity hospital where the premature birth occurred (χ^2=139.2, p<<0.01, r=-0.642, 95%CI) (tab. I).

There is also a significant correlation between the resuscitation method and subsequent respiratory support (r=0.543, p<<0.01). Thus, the frequency of cases that required CPAP in the NICU was significantly higher (56.9%) in infants who received T-piece PEEP in the delivery room, compared to those which received initial bag and mask ventilation during resuscitation (tab. I). The latter were more likely to require mechanical ventilation, either from the start, or due to CPAP failure.

The overall need for respiratory support after delivery room management was 59.5% in group 1 and 47.1% for infants in group 2. In group 3, the need to switch from non-invasive respiratory support (CPAP) to conventional mechanical ventilation (CMV) was noted in 11.8% of cases (n=4), and from CPAP to rescue high-frequency ventilation (HFV) in 8.82% cases (n=3).

There was a significant statistical correlation between the method of respiratory support and the level of care provided by the maternity hospital in which the babies were born. This correlation can be explained by the significantly increased frequency of CPAP use in group 1 newborns (38.4%) compared with rates found in group 2 (27.5%). Also, the need for CMV was of 7.1% of cases in group 2 and only 4.8% in group 1. Both in groups 1 and 2, GA was significantly lower in neonates who required invasive respiratory support.

The assessment of need for respiratory support in our study showed that 52.9% of infants in group 2 and 38.2% infants in group 1 did not require respiratory support during their first day of life. This had significantly changed afterwards: the frequency of infants in group 2 requiring respiratory support was significantly higher compared to group 1 (χ^2=33.12, p<<0.01, r=0.404, 95%CI). Meanwhile, newborns in group 3 became rapidly respiratory independent.

Respiratory support using CPAP showed significant differences between the study groups: in group 3 it was only necessary during the first day of life. For newborns in group 2, the use of CPAP in the first day of life was 34.2% compared with 47.7% in group 1, and increased significantly later, after being transferred to a level III facility (χ^2=36.203, p<<0.01, r=0.321, 95%CI).

TABLE I

Estimated parameters for the methods of respiratory support in preterm infants

		Totals	Premature infants			Univariate analysis
			Group 1 Level III (n=476)	Group 2 Level I, II (n=240)	Group 3 Level III + LISA (n=34)	
Respiratory support in the delivery room	Without	224 (29.87%)	133 (27.94%)	91 (37.92%)	0 (0%)	$\chi^2_{Pearson}$=139.29, p<<0.001* $r_{Spearman}$=-0.642
	CPAP	194 (25.87%)	138 (28.99%)	22 (9.17%)	34 (100%)	
	PPV	56 (7.47%)	41 (8.61%)	15 (6.25%)	0 (0%)	
	Nasal cannula	276 (36.80%)	164 (34.45%)	112 (46.67%)	0 (0%)	
Subsequent respiratory support	Without	320 (42.67%)	193 (40.55%)	127 (52.92%)	0 (0%)	$\chi^2_{Pearson}$=53.86, p<<0.001* $r_{Spearman}$=0.2588
	CPAP	276 (36.80%)	183 (38.45%)	66 (27.50%)	27 (79.41%)	
	CMV-HFV-CMV-CPAP	31 (4.13%)	21 (4.41%)	10 (4.17%)	0 (0%)	
	CMV-CPAP	40 (5.33%)	23 (4.83%)	17 (7.08%)	0 (0%)	
	CPAP-CMV-CPAP	47 (6.27%)	30 (6.30%)	13 (5.42%)	4 (11.76%)	
	CPAP-HFV-CMV-CPAP	36 (4.80%)	26 (5.46%)	7 (2.92%)	3 (8.82%)	
Complications of newborns during admission in the NICU		335 (44.7%)	189 (39.7%)	135 (56.3%)	11 (32.3%)	$\chi^2_{Pearson}$=21.57, p=0.00141*, $r_{Spearman}$=0.489
Deaths in the NICU		90 (12%)	57 (11.9%)	31 (12.9%)	2 (5.9%)	$\chi^2_{Pearson}$=12.39, p=0.034* $r_{Spearman}$=-0.543
		Gestational age				
Subsequent respiratory support	Without	31.5±1.9	31.5±1.9	31.4±2		$H_{Kruskal-Wallis}$=16.24 p<0.01*
	CPAP	30±2.3	29.9±2.2	30.5±2.5	29.3±1.6	
	CMV-HFV-CMV-CPAP	28.1±2.2	27.8±2.2	28.7±2.3		
	CMV-CPAP	28.5±2.7	27.7±2.6	29.5±2.5		
	CPAP-CMV-CPAP	28.5±2.5	28.2±2.2	29.5±3	27.8±1.3	
	CPAP-HFV-CMV-CPAP	29.2±2.2	28.8±2.2	30.4±2.1	29.7±2.1	
		Resuscitation method	No resuscitation	T-piece PEEP	Bag-and-mask PPV	
Subsequent respiratory support	Without		249 (66.76%)	26 (12.44%)	45 (26.79%)	$\chi^2_{Pearson}$=209.98, p<<0.001* $r_{Spearman}$=0.548
	CPAP		95 (25.47%)	119 (56.94%)	62 (36.90%)	
	CMV-HFV-CMV-CPAP		4 (1.07%)	13 (6.22%)	14 (8.33%)	
	CMV-CPAP		6 (1.61%)	19 (9.09%)	15 (8.93%)	
	CPAP-CMV-CPAP		11 (2.95%)	14 (6.70%)	22 (13.10%)	
	CPAP-HFV-CMV-CPAP		8 (2.14%)	18 (8.61%)	10 (5.95%)	

Continuous variables: mean ± standard deviation; categorical variables: number (%) *p-value <0.05 was statistically significant, LISA - Low Invasive Surfactant Administration, CPAP - Continuous positive airway pressure, CMV - Conventional mechanical ventilation, HFV - High-frequency ventilation, PPV - Positive pressure ventilation, PEEP - Positive end-expiratory pressure

Variations between study groups 1 and 2 were found regarding CMV after 7 days of life, with a significantly higher frequency of newborns requiring CMV in group 2 compared to group 1 (χ^2=25.83, p=0.0002, r=0.573, 95%CI).

Respiratory support provided by HFV was frequently found in group 1 neonates within 7 days of life (χ^2=450.81, p<<0.001, r=0.202, 95%CI), while in group 2 it was used more often after the first week.

Our study showed that mechanical ventilation has been particularly used in cases with lower gestational ages, in all study groups (H_{KW}=159.02, p<<0.01, 95%CI).

The multivariate analysis showed that the method of respiratory support throughout the stay in the NICU was significantly influenced by the method of resuscitation (OR=3.57, p=0.0007) and the level of maternity care of the hospital in which the preterm infants are born (OR=2.5077, p=0.0021). GA and birth weight were also important factors that influence the method of subsequent respiratory support, but to a lesser extent (OR=1.67, p=0.0045; OR =2.13, p=0.0028) (tab. II).

Regarding complications in the NICU, the multivariate analysis showed that the method of respiratory support (OR=4.25, p=0.007) and the level of maternity care (OR=3.19, p=0.002) are major predictive factors. Also, the method of resuscitation (OR=2.91, p=0.0014), GA (OR=1.91, p=0.015) and birth weight (OR=1.83, p=0.0012) are also important for the incidence of complications during NICU stay (tab. III).

TABLE II

Multivariate regression analysis - predictive factors for the method of respiratory support

Multinomial Logistics Regression (predictive factors)		Beta	SE	Wald	Sig. p-level	Exp(β) OR	95% CI for Exp(B)	
							Lower	Upper
Subsequent respiratory support	Resuscitation method	4.988	.236	4.190	.0007	3.579	2.571	5.804
	Level of maternity care	3.874	.612	8.251	.0021	2.507	1.314	4.852
	Birth weight	2.932	.367	6.781	.0028	2.137	1.591	4.871
	Gestational age	2.181	.104	5.834	.0045	1.674	1.135	3.783
CI - confidence interval, exp(β)=OR-(odd ratio), SE- standard error								

TABLE III

Multivariate regression analysis - predictive factors for complications of preterm infants in the NICU

LOGISTICS REGRESSION (predictive factors)		Beta	SE	Wald	Sig. p-level	Exp (β) OR	95% CI for Exp(B)	
							Lower	Upper
Complications of newborns in the NICU	Subsequent respiratory support	2.041	1.008	7.167	0.0014	4.252	1.563	5.067
	Level of maternity care	2.873	0.154	6.072	0.0027	3.199	1.217	4.023
	Resuscitation method	3.260	0.155	9.072	0.0078	2.912	1.182	4.697
	Gestational age	1.094	0.287	2.953	0.0156	1.911	1.148	3.223
	Birth weight	1.030	0.134	2.970	0.0128	1.837	1.287	3.514
CI - confidence interval, exp(β)=OR-(odd ratio), SE- standard error								

Mortality in the NICU is significantly influenced by the method of resuscitation (OR=3.74, p=0.002) and of subsequent respiratory support (OR=3.48, p=0.003). Also, the level of maternity care (OR=2.44, p=0.005), GA (OR=1.87, p=0.028) and birth weight (OR=1.9, p=0.025) are highly predictive for mortality in the NICU (tab. IV).

TABLE IV
Multivariate regression analysis - predictive factors for mortality of preterm infants in the NICU

LOGISTICS REGRESSION (predictive factors)		Beta	SE	Wald	Sig. p-level	Exp(β) OR	95% CI for Exp(B)	
							Lower	Upper
Mortality in the NICU	Resuscitation method	8.826	0.954	10.133	0.0028	3.746	2.185	6.729
	Subsequent respiratory support	5.407	0.090	11.378	0.0034	3.484	2.584	5.338
	Level of maternity care	3.961	0.692	7.146	0.0055	2.446	1.302	3.231
	Birth weight	2.705	0.918	5.453	0.0256	1.909	1.030	3.098
	Gestational age	1.801	0.773	5.607	0.0283	1.873	1.162	2.516
CI - confidence interval, exp(β)=OR-(odd ratio), SE-standard error								

DISCUSSION

Respiratory distress is one of the most common conditions encountered in preterm infants. Due to significant progress in medical technology, most of them will achieve full health with the assistance of varying levels of breathing support currently found in the NICU.

Currently, the most common form of respiratory support used in the delivery room is early CPAP with the aid of the T-piece resuscitator. This is a minimally invasive method that can provide a suitable PEEP for lung recruitment in a premature infant, thus maintaining an appropriate residual lung capacity and has been proven to be superior to positive pressure ventilation (10). However, this method is limited to infants with mild-to-moderate respiratory distress and spontaneous breathing and is not usually suitable for severe respiratory symptoms.

The early use of CPAP is associated with a significant reduction in the need for intubation (11). Various studies have pointed to the superiority of CPAP, with PEEPs of 5-7 cm H_2O to bag-and-mask ventilation in the delivery room (12). CPAP is also associated with lower rates of need for exogenous surfactant, respiratory failure and mortality (13). Surfactant administration is a costly intervention with limited availability. When CPAP is available in the delivery room, surfactant is no longer indicated for the prevention of RDS, only as early selective treatment once the symptoms of RDS occur (14). Our study also showed that the use of early CPAP in infants born in our level III hospital (groups 1 and 3) is associated with lower rates of later invasive mechanical ventilation. Unfortunately, not all level I and II maternity hospitals in the Republic of Moldova and Romania are equipped with T-piece resuscitators capable of providing CPAP in the delivery room. Also, the staff is reluctant to use such modern devices, despite their proven benefits.

Even when administered by an experienced physician, bag-and-mask ventilation

used during neonatal resuscitation can have deleterious effects, both due to the inadequacy and inconsistency of delivered pressures and volumes and the high concentration of oxygen that is used when gas blenders are unavailable (15).

However, the use of CPAP in the delivery room may fail in some infants, especially those of extremely low birth weight, as many of these require further endotracheal intubation followed by mechanical ventilation. This can probably be explained by the unnecessarily delayed surfactant therapy in these infants. In our study, about 23% of neonates required CMV after CPAP failure and some of them even required rescue HFV. Also, infants who fail early CPAP exhibit more complications, such as pneumothorax, chronic lung disease and intraventricular hemorrhage, and have a higher mortality compared to those in which early CPAP is used successfully (16).

Neonatal Intensive Care Units are increasingly adopting a gentle approach to ventilator support in preterm infants as standard care. Minimally invasive surfactant administration is part of this approach, as it does not require sedation, minimizes airway injury and avoids placing positive pressure ventilation on an immature lung. Moreover, it has been proven to be as effective as the moderately invasive technique of surfactant therapy (17). In our study, group 3 infants, who received surfactant through the LISA method, had a lower mortality rate, despite their lower GA.

The outcome of preterm infants requiring respiratory support depends on a multitude of factors, such as GA, the use of surfactant, the method, the duration or the concentration of oxygen used during respiratory support (8). In our study, the most important factor, for both the method of subsequent respiratory support and mortality is the level of maternity care of the hospital in which the infants are born. Our data are similar in this aspect to the findings of Marlow et al. (18). The level of care also impacts the methods used during resuscitation and the available technologies required for the long-term management of preterm infants, especially ELBW neonates.

The availability of new methods and techniques of management for various neonatal conditions is a crucial factor for the improved survival and outcome of preterm infants. Less Invasive Surfactant Administration is a somewhat revolutionary treatment method in neonatology with emerging benefits, such as pain reduction, increased comfort and low risk of complications due to intubation and mechanical ventilation (19). Our study also showed a significantly lower rate of complications and mortality in infants treated with LISA, compared to the other two groups.

CONCLUSIONS

The treatment of RDS in the preterm infant remains a challenge for neonatologists. Our study showed that due to the limited availability of modern therapeutic methods, the morbidity and mortality of preterm infants born in low-resource settings (level I and II maternity hospitals) are higher. Early use of non-invasive respiratory support such as CPAP is highly beneficial in such cases and leads to fewer and less extensive admissions to the NICU, less oxygen use, less lung damage caused by mechanical ventilation, and fewer complications. Also, surfactant administration by non-invasive methods, even when performed in extremely preterm infants is strongly correlated with a lower rate of complications and mortality.

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THE UTILITY OF SERIAL EVALUATION OF cTnI AND BNP IN EARLY DETECTION OF ANTHRACYCLINE-INDUCED CARDIOTOXICITY IN CHILDREN

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THE UTILITY OF SERIAL EVALUATION OF cTnI AND BNP IN EARLY DETECTION OF ANTHRACYCLINE-INDUCED CARDIOTOXICITY IN CHILDREN (Abstract): **Aim.** Early detection of chemotherapy cardiotoxicity in pediatric patients by using sensitive and specific methods. **Material and methods**: The study group included 34 patients with malignancies, aged between 3-18 years. We evaluate the patients through history, physical examination, electrocardiogram, 2D/ Doppler echocardiography, cardiac biomarkers - BNP (B-type natriuretic peptide) and cTnI (cardiac troponin I), at baseline and subsequently, in dynamic. **Results:** Cardiotoxicity was revealed in 24 patients (70.5%), with clinical manifestations in 2 cases (5.8%). Echocardiographic changes were found in 26 cases (76.4%) (16 cases-47% with systolic dysfunction, 26 cases-76.4% with diastolic dysfunction), electrocardiographically changes in 73.5% of cases. Elevated plasma BNP was found in 18 patients-52.9%, and cTnI levels were normal in all patients (cut-off 0.04ng/mL). Elevated levels of BNP have been correlated with the cumulative dose of anthracyclines and therapeutic protocol received. **Conclusions:** The study may give solutions for the reduction and prevention of cardiotoxicity, even when are used higher doses of anthracyclines with a higher rate of healing while improving the quality of life of survivors. **Keywords**: CARDIOTOXICITY, CYTOSTATICS, CHILD, DIAGNOSTIC, ANTHRACYCLINE.

Anthracyclines (ANT) are one of the most effective and commonly used antineoplastic agents in treatment of cancer in children. Their use has positively influenced the outcome, increasing substantially the survival rate. However, this benefit is encumbered by a high rate appearance of anthracycline-induced cardiotoxicity (1).

Over the years, many researchers have used various methods to assess the cardiotoxicity, to an early highlight and initiation of preventive measures. Cardiotoxicity detection by imaging methods (echocardiography, radio-nuclear angiography) has major limitations, emphasizing late left ventricular dysfunction and allowing objectifying the toxic effects only when is enough damage on the heart to cause functional disorders. Recent studies on cancer patients showed that elevated levels of B-

type natriuretic peptide (BNP) and troponin I(cTnI) may serve as a predictor of infraclinical phase of cardiotoxic effect of chemotherapy, allowing the stratification of cardiac risk in a very early phase, long before heart function decreasing and symptoms appearance, when many of preventive therapeutic strategies are effective (2, 3).

In this article, we expose the results of a prospective study which aimed to assess the sensitivity of cardiac biomarkers (BNP, troponin I) in the early detection of cardiotoxicity and to establish possible strategies in identifying patients at elevated risk of developing anthracyclines cardiomyopathy.

MATERIAL AND METHODS

The study group included 34 patients, 16 girls and 18 boys, aged between 3 and 18 years, admitted in a pediatric oncology-hematology clinic for one year period. The children have followed therapeutic protocols for various malignancies that included also anthracyclines.

The study was approved by the ethics committee of our hospital. Patients were included in the study only after informed consent was obtained in writing, from legally belongs of the children.

The median dose of anthracyclines (doxorubicin) administered during the study was 226 mg/m², with the minimum-maximum range of 50–920 mg/m². Additional to anthracycline treatment, patients received other cardiotoxic chemotherapy, which increased the risk of cardiotoxicity.

Cardiac assessment included clinical examination, cardiothoracic radiography (with cardio-thoracic index calculated), electrocardiogram (EKG), echocardiography (ECHO) 2D/Doppler and biochemical markers dosing (cTnI, BNP). No patient presented cardiac disease prior to the enrollment in this study. No cardioprotective treatment was administered during the study, except for two patients who developed heart failure, in which specific treatment required.

Echocardiographic evaluation was performed by a single pediatric cardiologist. Echocardiographic evaluation was performed before treatment and, subsequently, after each cycle of chemotherapy that included ATC, at 6 and 12 months after initiation of chemotherapy. The followed parameters were: ejection fraction (EF) and shortening fraction (SF) for systolic function, the peak early filling (E-wave) and late diastolic filling (A-wave) velocities, the E/A ratio, isovolumic relaxation time (IVRT), peak systolic (S) velocity, peak anterograde diastolic (D) velocity, the S/D ratio, isovolumic contraction time(ICT) for diastolic function, mixed parameters for systolic and diastolic function and myocardial performance index- Tei index. The average number of echocardiography for each patient was 6, with certain intervals: at baseline and then after each of the first 3 cycles of anthracycline, and additionally as needed.

The assessment of specific BNP and cTnI biomarkers was done at baseline (before initiating therapy) and in dynamic, depending on the time of administration of ATC - before and after administration of cytostatic infusion-, for a period of 12 months. The method used for detection was Microparticle Enzyme Immunoassay (MEIA). The device used was ® System AxSYM-Abbott Laboratories. We used the cut-off values suggested by the manufacturer: quantitative determination of cardiac troponin I - cTnI in serum - cut-off value troponin I < 0.04ng/mL, respectively quantitative determination of BNP in plasma - BNP cut-off value 100pg/mL. Values obtained from analysis of plasma BNP and

cTnI are controlled using the controllers both during calibration and proper analysis (double-blind).

Statistical analysis was performed with *SPSS*, version 15.0. Descriptive and inferential statistical analysis was done. Correlations between variables were calculated using the correlation coefficient test of Pearson's. Regression analysis was also effectuated. Statistical significance was set for a p-value <0.05.

RESULTS

Characteristics of the study group are shown in Table I. From the study group, 27 patients were still alive at the time of study completion; death of seven patients was determined by the evolution of the underlying disease and not by a possible secondary damage of heart function.

TABLE I.
Characteristics of the study group

Parameters		Number	Percent %
Sex	female	16	47.1%
	male	18	52.9%
Age	< 6 years	16	47.1%
	6-10 years	8	23.5%
	11-18 years	10	29.4%
Diagnostic	ALL	18	52.9%
	NHL	6	17.6%
	Sarcom	2	5.9%
	AAML	2	5.9%
	HL	5	14.7%
	others	1	2.9%
Echocardiographic changes	yes	26	76.5%
	no	8	23.5%
Decreased EF >10%	yes	18	52.9%
	no	16	47.1%
Decreased SF >10 %	yes	23	67.6%
	no	11	32.4%
EKG changes	yes	25	73.5%
	no	9	26.5%
BP changes	yes	6	17.6%
	no	28	82.4%
Cardiotoxicity	no	10	29.4%
	infraclinical	22	64.7%
	clinical	2	5.9%

The cardiotoxicity manifestations were grouped into two: 1) clinical type – with clinical signs and symptoms of heart failure (dyspnea, fatigue) and changes in paraclinical investigations (echocardiogram, EKG, cardiac biomarkers) and 2) subclinical - asymptomatic patients, but with significant changes highlighted in systolic or diastolic function. 22 cases (64.7%) were asymptomatic and 2 cases of heart failure occurred (5.9%). Other cardiological changes during evaluation were variations in BP values (6

cases), electrocardiographic changes (73.5% cases). EKG alterations were represented by minor changes in left ventricle repolarization, QT prolongation, supraventricular tachycardia (24 patients) and 1 case of AV block grade III.

Echocardiographic changes were seen in 76.5% of cases; most of them were transient, occurring immediately after taking cytostatic, later return to normal baseline. Decrease by> 10% of the EF or SF was found in 53%, respectively, 67.7%of cases, although the actual value of the FE and/or SF has not dropped below the lower limit of normal. In 1 case EF was <40% and 14 cases SF <28%. This is considered more representatives in appreciation of cardiotoxicity events. Changes in diastolic function were more common, 26 cases and Tei index increased was found in 14 cases (tab. II).

TABLE II.
Cardiac modification after ATC

Cardiac Assessment	Cardiac modification	Number	Percent %
Echocardiography	normal	8	23.5
	decreased EF by > 10%	18	52.9
	decreased SF by>10%	23	67.6
	modification of LV diastolic function	26	76.5
	modification of LV systolic function	16	47.1
EKG	normal	9	26.5
	modified	25	73.5
BNP levels	< 100 pg/mL	16	47.1
	100 - 200 pg/mL	6	17.6
	200 - 500 pg/mL	9	26.4
	> 500 pg/mL	3	8.8
BNP categories	A- normal	16	47.1
	B - transient increase further normalization	12	35.2
	C- increased	6	17.6
cTnI levels	< 0,04 ng/mL	34	100
	> 0,04 ng/mL	0	0
BP	normal	28	82.4
	high	6	17.6
Cardiotoxicity	no	10	29.4
	infraclinical	22	64.7
	clinical	2	5.9

BNP's elevated levels in plasma, above the cut-off of 100 pg/mL, were funded at 52.9% of cases. According to the BNP values changes during the study, they were divided into 3 groups: group A normal levels, group B - BNP levels were elevated, but later returned and were maintained in the normal range, and group C- BNP levels have remained elevated. The increased plasma levels of BNP were positively correlated with the administered dose of anthracyclines and type of chemotherapy followed protocol (fig.1, 2).

Serum cTnI levels did not exceed the cut-off value of 0.04 ng/mL in any patient in the study group, both baseline and in dynamics. Only 3 patients had cTnI value of 0.04 ng/mL, considered be the normal value.

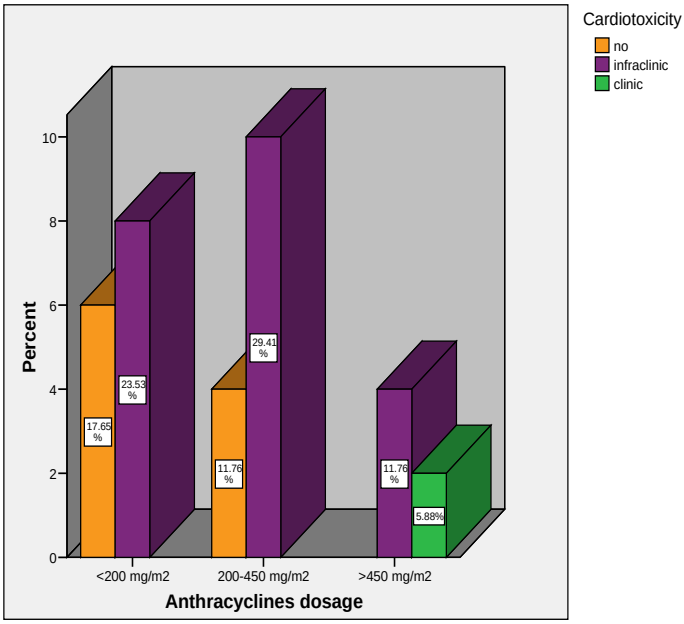

Fig.1. Correlations between the cumulative dose of anthracycline and emergence of cardiotoxicity

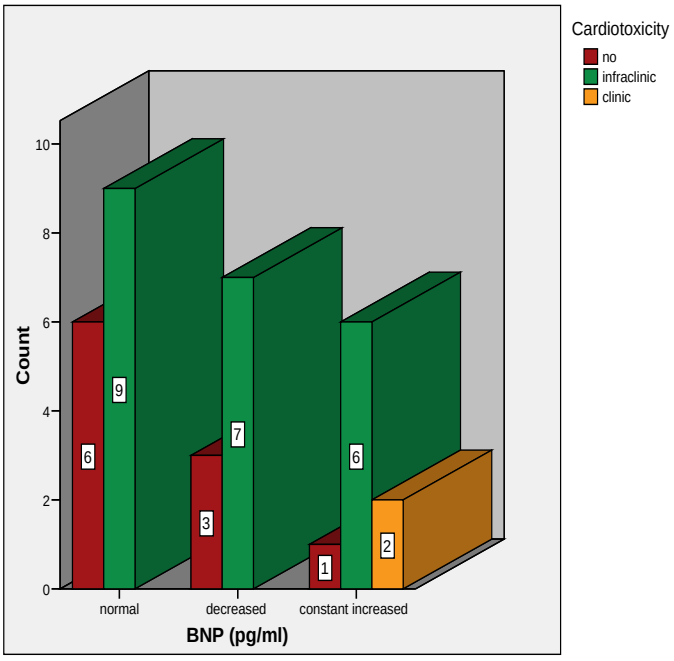

Fig. 2. Correlations between the BNP dynamics and emergence of cardiotoxicity

In the current study, cardiotoxicity was positively significant correlated decreased EF> 10% (r = - 0.457, p = 0.007), decreased SF >10%(r = -0.508, p = 0.002), pathological BP (r = - 0.341, p = 0.048), higher cumulative anthracycline dose (r = 0.505, p = 0.02) and higher serum BNP levels (r = 0.465, p = -0,006).

Scatterplot diagram of the relationship between the cumulative dose of anthracyclines and BNP value suggested a positive linear correlation. Accurately predicting the BNP levels depending on the cumulative anthracyclines dose is made by the relation:

Y = - 49 657 (1,099 x) in which x = the individual cumulative dose of anthracyclines and Y = the best predictive value for the individual BNP.

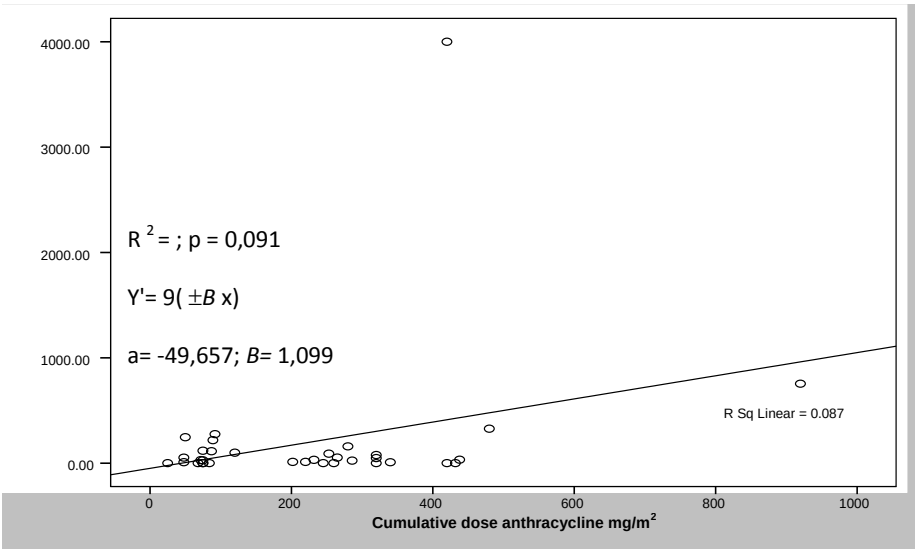

Fig. 3. Linear regression analysis between BNP levels and cumulative anthracycline dose

DISCUSSION

The study highlights the presence of anthracycline cardiotoxicity in pediatric oncologic patients with a considerable 70.6% incidence. The data obtained in this study correlate with the results referenced studies published in the literature (2, 3, 4). It stands high percentage of infraclinical cardiotoxicity (64.7%). Many of the clinical manifestations are nonspecific and may be misinterpreted. They are routinely assigned to the malignant disease, leaving laboratory investigations to early highlight the changes (2).

The first echocardiographic changes in our patients were those of diastolic function subsequent appeared and the systolic function. We did not found positive statistically significant correlation between electrocardiographic changes and the emergence of cardiotoxicity; this result is consistent with the results reported by other authors (5, 6, 7, 8).

As shown in other studies (5, 6, 7, 8), paraclinical method of choice is to evaluate serum levels of BNP and cTnI, that correlates with echocardiographic assessment. Evaluation of serum BNP and cTnI is a non-invasive, easily reproducible, with substantially reduced cost compared echo-

cardiogram or other imaging method.

The results obtained in the present study confirm the data reported in the literature, that there is a significant relationship between increasing plasma BNP levels and cardiac dysfunction both in symptomatic patients, asymptomatic and treated with anthracyclines.

Clinically asymptomatic patients, but which remained elevated BNP's during the entire period of the study, can be considered at elevated risk of developing anthracycline cardiomyopathy. In a relatively small number of cases were highlighted elevated BNP levels prior to anthracyclines treatment initiation which may suggest that there are other factors that influence myocardial wall stress (anemia, tachycardia), but these factors were not seen in the time in the group patients. The great marginal significance prognosis correlation between anthracyclines cumulative dose and higher levels of BNP ($r = 0.295$, $p = 0.091$), in terms of a strong relationship between elevated BNP and cardiotoxicity, as well as a strong relationship between higher anthracyclines cumulative dose and cardiotoxicity, suggests that other conditions which influence the BNP values deserve to be considered for further studies. In some patients, changes in biomarkers and echocardiography were transient. This emphasizes the need of monitoring initial and in dynamic status, to confirm whether patients with subclinical changes will develop cardiomyopathy over time and where is appropriate to start a cardioprotective treatment

The results on studying serum cTnI levels were according to the data obtained from other important studies in the literature (2, 3, 4), but larger studies are needed to establish whether this biomarker can be used to predict cardiotoxicity.

It is important to note that there is an individual variability in the relation between cumulative dose - cardiotoxic response, so efforts aimed to prevent cardiovascular morbidity due to anthracyclines should be channeled more on early detection of cardiotoxic effects than in chemotherapeutic protocols changes (9, 10).

CONCLUSIONS

Early detection of cardiotoxicity after anthracycline therapy and highlighting the patients at risk is a goal of many researchers in the field. Our results showed that there is a significant relationship between increasing plasma BNP levels and cardiac dysfunction both in symptomatic and asymptomatic patients treated with anthracyclines. BNP assessment at the initiation of treatment and subsequently in dynamics is useful in highlighting patients at increased risk of developing cardiotoxicity, and to establish therapeutic strategies to prevent installation of severe heart damage, improving the overall outcome of these patients. Studies are needed on large groups of patients, to have a proper perspective of the data obtained.

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NEWS

PATHOLOGY OF ISCHEMIC OPTIC NEUROPATHY

Ischemic optic neuropathy (ION) refers to infarction of any portion of the optic nerve from the chiasm to optic nerve head. Clinically, it is divided into anterior and posterior forms by the presence or absence of swelling of the optic nerve head, respectively. Both forms are further classified into arteritic when secondary to vasculitis and nonarteritic when caused by other entities. Nearly all cases of arteritic ION are due to giant cell arteritis. The pathogenesis of nonarteritic anterior ION is unsettled, but the clinical diagnosis encompasses several distinct, albeit uncommon, sources of injury including thromboembolism, systemic hypotension, and atherosclerotic vascular occlusion. Systemic hypertension, diabetes mellitus, and sleep apnea have been associated with nonarteritic anterior ION, but in most cases no direct underlying cause for vascular insufficiency is found. Occasionally referred to as ‘‘common’’ or ‘‘idiopathic,’’ nonarteritic anterior ION tends to affect optic discs with a small-diameter scleral canal and optic cup, the socalled disc at risk. Based on conventional use of the term, ischemic optic neuropathy refers to the acute stages of ischemic injury before optic atrophy develops. Ischemic optic neuropathies are common clinical conditions, with the incidence of nonarteritic anterior ION between 2.3 and 10.3 per 100 000 population per year. Despite the overall prevalence of ION clinically, eyes with acute ION are rarely encountered in the pathology laboratory, and then usually in the context of autopsy as an incidental finding, or in specimens removed surgically for other primary reasons (Patel HR, Margo CE. Pathology of Ischemic Optic Neuropathy. *Arch Pathol Lab Med*. 2017; 141:162–166).

Doina Butcovan

THE ROLE OF KINESIOTHERAPY IN THE MANAGEMENT OF OSTEOPOROSIS DURING POSTMENOPAUSE

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THE ROLE OF KINESIOTHERAPY IN THE MANAGEMENT OF OSTEOPOROSIS DURING POSTMENOPAUSE (Abstract): Bone functional adaptation is influenced by many risk factors, such as the lack of estrogen during menopause, surgical menopause, premature menopause, a low weight to waist ratio, trauma, excessive alcohol use, tobacco, coffee, certain drugs (corticosteroids, anticonvulsants), food deficiencies (especially low intake of calcium), immobilization, sedentary lifestyle, hereditary and racial factors, various diseases (hyperthyroidism, diabetes). **Material and methods**: The study group included 115 postmenopausal women treated in the 1st Rheumatology Clinic of Clinical Rehabilitation Hospital of Iasi between 1 June 2014 and 31 March 2016. Data gathering, processing and analysis were done by epidemiological, statistical and mathematical methods, using Epi Info 5, a software provided by the Epidemiology Program Office, Centers for Disease Control and the Global Programme on AIDS, World Health Organization. **Results and discussion**: The selection of patients was made based on a diagnosis of established postmenopausal osteoporosis (the diagnosis was made following clinical and radiographic exams and dual energy X-ray bone densitometry). The selected female patients had a bone mineral density (BMD) of less than - 2.5 SDs, dorsal lumbar spine pain and limited joint mobility due to paravertebral muscle contraction. Patients in the study group received kinesiotherapy (local electrotherapy, diadynamic currents, interferential current, etc.). **Conclusions**: Kinesiotherapy was shown to play a major role in rehabilitation, relieving pain, correcting functional disorders of spine (spine mobility, defective spine statics, altered muscle balance), re-educating gait and balance and preventing the risk of falling. **Keywords**: OSTEOPOROSIS, KINESIOTHERAPY, ASSESSMENT, TREATMENT.

Postmenopausal osteoporosis occurs in the first 10 years of lack of menstruation and is caused mainly by estrogen deficiency and other endocrine, metabolic and inherited disorders (1).

Postmenopausal osteoporosis is associated with physiological bone loss (occurring at around the age of 50 years), when women lose approximately 35-40% of cortical bone and 55-60% of trabecular bone, while men lose about 1/3 less (1, 2).

The risk of osteoporosis is mainly determined by two factors: bone mineral density at the beginning of menopause and the speed of bone loss after menopause, dependent on the rate of bone loss and on the years spent in post menopause (3, 4).

The present study aims to standardize an

effective kinesiotherapy program and to assess its effectiveness on bone mineral density, pain, spinal functionality, stability, coordination, balance and gait, and on the quality of life in osteoporosis patients. Paraclinical methods (bone densitometry), clinical analytical methods (joint and muscle assessment), semi global (tests for balance and gait) and global approaches were used (3).

MATERIAL AND METHODS

The study group included 115 postmenopausal women treated in the 1st Rheumatology Clinic of Clinical Rehabilitation Hospital of Iasi between 1 June 2014 and 31 March 2016.

The 115 cases were divided into 4 groups: 16 female patients (13.91%) treated with estroprogestatives, 31 female patients (26.95%) treated with ibandronic acid, 48 female patients (41.75%) treated with alendronic acid and 20 female patients (17.39%) treated with calcium and vitamin D. The results of the therapy were assessed by measuring L4 bone mass density (BMD), the total BMD before the treatment and 2 years after and by comparing T and Z scores at the same time with student's *t*-test. Increases or values of BMD, T and Z scores and the number of female patients who showed an increase or decrease in bone mass density following the four therapeutic approaches were also expressed as a percentage (fig. 1).

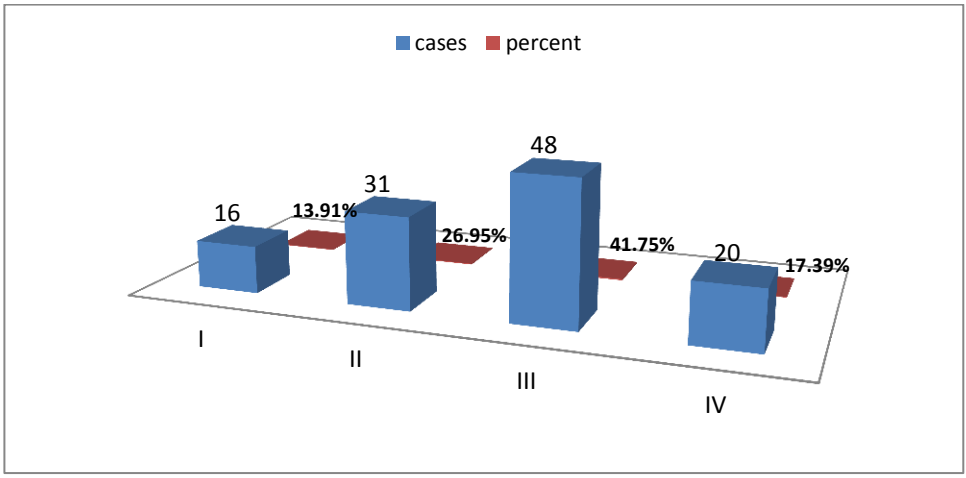

Fig. 1. Distribution of the 115 female patients in the four study groups

RESULTS AND DISCUSSION

According to their residence, most of the female patients were of urban origin (63%) as compared with the female patients of rural origin (37%). This difference is explained by better access to information and medical care in women from urban areas.

The medical history of each female patient provided relevant data for a positive diagnosis of osteoporosis. The patients' history showed major risk factors for osteoporosis.

Five age groups were identified at the beginning of the treatment (under 41 years old, 41-50 years old, 51-60 years old, 61-70 years old and 71-80 years old). The highest number of cases (48, 41.75%) of the group treated with alendronic acid were

found in patients aged between 51 and 60, followed by the age group 61-70. There were more cases in the age group 51 - 60 and 61-70 of the group treated with bisphosphonates. It was found that a slightly younger age of onset was associated with early menopause onset and earlier initiation of therapy.

There is a balance between bone resorption and bone formation in both sexes between the age of 30 and 35. After this age, the bone mass tends to decrease at a rate of 0.5-1% / year. At all ages, women have less bone mass than men, leading to higher rates of osteoporosis and higher risk of fractures with age (5, 6) (tab. I).

TABLE I

Distribution of the four age groups at the beginning of treatment

Age groups (years)	No. of cases	%	Therapy
41-50	16	13.91	Estroprogestatives
51-60	31	26.95	Ibandronic acid
61-70	48	41.75	Alendronic acid
71-80	20	17.39	Calcium and vitamin D

At the beginning of treatment, the patients' age was 31-54, with an average age of 46.67 +/- 7.553 years, but women treated with alendronic acid had the highest average age of onset most advanced (an average age of 61.81 years). This can be explained by the fact that the alendronic acid was administered in cases with lower bone mass density, typically at a T-score of less than -2.5 SDs and most often at a T-score between -3 SDs and -3.5 SDs. Alendronic acid was used as a cure rather than a preventive measure.

The age of first menstruation was not significantly different between the groups treated with calcium and vitamin D, alendronic acid and ibandronic acid; the group treated with estroprogestatives showed a two-point higher average without a plausible explanation.

The age of the last period was significantly lower (an average age of 41.51 years) in the group treated with estroprogestatives. As the age of menopause onset, defined as the age of the last period, was significantly lower in the group of women treated with estroprogestatives, this therapy was favored by patients with early menopause. Younger women accept treatment with estroprogestatives mostly for subjective reasons, including the need to achieve optimal vaginal trophicity and to combat vegetative disorders caused by estrogen deficiency. These patients disregard possible metabolic cardiovascular side effects of estroprogestatives and cancerphobia is less common in this group.

The duration of estrogen deficiency in the four groups cannot be compared with one another. Women treated with estroprogestatives had a duration of estrogen deficiency of 5.13 +/- 4.037 years. This is directly related to the fact that this therapy was administered early after the menopause. The therapy with ibandronic acid was accepted due to the fact that there is no potential risk of inducing estrogen-dependent neoplasia and breast diseases during therapy with estrogen receptor modulators. The alendronic acid was introduced after a long period of estrogen deficiency (15.81 +/- 8.314 years) in women with a severe decrease in bone mass density, but who were generally older. The female pa-

tients with background therapy with calcium and vitamin D were older women who did not agree to hormonal treatments or had a digestive intolerance.

The anthropometric measurements of waist and weight showed close values as average, minimum and maximum. Overweight female patients have a lower exercise capacity, as the excess weight overloads the joints with degenerative changes, leading to various conditions that worsen the health status of female patients. Height is one of the factors that cannot be influenced.

Overweight can aggravate spine deformities and stress the major joints, especially hips and knees. That is why it is important to maintain a healthy weight through a balanced diet and to achieve good muscle tone through regular physical exercise in osteoporosis patients.

The group treated with estroprogestatives had lower height and weight, due to the fact that some patients were suffering from Turner syndrome (reduced height and weight). The study groups treated with calcium and vitamin D, alendronic acid and ibandronic acid were slightly overweight.

Physical activity performed by the patients was mostly moderate, as most women were sedentary and before retirement most of them had sedentary jobs.

Although osteoporosis has a long asymptomatic period, vertebral pain was a common symptom and the main reason for hospitalization.

The required parameters (BMD of the lumbar vertebra = L4 BMD; total BMD; the T-score of the 4th lumbar vertebra - TL4; total T-score - Tt; total Z score - Zt) were used *for analyzing bone mass density*. These parameters have also been investigated at the beginning of the treatment. There was no significant difference between the group treated with calcium and vitamin D and the group treated with estroprogestatives; the group treated with ibandronic acid had a slightly lower L4 BMD; the group receiving alendronic acid had a significantly lower BMD.

Total BMD was correlated with L4 BMD at the beginning of the treatment and it was minimal in the group treated with alendronic acid. It had intermediate values in the group treated with ibandronic acid and the highest averages in the groups receiving calcium and vitamin D, and estroprogestatives, respectively. Women in all study groups had a T-score of less than -2.5 SDs in the L4 vertebra, confirming the diagnosis of osteoporosis. The group treated with alendronic acid had the most severe score (-3.384).

The total T-score at the beginning of the treatment was comparable in the groups treated with estroprogestatives and calcium with vitamin D. It was less severe than in women treated with alendronic acid and ibandronic acid. The correlation between the therapeutic choice and the L4 T-score and the total T-score showed that either alendronic acid or ibandronic acid was preferred when this score showed a higher risk of osteoporosis and a more severe form of osteoporosis (7, 8).

The Z-score shows a negative difference between a subject in the study group and a subject of the same age in terms of bone mass density. It was found that women had a more severe bone mass loss in relation to the average of the same aged female patients as compared with those treated with calcium and vitamin D (7, 9).

It was found that women treated with alendronic acid and ibandronic acid, respectively showed a more severe deficit in BMD in relation to the average of the same aged women as compared with those treated with calcium and vitamin D.

The results showed that the values of the total BMD and of the T-score tend to be lower in patients who are older at the beginning of the treatment. The same applies if the period of estrogen deficiency is prolonged: at the beginning of the treatment, L4 BMD, total BMD and total T are lower when there has been a longer duration of estrogen deficiency.

The results of therapeutic regimens were assessed after two years by analyzing specific parameters. L4 BMD values had a moderate increase in all study groups. The highest increase was found in the group treated with alendronic acid. A low increase occurred in groups receiving calcium and vitamin D, ibandronic acid and estroprogestatives.

The L4 T-score has improved in groups treated with calcium and vitamin D, ibandronic acid and estroprogestatives, with a significant increase in the group treated with alendronic acid. The total T-score has improved in all study groups. The highest increase of the Z-score was found in the group treated with alendronic acid. More moderate increases were seen in the other study groups.

Physical therapy aimed at achieving pain relief, spine mobilization, toning paravertebral muscles, correcting the posture and regaining the ability to exercise.

Local electrotherapy was used for pain relief and for reducing muscle contracture, which is the main cause of pain. The transverse galvanization had an antalgic and anticontractile effect. It is important that the size of the electrodes entirely covers the area to be treated, the intensity of the current fits the threshold and the duration of treatment is long enough (more than 20 minutes).

Diadynamic currents act therapeutically through muscle contractions induced by the excitomotor system. Here too, it is important to adjust the intensity of the current and the duration of treatment for a therapeutic effect.

The interferential current in static and particularly in dynamic mode with excitomotor frequencies is essential in preparing the muscles for the sessions of kinesiotherapy.

Kinesiotherapy is not synonymous with therapeutic exercises. Its scope is more limited, both in terms of resources needed and goals (12).

The use of various exercises, techniques or methods for the functional rehabilitation was based on the knowledge of the functional deficit through a joint and muscle assessment. This provided the angle of movement, muscular endurance and strength, changes in prehension and gait and a thorough physical examination, leading to a scientific validation of the kinesiotherapy protocol.

The efficacy of the rehabilitation treatment and the results were assessed through measurements with goniometer, centimeter and a series of tests that could estimate the range of motion in the damaged joints. Balance and gait were also assessed by using the Tinetti scales.

As in other studies (12), patients in our study groups received the following procedures: re-education of posture and correction of the alignment of body segments (postural disorders of the lumbar spine: kyphosis, lordosis, scoliosis) through self-correcting exercises, muscle stretching for contractured muscle groups, the use of lumbar orthosis, the increase in muscle strength and endurance, improved mobility of lumbar spine through extension exercises, stretching exercises for the muscle groups with a tendency to contraction-retraction: pectoral muscles paravertebral

muscles, the iliopsoas muscles; improvement of balance and coordination; effort training through aerobic exercises, walking, treadmill exercises, ergometer bicycle, jogging, sports, rhythmic gymnastics; respiratory re-education.

Similar to the results in the literature (11, 13, 14, 15) and based on a comparison of data from initial measurements with final measurements and with the control group, we have found a major improvement both in joint range of movement and in muscle strength. The assessment of pain showed a positive outcome both in the control and in the study group.

BMD assessment showed no statistically significant differences compared to the beginning of treatment, but the positive outcome of female patients (joint mobility, muscle strength, improved quality of life, decreased risk of fracture) leads to the conclusion that, even if kinesiotherapy does not replace medication, it may bring significant benefits as additional therapy.

CONCLUSIONS

Our study shows the importance of early detection of risk factors for osteoporosis, as a key element in diagnosing and in adopting a healthy lifestyle.

The results of our study confirm that kinesiotherapy plays a major role in the rehabilitation of patients with postmenopausal osteoporosis, as it relieves pain, it manages spinal functional disorders (spine mobility, defective spine statics, altered muscle balance) and it re-educates gait and balance, preventing the risk of falling.

Rehabilitation and kinesiotherapy must be adapted to each patient, considering individual needs and associated comorbidities; the exercise protocol should be varied, in order to gain patient's interest, adherence and active involvement.

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NEWS

SHORT- AND LONG- TERM RESULTS OF RADIAL ARTERY AND SAPHENOUS VEIN GRAFTS IN THE RIGHT CORONARY SYSTEM: A PROPENSITY- MATCHED STUDY

Cardiovascular disease is the leading cause of death worldwide attributed with more than 31% of global deaths (17.5 million people/year) and an estimated 7.4 million deaths due to coronary artery disease (CAD) alone. Coronary artery bypass grafting (CABG) is nowadays one of the most frequent surgical interventions in Europe (between 18 and 91 per 100.000 inhabitants) and according to both European and American societies' guidelines, is associated with an increase in quality of life and survival in patients with unprotected left main (or equivalent) and multi-vessel disease but the ideal grafting technique has not been established. The research group led by dr. Shohei Yoshida aimed to identify the optimal graft for the right coronary territory. Their study evaluated the short- and long-term outcome of radial artery (RA) grafts and saphenous vein grafts (SVGs) to the right coronary artery through propensity score analysis. Superior survival was noted in the unmatched RA group, but after propensity-score matching for late outcomes analysis there were identified 91 patient pairs similar in the two groups. Graft failure did not correlate with overall mortality, but displayed a strong correlation with cardiac events in all patients (RA and SVG). The predictors of graft occlusion in the RA group were mild proximal stenosis and low indexing glomerular filtration rates for body surface area, whereas those in the SVG were female gender and off-pump coronary artery bypass grafting. The authors concluded that there were no significant differences in long-term outcomes between the RA and SVG groups but predictors of graft occlusion differed between the two groups. Renal dysfunction in particular impaired RA patency, emphasizing the importance of careful graft selection for preventing long-term cardiac events. (Yoshida S, Numata S, Tsutsumi Y, Monta O, Yamazaki S, Seo H, et al. Short- and long-term results of radial artery and saphenous vein grafts in the right coronary system: a propensity-matched study. *Surg Today* 2017; 47(3): 335-343)

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ETHIOPATOGENIC HYPOTHESES IN LICHEN PLANUS

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ETHIOPATOGENIC HYPOTHESES IN LICHEN PLANUS (Abstract): Lichen planus is a mucocutaneous inflammatory dermatosis characterized by a papular rash; the disease is self-limited, has several clinical subtypes and follows a chronic or subacute clinical course. This article presents some etiological hypotheses: stress, genetic predisposition, systemic diseases, viral infections, materials used in dentistry. Also, medicines or contact allergens can cause lichenoid reactions, which are the main differential diagnosis. Autoimmune hypothesis can be supported by the association with other autoimmune diseases, such as ulcerative colitis, alopecia areata, vitiligo, dermatomyositis, morphea, lichen sclerosus, myasthenia gravis. This disease seems to be mediated through an antigenic mechanism of cytotoxic T lymphocyte activation, and production of proinflammatory cytokines, cascade of events that causes apoptosis of basal keratinocytes. A good understanding of the pathogenesis, clinical presentation and early diagnosis of lichen planus is critical in determining the appropriate therapeutic management. This present article aims to present and discuss the various etiopathogenetic concepts of lichen planus. **Keywords**: LICHEN PLANUS, ETIOLOGY, PATHOGENESIS.

Lichen planus is a noncontagious pruritic, papular inflammatory dermatosis of unknown etiology mainly affecting middle-aged adults (1).

The term "lichenoid" is used by clinicians to describe a flat-topped, glossy, papular lesion resembling lichen planus, or by histopathologists to describe a type of tissue reaction mainly consisting of basal cell liquefaction and an infiltrate of inflammatory cells that fills the papillary dermis in a band-like fashion. The name of lichen refers to the rough, dry appearance similar to that of lichens (symbiotes, fungi/algae that grow on the bark of trees) of the affected skin (1).

Lichen planus has different clinical subtypes based on lesion morphology and involved region. It can affect the skin, mucous membranes and skin appendages. Skin lesions are classically described by the 5 "Ps": papules, planar, polygonal, purple, pruritic, mainly located on the flexor aspects of the forearms, dorsal surface of the hands, lumbar region, ankles and genitally in males (2). At mucosal level, reticular,

papular lesions, erosive, atrophic or bullous plaques can be seen (3). Nail changes consist in onychorrhexis, onycholysis, onychoschisis, anonychia, trachyonychia, irregular depressions, subungual hyperkeratosis, and pterygium (4). On the scalp, it may cause scarring alopecia (5). Wickham striae (fine, lace-like network of white lines and points) can be spotted on the surface of the lesions. The linear configuration of lesions suggests they were induced by scratching (Köbner sign) (2).

The etiopathogenesis of lichen planus is still uncertain, but some evidence reveal that the disease is an immune process triggered by an antigen that alters the keratinocytes in the basal layer, making them susceptible to cell immune response. It induces the activation of CD4 + and CD8 + T lymphocytes, production of pro-inflammatory cytokines, such as interleukin-2 (IL2), interferon-gamma (IFN-gamma), tumor necrosis factor (TNF), cascade of events that causes basal keratinocyte apoptosis and rupture of basement membrane. The antigen responsible for inducing the immune response is still unknown, and its intrinsic or extrinsic origin is a matter of controversy. In some situations, especially in oral lichen planus, certain extrinsic antigens, included in the substances used in dental practice, dental amalgams, medicines (especially antimalarials, agents used in cardiovascular diseases, gold salts, non-steroidal, anti-inflammatory drugs and hypoglycemic agents) were identified. In such cases, although the lesions are very similar clinically and histologically to the idiopathic lesions, a lichenoid reaction is the preferred term. Thus, history and physical examination should also aim at establishing a cause-effect relationship in the pathogenesis of the disease (6).

ETIOLOGIC FACTORS

Genetic predisposition

The reported incidence of familial lichen planus varies widely in the literature and ranges from 1% to 15% for all forms of lichen planus. Genetic predisposition may influence the mucocutaneous reactivity to etiological factors. Genetic polymorphism of some HLA markers (especially HLA-A3, HLA-DR1, HLA-DQ1) as well as of some inflammatory cytokines, have been associated with the presence of lichen planus. Familial lichen planus is characterized by early onset and development of atypical forms with mucosal involvement. The lesions are recurrent and do not respond immediately to treatment (4).

Environmental factors

1. *Infectious factor*. The increased prevalence of liver disease among patients with lichen planus is still debated. Recent studies suggest testing for hepatitis C virus markers, especially in oral erosive lichen planus. Seroprevalence of hepatitis C in patients with lichen is up to 25%, while in the general population is about 5%. The prevalence of lichen planus in chronic hepatitis C is 5% and 1% in the general population. In 21-34% of lichen planus patient's liver function is impaired (7).

Recent studies developed in Mediterranean populations, United States, Saudi Arabia, Taiwan and Nigeria have confirmed the link between hepatitis C virus (HCV) infection and lichen planus, especially the oral form. This association can be explained by the ability of HCV to infect cells other than hepatocytes, epidermal cells, respectively, and by the frequent mutations of the virus that cause repeated activation of immune cells, increasing the risk of autoimmune diseases. In addition, treatment with interferon gamma (INF-

gamma) administered to hepatitis patients may account for lichenoid eruptions. It seems that oral lichen planus associated to HCV infection is a distinct subtype, several studies reporting a much higher frequency of a HLA-II allele, namely HLA-DR6 compared with patients with oral lichen planus without HCV infection (7).

Several viral infections have been associated with oral lichen planus, particularly those of the herpes virus family: herpes simplex, Epstein-Barr virus, cytomegalovirus and herpes virus 6. But there are still doubts whether these agents are responsible for the occurrence of lichen planus or cause a superinfection of preexisting lesions. The best investigated virus, after HCV, was the human papillomavirus (HPV). Syrjanen S et al. show a strong link between HPV and oral lichen planus. Moreover, HPV prevalence gradually increases with severity of lesions, and may also be associated with progression to malignancy. On the other hand, ulcerated lesions are believed to increase the risk of HPV infection and chronic corticosteroid treatment may favor virus replication. Thus, it is unlikely that the isolated presence of the virus to trigger the disease, but it may aggravate or favor the lesions and increase the risk of malignant transformation (8).

Recently, it has been suggested that *Helicobacter pylori* is involved in the etiology of lichen planus. During a duodenal ulcer or gastritis, *Helicobacter pylori* induces a significantly increased secretion of interleukin 1-beta and TGF-beta, cytokine involved in the pathogenesis of lichen planus. Eradication of *Helicobacter pylori* infection can result in the improvement of lichen lesions (3).

2. *Endogenous antigens.* Of the involved endogenous antigens, heat shock proteins are the ones that stand out. They are expressed by all types of cells and play a role in intercellular communication, cell differentiation and growth, signal transduction and apoptosis. Increased expression of these proteins may be in response to multiple exogenous agents such as sudden temperature changes, medications, viruses, nutritional deficiencies and growth factors. In oral lichen planus, this protein is expressed in large amounts, suggesting that it may be an autoantigen of the disease. Identification of an autoantigen associated with the other disease features, such as chronicity, association with other autoimmune diseases, preference for female gender, presence of T lymphocytes in lesions and resolution after the administration of immunosuppressive treatment, represents the full picture of autoimmune theory. However, as shown above, heat shock protein overexpression may occur because of multiple exogenous agents, representing an intermediate step and not the causal factor (9).

3. *Vaccines*. Various cases of lichen planus following HBV (hepatic B virus) vaccination have been reported. The interval between the administration of the initial vaccine dose and the development of lichen planus lesions ranges from a few days to 5 months. In a study of 18 pediatric patients, 5 developed bullous lichen planus after vaccine administration. It is possible that T lymphocytes formed following vaccine administration to cross-react with different epitopes expressed on keratinocytes. Patients who develop lichen planus are recommended to discontinue vaccination schedules due to the increased risk of developing severe lichen planus lesions (10).

4. *Neuropsychiatric factor.* Neuropsychiatric disturbances, such as depression, anxiety and stress were investigated in the etiology of lichen planus, the patients re-

porting the onset or exacerbation of lesions during high emotional stress conditions. Studies on this topic are quite controversial. Girardi C et al found a positive association between lichen planus and emotional states, while others did not. The use of different questionnaires, subjectivity and lack of standardization between studies are responsible for the controversial results. The pathology being complex, it is obviously the result of the interaction of multiple factors. It is likely that stressful situations influence the course of disease, by the alteration of immune functions and Th1/Th2 cytokine balance with increased Th2 response (11).

5. *Medications.* Numerous drugs are involved in the occurrence of cutaneous mucosal lichenoid eruptions and/or of the mucous membranes, especially of the oral ones. Sometimes, lichenoid reactions can be located only on genital mucosa. We emphasize that lichenoid reactions occur after a latency period of several months to years, unlike most drugs side effects which occur after a short period of treatment (12).

The mechanism by which drugs induce lichenoid reactions (tab. I) is unknown, the most plausible theory being that they occur following the cytotoxic T cell-mediated immune response against a Class 2 MHC antigen complex/drug, so that keratinocytes and Langerhans cells are seen by the immune system as "non-self". Macrophage migration inhibitory factor is significantly increased (12).

TABLE I

Medications causing lichenoid reactions (13)

Cutaneous reactions	Mucosal eruptions
Antimalarials	Gold salts
Gold salts	Nonsteroidal anti-inflammatory drugs
Isoniazid	Ketoconazole
Beta-blockers	D-penicillamine
Piritinol	Methyldopa
Thiazide diuretics	Sulphonyl urea
Levamisole	Allopurinol
Captopril	Inhibitors of angiotensin converting enzyme
Lithium carbonate	
Phenothiazines (Metopromazine, Levomepromazine)	
Methyldopa	
Antimitotic drugs (Hydroxyurea, Methotrexate)	
D-penicillamine	
Phenylenediamine salts	

6. *Color film developing agents and methacrylic acids.* Lichenoid eruptions have been reported in almost 25% of the people exposed to chemicals in color film developing agents. The responsible chemicals are the paraphenylenediamines. The current automatic film processors minimize the contact with these chemicals, this type of lichenoid eruptions being rare today. There have been reports of lichenoid eruptions in workers exposed to methacrylic acid used in the automotive industry. Also, because dental devices also contain methacrylic acids, they could be considered causative factors in oral lichen planus. Other materials that could induce lichenoid

reactions are dental materials, nickel, and gold aminoglycoside antibiotics (12).

7. *Dental fillings*, especially the mercury in the dental filling materials. Between 87 and 97% of the patients with oral lichen planus associated with dental fillings experienced improvements after dental filling removal, but only 28-39% of these patients had positive patch test to mercury. Patients who experienced improvements after amalgam removal but negative patch tests were supposed to have allergic reactions to dental fillings. A recent study reported that not the presence of fillings or gold is associated with a higher risk of oral lichen planus, but rather filling corrosion and a galvanic effect by the dissemination of metals in direct contact with oral mucosa (9).

8. *Graft-versus-host reaction*. Cutaneous Graft-Versus-Host Reaction (cGVHR) includes all inflammatory manifestations that may arise at variable intervals after transplantation of an organ containing lymphoid cells. Chronologically there:

- acute graft-versus-host reaction- occurs after 7-21 days and is mainly induced by donor's mature T lymphocytes; the main clinical manifestation is macula-papular exanthema;
- chronic graft-versus-host reaction- it may be an extension of acute form of may occur de novo. The average onset is after 4 months but it may also develop after day 40. Both donor's T cells and autoimmunity are involved. The cutaneous manifestations consist in lichenoid and/or sclerodermiform eruptions. Erythroderma and fasciitis are rare manifestations (3).

PATHOGENETIC MECHANISMS

The intimate etiopathogenetic mechanisms on lichen planus are not fully elucidated. Recent data suggest that lichen planus is a cell-mediated autoimmune disease, hypothesis supported by its possible association with other autoimmune diseases: primary biliary cirrhosis, chronic active hepatitis, Gougerot-Sjogren syndrome, dermatomyositis, lupus erythematosus, morphea, vitiligo, alopecia areata, autoimmune thyroid disorders, ulcerative colitis, myasthenia gravis, Biermer anemia, Addison's disease. Vesicular forms can coexist with certain cancers (6).

Multiple cell types, extracellular matrix proteins and chemokines, contribute in different ways to the development of lichen planus. The presence of the cells that induce T lymphocyte migration and activation and keratinocyte destruction triggers a cell-mediated antigen-specific immune response. On the other hand, metalloproteinases, chemokines and mast cells are responsible for the non-specific immune response. Finally, anti-desmoglein 1 and 3 circulating antibodies, along with the identification of immunoglobulin (Ig) M and IgA in the lesion suggest that humoral immunity plays a role in the pathogenesis of lichen planus (6).

The cells involved in the pathogenesis of lichen planus are: keratinocytes, CD4+ helper lymphocytes, CD8+ cytotoxic lymphocytes, dendritic cells, mast cells and macrophages, their levels varying per lesion stage. In the early stages, the levels of helper T lymphocytes, macrophages and dendritic cells are higher than in more advanced lesions, the key role being played by suppressor T cells. This distribution suggests that in the initial stages antigen-presenting cells as well as the cells responsible for the induction of the inflammatory response are the predominant cell population, while suppressor cells appear in more advanced stages, causing keratinocyte apoptosis (6).

Keratinocytes, cells that form the epithelium, are responsible for the secretion of type IV collagen and laminin 5, major structural proteins in the basement membrane, which in its turn is essential for maintaining keratinocyte viability, with dependence and close relationship between these two. In lichen planus, the keratinocyte is the target cell, the cell that undergoes apoptosis. For this phenomenon to occur, the cell must present an antigen (still unknown) since the onset of the pathogenic process. Once activated, keratinocytes secrete chemokines, which attract lymphocytes and other immune cells and trigger the pathogenic mechanism and favor the chronic nature of the disease (9).

Dendritic cells play an important role in immune response, activating T lymphocytes by antigenic stimulation. Studies show an elevated number of dendritic cells in lichen planus, especially Langerhans cells, stromal and plasmacytoid dendritic cells, with a role in promoting the inflammatory response. Of these, Langerhans cells have been most extensively studied. They are in the upper basal layer of the epithelium higher, and their function is to capture antigens. When this process takes place, the cells are activated, migrate to regional lymph nodes and activate T lymphocytes, eliciting a primary immune response. When Langerhans cells recapture the same antigen, they will be recognized by memory T lymphocytes, which will induce a secondary immune response. In lichen planus lesions, large numbers of Langerhans cells are present in the basal layer of the epidermis. It is assumed that they present antigens to T lymphocytes by molecules of the major histocompatibility complex class II (MHC class II), generating both an initial sensitivity to that antigen (primary immune response) and a secondary immune response leading to the occurrence of clinical signs (14).

CD4+ T lymphocytes are cells able of activating B lymphocytes, macrophages and CD8+ T lymphocytes, thus being responsible for the recruitment of various cells involved in the immune response. CD4+ T lymphocytes are classified into three subtypes: Th1 (lymphocytes T helper), Th2 and Th17, morphologically indistinct, but distinct by the cytokines they secrete. Th1 subtype is characterized by the production of IFN-gamma, IL-2 and TNF-alpha, cytokines which activate macrophages and cytotoxic T lymphocytes. Th2 subtype secretes IL-4, IL-5, IL-10 and IL-13, crucial for antibody production. The recently discovered LT Th17 produce IL-17, IL-22 and IL-26. IL-17, with an important role in the inflammatory response, is also associated with other autoimmune conditions, such as multiple sclerosis, psoriasis, and lupus. The number of Th1 and Th17 cells and IL-17 serum level were significantly increased in patients with lichen planus compared to the control group, especially in oral atrophic-erosive lichen planus, suggesting that Th17 and IL-17 may play important roles in the pathogenesis of lichen planus, especially in the oral type. CD4+T lymphocytes attach as a sub epithelial band in the lamina propria. However, they are not present near the basement membrane rupture. CD4+ T lymphocytes are activated by MHC class II antigens presented by the Langerhans cells or keratinocytes. From this moment on, LT CD4+ activates the cytotoxic T lymphocytes through binding of RCA (Request to Cytotoxic Activity) the receptor on the surface of CD8+ T lymphocyte, or secretion of Th1 cytokines, such as IFN-gamma, IL-2 and TNF-alpha. In addition, local production of

IFN-gamma maintains keratinocyte MHC class II expression, contributing to the chronicity of lesion (15).

In lichen planus, CD8+ T lymphocyte infiltrate is located primarily intraepithelial near basement membrane rupture. Keratinocytes are destroyed in several ways: by activation of perforins and granzymes or by expression of FasL receptor (CD95), which interacts with the Fas molecule on the surface of keratinocytes, or by secretion of TNF-alpha, which causes apoptosis by binding to TNF receptor on keratinocyte (6).

Mast cells can activate T lymphocytes and to produce several mediators which modulate the inflammatory response. In lichen planus, there is an increase in mast cell density in the lamina propria, near blood vessels and nerves, and especially near the basement membrane rupture, suggesting that it might play a direct role in the basement membrane destruction and intraepithelial migration of CD8+T lymphocytes. Mast cells also releases a series of cytokines, such as IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-16, TNF-alpha and Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES), through which, by a cyclic mechanism an interaction between T-lymphocytes and mast cells is established, interaction that may maintain the chronicity of inflammation (6).

Immunofluorescence studies also reveal the involvement of humoral immunity, globular IgM (occasionally IgG and IgA) deposits, corresponding to colloid bodies, being discovered. Also in support of a humoral autoimmune mechanism comes the finding of high serum level of anti-desmoglein 1 and 3 autoantibodies in patients with oral erosive lichen planus compared with patients with reticular lichen planus or recurrent thrushes. Of course, the increased level of these autoantibodies could be the consequence of the erosive nature of lichen planus (6).

Other researches show that the TNF-alpha serum level is significantly elevated in lichen planus patients. Also noted was the increase of metalloproteinases MMP-1 and 3 in patients with oral erosive lichen planus, these two being involved in the induction of apoptosis and basement membrane degradation (6).

CONCLUSIONS

We observed that to understand the ethipathogenic mechanisms of lichen planus, recognize its clinical subtypes and differentiate it from other diseases is of critical importance. The interaction of various factors is responsible for the initiation, persistence and worsening of lichen planus lesions. So, given the multifactorial nature of lichen planus, an accurate early diagnosis is essential for an adequate therapeutic approach.

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NEWS

A HIGHLY DURABLE RNAi THERAPEUTIC INHIBITOR OF PCSK9

Proprotein convertase subtilisin-kexin type 9 (PCSK9) is known as a target for LDL cholesterol-lowering therapy. Inclisiran is a long-acting RNA interference (RNAi) therapeutic agent, subcutaneously delivered, that inhibits the synthesis of PCSK9. In a study by Fitzgerald *et al.* healthy volunteers received a subcutaneous injection of inclisiran or placebo in either a single-ascending-dose phase (at a dose of 25, 100, 300, 500, or 800 mg) or a multiple-dose phase (125 mg weekly for four doses, 250 mg every other week for two doses, or 300 or 500 mg monthly for two doses, with or without concurrent statin therapy). It was showed that the reported adverse events (cough, musculoskeletal pain, nasopharyngitis, headache, back pain, diarrhea) were mild or moderate. Inclisiran doses of 300 mg or more reduced the PCSK9 level, and doses of 100 mg or more reduced the LDL cholesterol level in the single-dose phase. Reductions in the levels of PCSK9 and LDL cholesterol were maintained at day 180 for doses of 300 mg or more. All multiple-dose regimens reduced the levels of PCSK9 and LDL cholesterol. The study concluded that no serious adverse events were observed with inclisiran and doses of 300 mg or more significantly reduced levels of PCSK9 and LDL cholesterol for at least 6 months (Fitzgerald K, White S, Borodovsky A, et al. A Highly Durable RNAi Therapeutic Inhibitor of PCSK9. *New England Journal of Medicine* 2016, DOI: 10.1056/NEJMoa1609243).

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INTERRELATIONSHIP BETWEEN PATHOPHYSIOLOGICAL MECHANISMS OF VITAMIN D DEFICIT AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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INTERRELATIONSHIP BETWEEN PATHOPHYSIOLOGICAL MECHANISMS OF VITAMIN D DEFICIT AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE (Abstract): Several similarities have reported between patients with suboptimal level of vitamin D and patients with Chronic Obstructive Pulmonary Disease (COPD): both conditions are more prevalent after the 6th decade and are associated with impaired defense mechanisms, with skeletal muscles dysfunction and with an early ageing process. We have reviewed the literature concerning the interrelationship between the main pathophysiological processes of both conditions, in order to emphasize the influence of vitamin D deficit on COPD development and evolution. Epidemiological, clinical and experimental data supporting this association are presented. We concluded that the identification of common pathophysiological mechanisms for the vitamin D deficiency and COPD substantiates the hypothesis of the involvement of this hormone in the chronic obstructive pulmonary disease. **Keywords**: VITAMIN D, COPD, PATHOPHYSIOLOGICAL MECHANISMS.

Vitamin D is well-known for its essential role in bone metabolism. Nevertheless, the perspective on vitamin D has changed significantly over the past years and new actions have been described. The activation of vitamin D takes place in the tissues which express 1-α-hydroxylase (CYP27B1), an enzyme that is present, beside other localizations in the pulmonary cells, striated muscles (including the respiratory muscles), endothelium and in the cells involved in the processes of specific and non-specific defense (1). Several similarities have been noticed between adults with suboptimal level of vitamin D and Chronic Obstructive Pulmonary Disease (COPD). Both conditions mostly affect adults over 60 years old. The suboptimal level of vitamin D has 59 % prevalence in Romania, with a peak in elderly females (2). In cer-

tain risk groups, such as people with osteoporosis, the level can reach over 80% (3). The estimated prevalence of COPD in Romania is 8.3% and is higher in males aged over 60 (4). The level of vitamin D has seasonal variation and reaches its peak in the cold season, when there are also the most frequent exacerbations of COPD. A recent meta-analysis showed that low levels of vitamin D correlate with the COPD severity (6) and is associated with a decrease of the ventilatory function, irrespective of other factors (5).

IMPAIRMENT OF THE DEFENSE MECHANISMS

Impairment of the non-specific inflammatory response is a classical pathophysiological mechanism of COPD, which relates to the chronic inhalation of irritants that activates the pattern recognition receptors, such as Toll-like receptors and increases the transcription nuclear factor kB (NF-kB) and of the mitogen-activated protein kinases (MAPK) in the pulmonary neutrophils and macrophages. The chronic inflammatory processes and the oxidative stress lead to mucus hypersecretion, destruction of the elastin fibers and fibrosis.

Vitamin D suppresses in macrophages the formation of inflammasome by preventing the activation of the TLR-NF-kB pathway by the bacterial endotoxins. It also increases the IL10 production by enhancing transcription and via a calcium-dependent mechanism (7, 8). Vitamin D inhibits the maturation of dendritic cells, which modulates the function of T helper cells (Th), increasing the efficiency of the defense mechanism. Therefore, in vitamin D deficit, there is an impairment of the nonspecific host defense with increase of the inflammatory processes and a decrease of the reparatory, limitative ones. Vitamin D (1.25-dihydroxyvitamin D3) receptors (VDR) complex translocate in the nucleus, inducing the expression of *cathelicidin*, an antimicrobial and anti-inflammtory peptide. Cathelicidin is expressed in cells involved in the primary line of defense (keratinocytes, leukocytes, monocytes, neutrophils) as well as in T and B or NK cells.

In macrophages, the activation of Toll-like receptors up regulates the expression of VDR and of the genes expressing 25(OH)D3-1α-hydroxylases, inducing the expression of cathelicidin and the intracellular destruction of Mycobacterium tuberculosis (9, 10). In aseptic inflammation, cathelicidin plays a role in chemokine expression, stimulates angiogenesis and apoptosis and favors wound healing (10). Therefore, the chronic bacterial colonization and the decrease of the defense ability against pathogens through an impairment of the macrophage and neutrophils function in COPD patients could be magnified by the low level of cathelicidin secondary to vitamin D deficiency.

The actual mechanism of the specific immunity activation in COPD is not known. An exacerbated immune activation is noted, along with the increase in the number of T lymphocytes (L) and BL in the lungs. Lymphocytes organize themselves in lymphoid follicles, with the increase of the number and the activation of dendritic cells, which induces a predominantly differentiation in Th1 type. This increases the level of TNF-α, IL-1β, and IL-6, cytokines with pro-inflammatory effect, of the TGF-β and other growth factors for fibroblasts. These processes end up with the fibrosis of the small airways and a permanent inflammatory infiltrate of monocytes and Th1 cells. Vitamin D suppresses

both the proliferation and the secretion of pro-inflammatory Th1 cytokines: IL2, IFNγ, TNFα and promotes the synthesis of anti-inflammatory cytokines by Th2 lymphocytes (IL3, IL4, IL5, IL10) (10, 11, 12). As consequence, in vitamin D deficit, the pathogenic mechanisms of COPD, previously described, are favored (fig. 1).

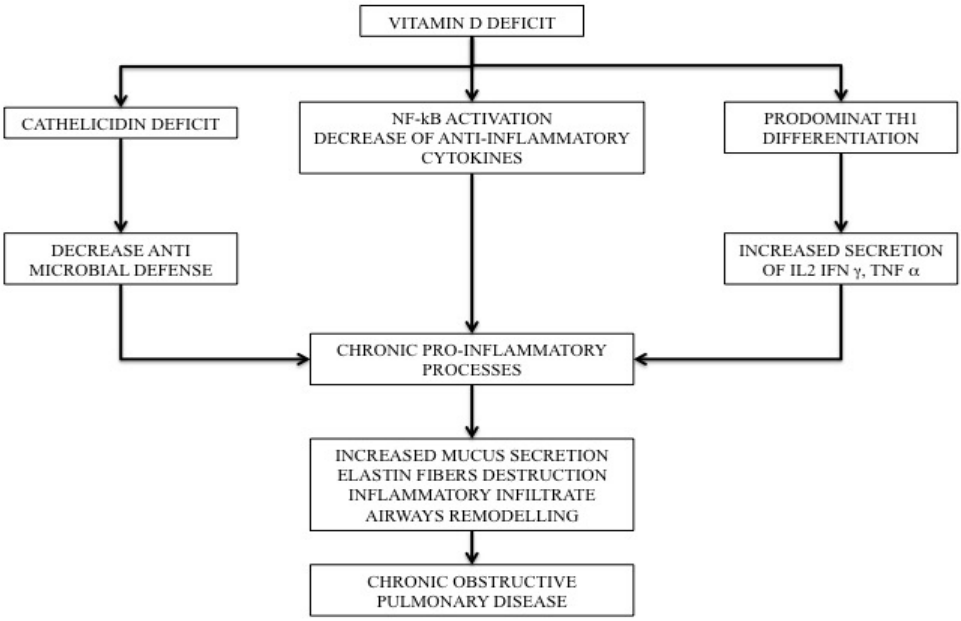

Fig. 1. Defense mechanism impairment in vitamin D deficit and COPD

THE DYSFUNCTION OF THE RESPIRATORY MUSCLES

The respiratory muscles are made up of 2 very thin muscle layers located in the intercostal space and by the diaphragm; the contraction of the later one ensures 70% of the ventilation/minute. In COPD, hyperinflation causes the flattening of the diaphragm and reduces the contraction force by altering its radius; hyperinflation also changes the normal relationship between the ends of the myosin fibers and the actin filaments and decreases the pressure generated for the same tension developed within (13). As an adaptation to permanent overload, type II fibers in the respiratory muscles change into type I fibers (14, 15), increasing the diaphragm resistance to fatigue but decreasing its ability to generate contraction force. In COPD, there is also a reduction in number and length of sarcomeres that moves the curve tension length to the left, as an adaptation to a lesser inspiratory extension determined by hyperinflation. However, reduction of the number of sarcomeres will end up reducing the force of muscle contraction. The respiratory muscles have to work 10 – 14 times more in COPD patients, as compared to healthy people, due to the chronic hypoxemia (16) and the adaptive phenomena described so far become inefficient. Even in moderate forms of COPD, the force of the inspiratory muscles is more severely

reduced than that of the expiratory ones; this is associated with a decrease of the proximal muscle force in the peripheral muscles (17). The long-term effects of COPD on respiratory muscle are muscle atrophy, changes in titin structure and an increase of sarcomere susceptibility to the harmful action of external factors (18).

Vitamin D has a regulatory role in muscle development and in the contraction force, increasing the transcription of myosin and of other calcium binding proteins. The association between the vitamin D deficiency and the proximal myopathy is clinically demonstrated by the improvement of muscular force after the administration of vitamin D to adults with a deficit of this vitamin. The preferential atrophy of type II muscle fibers may occur in the vitamin D deficiency with a similar effect to the one found in age-related sarcopenia and COPD (19) and it is reversible after vitamin D administration (20). The interaction of vitamin D with VDR stimulates adenylate cyclase, phospholipase C, D and A2 and increases the MAPK signal and inositol 3 phosphates (IP3). The intracellular calcium rises by influx through calcium voltage-dependent channels activated by proteinkinase A and by release from endoplasmic reticulum system IP3. The MAPK and Akt signal influences cell development, mitosis and differentiation of the myoblasts. Vitamin D deficiency will trigger a reduction of the contraction force through calcium deficit and impaired muscle fibers formation (21) and a decrease of the phosphate transport into the muscle cell, essential for ATP and protein synthesis.

Vitamin D releases the arachidonic acid from the phosphatidylcholine bound to the cell membrane. This polyunsaturated fatty acid modulates the insulin receptors and the glucose uptake (22, 23). Reduction of glucose transport in muscle cell under low level of vitamin D condition has major metabolic implications and reduces the ATP production, contributing to the reduction of the contraction force (fig. 2).

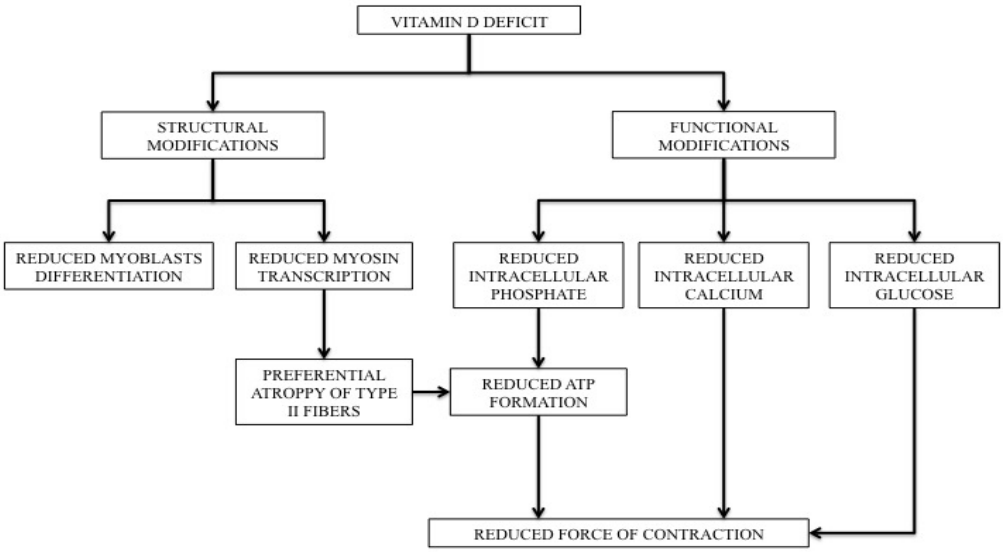

Fig. 2. Reduction of the contraction force of the respiratory muscles in vitamin D deficit

THE PREMATURE AGEING PROCESS OF THE CELLS IN THE BRONCHIAL MUCOSA

Forced expiratory volume in one second **(**FEV_1) normally decreases with age from a peak reached around 25 years old. Patients with COPD present an accelerated yearly decrease of FEV_1, generally fewer than 60% of the value corresponding to age (24). Subjects who reach a maximum of initial FEV_1 lower than the medium one may develop symptoms even if no other risk factors are present, through the normal age reduction of the pulmonary function. Genetic factors, reduced physical activity during childhood/adolescence, treatment with glucocorticoids and malnutrition are other possible causes of low initial maximum FEV_1.

The premature ageing process in COPD is related to the reduction of the expression of sirtuins compared to the age. Oxidative stress, characteristic for the chronic inflammation in COPD, blocks sirtuins activity. Vitamin D also affects both the acetylation of histones and the methylation of the DNA. The VDR/RXR complex, in the absence of 1.25-D3, can bind to co-repressors which attract histone-deacetylases. By binding of vitamin D to VDR, the co-repressors are replaced by co-activators as histone acetyl transferases; these might lead to similar effects with the decrease or blockage of the sirtuins.

The decrease of autophagy and the increase of mitophagy contribute to premature ageing of the respiratory mucosa in COPD. The mitophagy, enhanced by many compounds from the cigarette smoke, has the opposing effect to autophagy, triggering apoptosis. Vitamin D takes part in the regulation of autophagy through calcium, PI3k, the expression of becline-1 (25, 26) and of cathelicidin (27). In the absence of vitamin D, the physiologic processes of autophagy are reduced and the cell destruction phenomena are emphasized (25, 26).

The level of vitamin D decreases after the 6th decade. A conclusion regarding the preventive effect of vitamin D on the ageing of bronchial cells in COPD, although conceivable, has not yet enough scientific proofs.

CONCLUSIONS

We concluded that the vitamin D and COPD relationship is very complex. Since the epidemiologic and experimental data support the relationship between vitamin D deficiency and COPD, it is expected that future research will bring important insights in the understanding of these processes. The identification of common pathophysiological mechanisms for the vitamin D deficiency and COPD substantiates the hypothesis of the involvement of this hormone in the chronic obstructive pulmonary disease.

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SCORING IN ACUTE PANCREATITIS: WHEN IMAGING IS APPROPRIATE?

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SCORING IN ACUTE PANCREATITIS: WHEN IMAGING IS APPROPRIATE? (Abstract). Acute pancreatitis (AP) is a frequent presentation to the emergency departments with a rising incidence and a great variability in clinical severity and outcome. The aim of this review is to offer a succinct presentation on acute pancreatitis scoring systems and the use of different imaging methods in severity prediction: Ranson criteria, Glasgow criteria, Hong Kong Score, Acute Physiology and Chronic Health Evaluation II (APACHE II), computed tomography scoring systems, Bedside Index of Severity in Acute Pancreatitis (BISAP) score, Panc 3, Japanese Severity Score (JSS), Harmless Acute Pancreatitis Score (HAPS), Pancreatitis Outcome Prediction (POP), Sequential Organ Failure Assessment (SOFA). This article also describes the Revised Atlanta Classification of AP (2012) and the correlation with computed tomography. **Keywords:** PANCREAS, SCORING SYSTEM, PROGNOSIS

Acute pancreatitis (AP) is defined as an acute inflammatory disease of the pancreas which may involve the peripancreatic tissue and remote organ systems (1). The incidence of AP ranges in different studies from 13 to 45 per 100,000 (2). AP can result in a systemic inflammatory response syndrome with significant morbidity and mortality in 15-20% of patients (3, 4). The most common causes of acute pancreatitis are gallstones, idiopathic and alcohol; occult microlithiasisis probably the main cause of idiopathic pancreatitis (5,6).

Most patients present a mild and self-limiting disease (80-85%), and the remaining ones develop complications (major local or systemic), which lead to multiple organ failure and death. The mortality rate for the severe form of AP (SAP) is high, up to 30% (7). The possibility of selecting patients with SAP is pivotal for proper treatment, observing closely the course of disease and relying on clinical decision-making.

REVISED ATLANTA CLASSIFICATION

The *revised Atlanta classification* (11) divides AP into interstitial edematous pancreatitis and necrotizing pancreatitis, each with an early phase (1st week) and a late phase (after 1st week). Characteristically, the early phase of treatment is based on clinical parameters and in the late phase of treatment decisions are made by a combination of clinical parameters and morphologic criteria (peripancreatic fluid collections, pancreatic necrosis, other complications) established on the basis of computed tomographic (CT) findings (10).

Contrast-enhanced CT is the primary tool for assessing the morphologic criteria and is suited for patients who develop or are likely to develop SAP. The ideal time to visualize complications of AP with CT is 72 hours after the onset of symptoms.

In the revised Atlanta classification (11), necrotizing pancreatitis is subdivided into parenchymal necrosis alone, peripancreatic necrosis alone and a combined type (parenchymal and peripancreatic necrosis). The terminology for fluid collections is also revised, designated as acute peripancreatic fluid collection and acute necrotic collection within the first 4 weeks, and in pancreatic pseudocyst and walled-off necrosis after 4 weeks, respectively (fig. 1, 2).

The revised Atlanta classification (11) is not necessarily a predictor of severity, but it classifies disease severity based upon clinical and imaging features.

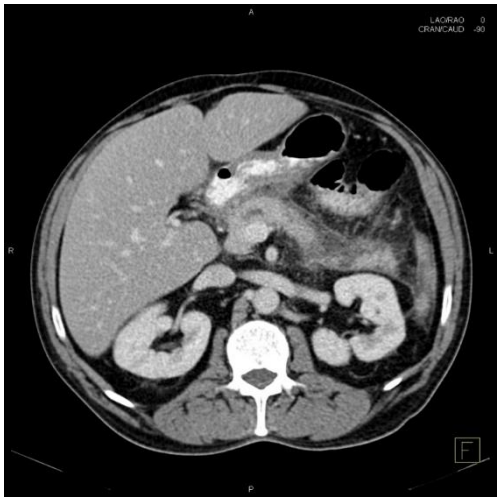

Fig. 1. Contrast-enhanced CT scan in a patient with interstitial edematous acute pancreatitis developing an acute peripancreatic fluid collection around body and tail (Collection of Radiology and Medical Imaging Laboratory, Iaşi"Sf. Spiridon" University Hospital, Prof. dr. D. Negru).

PROGNOSTIC SCORING SYSTEMS

The correct identification of SAP only by clinical assessment on admission is reported in 40-64% of patients. The requirement for an early objective measure of AP severity was recognized in the mid ′70s by Ranson who established the characteristics of the ideal prognostic method: objectivity, accuracy, simplicity, availability at diagnosis, non-invasiveness, quantitativeness, independence with regard to etiology and pre-existing disease, complication specificity, usefulness for disease course monitoring (13).

Fig. 2. Contrast-enhanced CT scan in a patient with acute necrotic pancreatitis, in late phase (after 4 weeks), with a pancreatic and peripancreatic fluid collection which meets the criteria for walled-off necrosis (inhomogeneous content, well defined wall). (Collection of Radiology and Medical Imaging Laboratory, Iaşi "Sf. Spiridon" University Hospital, Prof. dr. D. Negru).

Prognostic assessment by means of single laboratory markers is easier to use than scoring systems which are often difficult to apply and have a high degree of complexi-

ty. There are many analyses used in the prognostic evaluation of patients with AP (e.g. amylase, calcium, white blood cell count, blood glucose, hematocrit, phospholipase A2, coagulation factors, polymorphonuclear elastase, arterial hypoxemia, acidosis, ribonuclease, endothoxin, trypsinogen activator peptide, pancreatitis-associated peptide, neopterin, interleukin 6, interleukin 8, C-reactive protein (CRP), procalcitonin, creatinine, blood urea nitrogen -BUN) without confirmation of their validity. CRP usually peaks 72 hours after the onset of symptoms, at the end of therapeutic window, but it is an important marker for necrosis. BUN elevation on admission or rising 24 hours after the onset is associated with higher mortality (14).

Numerous *multifactorial prognostic scoring systems* (15, 16) have been proposed in the last 40 years, incorporating physiologic, laboratory, imaging parameters, performing with moderate sensitivity and high negative predictive value; also the positive predictive value is suboptimal, but there are debates regarding the design for initial and sequential studies, many studies that have used or assessed these scoring systems having limitations due to the small number of enrolled patients with SAP.

Ranson criteria (18) are divided into two parts: on admission, when included are: age, white blood cell count (WBC), glucose, lactate dehydrogenase (LDH), aspartate transaminase (AST), and after 48 hours there are evaluated hematocrit (Hct), calcium, blood urea nitrogen (BUN), base deficit, fluid loss, pO_2.

Glasgow scoring system uses pO_2, albumin, calcium, WBC, AST, LDH, glucose, and BUN. Hong Kong system requires just BUN and glucose (18). APACHE II is a scoring system used in intensive care unit based on multiparametric evaluation: temperature, heart rate, mean arterial pressure (MAP), respiratory rate, WBC, Hct, plasma Na+, plasma K+, creatinine, arterial pH, venous bicarbonate, pO2, alveolar–arterial pO2 difference, age, Glasgow Coma Scale (GCS), Chronic Health Score (CHS) (19).Other multifactorial prognostic scores are described, such as *Marshall* (based on MAP, FiO_2/pO_2, GCS, platelet count, creatinine), *BISAP –bedside index for severity in acute pancreatitis* (BUN, impaired mental status, SIRS, age, pleural effusion), *Panc 3* (Hct, body-mass index, pleural effusion), *JSS –Japanese severity score* (base excess, pO_2, BUN, creatinine, LDH, platelet count, calcium, CRP, SIRS, age), *HAPS –harmless acute pancreatitis score* (abdominal tenderness, Hct, creatinine), *POP –pancreatitis outcome score* (age, MAP, FiO_2/pO_2, arterial pH, calcium, BUN), *SOFA –sequential organ failure assessment* (MAP, FiO_2/pO_2, GCS, platelet count, creatinine, urinary output, bilirubin) (20, 21, 22, 23, 24).

The most widely used multiparametric scores are APACHE II score, Ranson and Glasgow criteria; APACHE II score initially developed for intensive care units requires a large number of variables being cumbersome in clinical practice, but can be used since admission; Ranson and Glasgow scores take 48 hours from admission, but require less amount of variables. Bedside index for severity in acute pancreatitis (BISAP) score is easier to use, requires those variables (vital signs, laboratories and imaging) commonly obtained in the emergency department, and can predict in-hospital mortality with higher accuracy (25).

Imaging prognostic scoring systems

These systems are based on computed tomography, a valuable method to depict pancreatic necrosis. Pancreatic necrosis is

more likely associated with severe clinical outcome and necrotizing pancreatitis and severe acute pancreatitis are practically interchangeable terms (26). Identifying pancreatic necrosis requires intravenous iodine-contrast administration and a delay in CT examination at 72 hours when necrosis is fully accomplished, to prevent underscoring.

Balthazar scoring system (27) developed in 1985 is based on the presence of pancreatic changes and the delay from onset of symptoms is 10 days. Balthazar scoring system uses a five-grade scale (from A to E) assessing the presence of pancreatic and peripancreatic inflammation, and fluid accumulation.

PSI –pancreas severity index (28) evaluates the pancreatic modifications with a delay of 72 hours; it is calculated by multiplying the largest anteroposterior diameter of the head by the largest anteroposterior diameter of the body. A PSI higher than 10 cm^2 is associated with severe acute pancreatitis.

CTSI –computed tomography severity index (1990) is based on Balthazar system, but adds necrosis of pancreatic parenchyma, requires intravenous contrast administration, and the delay is minimum 72 hours. CT severity index (CTSI) depends on the presence and degree of pancreatic and peripancreatic inflammation, and fluid accumulation in combination with the amount of pancreatic necrosis, using a 10-grade scale (29). It demonstrates a good correlation between necrosis, length of hospital stay, development of complication, and death, indicating no significant relationships between pancreatic necrosis and organ dysfunction, extrapancreatic parenchymal complications, and ultimate clinical outcome (30).

Modified CTSI (m-CTSI) (31) requires intravenous contrast administration, aggregates pancreatic inflammation, pancreatic necrosis and extrapancreatic complications (pleural effusion, ascites, thrombosis in portal venous system, arterial pseudoaneurysm, gastrointestinal tract involvement) on a 10-grade scale. m-CTSI shows a good correlation between calculated severity of pancreatitis and the outcome parameters, and with organ dysfunction.

Extrapancreatic inflammation on CT (EPIC) (32) score combines radiological manifestation of SIRS and organ dysfunction, without considering necrosis; incorporates pleural effusion, ascites, retroperitoneal and mesenteric inflammation.

Mesenteric edema and peritoneal fluid (MOP) score is based on the predictive value of intraabdominal inflammatory changes and severity of acute pancreatitis (33).

Simple prognostic score (SPS) combines biochemical and radiological parameters for risk stratification and prognosis of AP. SPS is based on quantifying serum lactate dehydrogenase (LDH > 900 IU/L), blood urea nitrogen (BUN >25 mg/dL), and the presence of pancreatic necrosis assessed by contrast-enhanced CT performed within 2 days after admission (34).

CONCLUSIONS

Concluding imaging prognostic scoring systems should be used for mortality prediction and for therapeutic guidance after the first 24-72 hours from admission, while nonspecific illness scoring systems (BISAP, APACHE II) are more suitable for the risk stratification within first 24 hours. Different prognostic scores should be applied to the different phases in acute pancreatitis, the goal being the correct identification of patients at risk to develop major complications or fatal outcome. Clinical scoring systems accurately correlate with

mortality and systemic complications, while imaging scoring systems diagnose clinically severe disease and have a better correlation with pancreatic infection and the need for intervention.

In our opinion we consider that on admission the selection of patients with mild AP should be based on the identification of pre-existing risk factors (obesity, advanced age), together with the common laboratory markers (BUN, creatinine) and chest-x-ray. Assessment of patients with a multifactorial scoring system (BISAP) at 24 hours after admission is important for identifying the group at risk for persistent organ failure. After 48-72 hours the group of patients at risk for organ failure should be checked by a prognostic score system plus CRP and a radiological score (CTSI, mCTSI). Monitoring in the late phase of AP must be based on CRP values, an organ failure score (Marshall) and repetition of contrast-enhanced CT should be used to diagnose the occurrence of complications and the changes in the extent of pancreatic necrosis or peripancreatic fluid collections.

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NONSELECTIVE BETA-BLOCKERS IN PATIENTS WITH CIRRHOSIS: "THE THERAPEUTIC WINDOW"

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NONSELECTIVE BETA-BLOCKERS IN PATIENTS WITH CIRRHOSIS: "THE THERAPEUTIC WINDOW" (Abstract): For over 30 years, nonselective beta-blockers (NSBB) have been successfully used for preventing variceal bleeding in patients with cirrhosis and portal hypertension. Nevertheless, recent studies suggest that NSBB may be effective only within a particular "therapeutic window" in patients with advanced liver disease. Outside of this window, in early stages of cirrhosis and in very advanced cirrhosis, NSBB may be ineffective and even potentially harmful. In this paper we review the beneficial effects and potential harms of beta-blocker therapy in cirrhosis and underline the most recent recommendations for their use in very advanced cases of liver disease. **Keywords:** LIVER CIRRHOSIS; BETA-BLOCKERS; THERAPEUTIC WINDOW.

Pathophysiological basis for using nonselective beta-blockers (NSBB) in patients with cirrhosis. The development of portal hypertension in cirrhosis is mainly due to:

a) An increase in resistance to portal blood flow, because of architectural modifications in the liver, and

b) An increase in portal territory blood flow, due to splanchnic arteriolar vasodilatation.

Therefore, NSBB goal in cirrhosis is to reduce portal pressure by two essential mechanisms:

a) *ß1- adrenergic blockade* , which decreases cardiac output, leading to reduced portal blood flow, and

b) *ß2- adrenergic blockade*, followed by an increase in arterial splanhnic resistance.

How did NSBB occur in the treatment of cirrhotic patient. The study of Lebrec et al. (3) published in 1981 in New England Journal of Medicine was the key-point in establishing the role of NSBB in the secondary prevention of variceal hemorrhage.

In 1987, the study of Pascal et al.(4) underlines the role of NSBB in the primary prophylaxis of variceal bleeding.

In 1991, Poynard et al.(5) meta-analysis together with other studies establishes that NSBB represent the first line therapy both in primary and in secondary prophylaxis of variceal hemorrhage.

The most important studies supporting NSBB usage in the treatment of cirrhotic patient are summarized by Phillip S. Ge., in

Journal of Hepatology from 2014 (1).

The indications of NSBB in cirrhosis

1. Primary prevention of variceal hemorrhage:

a) In cirrhotic patients with medium or large esophageal varices, NSBB lower the risk of first variceal bleeding (from 30% to 14%, compared to placebo) (6).

b) Primary prevention is not recommended in cirrhotic patients without esophageal varices, whatever the value of hepatic venous pressure gradient (HVPG).

2. Secondary prevention of variceal hemorrhage: NSBB reduce the rate of variceal rebleeding from 60% to approximately 40%.

3. Other benefits: NSBB might prevent the development of spontaneous bacterial peritonitis (SBP), by reducing intestinal permeability and bacterial translocation.

I. Pre-primary prophylaxis (to prevent the development of esophageal varices)

There are no data to confirm the benefit of using NSBB to prevent the development of esophageal varices, the most efficient method being the treatment of liver disease.

Studies showed that in patients without esophageal varices, NSBB (like *timolol*) have no role in preventing the development of esophageal varices, and the side effects are more frequent (7).

In conclusion, NSBB are not recommended in this set of patients.

II. Primary prevention (the prevention of first episode of variceal bleeding)

An important meta-analysis of 11 trials including almost 1200 patients evaluated the role of NSBB (*propranolol* or *nadolol)* in the primary prophylaxis of variceal haemorrhage (8) .

The results showed that in cirrhotic patients with medium or large esophageal varices NSBB lower the risk of first variceal bleeding (from 30% to 14%, compared to placebo) (6).

Other studies show that one bleeding episode is avoided for every ten patients treated with NSBB. In conclusion, NSBB reduce the rate of bleeding-related mortality compared to the control group (9).

Upon the size of esophageal varices, the recommendations are as follows:

According to Baveno VI Consensus, in cirrhotic patients with *medium* or *large* esophageal varices NSBB are recommended to prevent the first episode of variceal hemorrhage (6).

Still, the risk of bleeding depends not only upon the size of esophageal varices, but also upon the presence of the marks of bleeding (”red signs”) on the varices and on the severity of liver disease.

In patients with compensated liver disease (Child-Pugh A) and small esophageal varices without red signs, the risk of bleeding is 6%, while the patients with Child-Pugh C cirrhosis, with large esophageal varices with red signs have a risk of bleeding of 76% (10).

The set of cirrhotic patients with *small* esophageal varices are classified in two groups depending on the other factors which influence the risk of bleeding:

1. In patients with small esophageal varices and *low risk* of bleeding, which means without red signs and in the absence of severe liver disease (CHILD A), there are insufficient data that the use of NSBB would prevent the progression of esophageal varices. These patients may use NSBB to prevent the esophageal haemorrhage just in some individualized cases. The patients with small esophageal varices without therapy with NSBB must have endoscopy every 2 years, to evaluate the progression of

esophageal varices (11-13).

2. In cirrhotic patients with small esophageal varices and *high risk* of bleeding, meaning the presence of red signs and/or the presence of severe liver disease (CHILD-PUGH B or C), the treatment with NSBB is recommended (6,13).

III. Secondary prophylaxis of variceal bleeding

Secondary prevention means the prevention of rebleeding from esophageal varices, being addressed to those cirrhotic patients who have already had an episode of variceal haemorrhage.

Studies showed that patients who survive an episode of variceal haemorrhage have a high risk of rebleeding and death. In this set, untreated patients have a rebleeding rate of 60% at 1-2 years, with a mortality of 20-50% (14).

Besides, the prevention of rebleeding from esophageal varices was the first confirmed indication for using NSBB in the treatment of cirrhosis (the study of *Lebrec et al.* from 1981) (3).

It was demonstrated in trials that NSBB reduce the rebleeding rate from 60% to almost 40% (3,5,8).

According to the last guidelines, the combination of *NSBB* and *band ligation* is considered the maximal therapy for preventing rebleeding from esophageal varices. This provides the lowest rates of rebleeding, without major differences between the survival rates (compared to the patients treated only with NSBB). The early administration of NSBB may reduce the risk of bleeding till endoscopic band ligation. (6,15).

Other benefits of NSBB in cirrhosis include the prevention of other complications, like bleeding from portal gastropathy, the occurrence of ascites and of SBP (3,17).

The control of the efficiency of NSBB in cirrhosis targets:

a) *A hemodynamic response to NSBB* means a decrease in the HVPG below 12 mmHg or of 20% of the baseline value;

b) *The adrenergic response to NSBB* is defined as a decrease in the heart rate (measured 12-24 h after drug administration) of 25% of the baseline value;

c) *The dose of NSBB is decided* according to: a decrease in the heart rate (HR) of 25% of the basal value; the value of HR at rest not below 55/min; pay attention to the occurrence of side effects (3).

The side effects of NSBB

a) *Cardiac:* the exacerbation or precipitation of heart failure, symptomatic bradycardia, high grade heart block. The acute withdrawal of beta-blocker therapy can lead to accelerated angina, myocardial infarction and sudden death!

b) *Pulmonary:* increased airways resistance in patients with bronchospasm

c) *Circulatory:* the exacerbation of peripheral artery disease (claudication, absent pulses, cyanosis and gangrene)

d) *Metabolic:* in patients with diabetes mellitus, risk of severe insulin-induced hypoglycemia

e) *Other:* depression, fatigue and sexual dysfunction.

Contraindications to NSBB in cirrhotic patients

These may be classified as follows:

1) *General contraindications*

a) Absolute – severe heart failure, advanced heart block;

b) Relative – moderate chronic heart failure, asthma, chronic obstructive pulmonary disease, peripheral arterial disease, Raynaud syndrome, insulin-treated diabetes mellitus.

2) *Cirrhosis-related contraindications*
a) Portopulmonary hypertension;
b) Refractory ascites.

Is the therapy with NSBB in cirrhosis safe? How did this question occur? The same authors whose studies established the use of NSBB in the treatment of cirrhosis raised this problem!

In 2010, the study of Serste et al. (17,18) published in Hepatology concluded that NSBB should be contraindicated in cirrhotic patients with refractory ascites, leading to a shortening of survival.

Other authors, Wong and Salerno (19) showed that, regardless the design of the study, the safety of the treatment with propranolol in cirrhotic patients with refractory ascites is a problem.

In 2011, another trial of Lebrec et al. (3) postulated that NSBB are associated with the paracentesis-induced circulatory dysfunction (PICD), leading to a reduced survival rate in this group of patients.

"The therapeutic window" hypothesis. Recent studies of Krag et al. (20) suggest that the efficiency of NSBB manifests just during a window in the disease, named "*therapeutic window*" , in those patients with advanced cirrhosis.

Outside of this window, the therapy with NSBB may be ineffective in early stages of cirrhosis and potentially harmful in advanced cirrhosis (1).

Defining the term of "the therapeutic window". In early stages of cirrhosis, NSBB have no beneficial effect on survival and the adverse events may be more frequent (7).

These stages are defined by the following parameters: the esophageal varices are small or absent; intact cardiac reserve; sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS) have baseline activity; the risk of gut bacterial translocation is low, and the risk of mortality is also law (7).

In decompensated cirrhosis, NSBB improves the survival rate, lowering the rate of esophageal bleeding and of bacterial translocation.

This stage of cirrhosis has the following characteristics: medium or large esophageal varices; ascites; the cardiac reserve is intact, but declining; SNS and RAAS have increased but not maximal activity, and the risk of bacterial translocation and of mortality are increased (1).

The final (terminal) stages of cirrhosis are characterized by refractory ascites; impaired cardiac reserve, with baseline hypotension; SNS and RAAS are maximally stimulated and can no longer maintain adequate blood pressure (BP); high risk of gut bacterial translocation and of mortality (7).

In these stages, NSBB decrease the rate of survival as a result of the negative impact on cardiac reserve, lowering the perfusion of vital organs and precipitating the hepatorenal syndrome (HRS).

Why NSBB are not indicated in refractory ascites? The study of Serste et al. (18) from 2011 show that in cirrhotic patients with refractory ascites NSBB may cause a higher risk of paracentesis-induced circulatory dysfunction, thus reducing the rate of survival (2). NSBB (like *propranolol*) would prevent the adaptive hemodynamic response after large volume paracentesis, subsequently inducing PICD.

The factors which close the "therapeutic window" are:

a) The occurrence of refractory ascites and of SBP;

b) Systolic BP below 95 mmHg;

c) The presence of symptoms of orthostatic hypotension;

d) The occurrence of septic episodes;

e) Increased levels of serum creatinine.

The presented data show that the presence of medium or large esophageal varices opens the window of opportunity, which will be closed by the occurrence of refractory ascites, decreased medium BP and/or HRS. Taking into consideration the duration relatively limited of the ”therapeutic window”, some authors agree that NSBB should not be recommended in patients with low medical compliance and/or poor medical follow-up (21,22).

The initiation and continuation of NSBB therapy is ideal in those patients with home monitoring of BP and HR and who will have a close follow-up meaning frequent medical visits (23).

Concerning the ”therapeutic window” hypothesis, the results from studies and the existing evidences are still conflicting.

For example, the study of Galbois et al. (24) published in Hepatology contradicts some of the previous data, suggesting that NSBB are not associated with an inadequate hemodynamic response in patients with cirrhosis and severe sepsis.

For now, the recommendations of **Baveno VI Consensus (6)** are the following:

1) The safety of beta-blockers administration in cirrhotic patients with *refractory ascites* and/or *hepatorenal syndrome* was not demonstrated;

2) The patients with *refractory ascites* must be intensively monitored; if systemic hypotension or azotemia occur, we must reduce the dosage of NSBB or even discontinue the BB therapy;

3) If NSBB therapy is discontinued, the patient will need esophageal band ligation for preventing variceal bleeding;

4) Until practical data from randomised trials arrive, NSBB must be discontinued or the dose must be reduced in patients with refractory ascites if: systolic BP is below 90 mmHg; serum creatinine is raising and/or hyponatremia below 130 mmol/l occurs;

5) The consequence of discontinuing the BB therapy on the prevention of esophageal bleeding is not known;

6) If variceal bleeding or SBP occur after discontinuing the BB therapy, we may restart the treatment if the above parameters became normal.

CONCLUSIONS

In conclusion, the therapy with NSBB requires special attention in two clinical settings:

a) Decompensated cirrhosis in terminal stage, and

b) Refractory ascites.

It remains unclear if, after closing the ”therapeutic window” of NSBB in cirrhosis, this would be a definitive closure or not, referring to the possibility of restarting the beta-blocker medication after a period of time.

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THE PERSONAL REPRESENTATIVE - THE "ACHILLES HEEL" OF THE ROMANIAN MENTAL HEALTH LAW SYSTEM

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THE PERSONAL REPRESENTATIVE-THE "ACHILLES HEEL" OF THE ROMANIAN MENTAL HEALTH LAW SYSTEM (Abstract): The psychiatric law system was created with the sole purpose of protecting patients with mental illnesses, a vulnerable segment of the population. The laws regarding voluntary and non-voluntary admission of mental patients have been reformed in many European countries. After 1989, Romania starts to gradually benefit from a law system that meets the requirements of modern society. From this general context the Mental Health Law takes form, with the main purpose of ratifying the relationship between patient and psychiatrist. This system provides a new, more modern approach but also proves to have certain flaws. One of the most obvious is the aspect regarding the personal representative, which generates both ethical and professional conduit problems, difficult to manage from the standpoint of modern medicine. Minimizing the effects of such an error is possible by simply considering the small number of solutions suggested by the author in the end of this article. **Keywords**: MENTAL HEALTH LAW, PERSONAL REPREZENTATIVE, PSYCHIATRIC.

Mental health (MH) is an important issue not only in Romania, but also worldwide. In psychiatry, like in many other medical fields, ethical problems are a common fact, both in research and in clinical practice. Regarding psychiatric assistance, there are two ways of providing it: voluntary admission and treatment, at the patient's request or non-voluntary admission. In psychiatric institutions, non-voluntary admission for patients with MH problems is allowed only after all other means of voluntary admission have been properly taken into consideration. Non voluntary admission is one of the most complex problems in psychiatry (1).

The high complexity of cases addressed by psychiatry, the increased number of causes and effects treated, represent a fertile ground for both ethical and deontological problems. Many of these issues exist due to the high subjectivity present regarding important aspects like socio-economic status, psychological status and biological

variables. Regarding the results of issues, Constatin B. (2) states that, from the total number of complaints, coming from patients, about abusive non-voluntary admission, approximately 90-95% are unjustified. According to World Health Organization, 78% of countries worldwide, which represent about 69.1 % of the entire population of the planet, have a law system which deals with mental health (1). WHO reports show that 57.1% of the Middle Eastern countries have laws which deal with these issues, while the percentage for European countries is 91.8%. Over 50% of MH laws in effect today date as early as 1990, 25% having been introduced after 2000. Approximately 16% of laws are older than 1960. Although a legal system dedicated to MH exists, that doesn't automatically mean that human right are always respected. As a result in countries with an obsolete law system, breaking human rights is often more common that respecting them. Currently, guiding all actions according to the human rights agenda is an ethical obligation especially in countries part of international agreements. The European Court for Human Rights, supported by the European Union court, is a strong advocate for patients with MH problems, especially in member countries (1, 2).

In many cases, the parties involved are individuals whose knowledge about these ailments varies from high to none. This is why, frequently, it is hard to cover all aspects and issues of MH pathology, because it is encountered in small part of the population.

Regarding minorities, Buda O (3) says that "they can be considered individuals which are disadvantaged, almost continually or even traditionally, according to rules of certain communities or to popular views, by dominant individuals, through discrimination, scrutiny, punishment". Stigma also contributes substantially to the diversification and amplification of possible errors in a discipline where ethical limits are difficult to establish. Between those involved in MH care and stigma, a complex relationship exists: they can be a discriminating agent, they can fight against it or they became themselves a target (4, 5). Stigma in a society can be reduced either through education, protest and a new law system, through self-management of stigmatization or through social support (4, 6). Social stigma and labeling is a violation of freedom and human rights (7). The subject of nondiscrimination in Romania, a European Union member (8, 9), is a great challenge and amongst those who provide psychiatric care, stigma is an important issue.

THE PSYCHIATRIC PATIENT'S PERSONAL REPRESENTATIVE

In Ireland (10, 11), non-voluntary admission in psychiatric institutions is requested by husband / wife or another close relative, a law representative or member of the police. The admission has a validity period of 21 days, after which, if it is renewed, the admission can last as long as 3 months, or a maximum of 6 months. Periods can be extended, but they should not exceed 1 year. A patient's non-voluntary admission or its extension is automatically managed by a MH committee. The committee provides each mentally ill patient with a legal representative for free legal support. Currently, the main focus of the Irish Parliament is the Mental Capacity and Legal Representative Irish Act. A new legislation with new stipulations regarding non-voluntary admission is considered necessary in Ireland, to protect the rights of pa-

tients with MD. According to studies, one year after their discharge, 60% of patients admitted through non-voluntary means, felt that their coercive admission was justified. Although MHA 2001 in Ireland benefits from new stipulations regarding mentally ill patients, the advance directive represents an issue that needs to be considered further.

The economical aspect is an issue that has not been discussed and investigated thoroughly in studies involving individuals with MD. Specialty literature has paid little attention to this subject also. Financial issues are very common, especially amongst patients with severe MD, such as schizophrenia patients. Thus, establishing a financial representative would be very useful for some patients with serious psychiatric disorders (12). The medical team may adopt a paternalistic attitude if the patient is considered unable to make decisions on his own. From a legal standpoint, a patient can be considered unfit to make a medical decision and, thus, a third party is required (usually a family member). A paternalistic attitude of the medical team is not always justified; therefore, some studies support the involvement of a third party (lawyer, relative) in psychiatrist-patient relationship (13). When the patient is considered incapable, a legal or personal representative may give his consent for both healthcare and research. Clinical research can include subjects with MD which are not able to make a decision, and, in these cases, the legal or personal representative can decide for them. The mental capacity of patients with MD is a subject that involves many ethical and legal discussions, especially in situations that require coercive measures (14, 15). The patient should be represented by a legal representative, who can be appointed if the patient can not represent themselves. If the patient lacks judgment, consent will be given by the patient's legal representative.

Individuals incapable of self-autonomy, relative or absolute, are considered vulnerable persons. There are two vulnerable population groups: the first group includes some patients with MD, children, coma patients, individuals who lack self-autonomy. This group lacks the ability to decide for themselves, and need a permanent representative to protect their interests. The second group incorporates individuals who can be manipulated, coerced to participate in human research studies. This vulnerable group is characterized by an impaired reasoning ability and not a lack of judgment. This group can also include patients found in extreme suffering or poverty, etc. (16, 17).

In Romania, there have been numerous efforts to decrease the degree of subjectivity specific to psychiatry, often found in relationships with other medical specialties, institutions or forums. This issue is covered by the Mental Health Law, the law 487 of 11 July 2002 (129/2012), developed by the Romanian Parliament (18, 19).

LAW 487/2002 VS. LAW 129/2012

The subject of this article covers some aspects coming from the imprecise definition of certain terms used by the aforementioned Law, which can bring forward interpretations that may cause dysfunction and / or abuse for all parties involved. Article 5 of Law 487/2002 (law 129/2012) defines patients with MD. Thus, an individual with MD is "a mentally ill person, a person with mental imbalance or mentally underdeveloped or alcohol and drugs addiction and the person who exhibits other disorders that can be classified, in accordance with cur-

rent diagnostics used in the psychiatric medical practice." The law specifies degrees of MD, also referring to a person with serious MD, characterized by the fact that "he is not able to understand the meaning and consequences of his behavior, thus requiring immediate psychiatric help". An important aspect is that, of all mental pathology, between 20% and 30% of cases can be considered patients with severe MD. Given their complexity, these cases are assisted by a therapeutic team, which is defined by law as "all MH professionals involved in providing mental health care, medical assistance and the caring of a person with MD, such as: physician, psychiatrist, psychologist, nurse specialist, social worker, occupational therapist and paramedical personnel". Regarding people with MD, the Law promotes the concept of mental capacity, meaning the "attribute of the mental state to be compatible, at a time given, with the exercise of civil rights or specific activities." It is obvious that it mainly addresses the legal aspects of mental capacity and focuses less on the medical ones, not covered by the definition. Based on this type of definition, only two categories exist, scarcely seen: the first one compatible with the exercise of human civil rights and other specific actions, and the second one incompatible. Almost all patients with severe MH problems belong to the second.

Law 487/2002 republished, makes a number of clarifications regarding consent, starting from its definition: "the agreement of a person regarding admission procedures, diagnosis and treatment, which must be free from any coercion and preceded by understandable and full access to information, showing the advantages, disadvantages and alternatives procedures, which can be further confirmed whenever needed or at the initiative of the person concerned ". In conclusion, valid consent can legally be obtained from individuals whose psychological distress is placed in the first category and not in the second.

The law states that the patient or his legal representative has the right to retract given consent and, in this case, the psychiatrist is obligated to inform the patient or his legal representative regarding the consequences of discontinuing treatment. But, if the specialist decides that calling off treatment could pose a danger to both patient and the individuals around him, because of his impairment, he has the right to continue with appropriate medical therapy for as long as strictly required, with the condition of notifying and presenting the case to a specialized procedural committee. If we consider informed consent an action, some patients will be capable to understand both content and consequences while others won't. Judgment, legally speaking, represents "that aspect of mental capacity which deals with a specific event and the individual's capacity to understand its significance and results".

Even now, in Romania, the law defines two categories of legal representatives for a patient, but does not take in consideration the mental capacity of that said individual: the legal representative ("appointed person, by law, to represent the interests of a mentally ill person) and the personal representative ("a person, other than the legal representative, who accepts to represent the interests of a mentally ill patient, chosen by the person in question"). While the legal aspects of the legal representative are amply and clearly covered, the legal framework regarding the attributions and the appointment of the personal representative

are loose, thus allowing a number of interpretations adverse to psychiatric practice. One of the most important issues, not covered by context, is the fact that a personal representative should have a mental capacity compatible with exercising civil rights or specific actions. This flaw often leads to situations in which a patient with severe mental issues exercises his right to appoint a personal representative and chooses an individual who also suffers from severe mental problems. He is given by law, as we will see a lot of decision-making power. A second shortcoming is the fact that the moment of appointment of the personal representative is overlooked (before, at or during hospitalization) or the period of time he is allowed the exercise the power with which he was invested. A third issue is created by the absence of information concerning the existence of a legal document that mentions the personal representative named by the patient, which can be used in relation with the institutions that assist them. The absence of such a document favors an opportunistic attitude coming from the patient, who can– depending on the situation - occasionally appoint more than one personal representative to serve his interests. Patients are informed about this right upon admission, when they are made aware that the content of the informed consent template, including the Mental Health Act, as an annex. For non-voluntary admission cases, the law provides extended information, offering a wide and varied range of possibilities to apply for admission, even if the solicitor it not the patient's legal or a personal representative. According to law 487/2002, republished (Law 129/2012), non-voluntary admission is "against the will of the patient or without his consent". Not being able to realize the severity of his disease, the patient often considers admission useless or even an abuse. Hence the interpretation, easy to deduce, that those who requested admission do not represent the interests of the patient, the later one refusing their representation and soliciting another representative (which he considers more "reasonable").

Regarding the evaluation of MH state and the procedures needed to diagnose mental illnesses, in chapter 3 the law brings forward again the two types of patient representatives. So, according to article 10, law 487/2002 republished, "a patient's mental state can only be evaluated directly by a trained specialist". It is also stated that "MH evaluation can be performed after the subject has been properly informed and after he has given his consent, with the exception of specific situations, established by law, when an individual presents difficulties understanding direct consequences of such a decision, situations when a legal or personal representative is required" according to article 11, law 487/2002 republished (law 129/2012). According to article 15 law 487/ 2002 (law 129/2012), the results of MH evaluation must be recorded in official medical files and also made available to the patient, his legal representatives or at the express request of proper authorities. Also, the treatment plan a psychiatrist is obligated to establish whenever a mental illness is diagnosed, must be presented to both patient and legal representative. Furthermore, "to establish and apply a treatment plan, the psychiatrist is obligated to have proper consent and to respect the patient's rights to assistance when it comes to making such a decision".

However, the law refers to situations in which the psychiatrist can establish treat-

ment without the patient's consent: the patient's behavior is an immediate danger to himself or those around him; the patient lacks mental capacity to understand his disease and the need of medical treatment; the patient has a legal guardian established by law or court ordered; the patient is underage thus the psychiatrist is required to ask and obtain consent from his legal or personal representative.

When the psychiatrist cannot obtain the patient's consent (20) or consent from his legal or personal representative, he can act "on his own responsibility, establishing a diagnosis and a treatment plan he deems necessary, for a period of time strictly necessary to achieve the aim pursued" according to MH law. However, the law requires that such cases must be brought to the attention and scrutiny of a specialized procedure committee. Whenever a patient and his legal or personal representative are in a conflict of interests, the psychiatrist has the right to ask proper authorities for a new legal representative. This legal aspect, gives the psychiatrist the opportunity to correct a possible wrong decision made by the patient when he chose a personal representative, but is a procedure that consumes a lot of time and resources. Furthermore, such a procedure lacks a proper protocol in accordance with the situation, established by law, thus making it more difficult to apply.

According to articles 34 law 487/2002 republished (law 129/2012), "any mentally ill patient or former patient or his legal/personal representative can submit a complaint whenever a patient's rights are violated". This article gives a patient's legal representative the possibility to delay any treatment plan. To resolve such an issue a certain amount of time is required, during which the patient is not subjected to any treatment or procedures, established by law, for serious medical conditions or relapses. In section 3, "The rights of mentally ill patients", the law gives the legal representative a list of actions he can choose from, interventions that can trigger lengthy and complex legal procedures, which may impair the quality and speed of psychiatric treatment. According to article 44 MH laws republished, "from the moment he is admitted in a mental healthcare institution, every patient must be informed, as soon as possible, clearly and understandably, about his rights, what they mean and how he can act according to them, in accord to legal provisions. If the patient is unable to understand this information and he will incapable to understand them in the near future, his right will be brought to the attention of his legal or personal representative". According to article 62 law 129/2012 "the patient or his legal/personal representative can apply for a forensic psychiatric examination or any other procedure, according to law".

CONCLUSIONS

Aspects illustrated previously around legal stipulations, when the patient and his legal/personal representative represent a dysfunctional element in psychiatric practice, can be corrected by establishing a more precise legal role for the legal representative.

As a whole, this article illustrates one of the main issues of Mental Health Law, tailored to current international legal models. The issues discussed in the article are some that obligates the psychiatrist and the therapy team to ethical and professional approaches that have more of a Hippocrates 'oath base than a legal base, which would

be beneficial for all parties involved.

Based on these considerations and continued comparative analysis of the laws of different countries, we consider the following issues as requiring modifications, additions or revisions in Romanian law: implementing an advance directives legal system in which the patient may express wishes for the future of his psychiatric care, including aspects regarding the personal representative.

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NEWS

TWO SPATIOTEMPORAL PATTERNS OF ^{123}I-IOFLUPANE SCINTIGRAPHIC UPTAKE IN PARKINSON'S DISEASE

To assess correlations between the degree of dopaminergic depletion measured using single-photon emission computed tomography (SPECT) and different clinical parameters of disease progression in Parkinson's disease (PD) was conducted a retrospective study including 411 patients (60-80 years,of age) undergoing (123)I-ioflupane SPECT scans between 2003 and 2013. According to their clinical diagnosis, there were 301 patients with PD (163 men) and 110 patients with nondegenerative conditions included as controls (55 men). Comprehensive and operator-independent data analysis included spatial normalization into standard space, estimation of the mean uptake values in the striatum (caudate nucleus + putamen) and voxel-wise correlation between SPECT signal intensity and disease stage as well as disease duration in order to investigate the spatiotemporal pattern of the dopaminergic nigrostriatal degeneration. Increasing disease stage was associated with an exponential decrease in (123)I-ioflupane uptake ($R(2) = 0.1501$) particularly in the head of the ipsilateral caudate nucleus ($p < 0.0001$), whereas increasing disease duration was associated with a linear decrease in (123)I-ioflupane uptake ($p < 0.0001$; $R(2) = 0.1532$) particularly in the contralateral anterior putamen ($p < 0.0001$). These two distinct spatiotemporal patterns of posterior to anterior dopaminergic depletion associated with disease stage and disease duration in patients with PD can be used for clinical and research applications. (Badoud S et al. Distinct spatiotemporal patterns for disease duration and stage in Parkinson's disease. *Eur J of Nucl Med and Molec Img* 29.08.2015 Doi:10.1007/s00259-015-3176-5, 1-8)

Roxana Gherasim

INTERVENTIONAL CARDIOLOGY AND ELDERLY PATIENTS: WHEN IS IT TOO LATE?

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INTERVENTIONAL CARDIOLOGY AND ELDERLY PATIENTS: WHEN IS IT TOO LATE? (Abstract): **Aim:** To analyze the incidence of complications associated with interventional cardiology (myocardial revascularization techniques or permanent cardiac pacing) in hospitalized elderly patients. **Material and methods:** Retrospective study of 40 patients aged ≥ 65 years, with a history of interventional cardiology: percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG) or permanent cardiac pacing who were admitted to the Geriatric Clinic in the interval January-December 2014. The main causes of current readmission were investigated. Comprehensive geriatric assessment (CGA), including nutritional, functional and psychological assessments, were performed. **Results:** Most cardiovascular procedures were performed in patients aged ≥ 70 years. Current hospital admission was mainly the result of non-adherence to medical treatment. None of the patients were admitted due to complications of any of the above mentioned procedures. CGA performed in these patients revealed: increased autonomy at home reduced cognitive impairment, decreased rate of mild depression and risk of undernutrition. Psychological examination confirmed that quality of life improved in all patients after interventional cardiology. **Conclusions:** Interventional cardiology confers major benefits in elderly patients, with low rates of late complications, and influences prognosis and quality of life. **Keywords:** INTERVENTIONAL CARDIOLOGY, ELDERLY, QUALITY OF LIFE.

One of the major cardiovascular risk factors is ageing itself, mainly due to inexorably advancing atherosclerosis but also to the inevitable ageing of the cardiovascular system and long period of time for other risk factors to negatively influence the cardiovascular condition (1). Moreover, elderly patients frequently develop cognitive and functional impairments (sleep disorders, chronic pain etc.), which would require complex drug administration and special care in establishing a therapeutic plan. Given the high prevalence of cardiovascular diseases among the elderly, special attention for this group of patients is granted in the latest ischemic heart disease guidelines but information provided derive mostly from carefully selected randomized trials with

carefully selected elderly groups. Patients aged ≥ 80 years are almost completely absent in trials, and when included, it would be only those without co morbidities, which are the hallmark of their age. We face an interesting paradox: the patients at the highest cardiovascular risk should benefit most from the treatment recommended by the guidelines, but this category is not really part of data supporting the guidelines (2).

The recommendations for interventional cardiology in the elderly population have permanently been updated in the past decade. The 2012 and 2013 guidelines regarding the management of stable angina and acute coronary syndromes emphasize that older patients are more likely to have diffuse and severe coronary atherosclerosis with a higher prevalence of three-vessel and left main coronary artery disease. The guidelines acknowledge the less frequent use of evidence-based therapies in older adults, due to therapeutic challenges secondary to age-related changes: altered pharmacokinetics (due to reduced muscle mass, renal and/or hepatic dysfunction, and reduced volume of distribution) and pharmacodynamics (increased risks of hypotension and bleeding) (2), and the risk of polypharmacy and iatrogenesis. Therefore, there is a certain degree of reluctance in the application of guideline recommendations in older patients, often based on a subjective risk evaluation.

This article aims to explore the benefits and limitations of interventional cardiology in the geriatric population.

MATERIAL AND METHODS

We retrospectively analyzed a group of 1219 patients aged ≥ 65 years old who were admitted to the Geriatric Clinic of the Iasi "Dr. C.I. Parhon" Hospital, Romania in the interval January-December 2014. Of the total number of patients, 40 patients (3%) had undergone at least one of the following procedures: percutaneous coronary intervention (PCI), permanent cardiac pacing, and coronary artery by-pass graft (CABG). Of them, 4 patients (10%) underwent surgery for hip fracture and were admitted for postoperative assessment and 2 patients (5%) were recovering from oncological surgery.

Patients were divided into two groups in order to assess the significant gender differences. The parameters of interest were: baseline demographics (age, gender, and social origin), causes of current admission (medication non-adherence included), symptoms and signs (angina equivalents included), complications of cardiovascular procedures, course, and discharge status.

Every patient had been subjected to a comprehensive geriatric assessment (CGA) and a psychological examination, which included: ADL (Activities of Daily Living) (3), IADL (Instrumental Activities of Daily Living) (4), MMSE (Mini Mental State examination) (5), GDS (Geriatric Depression Scale) (6) and MNA (Mini Nutritional Assessment) (7).

Data were collected from the patient medical records by the medical staff involved in this study. The collected data were analyzed by SPSS 18.0 software, using the Paired T Test for quantitative analyses and Chi-square Test for differences in proportions. The possible influence of every parameter in each of the two groups (male vs female patients) was evaluated. When the study of correlations indicated a degree of influence, we analyzed if there was a statistically significant relationship between that parameter and its dependent variables. The statistical significance was defined in a 95% confidence interval ($p<0.05$). For the geriatric assess-

ment we carried out an overall descriptive group analysis.

RESULTS

The analysis of the demographic parameters showed that the mean age was 77.5 years (range 65 - 90 years). The cohort included mainly old (52.5%) and young-old (35%) patients, with no significant difference between genders (p=0.993). As to the area of residence, 55% of patients lived in urban areas and 45% in rural areas, with no significant difference between genders (p=0.923).

In conclusion, the study group was homogenous in terms of gender, age group and area of residence (tab. I).

The main cardiac procedures were permanent cardiac pacing (42.5%) and coronary angioplasty (40%). The mean age at the time of intervention was 71.6 years. CABG was more frequently performed in males, and in this group there was a threefold increased relative risk of readmission.

TABLE I

Analysis of the demographic parameters

Parameters	All cohort (n=40)	Male (n=23)	Female (n=17)	p
Age (years) mean±SD *limits	77.5 *(65-90)	77.21±6.45	76.41±4.74	0.300
Classification				
Young-old (65-74 y)	14 (35.0%)	8 (34.8%)	6 (35.3%)	0.993
Old (75-84 y)	21 (52.5%)	12 (52.2%)	9 (52.9%)	
Oldest old (85+ years)	5 (12.5%)	3 (13.0%)	2 (11.8%)	
Area of residence				
Rural	14 (45.0%)	10 (43.5%)	8 (47.1%)	0.923
Urban	21 (55.0%)	13 (56.5%)	9 (52.9%)	

The symptoms at admission were various, but most patients presented with dyspnea of cardiac origin (72.5%), mainly due to discontinuation of treatment (47.5% of the patients). None of the symptoms were due to complications following cardiac procedures (e.g. stent/graft occlusion, pacemaker dysfunction) (tab II).

The geriatric assessment showed that most patients had increased autonomy at home. ADL test showed that 31 patients (77.5%) had full function, 9 patients (22.5%) had moderate impairment, and no patient had severe impairment. The IADL test showed that all patients were independent; none of them needed any assistance at home. CGA and psychological examination concluded that 34 patients (85%) had normal cognitive function and only 6 patients (15%) had mild cognitive impairment. There were no signs of depression in 14 patients (35%), while mild depression was detected in 17 patients (42.5%) and severe depression in 9 patients (22.5%). The MNA scale showed that most of our patients were at risk for malnutrition (26 patients, 65%), 8 patients suffered from malnutrition (15%), while only 8 patients (15%) had a good nutritional status.

Patients who underwent surgery for hip fracture had a good postoperative outcome and their recovery period was within normal limits, since rehabilitation was initiated after the same period of time as in patients without cardiac surgery.

TABLE II

Clinical history and medical status

*** increased relative risk in the female population**

Parameters	All cohort (n=40)	Male (n=23)	Female (n=17)	p	RR (IC95%)
Type of intervention					
▪ PCI	16 (40.0%)	9 (39.1%)	7 (41.2%)	0.845	0.95(0.44÷2.04)
▪ Pacemaker	17 (42.5%)	9 (39.1%)	8 (47.1%)	0.859	0.83(0.41÷1.70)
▪ CABG	5 (12.5%)	4 (17.4%)	1 (5.9%)	0.546	2.96(0.36÷24.1)
▪ PCI+ pacemaker	1 (2.5%)	1 (4.3%)	0 (0.0%)	0.878	-
▪ CABG + pacemaker	1 (2.5%)	0 (0.0%)	1 (5.9%)	0.876	-
Mean age at intervention (years) ±SD	71.69±6.37	70.35±6.86	73.20±5.61	0.105	-
Symptoms at admission					
▪ breathlessness	29 (72.5%)	19 (82.6%)	10 (58.8%)	0.191	1.40(0.90÷2.18)
▪ angina	5 (12.5%)	2 (8.7%)	3 (17.6%)	0.717	2.03(0.38÷10.8)*
▪ atypical chest pain	11 (27.5%)	2 (8.7%)	9 (52.9%)	**0.006**	6.09(1.50÷24.6)*
▪ ankle swelling	10 (25%)	6 (26.1%)	4 (23.5%)	0.853	1.11 (0.37÷3.33)
▪ fatigue	4 (10%)	0 (0.0%)	4 (23.5%)	**0.026**	-
▪ palpitations	9 (22.5%)	3 (13.0%)	6 (35.3%)	0.200	2.71(0.79÷9.31)*
▪ headache/dizziness	15 (37.5%)	3 (13.0%)	12 (70.6%)	**0.001**	5.41(1.80÷16.2)*
▪ fever	2 (5.0%)	2 (8.7%)	0 (0.0%)	0.608	-
▪ cough±sputum	11 (27.5%)	10 (43.5%)	1 (5.9%)	**0.023**	7.39(1.04÷52.4)*
▪ nausea/abdominal pain	7 (17.5%)	1 (4.3%)	6 (35.3%)	**0.034**	8.12(1.07÷61.3)*
▪ other	13 (32.5%)	6 (26.1%)	7 (41.2%)	0.505	0.63(0.26÷1.55)
Causes for readmission					
▪ medication non-adherence	19 (47.5%)	10 (43.5%)	9 (52.9%)	0.785	0.82(0.43÷1.57)
▪ infection	8 (20.0%)	6 (26.1%)	2 (11.8%)	0.472	2.22(0.51÷9.67)
▪ hypertensive crisis	5 (12.5%)	3 (13.0%)	2 (11.8%)	0.717	1.11(0.21÷5.92)
▪ others (arrhythmia, anxiety, anemia, excessive physical exertion, etc.)	8 (20.0%)	3 (13.0%)	5 (29.4%)	0.379	0.44(0.12÷1.61)
Discharge status					
▪ recovered	36 (90.0%)	22 (95.7%)	14 (82.4%)	0.394	1.16(0.92÷1.47)
▪ transferred	1 (2.5%)	0 (0.0%)	1 (5.9%)	0.878	-
▪ dead	3 (7.5%)	1 (4.3%)	2 (11.8%)	0.795	0.37(0.04÷3.75)

DISCUSSION

Interventional cardiology is often controversial, and strong debates argue the optimal therapeutic indications in patients ≥ 75 years old who require myocardial revascularization and/or cardiac pacing treatment.

Several studies suggest an increased mortality rate in older patients compared with young adults undergoing a myocardial revascularization procedure (interventional or surgical) (8), but the data on this issue are often contradictory.

The CRUSADE Initiative points out that 7% of patients presenting with STEMI were not considered for myocardial revascularization because of their advanced age (9). If coming to choose between techniques, a larger number of geriatric patients

seem to benefit from PCI compared to CABG, but their age should not be the sole criterion for the type of revascularization. Their selection should be based on the assessment of frailty, co morbidities, life expectancy and personal desire (2, 10, 11).

Evidence from the medical literature shows that PCI should be a viable treatment option in elderly patients (12-15). PCI is a low-invasive procedure and carries a small likelihood for cognitive impairment. In carefully selected patients with clinically relevant coronary stenosis, PCI may provide greater symptomatic relief than medical therapy. Patients presenting with ST elevation myocardial infarction (STEMI) who are expected to live an autonomous life style after discharge are the best candidates for PCI in order to secure vessel patency and achieve myocardial salvage. Still, PCI is frequently avoided in elderly patients because age is a potent and independent negative predictor of death, myocardial re-infarction, stent thrombosis, and re-hospitalization (11). Hence, a careful follow-up of these patients, associated with maximizing their medical treatment and therapeutic education to prevent non-adherence are mandatory.

The determination of geriatricians to assess the benefit of interventional cardiology in the elderly patients is reflected by studies that included specific subgroups, such as octogenarians (10) and nonagenarians (11, 12). Liu et al. (16) compared 112 octogenarians and 336 young adults who underwent PCI between 1993 and 2010 and found no significant differences with regard to procedural success, in-hospital complications and major cardiovascular events 1 year after the procedure. The authors concluded that PCI remains a reliable treatment for carefully selected elderly patients, and complying with the above mentioned recommendations, such as having a good radial or ulnar access site, limiting contrast load and optimizing fluid status to prevent contrast associated nephropathy, and favoring bare-metal stents. However, other studies (17) showed that PCI was associated with lower mortality and morbidity on short-term, while CABG was followed by superior outcomes on the long-term. The same conclusions are found in other studies that included octogenarians: CABG poses a greater risk of short-term complications compared with PCI, but provides a better survival (18, 19).

Maintaining quality of life up to patient expectations should be an important parameter to take into account when managing geriatric patients (20), and, therefore, many studies analyzed if patient status after PCI correlated with a better quality of life. Thus, quality of life of octogenarians following PCI appears to be acceptable, with main improvements in their cognitive status (8). Spertus et al. (21) found that the only factors independently associated with a better quality of life 1 year after PCI were improvement of physical function and decrement of angina frequency.

Frailty, co morbidity and quality of life are highly prevalent in elderly patients undergoing PCI. These factors should be incorporated in current risk-prediction models along with traditional cardiovascular risk factors in order to assess the risk of patients undergoing PCI. For example, their inclusion improved the discriminatory ability of the Mayo Clinic risk score to evaluate the long-term prognosis of elderly patients after PCI (22). Long-term mortality/myocardial infarction were significantly higher in patients considered to be frail.

Hip fracture is highly common among

geriatric patients. For the management of these patients, Papp (23) developed a useful algorithm to assist the surgeon in gathering important information and developing perioperative approaches to treatment according to the personal medical profile of the patient.

While the incidence of acute myocardial infarction in younger adults is increasing, the conditions that require a pacemaker implantation (complete atrioventricular block, atrial fibrillation with a spontaneous heart rate < 40 bpm, sick sinus disease, etc.) are prevalent in older patients. Some statistics estimate that 70-80% of pacemaker implantations are performed in patients aged ≥ 65 years (24).

The most common complications associated with a pacemaker implantation are: pocket hematoma, infection of the pocket, pneumothorax – at the time of implantation, and late complications: lead displacement/failure, lead endocarditis, pocket fibrosis and myocardial rupture. Ozcan et al. (25) published in 2013 a prospective study in which they compared the incidence of these complications in older patients with younger patients. Their results showed that pacemaker implantation in elderly patients is a safe procedure when performed by an experienced team, and patients over 70 years old may even have fewer complications than younger patients. The explanation could be the higher proportion of dual chamber pacemaker implantation in younger patients, which is frequently associated with atrial lead dislodgement. None of the patients included in our study presented with late complications of pacemaker implantation.

CONCLUSIONS

Our study, even though a single-center study on a small number of patients for the prevalence of complications related to past cardiac interventions, confirmed that interventional cardiac surgery is beneficial in elderly patients. The study group was homogenous in terms of gender, age group and area of residence, but the absence of statistically significant differences between the analyzed parameters should be taken with caution.

Late complications of PCI, CABG or pacemaker implantation were not recorded in our study. Although most procedures were performed in patients over 70 years of age, the cognitive, psychological and social status of our patients had a positive impact on the outcome of these procedures. Hence, according to current evidence, the decision to perform an invasive cardiac procedure should not be based on chronological age alone, but rather on each patient's general eligibility and the clinical circumstances as a whole.

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MYTHS AND CONTROVERSIES IN HYPOGONADISM TREATMENT OF AGING MALES

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MYTHS AND CONTROVERSIES IN HYPOGONADISM TREATMENT OF AGING MALES (Abstract)**:** Low T is an independent risk factor for metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM) development; patients with these clinical conditions should be screened for hypogonadism. Testosterone replacement therapy (TRT) ameliorate**s** libido, improves bone mass, improves insulin resistance, reduces fat mass and increases lean body mass with no change in body weight. There are no evidences that testosterone therapy increases the risk of prostate cancer but it is certain that testosterone stimulates growth of metastatic prostate cancer. TRT has an antiarrhythmic and a vasodilator effect, independent of the nitric oxide effect. Patients with heart failure have low levels of testosterone**,** and TRT improves exercise capacity. Men with low testosterone have risk for premature death. Cardiovascular adverse effects of testosterone therapy are under discussion. We need large prospective placebo-controlled randomized trials to determine definitively the cardiovascular risks of TRT. **Keywords**: MALE HYPOGONADISM, TESTOSTERONE REPLACEMENT THERAPY, DIABETES MELLITUS, METHABOLIC SYNDROME, CARDIOVASCULAR RISK, MORTALITY.

Late onset hypogonadism (LOH) is a clinical and biochemical syndrome associated with advancing age and characterized by symptoms and a deficiency in serum testosterone level; it may cause a significant deterioration in quality of life and functional alterations of multiple organs (1,2). In the Massachusetts Male Aging Study (MMAS) after the age of 40 the average age-related reduction in total testosterone is 0.8% per year, whereas free testosterone declines by 1.7-2.8% per year; the prevalence of syptomatic hypogonadism is between 6-12% (3). In European Male Aging Study (EMAS) the prevalence of hypogonadism is 2.1% and increase with age from 0.1% for men 40-49 years of age to 5.1% for those 70-79 years (4). The odds of having low testosterone levels is 2.4 higher for obese men, 2.1 times higher for men with diabetes, 1.58 times higher for men with hypertension, and 1.5 times higher for those with dyslipidemia or chronic obstructive pulmonary disease (1,5).

Clinically, the syndrome is characterized by:

- reduce sexual desire, low libido, erectile dysfunction, reduction of spontaneous erections

- breast tenderness, gynecomastia
- corporeal hairiness reduction, reducing the frequency of shaving
- mood changes with reduced intellectual activity, cognitive functions, spatial
- orientation ability and memory
- fatigue, depression and irritability, depressed mood
- sleep disorders, hot flushes , sweating
- decrease muscle mass and muscle strength
- increased body fat mass, increased BMI
- osteopenia / osteoporosis and increased risk of fracture
- mild anemia (Hb and Ht normal values for women) (6, 7).

The MMAS has shown that a lot of co morbid conditions are strongly associated with declining testosterone levels; in these cases it is suggested measurement of testosterone level:

- metabolic syndrome
- obesity
- hyperlipidemia
- hypertension
- elevated fasting glucose and serum insulin elevated C reactive-protein
- diabetes mellitus type 2
- chronic obstructive pulmonary disease
- cardiovascular disease (including aortic atherosclerosis)
- inflammatory arthritic disease
- HIV-associated weight loss
- end stage chronic kidney disease
- low trauma fracture
- sellar mass, radiation in the sellar region
- chronic use of glucocorticoids and opioids
- hemochromatosis (2, 8, 9).

The diagnosis is made by subnormal values of total testosterone and the combination of characteristic symptoms.

Testosterone circulates bounded to Sex Hormone Binding Globulin (SeHBG) (68%), loosely bound to albumin (30%) and only 0.5-3% is free. Total testosterone levels may vary depending on the SeHBG value.

Total Testosterone is determined in the morning, 8-11 AM, by RIA, immunometric methods or ideal liquid chromatography tandem mass spectrometry (LC-MS), 2 determinations at intervals of at least 1 month (10). The dosage of **free testosterone** is expensive; the only method validated is by equilibrium dialysis. **Bioavailable Testosterone** (free plus the loosely bound to albumin) is determined by ammonium sulphate precipitation method, or is calculated from total testosterone, SeHBG and albumine level.

SeHBG - binds testosterone with higher affinity than estrogen. The values are increased in elderly, liver cirrhosis, hyperthyroidism, anticonvulsant therapy, estrogen, HIV and decreased in moderate obesity, hypothyroidism, nephrotic syndrome, acromegaly, corticosteroid use, androgen treatment, progesterone (10, 11).

It is not recommended a population screening for androgen deficiency (2); in certain circumstances (see above), there is a high prevalence of low testosterone levels and it is suggested measurement of serum testosterone (tab. I).

Testosterone treatment is recommended:

- at patients with typical clinical signs of androgen deficiency and biological confirmation
- for a limited time for patients with HIV infection, low testosterone and weight loss to increase and maintain lean mass (3-6 months)
- short chronic glucocorticoid therapy (prednisone equivalent 5-7.5mg/day) due to inhibition of gonadotropic axis (2,12).

TABLE I

Biochemical definitions of hypogonadism

Society	Serum testosterone values		
EAA, ISA, ISSAM	<3.4 ng/ml	<340 ng/dl	<12 nmol/l (mild)
EAU, ASA, ISSM	<2.31 ng/ml	<231 ng/dl	<8 nmol/l (severe)
ES	<3.0 ng/ml	<300 ng/dl	<10.4 nmol/l
AACE	<2.0 ng/ml	<200 ng/dl	7 nmol/l

EAA = European Academy of Andrology, ISA = International Society of Andrology, ISSAM = International Society for the Study of Aging Male, EAU = European Association of Urology, ASA = American Association of Urology, ISSM = International Society for Sexual Medicine, ES – Endocrine Society, AACE = American Association of Clinical Endocrinologists

The goal of treatment (2, 11) is to safely restore testosterone to normal physiological levels, thereby ameliorating symptoms associated with TD and improving patient health and well being.

Options for testosterone therapy include oral preparations (2-3 doses per day), buccal agents, injections (at 2-3 weeks or at 12-14 weeks), transdermal systems (patches, gels, solutions) or subcutaneous pallets (tab. II).

TABLE II

Route of administration, dosage, adverse events and monitoring of treatment with testosterone

Testosterone	Administration/Dose	Adverse events/ Disadvantages	Monitoring
Testosterone enantat/ cipionat	75-100 mg / 7 days or 150-200 mg / 14-21 days i. m.	wide fluctuations in serum testosterone levels	testosterone level at the middle interval between 2 injection
Testosterone undecanoat inj.	1000 mg i. m every 12-14 weeks	an enhancement at dose administration	immediately until the next injection
Testosterone non- scrotal patches	5-10 mg/ day	local irritation	at 3-12 hours after application
Testosterone gel	50-100mg/day	transfer during close contact	anytime (> 7 days after initiation)
Buccal testosterone	30mg /day	taste changes	immediately
Oral Testosterone Undecanoat	40-80mg x 2-3/day, during meals	digestives effects	at 3-5 hours after administration
Testosterone implants	4-6 implants of 200 mg, every 4-6 month	risk of extrusion or infection	at 3-5 hours after administration

Monitoring of testosterone treatment is recommended:

- first, at three months from therapy initiation, and then annually;

- determination of hematocrit (Ht); Ht > 52%-54% during the treatment is an indication of stopping the administration of testosterone, assessment of hypoxia and sleep-apnea**,** and after normalization of Ht restarting with lower doses of testosterone (2,9).

- BMD by DXA is repeated every 12-24 months in hypogonadal men with osteoporosis or fragility fractures;

- digital rectal examination and PSA checking are performed before treatment, at 3 months, and then in accordance with guideline for prostate cancer screening depending on age; urological evaluation is necessary if :

- PSA > 4 ng / ml

- PSA increase of more than 1.4 ng / ml within any 12 month period of testosterone treatment
- PSA velocity of more than 0.4 ng / ml / year, using PSA level after 6 month of testosterone administration as reference (only if PSA data are available for at least 2 years)
- Score for prostatic symptoms > 19 (AUA- American Urology Association or IPSS-International Prostate Symptoms Score - Europe)

Positive effects of testosterone administration are translated by improving mood, well-being and erectile function, or erectile response at phosphodiesterase 5 inhibitors administration, increasing of muscle mass and strength, especially in the upper limbs, decreasing of abdominal fat, increasing of bone mass. Short-acting testosterone preparations are preferred because it can be immediately interrupted if side effects occur.

Testosterone therapy in ageing men with hypogonadism is indicated only if the positive effects outweigh the risks, and after patient information about treatment risks and benefits.

Conditions in which testosterone administration is associated with increased risk of adverse effects:

- very high risk of serious adverse outcomes in metastatic prostate cancer and breast cancer
- moderate to high risk of adverse outcomes in unevaluated prostate nodule or indurations, or unexplained elevations of PSA (>4 ng/ml), Ht >50%, severe lower urinary tract symptoms, uncontrolled or poorly controlled congestive heart failure (class III, IV), severe, not treated sleep apnea (1,2).

In young patients, the goal testosterone concentration is midlevel of normal; at ageing patients, the goal is lower level of normal. There is no basis for dose escalations in pursuit of greater efficacy, and the practice may increase the risk of side effects. The vitality, energy and libido are ameliorated in the first 3 months of treatment, but the effect on body composition is evident latter, and is related directly with the testosterone level.

Adverse events for which there is evidence of association with testosterone administration:

- male pattern balding, acne
- gynecomastia - may exist before treatment
- induction or worsening of obstructive sleep apnoea
- worsening of Benign Prostate Hyperplasia (BPH)
- detection of subclinical prostate cancer, growth of metastatic prostate cancer;
- increased hematocrit
- reduced sperm production and infertility
- cardiovascular events (1, 2, 10).

EFFECTS OF LOW TESTOSTERONE AT VARIOUS ORGANS AND SYSTEMS; POSITIVE AND NEGATIVE EFFECTS OF TRT IN AGING MALES

Sexual function

Low testosterone reduces libido but the effect on erectile function is less obvious; erectile dysfunction is associated with low testosterone only among men with high luteinizing hormone (LH) levels. There are evidences suggesting therapeutic synergism with combined use of testosterone and phosphodiesterase-5 (PDE) inhibitors in hypogonadal or borderline eugonadal men (9, 13, 14).

Prostate cancer and BPH

There is no conclusive evidence that testosterone therapy increases the risk of prostate cancer. It is certain that testosterone stimulates growth and worsening of

symptoms in patients with advanced or metastatic prostate cancer. On the other hand, there are studies indicating that the aggressiveness of prostate cancer is higher in patients with low testosterone. For this reason, it is recommended at least one digital prostate exam and PSA evaluation before initiation of androgen therapy.

Also an IPSS > 21 or > 19 for AUA lower urinary tract symptoms (LUTS) are relative contraindication to androgen therapy. Contraindication is not maintained after treatment of prostatic pathology (14, 15). Rectal digital examination and PSA checking are indicated every 3-6 months at the beginning of treatment, and then annually.

Cognition, mood and energy

Some studies found benefits in improving spatial ability, verbal fluency, and working memory in elderly men. In a meta-analysis reviewing the effects on depression, testosterone therapy was found to have beneficial effects on mood and depression (10, 16).

Bone metabolism,
bone mass and fractures

The prevalence of osteoporosis in hypogonadal males is 2-fold increased compared with eugonadal males. Testosterone administration improves bone mass after aromatization to estradiol: spinal BMD increased by 8%. Effects on femoral neck are inconclusive. No fractures studies have been reported. Aging hypogonadism is associated also with vitamin D deficiency but the clinical significance of this relanshionship has to be investigated (10, 13, 17).

Body composition,
muscle strength and risk of falling

Interventional studies indicate an improvement in body composition with an increase in lean mass and decrease in fat mass after administration of testosterone in hypogonadal patients; it seems that the risk of falling by improving mobility is also reduced by administration of testosterone, although the mechanisms involved are not clear (10, 13).

Obesity

In MMAS study, average Body Mass Index (BMI) was 28.5 to 31.5 kg/m² for eugonadals and respective hypogonadals. 20-64% of obese men have low total and free testosterone levels, and low levels are associated with insulin resistance, metabolic syndrome and type 2 diabetes. The exact mechanism of the interrelationship between obesity and low testosterone is related to reduced abdominal lipolysis in case of androgenic deficit, increased visceral fat determining increase aromatase production, with increasing testosterone to oestrogen conversion and pituitary inhibition. Androgen replacement therapy reduces fat mass and increases lean body mass, with no overall changes in body weight (9, 10, 13, 18).

Lipid metabolism

Hypogonadal men have increased levels of total cholesterol and triglycerides (15). TRT decreases total cholesterol in men with lower testosterone levels, with no effect on triglycerides level (19); the effect of TRT in elderly men on LDL-C and HDL-C is controversial. Supraphysiological testosterone levels induce an increase in LDL-C and decrease in HDL-C levels, and increase the risk of cardiovascular disease.

Metabolic Syndrome
and Diabetes Mellitus

Epidemiological evidence from several longitudinal population studies shows that low testosterone (T) is an independent risk factor for the development of both MetS and T2DM. Interaction between insulin resistance, visceral adiposity, and hypo-

gonadism is not sufficiently elucidated, but seems related to the increased production of aromatase, leptin levels and inflammatory cytokines (15, 18). Veterans of U.S. with prostate cancer and anti-androgenic therapy have a higher incidence of type 2 diabetes, myocardial infarction, stroke and sudden cardiac death, and acute antiandrogen-therapy reduces insulin sensitivity and strongly impairs glycemic control of men with T2DM. Prospective studies have shown that men with higher T levels had a 42% lower risk of T2DM. In men with type 2 diabetes, metabolic syndrome and hypogonadism, testosterone therapy improves insulin resistance, as measured by HOMA-IR and reduces HbA1 by approximately 0.7% by 18 months.

Thus, a vicious circle ensues in which MetS suppresses T biosynthesis and conversely, reduced T concentrations predispose and contribute to the onset of development of MetS and in turn obesity (15, 18, 20).

Based on this epidemiological evidence, current guidelines suggest that patients with clinical conditions associated with insulin resistance (obesity, T2DM, and MetS) should be screened for hypogonadism.

TESTOSTERONE THERAPY AND CARDIOVASCULAR SYSTEM

Antiarrhythmic effect

Testosterone has an antiarrhythmic effect due to the observation that a low testosterone causes QTc prolongation (increased incidence of ventricular arrhythmias), reversible by testosterone administration. This effect is explained by increasing expression of Kv1.5 K+ channels and L calcium channels (15, 21).

Testosterone and atherosclerosis

It exist an inverse correlation between testosterone concentration and thickness of the intima (as marker for preclinical atherosclerosis): on the one hand, the metabolic effects, the increasing of the local inflammatory response (IL6, hCRP, TNF, IL-1B), apoptosis and lowering vascular smooth muscle cell stability of a low testosterone are cardiovascular risk factors, and on the other hand atherosclerosis determines low testicle perfusion and low testosterone secretion (15, 20).

Another effect of testosterone is as vascular vasodilator, independent of nitric oxide, possibly by inhibiting L-type and T-type calcium channels. (20,21). In the same way, men with lower levels of endogenous testosterone are more prone to develop coronary artery disease (CAD) during their lifetime, and it exists a correlation between the severity of CAD and the degree of testosterone deficiency (20,22). TRT is positively associated with a significant increase in treadmill test duration and time to 1mm ST segment depression in men with stable coronary artery disease. TRT improves the angina threshold (14, 20) and reduces the infarct size comparative with that in hypogonadal subjects not treated (21). Controversy, there are studies (in rats) that show that exogenous testosterone exacerbate atherosclerosis and it exist a proapoptotic effect of testosterone (21, 23). In his study, Glueck has demonstrated that men with coagulability problems (factor V Leiden mutation or elevated homocysteine) have an increased risk for thromboembolism at 3 months after androgen therapy initiation (24). Prospective epidemiological studies are needed to analyze in detail the association between coronary heart disease and endogenous bioavailable testosterone (20, 23).

Heart failure

A key feature of congestive heart failure (CHF) is metabolic shift toward catabolism,

with activation of neuroendocrine and inflammatory pathways. The result is worsening of exercise intolerance. Studies show that patients with heart failure have low levels of total and free testosterone, and that with worsening severity of cardiac failure, there is a significant stepwise decrease in testosterone concentration (20, 25). Furthermore, it was hypothesized that the only predictor of the degree of deterioration of exercise capacity (using peak VO2, peak-VO2 pulse) is the magnitude of testosterone levels reduction. This means that the association between serum testosterone and aerobic exercise capacity is independent of the severity of heart failure, the use of beta-blockers or chronotropic response to exercise (20). Testosterone therapy in cardiac failure improves exercise capacity by 0.5 DS, not by effect on left ventricular ejection fraction, but by a peripheral direct mechanism on the skeletal muscles, by causing a shift toward more type I muscle fibers in patients with CHF. Low testosterone in cardiac failure patients correlates negatively with exercise capacity and is associated with a worse prognosis and higher mortality, and TRT is associated with a significant improvement in exercise capacity (20, 25).

MORTALITY

Men with low testosterone are at risk of premature death; testosterone value below 8 mmol / l increases mortality three times, and five times the cancers risk (28), and a decreased testosterone with 2.1 DS is associated with an increased risk of cardiovascular mortality by 25% (20). Low testosterone is associated with increased mortality in diabetic patients, and TRT improves the survival (13, 20, 23, 26, 27, 28).

Cardiovascular risk and androgen therapy is a very controversial topic because an optimal level of testosterone is a biomarker for survival, but the androgen therapy may increases the risk of nonfatal MI. There are a lot of studies that find no increase in the cardiovascular events in case of testosterone replacement therapy, but pharmaceuticals companies support many of them. Studies that had alert about the cardiovascular risk are also critical because no one had as endpoint the cardiovascular risk and the TRT, most studies involved have small cohorts with a small number of events, the study population was old (over 70 years), with cardiac problems, the doses of testosterone were very high (100mg/day) (29), or the alternative study drug was an PDE inhibitor that provide cardiovascular-protective effect (30, 31), or the impact of symptoms as an indication for TRT prescribing was not analyzed because is well-known that the presence of erectile dysfunction is an independent cardiovascular risk factor (increase the incidence of CV events with more than 50%)(30, 31, 32).

This apparent discordance between low testosterone as cardiovascular risk factor and the potential cardiovascular risk of testosterone replacement therapy could be explained by Corrona's concept of **"low testosterone syndrome"** - an adaptive response in order to be more resilient to a pathological condition (cardiovascular frailty: T2DM, CVD); the low testosterone is only a marker for poor health, for saving energy (less physical and sexual activity) and to protect species (low fertility) (14).

Another explanation for these discrepancies between low levels of testosterone as cardiovascular risk factor and the cardiovascular risk in case of TRT, could be that endogenous testosterone is beneficial, but exogenous testosterone and its metabolites could be harmful, the degree of testosterone deficiency is not similar in late onset hypogonadism (LOH) versus Klinefelter

syndrome, or induced hypogonadism in prostate cancer, auto medication and self increasing doses (14).

If we want to respond to this debate: Is low testosterone level in CVD a positive consequence of the body trying to decrease unnecessary energy (and in this situation the treatment might be deleterious for overall and cardiovascular health) or it represent**s** a pathophysiological factor in the CVD (in this situation the treatment is recommended)**,** we need large prospective placebo-controlled randomized trials such as the Women's Health Initiative to determine definitively the cardiovascular risks of TRT. Until this**,** physicians and patients should have a conversation about the risks and benefits of using testosterone, especially in patients who have pre-existing heart disease (32, 33).

In conclusion**,** LOH is frequently co morbid with almost all severe and chronic diseases; clinicians have to look for hypogonadism in men with Met**S**, T2DM or CV disease**,** and conversely to look for metabolic and CV co morbidities when hypogonadism is found. TRT is able to improve central obesity (subjects with MetS), glycometabolic control and insulin resistance, ameliorate exercise capacity and survival. Appropriately prescribed testosterone is undoubtedly beneficial**,** but caution needs to be taken in certain situations**,** as the associated health/benefits of TRT **to outweigh** the potential increased risk of cardiovascular-related events.

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IMPLEMENTING EBUS TBNA: FIRST EXPERIENCE AND REVIEW OF LITERATURE

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IMPLEMENTING EBUS TBNA: FIST EXPERIENCE AND REVIEW OF LITERATURE (Abstract): Lung cancer has a very dismal prognosis and careful diagnosis and staging is of outmost importance. EBUS has become a cornerstone investigation for diagnosis and staging and current guidelines stress that there is a steep learning curve when introducing this technique in practice (only 30 procedures are considered necessary). Over a period of 10 months a total of 21 patients have been addressed to our unit for an EBUS TBNA procedure. Only three were referred for staging purposes (for lung, digestive and cervix cancers) the others being primary diagnostic approaches where simpler procedures had previously failed. Procedures were initially performed under local anesthesia (3 cases) then under general anesthesia and jet ventilation using a laryngeal mask approach. Mediastinal lymph node group 7 was the most frequent target (9 cases) followed by group 4R (8 cases) and peribronchial tumoral processes (7 cases); one case did not required any needle-aspiration. On average each examination resulted in the sampling of 1.4 targets. There were no significant procedure related severe adverse events. Although 21 G cytology needles were used, adequate histological samples were obtained for 11 cases and cytology was the examination of choice for 9 cases. The pathology/cytology results were retrospectively assessed as satisfactory for 15 cases (confirmed neoplastic or other disease) and inconclusive for 5 cases. Non neoplastic disorders were represented by sarcoidosis, tuberculosis and bronchogenic cyst (3 cases). The procedure can be considered fast and safe; trained pathology personnel play an extremely important role; presently referrals are rare for staging purposes. **Keywords:** LUNG CANCER, DIAGNOSIS, STAGING, EBUS TBNA, ENDOSCOPIC ULTRASOUND.

Lung cancer has a very dismal prognosis and careful diagnosis and staging are of outmost importance in choosing the optimum therapeutic approach - surgery, chemotherapy, radiotherapy or combinations. Assessing the N2 nodes status in non small cell lung cancer patients has become essential as multimodal therapeutic approaches became available. Despite significant advances in mediastinum imaging, careful clinical lymph node staging is preferable in guiding therapy as frequently radiologic criteria may prove unreliable. Overall sensitivities of 75% and 84% and

specificities of 82% and 89% were cited for CT and PET/CT respectively (1, 2). Therefore invasive (i.e. histological proven) mediastinal staging should be the norm if N2 or N3 disease is suspected (3).

Since the 1954 initial report mediastinoscopy quickly became the gold standard for invasive mediastinal assessment with a sensitivity of 81% and a negative predictive value of 91% (1, 4). It has been partially superseded by minimal invasive approaches as endoscopic ultrasound (EUS) and endobronchial ultrasound (EBUS). These became cornerstone investigations for diagnosis and staging as results are comparable to mediastinoscopy with a much lower adverse event rate and cost. A pooled specificity of 1.00 (95% CI 0.92 to 1.00) and sensitivity of 0.88 (95% CI 0.79 to 0.94) has been reported for EBUS TBNA as far as mediastinal lymph node staging is concerned (5).

Similar values have been reported for endoscopic ultrasound (EUS) approaches for non-small cell cancer staging(6). A combination of both techniques have been proved to be superior compared with mediastinoscopy with a combined sensitivity of 94 % *vs.* 79 % (7).

A recent meta analysis(8) reported a negative likelihood ratio of around 15 % a value suggesting that there is still a role for more invasive approaches in selected cases.

However EBUS/EUS is considered as the first intent approach - considering the fast patient recovery since only minimal anesthesia is required and no significant surgical trauma is involved. Most mediastinal lymph nodes groups can be sampled (with the exception of groups 5 and 6 (9, 10) which can be usually reached by VATS). Its role is clearly expanding as more potential targets are considered – such as left adrenal, left liver lobe or thyroid gland (11). Apart from versatility, ultrasound imaging may contribute with additional data to CT appearance to assess node involvement. Indeed, apart a small diameter over 10 mm and a round shape (features usually assessed on CT) heterogeneous echogenity and absence of central hilar structure were recently mentioned as predictors for malignant involvement (12). Furthermore EBUS TBNA can be readily repeated if inadequate samples are obtained or for the purpose of disease progression monitoring as no significant sequelae are expected. There were some concerns when comparing EBUS to mediastinoscopy as far as yield was concerned. Recent data suggests that a usual approach entailing at least 3 passes per target will return an acceptable sample for pathology and molecular examinations. This will ensure high (over 95%) sensitivity and accuracy values for mediastinal staging (13).

Current guidelines stress that there is a steep learning curve when introducing this technique in practice – on a yearly basis significant improvement is seen after 25 procedures and diminishing returns are encountered after 50 procedures(14).

Since the introduction of EBUS TBNA in our unit some particular issues and pitfalls became apparent and we report on our initial experience.

MATERIAL AND METHOD

A total of 21 patients have been assessed using an EBUS TBNA approach over a year starting with June 2013; this was performed using an OLYMPUS EUME1 on an OLYMPUS EVIS EXERA III infrastructure.

Only three patients were referred for staging purposes (for lung, digestive and cervix cancers). The rest of the procedures were primary diagnostic approaches where simpler less invasive procedures had previ-

ously failed or were judged to be potentially useless (mainly bronchoscopy and transthoracic approaches).

METHODOLOGY

Standard preprocedure requirements included – complete medical history, signed informed consent, coagulation assessment, EKG, chest CT, per month for 12 hours.

Main contraindications considered were: significant arrhythmias or cardiac conditions, high bleeding risk (thrombocytopenia, anticoagulant / antiplatelet agents, renal failure), acute respiratory failure. There were no absolute contraindication; two procedures were postponed until antiplatelet agents effects wore off (seven days). No antibiotic prophylaxis was routinely considered although seven cases received postprocedure antibiotherapy.

Initially potentiated local anesthesia was used (three cases) then a switch was made to general anesthesia under high frequency jet ventilation administered on laryngeal mask (this approach ensured good working conditions and patient comfort). Median working time was 60 minutes (anesthesia time included).

EBUS examination was preceded by standard bronchoscopy to exclude evident bronchial lesions as the EBUS scope has a lower resolution image and a narrow 30 degree angled field of view. Once the EBUS scope was passed into trachea the convex ultrasound probe was brought in contact with the lateral tracheal wall (an inflatable balloon was used to improve contact). Main lymph nodes groups were identified (hypo echoic and Doppler negative) and assessed (shape and size) using the following approaches/landmarks using a N3 to N1 approach:

2R, trachea, superior vena cava, brachiocephalic veins

2L, trachea, above the aortic arch

4R, trachea, superior vena cava, below the azygos vein (Fig. 1)

4L, trachea, aortic arch, pulmonary artery

7, right or left main bronchus

10R, right main bronchus, interlobar pulmonary artery branch (Fig. 2)

10L, left main bronchus, left pulmonary artery

11R, intermediary trunk, interlobar pulmonary artery branch

11L, distal left main bronchus, interlobar pulmonary artery branch

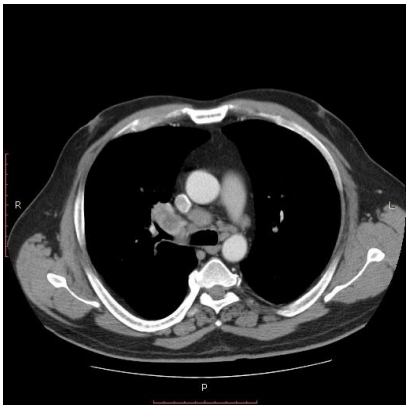

Fig. 1. Groups 10R and 4R lymph nodes – CT section

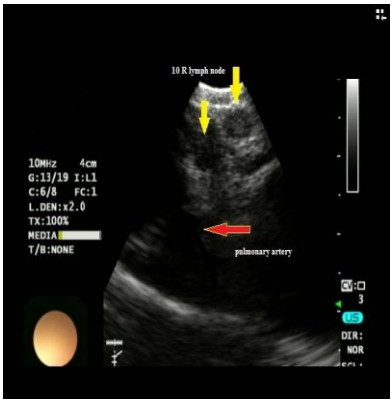

Fig. 2. Group 10 R lymph node – echographic view

Abnormal nodes were identified on CT. Identified lymph on the endoechographic images were punctured under ultrasound control using 21 G Olympus needles; at least three passes were done per target as recommended. Rapid on site cytology assessment although available was not considered necessary (16). Aspirated specimens were discharged in 90 degrees alcohol – this approach while convenient as it provided immediate feedback caused some difficulties for pathology personnel as instantly formed large blood clots obscured aspirated fragments. Therefore it was later changed to saline followed fixing by addition of the same volume of 97% alcohol. Cytoblocks were obtained after centrifugation and inclusion in a support matrix and then processed as regular biopsies for Paraffin embedding.

RESULTS

Mediastinal lymph node group 7 was the most frequent target (9 cases) followed by group 4R (8 cases) and peribronchial processes (7 cases). Targets also included groups 4L, 10 and 11 on both sides. Each examination involved an average of 1.4 targets to a total of 29.

There were no procedure associated adverse events in our group except for two cases. In first case the procedure was prematurely stopped due to anesthesia issues. The second was a foreign body situation - distal latex balloon disengaged; it was promptly recovered using a biopsy forceps and a video bronchoscope. Minor procedural bleeding was not considered a significant adverse event and did not require any specific intervention. Recovery was fast; all patients were returned to the ward by next day and usually discharged soon after.

Estimated expenditure was around 350 euro per procedure - mainly due to disposable equipment and standard short hospitalization costs.

Although 21 G cytology needles were used adequate histological samples, albeit small were obtained for 11 cases and cytology results were available for 9 cases.

For one case no invasive approach was needed as ultrasound examination revealed a small diameter adenopathy (suspicious on anterior CT scan).

The pathology/cytology results were retrospectively assessed as satisfactory for 15 cases (confirmed neoplastic or other disease) and inconclusive for 5 cases (non specific aspects except lymphoid tissue).

These were referred for more invasive approaches if there was a high index of suspicion.

Non neoplastic disorders were represented by sarcoidosis, tuberculosis and bronchogenic cyst (3 cases).

DISCUSSION

While the optimum number of stations to be assessed during an EBUS examination is still open to debate it is generally considered that sampling more than two may improve staging accuracy (17). Still as for our case series the average was 1.4 stations per procedure – a figure which must be interpreted taking account of the diagnostic purpose of the approach. More often than not the patient had an imagistically assessed stage IV disease which precluded or made inadequate invasive approaches such as thoracotomy and also made unnecessary N staging.

Group 7 was the most frequent target in our group and could have probably been reached by conventional TBNA – still EBUS was preferred as it allowed multiple

target assessment and cost was roughly similar. Furthermore recent data suggests conventional TBNA skill improvement after implementing EBUS training (18).

While peripheral lung nodules may not always be assessed by EBUS this approach proved useful in selected cases (seven for our group) as have been reported before; all seven peripheral pulmonary nodules/masses were positively diagnosed(19).

EBUS-TBNA complications were previously reported – agitation, post-procedural cough, hypoxia, laryngeal injury, fever, bacteremia/infection, pneumothorax, mediastinal abscess, laryngospasm/ laryngeal edema, bronchospasm; their frequency was estimated around 1.4%. The main risk factor was considered the presence of a concurrent transbronchial lung biopsy (20). There were no procedure related complications in our group. This may partly be explained by the general anesthesia/high frequency jet ventilation approach we used which circumvented upper airways reactions and total refrain from trans bronchial lung biopsies. Post procedure antibiotherapy was prescribed if purulent bronchial secretions had been found.

Fast recovery – due to either low anesthesia requirements or low surgical trauma involved may imply a special role for EBUS TBNA for frail patients for whom a more invasive approach may associate a significant vital risk. This is also suggested by the fact that no patient was refused once addressed as their concurrent conditions were not considered to be absolute contraindications. There was one case for which active watch was preferred as available clinical data was suggestive for reactive inflammatory adenopathy; chest CT repeated after three months showed complete resolution (21).

As far as results were concerned 15 procedures returned positive diagnosis – five were considered inconclusive. Given the low negative likelihood ratio (8) and the high suspicion threshold we decided to refer the patients for subsequent more aggressive approaches. Still we consider 15 positive diagnoses to be encouraging given the fact that histological samples were available in only 11 cases. This was probably possible because EBUS samples proved to be generally suitable to immunhistochemistry assays either as core biopsies or as cell blocks (22, 23).

There were some non neoplastic conditions in our group. While tubercular lymph node involvement and bronchogenic cysts are not usually explored by TBNA (mainly because late complications such as fistulae may develop) these were incidental findings while suspecting malignancy. On the other hand sarcoidosis node involvement was suspected and confirmed for one patient; EBUS TBNA was preferred as there were no significant endobronchial lesions and no infiltrates on chest CT. Because of group 4R involvement which is particularly difficult to assess on an unguided approach; current practice strongly encourages the use of EBUS TBNA over TBNA for these cases(24).

More while transbronchial lung biopsies seem to have the highest diagnostic yield as a standalone procedure adding EBUS TBNA may improve the figure to over 80% and is probably superior to conventional TBNA (25) as recent data suggests a particular appearance for sarcoid lymph nodes – distinct margins and granular appearance(26).

CONCLUSIONS

EBUS TBNA may be used for diagnos-

tic and staging purposes in bronchopulmonary carcinoma patients as it can reliable reach mediastinal node stations (excepting groups 5, 6, 8, 9) and peribronchial masses.

It can be considered a reasonably fast procedure with an excellent safety profile, acceptable cost as compared to mediastinoscopy and a particularly fast post procedure recovery.

General anesthesia under jet ventilation greatly improves comfort for both the patient and the medical team but the procedure may be performed reasonably well under local anesthesia.

Trained pathology personnel play an extremely important role and sample management protocol should be adequate for imunhistochemistry assays.

Although diagnostic yield is encouraging for both neoplastic and non neoplastic conditions more invasive approaches may still be necessary as negative predictive power is low.

The procedure is little known to other health professionals and thus referrals are rare for staging purposes – more publicity would probably be useful.

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RED CELL DISTRIBUTION WIDTH (RDW) - A POTENTIAL PROGNOSIS FACTOR IN MALIGNANT MELANOMA

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RED CELL DISTRIBUTION WIDTH (RDW) - A POTENTIAL PROGNOSIS FACTOR IN MALIGNANT MELANOMA (Abstract): Although malignant melanoma accounts for less than 5% of all cancers, it is one of the most aggressive types of cancer, associated with a poor outcome. Survival rates significantly vary according to stage and even within the same stage there are significant survival differences. The **aim** of this study was to assess the role of red cell distribution width (RDW) as a potential prognosis factor for malignant melanoma. Also, this analysis aims to determine average survival and the time until relapse/progression of the disease in patients with malignant melanoma treated at the Regional Institute of Oncology Iasi (IRO) between 2013 and 2016. **Materials and methods**: We performed a retrospective analysis of melanoma patients treated in the IRO between 2013 and 2016. We collected several parameters including age, sex, tumor localization, clinical and pathological stage, Breslow Index, Clark level, ulceration and detailed therapeutic management. We then analyzed each parameter to assess its impact on progression-free survival and overall survival. **Results**: We identified RDW as a significant prognosis marker, with worse outcome for patients with abnormal RDW values. Additionally, tumor localization, Clark level, Breslow index and cancer stage also showed prognosis value. **Conclusions:** RDW is a useful, inexpensive prognosis marker that can better predict survival. Prospective studies are required for confirming the findings of the present analysis. **Keywords**: MELANOMA, RED CELL DISTRIBUTION WIDTH, PROGRESSION FREE-SURVIVAL, OVERALL SURVIVAL.

Along with the worldwide population ageing trend (1), there is a progressive increase in the incidence of a large number of cancers, such as colon cancer, hepatocarcinoma (2), breast cancer and melanoma (3). Although malignant melanoma currently represents less than 5% of cancers, it is estimated that in 2014 this neoplasia was responsible for over 9,000 deaths in the United States alone (4), being considered one of the most aggressive types of cancer and generally associated with a bad prognosis (5). Furthermore, epidemiological reports from recent years have shown there is a tendency towards the decrease of the median age of diagnosis, together with a significant increase in melanoma incidence in males (6). In Europe, the countries with the highest number of melanoma cases are in Germany, Switzerland, Norway, Denmark and Iceland (5). In Romania, melanoma is the second most frequent type of

cancer in individuals aged 15-29 years, with an increase rate of 4% per year (7).

Traditionally, malignant melanoma is believed to be a cancer associated with an unfavorable outcome. With the improvement of surgical and radiotherapy techniques, along with the introduction of immune therapy and the systematic testing of the BRAF mutation, new therapeutic approaches have significantly improved the quality and life expectancy of patients with metastatic malignant melanoma (8). Five-year survival is estimated to be 62% for patients with lymph node invasion at diagnosis and 18% for those diagnosed directly in the metastatic stage (9), results which are now well above the epidemiological reports of previous decades. However, survival assessment remains difficult (10) and there are significant variations within the same disease stage (11). Also, similar to other types of tumors (12), there are certain clinical situations where diagnosis and staging are difficult due to lack of symptom specificity and aggressiveness of the disease. In addition to TNM staging (tumor size, lymph node invasion, and presence of metastasis) recommended by the American Committee Against Cancer (AJCC), which remains the most prevalent method of assessing prognosis (13) for many cancer types (14), there are certain "classical" prognosis factors considered to have an independent impact on survival in malignant melanoma. Of these, male gender, advanced age, localization on the head, neck or trunk, increased Breslow and Clark levels, presence of ulceration, and increased mitotic rate (15) are associated with a more bleak prognosis, regardless of the stage at diagnosis.

Together with a significant increase in the number of preclinical and clinical cancer studies (16) that aim to improve diagnosis or find new methods of treatment (17), , more and more new potential prognosis factors have been assessed which has led to the identification of some parameters that can predict significant modifications of the length of survival and have a predictive role in the response to treatment. Some of these, such as the presence of BRAF mutation, sentinel lymph node status, LDH level or S100 protein, are already used in the clinical setting (18), whereas others such as vitamin D (19), lymphocyte infiltration (20), or genetic modifications identified by means of "next generation sequencing" (NGS) techniques (11) have not yet gathered sufficient scientific evidence or cannot be implemented on a large scale due to significant additional costs. As such, there is still a need to identify new prognostic factors that are easy to assess for malignant melanoma to individualize patient prognosis.

Red cell distribution width (RDW) is a commonly-assessed parameter included automatically in the complete blood count of patients; it determines the presence of variations in the size or volume of red blood cells (21). In recent years, it has been assessed as a prognosis factor in several types of solid tumors such as lung (22), prostate (23), endometrial (24) or hepatic (25) cancer and it has been identified as a relevant prognosis factor for all these localizations. However, to the authors' knowledge, RDW has not been previously assessed as a prognostic factor for patients diagnosed with malignant melanoma.

The **aim** of this paper is to assess the role of RDW as a potential prognosis factor for malignant melanoma. Also, this analysis aims to determine average survival and the time until relapse/progression of the

disease in patients with malignant melanoma treated at the Regional Institute of Oncology Iasi between 2013 and 2016. One other aim is to confirm the value of established prognostic factors in assessing survival for patients included in the present analysis.

MATERIAL AND METHODS

We performed a retrospective analysis on all cases of cutaneous melanoma treated at the Regional Institute of Oncology (IRO) Iași between 2013 and 2016. IRO is a tertiary medical unit specialized in diagnosing and treating both hematological and solid malignancies that serves as a reference center for Moldova, the north-eastern region of Romania. In IRO, melanoma diagnosis is based of pathology assessment of tissue and staging is determined based on CT, MRI, or PET-CT imaging studies. All cancers diagnosed in IRO are staged according to AJCC's TNM system. Performance status is routinely assessed by means of the World Health Organization approved Eastern Cooperative Oncology Group (ECOG) PS scale.

Inclusion criteria for the present analysis were histological confirmation of cutaneous melanoma (incisional/excisional biopsy or surgery), receiving local or systemic treatment in IRO, a performance status ≤ 2 at the time of the diagnosis and being over 18 years of age.

Exclusion criteria were: a history of a prior malignancy (non-melanoma skin cancer excluded), chemo/radiotherapy prior to the initiation of melanoma treatment, non-cutaneous melanoma localization, doing treatment and follow-up in another medical unit and performance status > 2.

Patient records were accessed by means of the hospital's electronic database. We collected a series of patient's characteristics, tumor and clinical data for each case. Parameters were structured as follows: demographic data: sex, age, urban/rural residence, clinical data: tumor localization, clinical TNM stage, presence and localization of metastasis, ECOG performance status, biochemical data (before treatment): LDH, RDW, platelet distribution width (PDW), white blood cell count, hemoglobin, histology and molecular biology data: Clark level, Breslow index, presence/absence of ulceration, pathology TNM stage, S100, BRAF, treatment data: surgery, radiotherapy, first-line and second-line anti-cancer systemic treatment, toxicity of treatment, survival data: date of diagnosis, recurrence date (if applicable), overall survival

Statistical evaluation was performed using *SPSS version 20.0* software. Unpaired Student's *t*-test, Chi-squared, Kaplan-Meyer survival curve and Fischer exact test were used according to data type. For each collected parameter, we assessed its potential impact on overall survival. Statistical significance was defined as $p < 0.05$; variables were considered independent for the statistical analysis; continuous data was expressed as mean ± standard error.

RESULTS

Descriptive analysis

Of the 120 patients identified in the electronic database, 75% of the patients fulfilled the inclusion criteria. 25% of the patients were not included in the analysis because they had non-cutaneous melanoma or because they either refused treatment of received it in a different hospital.

In our study group, the male: female ratio was 1.04:1, with 51.1% male and 48.9% female. Average age at diagnosis was 58.56

years, with a median of 59 years. Most patients were younger than 65 at diagnosis (61 out of the 90 patients) and lived in the urban area (74.4%). Regarding localization, most primary tumors were in the lower limbs (32.2%), followed by posterior thorax (22.2%), anterior thorax (12.2%) and abdomen (11.1%). From a clinical staging point of view, most patients were diagnosed in stage II disease, with 18 patients being diagnosed in stage IIA, 13 in stage IIB and 12 in stage IIC. The rest were most often diagnosed in stage III (27.7% patients) or stage IV (7.77% patients) of the disease. Only four patients were diagnosed in stage I. Most frequent metastatic sites included brain, lung liver and bone.

Regarding performance status, most patients (91.1%) had ECOG = 1 at diagnosis, whereas 4.44% were ECOG = 0 and 4.44% were ECOG = 2. Biochemical assessment before treatment initiation indicated that 38.9% of the patients had increased LDH levels, 36.6% had lymphocytosis, 13.3% had a decreased monocyte count, and 24.4% had grade I or II anemia.

Pathology and molecular biology analysis of the biopsy/surgical piece revealed that most melanomas had a Breslow index of over 4 mm (44.4%) and only 2 patients had a Breslow index < 1 mm. Similarly, Clark levels were most often IV (27%) or V (25%), with only one patient being assessed as a Clark I. More than half of the patients (56.7%) presented ulceration at diagnosis and most cases were upstaged after tissue analysis (cTNM vs. pTNM). Regarding BRAF mutation, it was only tested in 15 patients, 6 of which were found to be BRAF positive. S100B protein was increased in 18.8% of patients, with normal levels in 74 patients.

Regarding treatment data, most of the patients underwent surgery as a first-line localized therapy option. Throughout the course of the disease, however, almost all patients underwent curative or palliative surgery and most of them benefited more than once from this approach (96.6% of patients with 152 surgical procedures performed). Adjuvant therapy included interferon in three cases and radiotherapy if positive margins, although most localized melanomas were simply followed-up periodically. Most patients in stage II or III disease quickly developed local or generalized recurrences and received either local therapy (second surgery and/or radiotherapy) or first-line systemic therapy. Radiotherapy was recommended in 23.2% of patients, either at the primary site (12.2% of patients) or for brain metastases (11.1% of patients). Dacarbazine was the preferred first-line chemotherapy choice in all cases. Second-line chemotherapy options were Carboplatin (10%), Cisplatin and Paclitaxel (4.44%) or Temozolamide (2.22%). One in four patients (24%) could not complete the projected number of cycles due to inacceptable drug toxicity/ One patient refused systemic treatment after being diagnosed with systemic recurrence six months after surgery.

Maximum progression-free survival recorded was 191 months; survival time had a minimum of one month and an average of 42 months. Average progression-free survival was 22.46 months.

Prognosis factors

After analyzing the potential relevance as a prognosis factor, five of the assessed parameters were found to be associated with prognostic variations: primary tumor localization, Breslow index, Clark level, stage and RDW values.

Primary tumor localization was associated with a better outcome if the tumors were in the limbs when compared with abdominal or thoracic melanoma (p=0,043 for limb vs. abdominal melanoma and p=0,036 and p = 0,021 for limb melanoma versus anterior and posterior thoracic melanoma). Average survival for a patient with melanoma on the abdominal cutaneous surface was 29 months and 26 months for thoracic melanoma. In contrast, lower-limb melanoma patients had an average survival of 36 months and upper-limb melanoma patients had an average survival of 77 months, a statistically significant difference.

Breslow index was found to be a significant prognosis factor, with better outcome for a Breslow < 1 mm when compared with a Breslow > 2.1 mm (p=0,008). Recurrence-free interval was 142 months for patients with Breslow < 1 and progression-free survival (PFS) abruptly decreases as the Breslow index is higher, with a 48-months value for a 1-2 mm Breslow index. Similarly, Clark level also showed prognosis value, with statistically different survival averages between level III when compared with level IV and level V (p=0,006, p=0,010). Progression-free survival was 142 months for Clark I, with a progressive decrease of survival as Clark levels grow (23 months overall survival for Clark V).

In our analysis, stage had a significant prognosis value. Patents with clinically noticeable lymph node invasion (N = 33) had a worse outcome when compared with patients with negative lymph nodes (p<0,016). Among those without node involvement at diagnosis, 42.2% quickly presented with local or systemic relapse. Average survival was 21 months for patients with lymph node involvement and 46 months for those without. Patients diagnosed directly with stage IV disease had a worse prognosis when compared with localized or locally advanced disease (p=0.00038), with an average survival of seven months for metastatic disease. Stage had a statistically significant impact on survival, with stage IV patients that were associated with worse prognosis when compared with other stages (p=0,006, p=0,016, p=0,013 when compared with stage I, stage II and stage III disease).

RDW also had a significant prognosis value - patients with an RDW > 15% (increased) had a decreased survival time when compared with those with normal RDW values at diagnosis (p=0,030). Average survival was 33 months for patients with abnormal RDW values compared with 64.97 months for those with normal RDW values (fig. 1).

Several other factors were assessed in this analysis and showed little or no influence on prognosis. Although a small benefit was noted for females, distribution according to sex did not show statistically significant differences. Similarly, age distribution, LDH and S100 values were not shown to have prognosis values. A small benefit of survival (38 vs. 37 months) was shown for patients without tumor ulceration, but it don't reach statistical significance. Since generalized BRAF testing was only available in IRO in 2015, we could not assess the effects of BRAF mutations on survival. All patients in non-metastatic stages received surgery and first-line chemotherapy was in all cases Dacarbazine, so no comparison could be performed. Second-line chemotherapy was not initiated in all cases and no statistical differences were found between treatment protocols.

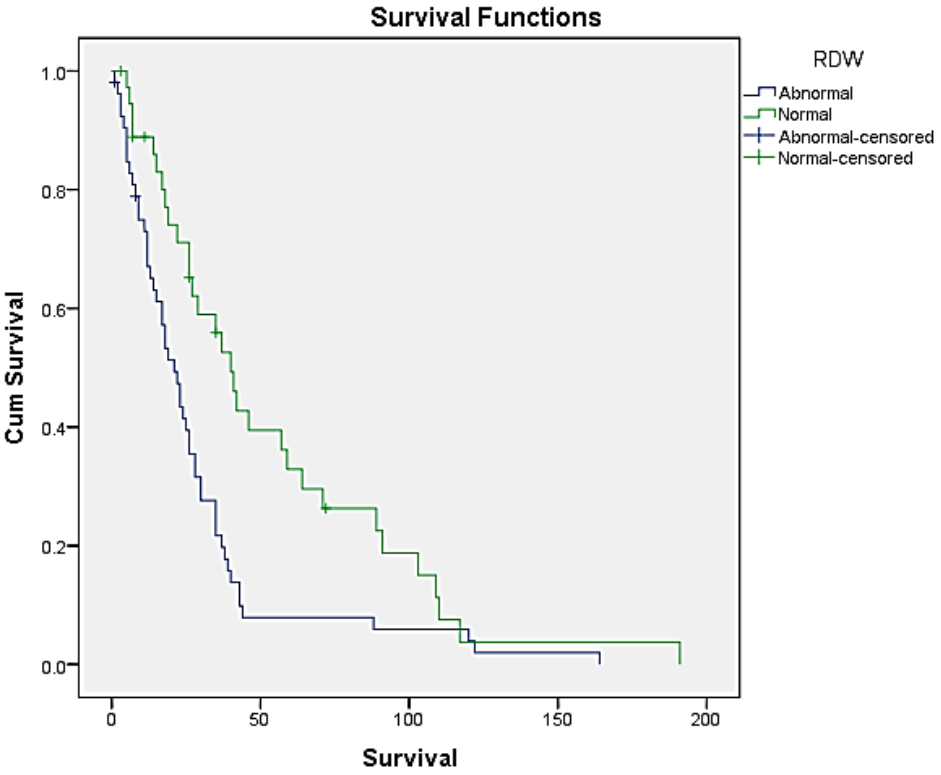

Fig. 1. Patient survival and RDW values before treatment initiation

DISCUSSION

The present retrospective study included 90 patients who received treatment in IRO Iasi. The maximum survival rate registered in this group was 191 months, with a mean survival of 42 months; progression-free survival was an average of 22.46 months. Gender did not have a significant influence on overall survival, although we noticed a numerical difference in favor of the female sex. Literature data is not consistent regarding the influence of gender on survival rate, although a study assessing 3,900 melanoma patients showed that women appear to have a better prognosis than males due to a protective factor that was not yet clearly identified (26). Our analysis did not identify a difference in gender survival, most likely due to the small number of patients included.

Regarding age as a prognostic factor, we did not identify any significant differences between patients. This is somewhat surprising, taking into account the fact that elderly have a tendency for self-neglect (27) and often have cognitive dysfunctions and other relevant comorbidities that can significantly delay and influence both diagnosis and treatment (28,29). However, we have interpreted this result because of patient selection - there is an inherent bias to choosing only patients that choose to receive treatment in a cancer institute and are fit enough to do so.

Similarly, the place of residence (urban versus rural dwellings) did not have a significant influence on survival, a result which is different from other literature data. According to several retrospective studies conducted in Canada, there was a higher incidence of melanoma reported in urban vs. rural areas. Researchers explained this by pointing out that people living in a city have easier access to see a dermatologist, which increases the chance of diagnosing atypical nevi with malignant potential and diagnosing melanoma in general, concluding that actually there is no real difference in prevalence in urban versus rural areas (30).

Primary anatomical localization was identified as a major prognosis factor, with significant statistical differences between melanoma of the extremities versus melanoma located on the abdomen and on the anterior and posterior thorax. In this analysis, we noted a significant survival advantage for patients diagnosed with melanoma in the limbs. These data is consistent with other literature data, considering that in a study of 2,500 patients, melanoma located on the neck, trunk and head had worse prognosis when compared with other sites (31).

Stratifying according to the Breslow Index showed a significant difference in survival rate, with longer survival for less invasive melanomas. An increase in the Breslow Index was associated with a worse outcome, with a steep decrease in recurrence-free survival for a 1-2 mm Breslow (48 months). According to the AJCC, in a study of 1.841 patients, the 10-year overall survival rate was 92% for Breslow Index 1, 80% for Breslow Index 2, 63% for Breslow Index 3 and 50 % for patients with the Breslow Index 4, consistent with the results of this analysis (32). Similar results were observed for the Clark level, with a progressively decreasing overall survival from Clark I (142 months) to Clark V (23 months).

Our data indicated the TNM stage was another relevant prognostic factor, with a significant increase in the risk melanoma recurrence or progression for those patients with positive lymph nodes at the time of diagnosis. Thus, average survival was 21 months for those with positive lymph nodes and 46 months for those with negative lymph nodes, regardless of the stage of the disease. According to literature data, lymph node invasion is a well-established prognostic factor with studies reporting that negative lymph node patients have a 76-90% PFS at 5 years, whereas those with positive lymph nodes have a PFS of 35-58% at 5 years (33). The presence of systemic disease at diagnosis is considered an independent prognosis factor with a mean survival of one year for stage IV patients according to one study (34). Our results fall in line with available data - in IRO, the survival of patients with metastatic malignant melanoma was 7 months.

RDW variations were associated with different outcomes. There was a statistically significant difference in survival for patients who had RDW above 15% (increased) when compared with those with normal RDW values. Average survival was 33 months for patients with increased RDW and 64.97 months for those with normal RDW prior to treatment initiation. In recent years, RDW has become an attractive prognosis factors and has been assessed in several types of tumors, showing that it can be used as an independent means for predicting survival. In a study of over 300 patients, Koma&colab. found RDW to accurately predict survival in lung cancer, not only for advanced, but also for localized stages of the disease (22). RDW was found to predict survival of patients

with pancreatic cancer in a study conducted in Turkey on a group of 104 patients (35). Also, in a Chinese study performed on a group of 608 patients, abnormal RDW values were correlated with a worse outcome in breast cancer patients (36).

Due to the characteristics of the present analysis, several factors considered predictive by international guidelines did not have any effect on survival. Thus, a definite correlation could not be made between the presence of ulceration and patient survival due to incomplete pathological staging (in some cases) and the small group of patients. In a study performed on a group of 423 patients in Spain, ulceration was significantly correlated with a decrease in both overall survival and PFS survival (37). Since routine BRAF mutation testing has been only recently introduced in IRO standards, the number of patients tested for BRAF mutation was too small to achieve a definite correlation. Also, we did not record significant differences in overall survival when stratifying by LDH or S100b protein level. A possible explanation for these findings would be reduced specificity of these markers (abnormal levels are also found in patients with hepatic, renal, various inflammatory and infectious diseases). Also, in this analysis we included patients diagnosed in all disease stages, whereas most studies that indicate these markers as prognosis factors were performed on stage IV patients. In a study on a group of 855 patients with melanoma, both LDH and S100b values were classified as independent prognostic factors for metastatic melanoma (38). However, other studies on these two markers were inconclusive (39).

The general research trend encourages and supports identifying new treatment methods, molecular biology or genetic risk factors and funds the development of novel drugs. However, some of the clinically used drugs and some of the already available markers might have surprising uses in different practical settings (40). Changing drug ratio for active combinations (41) or simply reanalyzing some of the markers at hand can lead to novel results and improve daily practice without additional costs.

Limits of the study

The design of this analysis is retrospective, and it was conducted in a single oncology institute. The number of patients included in the study was quite small (90 patients) and several types of analysis were performed irrespective of the patient's stage. We were not able to confirm several of the well-known prognosis factors for melanoma.

CONCLUSIONS

In our study, red cell distribution width is a promising new prognosis factor for patients with malignant melanoma. Alongside other parameters already in use, regularly assessing it can improve survival predictions and aid physicians working with cancer patients in decision-making and communicating with the family. Additional studies, preferably with a prospective design, are required to certify our analysis' findings.

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IS THERE A CARDIOVASCULAR RISK IN INFLAMMATORY BOWEL DISEASES?

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IS THERE A CARDIOVASCULAR RISK IN INFLAMMATORY BOWEL DISEASES? (Abstract): Inflammatory bowel diseases (IBD) are characterized by an increased thrombembolic risk, given the powerful relation between inflammation and thrombosis. Multiple studies showed that patients with IBD have an up to 3-fold higher risk for developing venous thrombembolic (VTE) complications compared to general population, this risk being more increased in the hospitalized IBD flares. Thus, latest consensus recommendations indicate prophylaxis for VTE in hospitalized patients with active IBD but with no clear indications for the management of IBD outpatients. Regarding atherothrombotic risk (myocardial infarction or stroke), up-to-date data are inconclusive. IBD is associated with subclinical atherosclerosis in patients without clinical manifestations of cardiovascular diseases (CVD). However, the results of major studies assessing the hypothesis that IBD is strongly associated with atherosclerotic macrovascular events prove to be divergent even if they show positive correlations with CVD especially on different subgroup analysis. These facts should lead in the future to more prospective studies with control groups that have the same cardiovascular risk profile as in IBD populations in order to admit definitively that patients with IBD are exposed to an increased cardiovascular risk. **Keywords**: INFLAMMATORY BOWEL DISEASES, CARDIOVASCULAR RISK, VENOUS THROMBEMBOLISM, MYOCARDIAL INFARCTION, STROKE

Cardiovascular diseases (CVD) still represent the main cause of mortality worldwide despite the implementation of major programs of primary prevention. More than 70% of total deaths caused by CVD are determined by complications of atherosclerosis (about 50% - coronary disease, 20% - stroke).

Atherosclerosis represents a chronic inflammatory disease which is characterized by general inflammation. The immune mechanisms, in combination with well known metabolic risk factors (e.g. hypercholesterolemia, smoking), are key components in the initiation, activation, propagation and complication of the atherosclerotic plaque that, by rupture and thrombosis, will finally lead to acute coronary syndromes, strokes or other acute manifestations (1). In the last years, there is a growing interest in the involvement of the inflammatory and immune actions in the process of athero-

genesis that will lead in the future to a translational medicine from the pathogenesis to clinical approaches of atherosclerosis. More biomarkers promise to play a great role in detecting subclinical activity, such as high-sensitivity C reactive protein (hs-CRP), cytokines (IL-6), proteases such as matrix metalloproteinase 9 or myeloperoxidase (2). The major problem is that in more than 30% of cases, the first clinical manifestation of atherosclerotic burden is a myocardial infarction. This is due to the fact that the disease acts undetected or the patient is wrong classified into a lower risk class with an inappropriate treatment and weak prevention measures.

Taking into consideration the upmentioned facts, it is with no doubt and it is well proved that chronic inflammatory diseases, such as systemic lupus erythematosus, rheumatoid arthritis or psoriasis, are associated with an increased cardiovascular risk. Regarding inflammatory bowel diseases (IBD), the relationship with atherothrombotic cardiovascular events is quite unclear, even if certain studies tend to suggest that there is a direct connection between these two diseases. Thus, our aim is to provide a concise overview over the current and latest studies regarding the relationship between inflammatory bowel diseases and the associated cardiovascular risk.

IBD include two main entities: Crohn's disease and ulcerative colitis, with an estimated prevalence of 2.2 million persons in Europe. Even if it is long studied, the precise pathological mechanisms that lead to the development of the diseases are not fully understood. Certain contributing factors are represented by cigarette smoking, family history of IBD and appendectomy (3).

However, given the inflammatory status in IBD, these patients possess a greater thrombembolic risk, the link between thrombosis and inflammation being well recognized. This may be due to different mechanisms such as microvascular injury, a state of hypercoagulability, increased platelet activation or higher incidence of hyperhomocysteinemia in IBD patients (4, 5).

Moreover, in patients without clinical manifestations of CVD, IBD is associated with subclinical atherosclerosis, with increased carotid intima media thickness, arterial stiffness, hs-CRP, homeostasis model assessment of insulin resistance (HOMA-IR) or atherogenic lipid profile, suggesting higher risk of atherothrombotic disease (6).

There is a general agreement that patients with IBD have an increased risk of thrombembolic manifestations with the predominance of venous rather than arterial complications.

Multiple metaanalysis have proven that patients with IBD have an up to 3-fold higher risk for developing venous thrombembolic (VTE) complications compared to general population, this risk being even more increased in the hospitalized IBD patients (7, 8).

In a large case-control study involving almost 100,000 cases, Grainge et al. prove that patients with IBD had higher risks of VTE events, with an up to 8-fold higher risk during the IBD flares (9). In IBD inpatients, the prevalence of venous thromembolism is 32% increased in patients with Crohn's disease than in those with ulcerative colitis, but overall two times higher than in subjects without IBD. This fact leads finally to higher treatment charges and mortality in IBD patients that associate thrombotic complications (10). Another multicenter study concludes that patients

with IBD and venous thrombembolism are more prone to develop recurrent venous thrombembolism. But the same study states that it is uncertain which patients would benefit from prolonged therapy with anticoagulant, taking into the balance both the risk of bleeding and the risk of recurrent thrombotic complications (11).

However, current consensus recommendations indicate prophylaxis for venous thrombembolism in hospitalized patients with active IBD. Thrombophylaxis proves to be safe in patients with IBD even during a flare when there is rectal bleeding. Despite this evidence, up to two thirds of gastroenterologists do not administer venous thrombembolism prophylaxis to patients hospitalized with acute colitis. There is a great unevenness among physicians regarding the necessity, the way the VTE prophylaxis should be performed and the long term follow of such cardiovascular complications (12). Future clinical trials are needed to confirm safety and effectiveness of venous thrombembolism prophylaxis in outpatients with active disease (13). This last statement is underlined by other recently published studies which confirm that approximately half of thrombembolic events in IBD patients occur after hospital discharge, especially in those with additional risk factors for VTE (e.g. after a hospitalization for an IBD flare or after surgery) (14, 15). Moreover, IBD subjects present in the first 30 days after surgery a double risk for developing deep vein thrombosis or pulmonary embolism compared to general population, but without higher additional risk for myocardial infarction or stroke (16).

Taking into account these considerations, in 2014 is released the Canadian Association of Gastroenterology guidelines regarding treatment and prophylaxis of venous thrombembolism in patients with IBD which makes the following main recommendations based on current evidence (17). For:

- hospitalized IBD patients with moderate-severe IBD flares without severe bleeding, it is recommended anticoagulant thromboprophylaxis with low molecular weight heparin, low-dose unfractionated heparin or fondaparinux;
- IBD patients, even in clinical remission, hospitalized for other medical indications unrelated to IBD, it is recommended anticoagulant thromboprophylaxis;
- IBD patients that underwent major general or abdominal-pelvic surgery, it is recommended anticoagulant thromboprophylaxis during hospitalization;
- outpatients with IBD flare who did not experience a previous venous thrombembolism, it is NOT recommended anticoagulant thromboprophylaxis;
- IBD outpatients with a previous venous thrombembolism and are no longer on anticoagulation, it is recommended anticoagulant thromboprophylaxis during moderate-severe IBD flares;
- IBD patients who undergo their first venous thrombembolism episode, even with IBD in clinical remission and in the absence of other provoking factor, it is recommended indefinite anticoagulant therapy with periodic reassessment of this indication;
- IBD patients with active disease, diagnosed with their first venous thrombembolism episode, it is recommended anticoagulant therapy at least until the IBD is in remission for 3 months or indefinite anticoagulant therapy.

Thereby, until new evidence regarding

anticoagulant treatment in IBD outpatients are released, an initial pragmatic approach is preferred in order to decrease the thrombembolic risk in IBD outpatients. This approach is represented, firstly, by an appropriate education of the patients for reducing and preventing thrombotic risk factors, recognizing early signs and symptoms of the venous thrombotic events and a regular clinical evaluation of the patients made by the specialist for the evaluation of possible venous complications (18).

While venous thrombembolic events have a higher incidence in subjects with IBD, the debate persists if IBD also correlates with atherotrombotic diseases including myocardial infarction or stroke.

A meta-analysis published in 2007, comprising 11 studies with more than 14000 participants concluded that IBD were not associated with increased CV mortality (19). In addition to this, Osterman at al. (20) conducted two retrospective cohort studies, with more than 25,000 patients with IBD with a mean follow-up of 4.5 years and found that neither Crohn's disease nor ulcerative colitis had an increased risk of myocardial infarction. Another case-control study made by Ha et al. (21) supported once again the idea that these patients did not have an overall increased risk of arterial thrombotic events even if some subgroup analysis revealed some positive correlations (e.g. women below 40 years had a two-fold higher risk for stroke).

Nevertheless, there are studies that show direct causal link between IBD and further CV events. Bernstein et al. (22) concluded on more than 8,000 IBD patients that these subjects were more likely to develop cardiac atherosclerotic events, regardless of sex, with a 32% higher incidence of stroke in patients with Crohn's disease. Moreover, in a cohort that included more than 8000 persons with Crohn's disease, only the subgroup with younger patients (aged less than 50 years) presented a 3-fold increased risk of stroke but with no increased risk in older patients (23). Yarur et al. (24) proved in another 4-year cohort study that patients with IBD had a higher risk of coronary artery disease (hazard ratio 2.85) despite associating a lower burden of cardiovascular risk factors.

Probably the most important study that supports a direct causal link between CVD and IBD is the Danish Nationwide Cohort Study conducted by Kristensen (25) which included more than 20,000 patients with IBD matched for age and gender with 200,000 controls. The study concluded that there was an increased risk of myocardial infarction, stroke and CV death in patients with IBD but only during episodes of flare or periods of persistent active disease, whereas the risk was the same as in the control population in patients with IBD remission. However, this study did not take into consideration other cardiovascular risk factors or different treatments that the subjects could have followed at that time. One recently published evidence is offered by Singh et al. (26) in a systematic review over 9 studies. The authors found a slightly increased risk of stroke and ischemic heart disease in patients with IBD, especially in women, but with no difference for peripheral artery disease. The CVD risk was similar both for Crohn's disease and for ulcerative colitis patients.

It is certain that IBD subjects are predisposed to an accelerated atherosclerosis process mostly secondary to chronic general inflammation. But with all the upmentioned inconsistent results, it is clear

there is a great heterogeneity in the population with IBD and with different degree of endothelial injury that finally leads to different CV risk. However, there are some categories that can be included into a higher risk, such as patients with extensive bowel involvement, longer activity or severe persistent disease (27).

In summary, IBD proves to be associated with an up to 3-fold increased risk of developing venous thrombembolic events. This leads to firm indication for anticoagulant thromboprophylaxis for IBD hospitalized patients. Regarding the atherothrombotic risk, there is good evidence that IBD have relevant subclinical atherosclerotic lesions. However, studies are divergent in concluding that IBD are strongly associated with macrovascular events, such as myocardial infarction or stroke, even if they prove positive correlations with CVD especially on subgroup analysis. There are needed more prospective studies with control groups that have the same cardiovascular risk profile as in IBD populations in order to conclude definitively that patients with IBD have an increased risk for developing CVD.

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INFLUENCE OF MONTELUKAST ON CISPLATIN-INDUCED EXPERIMENTAL ACUTE RENAL FAILURE

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INFLUENCE OF MONTELUKAST ON CISPLATIN-INDUCED EXPERIMENTAL ACUTE RENAL FAILURE (Abstract): **Aim:** To investigate the influence of Montelukast (MK) in acute renal failure (ARF) experimentally induced by cisplatin (CIS). **Material and methods**: The experiment was carried out in male Wistar rats (280-320g), divided into 4 groups of 12 animals each, treated as follows: Group I (Control); Group II (CIS): CIS, 5 mg/kbw, single dose, i.p.; Group III (CISMK): CIS, 5 mg/kbw, single dose, i.p and MK 2mg/kbw, p.o., 10 days; Group IV (MK): MK 2mg/kbw, p.o., 10 days. Blood and urine (24 h) samples were taken before the start of experiment (Io) and at 3, 7, 10 and 14 days after CIS administration. The following were determined: urea, creatinine, Ca, Mg serum levels and creatinine, proteins, Ca and Mg urinary levels. For histological evaluation, 3 rats of each group were sacrificed at 3, 7, 10 and 14 days. The severity of histopathological changes was evaluated using scores on a 1-4 point scale. **Results:** Both in CIS and CISMK group, serum levels of urea and creatinine and urinary protein levels were significantly higher compared with Io values and control group at 3 and 7 days, ($p<0.01$ and $p<0.05$, respectively). Urinary creatinine levels were significantly decreased at 3 and 7 days, ($p<0.01$) and ($p<0.05$) in CIS and CISMK group compared to Io and control group. The degree of severity of histopathological changes was similar in CIS and CISMK group. **Conclusions:** The obtained results showed that MK at a dose of 2mg/kbw had no protective effect in CIS-induced experimental acute renal failure. **Keywords**: CISPLATIN, MONTELUKAST, ACUTE RENAL FAILURE.

Cisplatin (cis-diamminedichloroplatinium II, (CDDP) (CIS) is an anti-neoplasic drug used in the treatment of solid tumors, such as brain, neck, lung, testicular or ovarian tumors. The main side effect which limits the use of cisplatin is its nephrotoxicity. One of the most common renal manifestations caused by cisplatin treatment is acute kidney injury. The kidney accumulates cisplatin more than other organs, the concentration of cisplatin in the epithelial cells of the proximal tube being 5 fold higher than the serum concentration (1). Cisplatin accumulates in the renal cells by active transport and passive diffusion through the cellular membrane. Transporter-mediated accumulation of cisplatin is the major intake mechanism in renal cells. Recent studies have identified 2 different membrane transporters capable of transporting cisplatin into cells: Ctr1 and OCT2. Ctr1 is a cooper transporter which also seems to

mediate cisplatin uptake; Ctr1 is found in a significant concentration in the kidney and localizes to the basolateral membrane of the proximal tube. OCT2 is an organic cationic transporter and is found predominantly in the kidney; recent studies showed that the deletion of OCT2 gene significantly reduces the urinary excretion of cisplatin and nephrotoxicity (2).

Montelukast (MK) is a selective cysteinyl leukotriene receptor 1 (CysLT1) antagonist used in the treatment of asthma. MK is an anti-inflammatory drug with antioxidant properties that interferes directly with leukotriene receptors. Some recent studies highlight that Mk is a highly free radical scavenger, balancing oxidant-antioxidant status and regulating the generation of inflammatory mediators (3).

In this study we investigated the influence of MK in CIS-induced experimental acute renal failure in rats.

MATERIAL AND METHODS

Sinplatin (cisplatin), 1mg/ml concentrate for solution for infusion (SINDAN PHARMA SRL, ROMANIA) and Montelukast film-coated tablets, 10 mg, (TEVA) were used.

Adult male Wistar rats (280-320g) were used in the study. The animals were housed in a temperature (22-24^{0}C) controlled room with a constant 12 h light/dark cycle, in plastic cages with free access to standard food and water. After a quarantine period, the animals were divided into 4 groups of 12 subjects each, housed in standard metabolic cages and treated as follows Group: I (Control); II (CIS): CIS, 5 mg/kbw, single dose, i.p.; group III (CISMK): CIS, 5 mg/kbw, single dose, i.p and MK 2mg/kbw, by gastric gavage, 10 days; group IV (MK): MK 2mg/kbw, by gastric gavage, 10 days.

Blood samples from the retro-orbital plexus were taken before the start of the experiment (Io) and at 3, 7, 10 and 14 days after the CIS administration. 24-hour urine samples were collected from all animals in individual metabolic cages, at the same time periods as blood samples. The urinary volume was measured. Serum levels of urea, creatinine, Ca, Mg and urinary levels of creatinine, proteins, Ca and Mg were determined. For histological evaluation, 3 rats of each group were sacrificed at 3, 7, 10 and 14 days under ether anesthesia and the kidney samples were taken. All biochemical parameters were determined using RANDOX diagnostic kits on Rx Daytona analyzer.

A semi-quantitative assessment of renal tissues was realized using a scale for the degree of injury severity in agreement with the criteria for laboratory methods. The scores for the severity of changes were measured on a scale from 1 to 4 as follows: (1) minimal (affecting < 25%); (2) mild (affecting 25-50%); (3) moderate (affecting 50-75%) and (4) severe (affecting > 75%). All kidney sections were evaluated for degeneration/necrosis of the proximal tubules (PT), distal tubules (DT), collector duct (CD) (with or without regeneration), intratubular casts, hyaline, cortex and interstitial inflammation. Two whole organ sections were examined and an average score was obtained.

The use of animals and the experimental protocol were in agreement with the "Grigore T. Popa" University of Medicine and Pharmacy guidelines for Research and Ethical Issue and also in compliance with the ethical standards of the European Community.

The results were statistically evaluated by one-way analysis of variance (ANOVA) and t-test. p-values <0.05 were considered statistically significant. All data are pre-

sented as mean±standard deviation (SD).

RESULTS

During the experiment the animals did not show significant signs of toxicity. The animals receiving CIS and CISMK showed weight loss (10%) in comparison with the MK and control groups. The 24h-urine volume in CIS and CISMK groups decreased significantly ($p < 0.05$) at 3 and 7 days compared to baseline values (Io) and increased significantly ($p<0.05$) at 14 days (tab. I). Serum urea and creatinine levels were significantly higher in CIS and CISMK groups at 3 and 7 days, ($p<0.01$ and $p<0.05$, respectively), compared to Io values and control group (tab. II). Mg serum levels significantly increased at 3 days, ($p<0.01$) in CIS and CISMK groups, compared to Io and control group (tab. III).

TABLE I

Urine volume (24h) in CIS, CISMK, MK and control groups (X±SD)

Parameter	Group	Io	3 days	7 days	10 days	14 days
24-h urine volume (ml)	CIS	24.71±9.06	14.85±6.49 *p<0.05	13.0±2.0 **p<0.01	30.66±5.77	34.00±4.5 *p<0.05
	CISMK	25.5±6.02	12.62±3.88 *p<0.05	15.5±3.95 *p<0.05	19.66±3.78	23.5±3.53
	MK	10.2±2.68	11.6±1.94	10.2±2.14	12.0±2.4	11.33±2.51
	Control	11.8±3.12	12.78±2.3	12.51±1.97	13.24±2.78	12.57±2.21

*p<0.05; **p<0.01 compared with Io

Urinary proteins increased significantly at 3 and 7 days, ($p<0.01$), ($p<0.05$) in the groups receiving CIS and CISMK, in comparison with Io values and control group. Urinary creatinine was significantly lower at 3 and 7 days in CIS and CISMK groups, ($p<0.01$ and $p<0.05$, respectively), compared to Io (tab. IV).

TABLE II

Serum urea and creatinine levels in CIS, CISMK, MK and control groups (X±SD)

Parameters	Groups	Io	3 days	7 days	10 days	14 days
Urea (mg/dl)	CIS	32.33±8.9	276.66±58.1 *p<0.01	96.52±6.85 *p<0.01	38.16±8.32	37.83±8.79
	CISMK	31.33±6.3	264.33±64.0 *p<0.01	93.67±7.63 *p<0.01	36.16±8.37	36.0±8.0
	MK	32.0±7.21	36.16±7.16	32.83±6.21	33.24±7.8	31.66±6.25
	Control	32.38±8.2	33.46±6.78	32.28±8.56	32.87±5.57	33.21±4.32
Creatinine (mg/dl)	CIS	0.57±0.05	2.98±0.43	1.25±0.04	0.63±0.09	0.62±0.05
	CISMK	0.58±0.05	2.93±0.64 **p<0.01	1.12±0.05 *p<0.05	0.62±0.06	0.61±0.07
	MK	0.59±0.05	0.59±0.07 **p<0.01	0.58±0.06 *p<0.05	0.59±0.08	0.58±0.03
	Control	0.61±0.02	0.59±0.06	0.59±0.07	0.62±0.07	0.60±0.03

*p<0.05; **p<0.01 compared with Io and control

TABLE III
Serum Ca and Mg levels in CIS, CISMK, MK and control groups (X±SD)

Parame-ters	Groups	Io	3 days	7 days	10 days	14 days
Ca (mg/dl)	CIS	10.71±0.3	12.05±1.32	11.44±0.49	11.37±0.35	11.53±0.3
	CISMK	10.78±0.2	10.81±0.11	10.83±0.21	11.34±0.18	11.14±0.15
	MK	12.07±0.9	11.95±0.83	12.51±0.37	12.72±1.02	12.6±0.59
	Control	11.87±0.7	11.32±0.73	12.02±0.83	11.98±1.02	11.72±0.95
Mg(mg/dl)	CIS	2.83±0.07	4.15±0.54	2.4±0.08	2.64±0.13	2.59±0.27
	CISMK	2.55±0.18	**p<0.01 3.75±0.6	2.68±0.41	2.6±0.39	2.65±0.14
	MK	2.25±0.26	*p<0.05 2.31±0.07	2.26±0.34	2.3±0.18	2.38±0.17
	Control	2.38±0.17	2.42±0.21	2.39±0.39	2.46±0.32	2.40±0.15

*p<0.05; **p<0.01 compared with Io and control

TABEL IV
Urinary protein and creatinine levels in CIS, CISMK, MK and control groups (X±SD)

Parameters	Groups	Io	3 days	7 days	10 days	14 days
Proteins(mg/dl)	CIS	0.41±0.03	2.49±0.51	1.04±0.07	0.59±0.11	0.64±0.09
	CISMK	0.35±0.07	*p<0.01 2.26±0.5	**p<0.05 0.98±0.07	0.62±0.08	0.72±0.03
	MK	0.45±0.09	*p<0.01 0.51±0.13	**p<0.05 0.44±0.11	0.48±0.09	0.47±0.02
	Control	0.41±0.07	0.43±0.09	0.42±0.07	0.42±0.09	0.43±0.03
Creatinine(mg/24h)	CIS	10.1±1.52	3.15±0.11 *p<0.01	6.93±0.98 **p<0.05	8.97±1.35	11.12±2.31
	CISMK	12.5±1.36	5.04±1.05	8.74±1.07	9.2±1.98	10.4±0.89
	MK	9.13±1.72	*p<0.01 9.16±1.53	**p<0.05 9.1±2.32	9.36±2.67	9.35±2.35
	Control	9.85±1.03	10.1±1.98	9.78±2.32	9.95±1.86	10.2±2.05

*p<0.01; compared with Io and control; **p<0.01; compared with Io and control

Urinary Mg and Ca levels were significantly lower at 3 days (p<0.001) in CIS and CISMK groups, compared with Io and control group. At 7 days, urinary Ca and Mg levels registered a significant increase (p<0.01), in comparison with the 3 day levels (tab. V). No significant differences in biochemical parameters between CIS and CISMK were detected.

The histopathological examination showed no differences between controls and MK group. In CIS and CISMK groups, degenerations and tubular necrosis of mild to moderate severity at 3 and 7 days were found. At 10 and 14 days the injury severity was 1 and 2. Cisplatin induced degeneration/ necrosis of the S3 segment of the renal proximal tubules. Tubular dilations of

the renal cortex of mild severity were also noticed in all animals. Also, mild regeneration was associated with degeneration/ necrosis.

TABLE V
Urinary Ca and Mg levels in CIS, CISMK, MK and control groups (X±SD)

Parameters	Groups	Io	3 days	7 days	10 days	14 days
Ca (mg/dl)	CIS	34.65±2.53	5.33±0.14	42.14±0.3	41.41±0.18	43.26±2.56
	CISMK	30.14±0.12	*p<0.001 5.83±0.28	**p<0.001 41.39±0.98	41.39±1.35	41.62±3.12
	MK	40.01±1.2	*p<0.001 39.3±1.07	**p<0.001 40.0±0.51	40.27±0.68	39.94±0.65
	Control	40.56±0.56	41.12±2.3	41.27±1.78	40.76±0.87	41.33±0.48
Mg (mg/dl)	CIS	39.91±1.24	0.99±0.35	60.02±1.19	46.53±2.77	49.26±2.56
	CISMK	43.13±0.37	*p<0.001 0.77±0.13	**p<0.001 43.36±0.54	54.83±0.97	*p<0.05 54.89±1.05
	MK	55.56±4.92	*p<0.001 54.34±6.28	**p<0.001 57.23±3.27	58.1±2.33	*p<0.05 58.46±1.27
	Control	52.24±3.19	53.64±5.29	56.41±4.78	55.36±2.95	56.38±4.52

*p<0.001compared with Io; **p<0.001 compared with 3 day levels

DISCUSSION

Conversion of cisplatin to toxic compounds takes place in the proximal tubular cells (4). On experimental models, cisplatin affects the proximal tubular cells of the internal renal cortex and outer medulla (S3 segment), the most damaged region by cisplatin. Depending on the dose, it can also damage the distal tubule and collecting duct. Morphological glomerular changes are not present (5, 6). 72 hours after cisplatin administration proximal and distal tubular reabsorption is impaired and vascular resistance is increased. The proximal tubule loses the epithelium responsible for favoring the flow of substances into its cells. This process involves the formation of concentrated urine, which leads to increased levels of toxic compounds in the tubular fluid.

Our results showed a good correlation, individually, between the presence of mild to moderate degeneration/necrosis at 3 and 7 days, higher serum urea and creatinine levels, higher urinary protein levels and lower urinary volume. The 2mg/kbw MK dose had no protective effect in acute renal failure induced by CIS.

ARF is associated with renal vasoconstriction, reduced GFR, decreased urine output and increased serum creatinine. Creatinine is secreted through organic cation pathway located in the basolateral side of the proximale tubules. The proportion of tubular creatinine secretion increases with worsening renal function, while urine creatinine excretion decreases and extra-renal secretion of creatinine increases. Serum creatinine can therefore overestimate GFR in advanced renal dysfunction (7).

MK is a well known anti-asthmatic drug, which also has other pharmaco-dynamic effects (8). There are data that show that the CysLT1 antagonist has an anti-ulcer activity (9, 10), as well as a protective effect in burn-induced oxidative injury of the skin (11). Some other studies suggest that MK has a

partially protective activity in experimental intoxication with carbon tetrachloride (12). There are studies that show that the CysLT1 antagonist at a higher dose than we used in our experiment had a positive effect in acute renal failure experimentally induced by cisplatin, gentamicine, amykacine (13, 14).

CONCLUSIONS

The results obtained in our study show a good correlation between the biochemical parameters that define ARF and the histopathological changes found, and demonstrate that CIS, 5 mg/kbw, i.p., induced an abrupt (within 48 h) reduction of kidney function in rats.

MK dose of 2 mg/kbw used in our experiment did not have a protective effect in CIS-induced experimental acute renal failure.

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THYROID FUNCTION DURING THE FIRST YEAR OF RECOMBINANT HUMAN GROWTH HORMONE THERAPY IN SHORT STATURE CHILDREN

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THYROID FUNCTION DURING THE FIRST YEAR OF RECOMBINANT HUMAN GROWTH HORMONE THERAPY IN SHORT STATURE CHILDREN (abstract) **Introduction:** The relationship between rhGH treatment and thyroid function has been the subject of numerous studies. Some say that rhGH treatment unmasks central hypothyroidism, other say that rhGH induces subclinical primary hypothyroidism. **Aim:** To assess the changes in thyroid function in short stature children in the first year of treatment with rhGH and the impact on growth velocity. **Material and methods**: We evaluated 37 patients treated with rhGH, 5 were excluded because developed side effects during treatment. We measured height, height velocity, and height standard deviation gain during treatment and thyroid function during the first year of treatment. **Results**: We observed a slight increase in the TSH level and no significant change in the f T_4 level after the first 3-6 months of treatment in all the groups; in GH deficiency (GHD) patients, we observed a statistically significant decrease of the f T_4 level after the first 3-6 months, without a significant increase of the TSH level. After the first year, thyroid function returned to baseline. There were no differences between height velocities in all the groups, except from the GHD patients. **Conclusions**: The slight increase in the TSH level and the decrease of f T_4 level might unmask a transient subclinical primary hypothyroidism but these changes do not influence the growth velocity in first year of rhGH treatment. **Keywords**: RECOMBINANT HUMAN GROWTH HORMONE (rhGH), THYROID FUNCTION, TSH, FT_4

Recombinant human growth hormone (rhGH) has been approved for the treatment of growth deficiency (GHD) in both children and adults since 1986 and also since then, the indications have been extended to other non-GH deficient diseases in children, such as idiopathic short stature, Turner syndrome, small for gestational age, Prader–Willi syndrome. rhGH can be given as a replacement dose (for GHD) or pharmacologic (for most other conditions).

In children with GHD, rhGH treatment is given in doses that reproduce the daily normal secretion of growth hormone. In pharmacological dose, rhGH stimulates growth-promoting pathways affected by the diseases or not functioning properly (1). In these cases rhGH is used in higher doses in order to overcome the relative GH or IGF-1 insensitivity. The response to treatment depends

on the sensitivity to GH, the dosage and the age of initiation of the treatment (2).

The relationship between rhGH and hypothalamus-pituitary-thyroid axis has been the subject of several studies with divergent results. Researchers showed that rhGH treatment induces a fall in serum total and free T_4 concentration, an increase in serum T_3 concentration, independent of the TSH action, which decreases or remains unchanged. Several explanations have been issued for these effects but most of them are contradictory (3-8).

The aim of our study was to evaluate the changes induced by the rhGH treatment on the thyroid function and the impact on velocity in short stature children in the first year of treatment with rhGH.

MATERIAL AND METHODS

We evaluated children treated with rhGH between 2009 and 2012, in our Department of Endocrinology. Patients were included in our study based on the inclusion criteria for rhGH treatment of the current national recommendations in the program of therapy of short stature children (9). Parental informed consent was obtained from all the participants in our study.

The retrospective analysis included 37 patients. We divided our patients in 4 subgroups: growth hormone deficient (GHD) 17 patients, Turner syndrome 4 patients, small for gestational age (SGA) 5 patients and idiopathic short stature (ISS) 6 patients (tab. I).

TABLE I

Auxological and hormonal data of the patients

Diagnosis		GHD	Turner Sdr.	SGA	ISS	p
Number		17	4	5	6	
Female/Male		7 / 10	4	3 / 2	2 / 4	
Age at GH start,years		10.46±3.5	10.25±3.59	9± 3.39	9.333± 1.42	>0.9
Target heigh,cm		171±9.8	165.5±2.04	168.4± 8.99	170.58± 4.85	>0.9
Bone age at GH start		6.72±3.33	10.653±2.08	5.906± 4.82	4.045± 1.66	0.0868
rhGH dose, µg/day		0.701±0.26	1.233±0.57	0.6± 0.25	0.725± 0.101	
I-st year velocity,cm/y		8.593±2.19	7.625± 0.47	7.88± 1.08	8.26± 0.737	0.129
Height SD gain		0.941±0.61	0.775±0.38	1.196± 0.46	1.192± 0.29	0.5981
Maxim GH, ng/ml	Clonidine	6.764±2.96			16.75± 2.93	
	Insulin	4.93±3.38			6.985± 6.6	
TSH, mUI/l (0,38-4,31)	Before	2.46±0.40	4.66±1.76	2.438±0.77	2.026±0.6	0.34
	3-6 mo	3.21±0.48	5.35±1.34*	3.31±0.55	1.65±0.39*	***0.039**
	1 year	2.28±0.39	3.842±1.39	3.42±0.17	2.32±0.57	0.5159
fT_4, ng/dl (0,82-1,63)	Before	1.39±0.084	1.31±0.11	1.20±0.04	1.28±0.046	0.5451
	3-6 mo	1.08±0.07*	1.22±0.088	1.40±0.03*	1.29±0.05	***0.0454**
	1 year	1.16±0.075	1.2±0.05	1.26±0.04	1.08±0.107	0.4274

*groups compared to one another, with statistical significant difference ($p<0.05$)

Patient height was obtained by direct measurement on a wall mounted stadiometer and standard deviation was assessed using Prader 1989 growth charts. Target height was calculated using the formula: (father's height+mother's height ± 13 cm)/2. Bone age was calculated according to Greulich-Pyle's 1959 standards (10) or Tanner Whitehouse 20 system. All the stimulation tests and blood samples were

performed during the morning hours.

Diagnosis of GHD was based on height below - 2.5 standard deviations (SD), bone age delayed with more than 2 years, low or normal IGF-1 for age and sex and at least 1 negative stimulation test for growth hormone. We performed clonidine stimulation test with 0.15 mg/m^2 or insulin tolerance test with 0.15 IU/kg. GHD was established if GH peak was less than 10 ng/ml. Severe GHD was assessed as peak GH less than 3 ng/ml.

Diagnosis of SGA was based on birth weight less than -2 SD or length less than – 2 SD compared to normal values for gestational age, height below – 2.5 SD at the age of 4.

ISS was diagnosed in the presence of a height less than - 3 SD, with normal nutritional status, no history of chronic diseases and after having excluded any other cause of growth failure.

Diagnosis of Turner syndrome was made on the clinical phenotype and confirmation by karyotyping of peripheral blood leukocytes. Diagnosis for Noonan syndrome was made clinically and Prader-Willi syndrome was diagnosed based on methilation analysis.

We excluded children less than 3 years of age and we did not start treatment until we excluded other possible secondary causes for growth failure like decompensated cardiac abnormalities, decompensated renal failure, malabsorption, congenital hypothyroidism, Cushing syndrome, decompensated diabetes mellitus, skeletal deformations, vitamin D deficiency, pituitary tumors, active malignant tumors or genetic disorders other than Turner syndrome, Noonan syndrome, Prader-Willi syndrome or Russel Silver (approved in our country for rhGH therapy).

Clinical aspects. The patients have been regularly followed up at our clinic.Before starting rhGH treatment; we assessed all the antropometric parameters, like birth height and weight, actual length and weight, target height, bone age and signs of puberty. We also obtained magnetic resonance images of the sellar region in order to exclude possible intracranial tumors. MRI showed partial empty sella in one girl with Turner syndrome, complete empty sella in one SGA boy, agenesis of corpus callosum in a GHD girl and pituitary hypoplasia in 2 children with GHD.

During each visit at 3, 6, 9 and 12 months after initiation of rhGH therapy, patients were closely checked for body height, weight, bone age, signs of pubertal development, complete blood count, fasting glucose levels and thyroid function – TSH, fT_4 and thyroid antibodies in case of hypothyroidism.

Treatment. Thyroid function was normal in most of the children (24 cases) but in the remaining 8 cases, 3 in GHD group, 3 in Turner group, one in SGA group and one in ISS group, levothyroxine was administered for primary hypothyroidism in order to maintain TSH and fT_4 values within the normal range and the dose was adjusted at each visit during follow-up. Three children had positive thyroid anti peroxidase antibodies.

All the patients except one girl were prepubertal at the time of diagnosis and no priming was necessary. The girl had Turner syndrome and conjugated estrogen therapy was started one year before initiation of rhGH therapy. Bone age was delayed in all groups with the exception of Turner patients with a bone age similar to the chronological one.

The dose of rhGH was adjusted in order to maintain an IGF-1 level within the normal range for sex and age.

Plasma TSH and fT_4 concentrations

were measured using an electrochemiluminescence immunoassay „ECLIA". Growth hormone concentrations were measured by IMMULITE® 2000 Growth Hormone. IGF-1 was assessed by an automated chemiluminescent assay system (IMMULITE®, Diagnostic Products Corp., Los Angeles, CA, USA) and the normal range was corrected for age.

Statistical analysis was performed in GraphPad prism 6, Demo Version. Numerical data are expressed as mean ± SD. We used *t*-test for paired data, Wilcoxon's test for non-parametric data with non-Gaussian distribution. When we analyzed the differences between more than three groups, we used ANOVA test. The level of statistical significance was at $p < 0.05$.

RESULTS

During follow up, we excluded 5 patients: one girl developed type 1 diabetes mellitus 8 months after rhGH treatment, one boy developed restrictive cardiomyopathy after 4 years of treatment, one boy presented high glucose levels 1 year after treatment, one girl with Prader-Willi syndrome presented aggravated obstructive sleep apnea 3 months after starting rhGH treatment and one boy was lost during follow-up. No other serious side effects were reported, except for a suspicion of epiphisiolysis in 14 years old, unconfirmed by the orthopedics.

We observed a slight increase without statistical significance in the TSH level and no significant change in the fT_4 level after 3, 6, 9 or 12 months of treatment in all the groups (tab. I, fig. 1A, B). The increase of the TSH level was highest after the first 3-6 months of treatment and during the first year it returned to pretreatment levels. The small changes of fT_4 level also stabilized after the first year of rhGH treatment (fig. 1C).

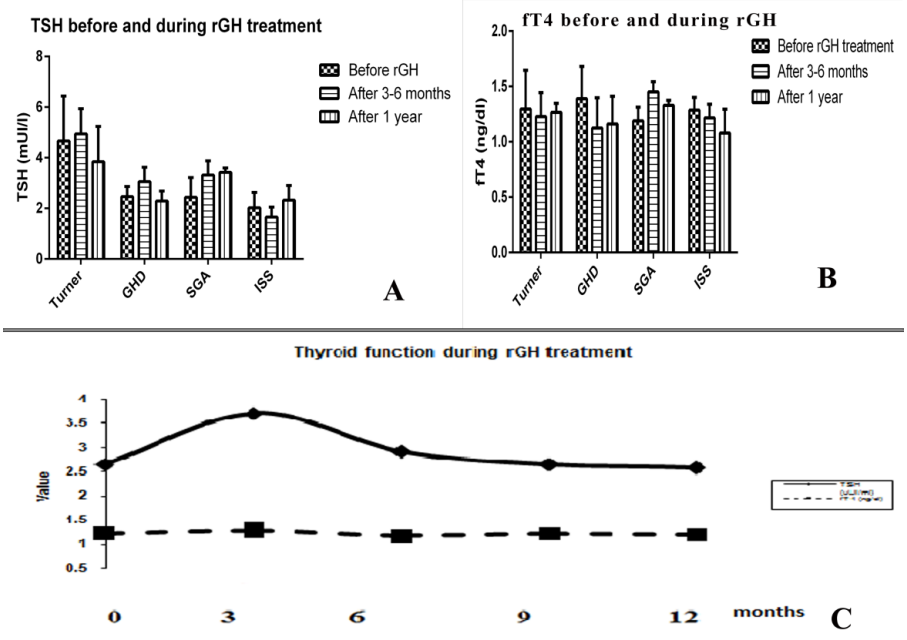

Fig. 1. Thyroid function evolution during the first year of rhGH treatment.

We compared TSH values between the 4 groups at the same time during monitoring. We observed a statistically significant difference of TSH values at 3-6 months only between Turner patients and ISS patients (tab. I).

Despite these changes, none of the patients from the euthyroid group needed levothyroxine supplementation as compared to four of the patients previously treated with levothyroxine who needed a small increase of the dose with an average of 25μg/day in order to maintain TSH values within the normal range.

In a subgroup analysis for GHD patients, changes of the thyroid function were more evident, with a significant fall in serum fT_4 levels after 3 and 6 months of rhGH therapy (fig. 2B). fT_4 was towards the lower limit but always within the normal range and we decided not to start levothyroxine substitution therapy. We did not find a significantly increase in the TSH level after the first months of treatment (fig. 2A) as we would have expected based on the fall of fT_4 levels.

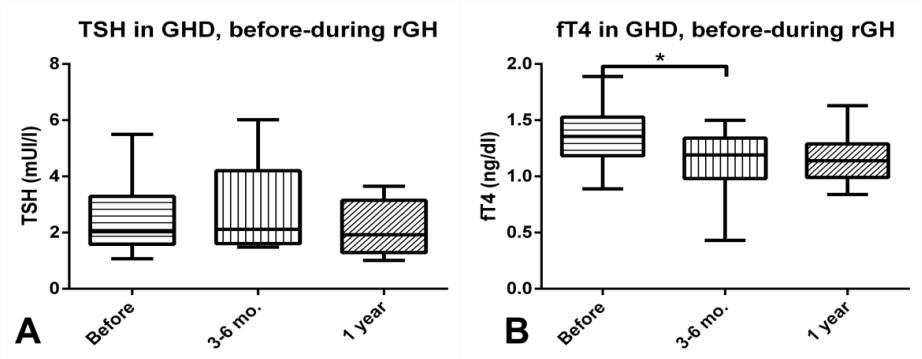

Fig. 2. Thyroid function in the GHD patients during the first year of rhGH treatment.

There were no statistical differences between height velocities and height standard deviation gain in the first year of rhGH treatment in all groups (tab. 1, fig. 3).

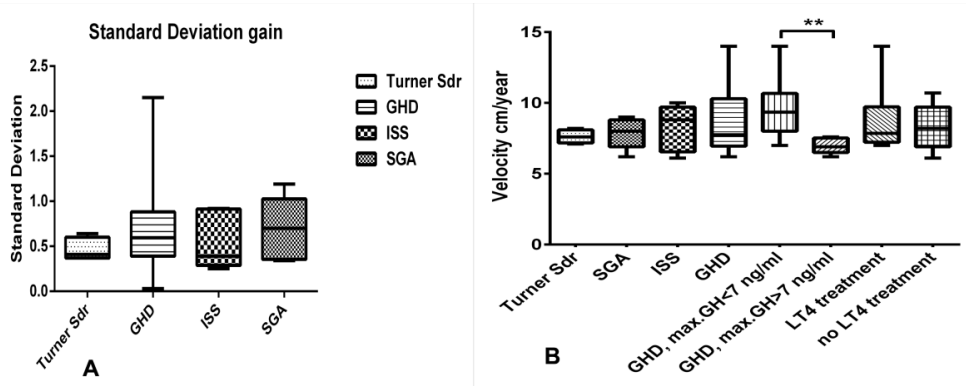

Fig. 3. Height velocity during the first year of rhGH treatment

The greatest height velocity was observed in the GHD group while height standard deviation gain was the most important in SGA group. The lowest gain was seen in Turner group. We did not notice any differences regarding velocity and height standard deviation gain between the euthyroid patients and those with previous levothyroxine substitution before rhGH therapy. In the GHD subgroup we observed a statistical difference in the height velocity between those who had a maximum GH during clonidine stimulation test less than 7 ng/ml versus those with maximum GH more than 7 ng/ml (fig. 3B).

DISCUSSION

This study demonstrates that during rhGH therapy for short stature children, thyroid function is influenced by a shift of fT_4 levels toward the lower limit and slightly elevated levels of TSH in all euthyroid children. The same changes were also seen in the patients with previous primary hypothyroidism but adequately substituted with levothyroxine.

Based on the results of our study, we issued several hypotheses. One of them is that rhGH treatment induces an increase in the activity of deiodinase type I and III which leads to increased peripheral conversion of T_4 to T_3. The increased levels of T_3 might lead to decreased secretion of TSH through negative feedback. Also, the decreased levels of f T_4 induce through negative feedback an increased secretion of TSH. Together, these might explain why TSH level remains relatively low as compared to the decreased level of f T_4, such as seen in central hypothyroidism. Of great interest would be to determine the effect of rhGH treatment on the TRH gene expression in the hypophysiotropic neurons and the activity of type 2 deiodinase (D II), the primary thyroid hormone activating enzyme in the mediobasal hypothalamus and also in peripheral tissues. Another hypothesis of ours is that rhGH induces inhibition of TRH gene expression by increasing the local thyroid hormone activation in the hypothalamus and leading to central subclinical hypothyroidism. In addition, we hypothesize that an increase of DII activity in peripheral tissues may also contribute to the altered peripheral T_3/ T_4 ratio.

Previous studies have shown that rhGH therapy might unmask a central hypothyroidism in the face of low fT_4 with inadequately elevated TSH levels (5). Others contest this explanation by showing that fT_4 changes induced by rhGH therapy are independent of TSH secretion and are caused by the increased peripheral conversion of T_4 to T_3 which will determine a blunt TSH secretion via negative feedback (6). Because of these effects on thyroid hormones, Portes et al suggest that serum T_3 levels have no use in the diagnosis of central hypothyroidism but they would be useful to indicate excessive thyroxine supplementation (6).

Since we could not measure the T_3 serum concentration to justify the physiological response of TSH secretion, we interpret the changes seen in our patients as a transient subclinical central hypothyroidism which resumed after the first year of rhGH therapy. A same hypothesis issued Seminara et al (7).

Despite these changes in the thyroid function, we did not start levothyroxine substitution in our previous euthyroid patients because none of the values of fT_4 were below the normal limit and none of our patients developed signs or symptoms suspicious of hypothyroidism. We in-

creased the dose of L- T_4 in only four patients with previous primary hypothyroidism because of increased TSH levels above the normal limit, especially in the group of patients with Turner syndrome. As it has been shown, children with Turner syndrome are predisposed to thyroid disorders, especially hypothyroidism induced by thyroid autoimmunity, leading to increased levels of TSH and low or normal levels of fT_4 (11). This mechanism also explains the significant statistical difference we observed when comparing the TSH value at 3-6 months between Turner patients and ISS patients.

When comparing fT_4 levels we observed a statistical difference between the lower fT_4 values in GHD patients compared to fT_4 levels in SGA patients. We explain this difference as a direct effect of rhGH supplementation in GHD patients and increased peripheral conversion of fT_4 to T_3, as compared to SGA patients.

We assume that the transient changes in TSH and fT_4 are a consequence of a new equilibrium established once rhGH therapy has been started, especially in those with growth hormone deficiency. The transient changes of thyroid function seen in our study were also reported by other researchers but the mechanisms remain unclear. Besides the increased peripheral conversion of fT_4 to T_3 through stimulation of deiodinase type I and III, and decreased conversion of T_4 to r T_3 (8), some say that the extracellular water increase might determine these shifts in thyroid hormone levels (12). Another possible explanation for the lack of significant changes in TSH level is the increased somatostatinergic tone through somatostatin, the natural inhitor of TSH during rhGH therapy (13).

Despite these changes, we did not notice any negative impact on growth velocity and height standard deviation gain in either of the studied groups. The positive growth response might be explained by the increased levels of serum T_3 actively acting in periphery. All patients had velocities expected for the treatment schedule. There were no changes in velocity when comparing euthyroid patients to those with levothyroxine substitution before the onset of rhGH therapy which further supports our hypothesis that these changes in the thyroid function are transient and there is no need for levothyroxine substitution unless there are clear changes suggestive for hypothyroidism, especially in those patients previously known with thyroid diseases.

CONCLUSIONS

During the first year of rhGH treatment, there are changes in the thyroid function, with a slight increase in the THS level and a decrease of fT_4 level. We suppose that these changes unmask a subclinical primary hypothyroidism but do not influence the growth velocity. There should be a careful decision for supplementation with levothyroxine in previous euthyroid children, since the changes in thyroid function are transient and return to baseline values within one year of continuous treatment with rhGH. Further studies are necessary in order to establish the effects of rhGH treatment at the hypothalamic and pituitary level and the impact on thyroid hormone metabolism.

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Declaration of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific paper.

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ANXIETY AND DEPRESSION IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE: AN OPEN AGENDA FOR RESEARCH

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ANXIETY AND DEPRESSION IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE: AN OPEN AGENDA FOR RESEARCH (Abstract): Depression and anxiety are psychiatric conditions often associated with poor survival rate and impaired social functioning in chronic illnesses, like chronic obstructive pulmonary disease (COPD). COPD is a major cause of chronic morbidity and mortality, being nowadays the fourth leading cause of mortality worldwide and the burden of this disease is increasing as the population is ageing and it is continuously exposed to risk factors. Common mechanisms for explaining the association of anxiety, depression and COPD include cigarette smoke exposure, physical inactivity, social isolation, multiple episodes of dyspnea and chronic hypoxia. BODE index and MMRC dyspnea score could be associated with anxiety and depression in COPD patients and the screening usually implies administration of simple questionnaires. Therapeutic options for anxiety include serotonin-reuptake inhibitors, which decrease the perception of dyspnea, while newer antidepressants, such as venlafaxine, duloxetine and mirtazapine are particularly useful in depression, since they do not produce respiratory depression. **Keywords**: CHRONIC OBSTRUCTIVE PULMONARY DISEASE, DEPRESSION, ANXIETY.

Depression and anxiety are psychiatric conditions that cause significant social disability being characterized by sleep disturbances, weight cycling, depressed mood, loss of interest in nearly all activities, and somatic symptoms of tension (psychomotor agitation, dyspnea, irregular heartbeats) (1, 2, 3). In chronic illnesses, depression is often associated with poor survival rate and impaired social functioning (1).

Chronic obstructive pulmonary disease (COPD) is a major cause of chronic morbidity and mortality, being nowadays the

fourth leading cause of mortality worldwide. COPD burden is increasing as the population is ageing and it is continuously exposed to risk factors such as smoking, occupational or environmental exposures to various particles (4).

Patients with this progressive respiratory disease often experience physical and role limitations, expressed as anger, frustration, physical and emotional dependency, and consequently guilt and embarrassment (5).

Anxiety and depression are considered major co morbidities in COPD, both associated with poorer prognosis, and a large number of studies provided relevant information (6).

THE IMPACT OF ANXIETY AND DEPRESSION IN COPD PATIENTS

The prevalence of generalized anxiety disorder in COPD patients is considered to be raging between 10% - 33%, with a prevalence of panic attacks up to 67% (7, 8). A small study comparing 20 subjects with COPD with a control group of 20 patients hospitalized for degenerative orthopedic conditions revealed that anxiety disorders among respiratory patients had a prevalence of 55%, compared with 30% in the orthopedic group and among anxiety disorder panic disorder was the most prevalent expression of psychiatric disease (9).

On the other hand, the prevalence of major depressive disorder in the same patients ranges between 37% and 71% (10). Two other prevalence studies identified clinically significant depressive symptoms in 42-57% of COPD patients (11, 12). A cross-sectional study guided in USA, assessed for depression 1736 patients with COPD and the results showed that 40% of the patients had three or more depressive symptoms. These symptoms were more common in patients with COPD than in patients with coronary heart disease, stroke, diabetes, hypertension and cancer (13).

A case-control study published in 2002, evaluated 676 subjects with COPD and showed that the prevalence of depression in patients with severe COPD was 25% compared to 19.6% in patients with mild to moderate COPD, and 17.5% in controls. The risk of depression was 2.5 times higher for patients with severe COPD than for controls, these results highlighting the importance of reducing depressive symptoms from the early stages of COPD (14). Worsening COPD status can be associated with higher prevalence of anxious symptoms (15). As well, it was revealed that depression after hospitalization for COPD is frequently associated with a significantly increase in mortality (16, 17).

Despite the magnitude of the problem, depression in COPD patients could be very difficult to recognize, due to the lack of specific symptoms, the depressive symptoms being often attributed to the underlying pulmonary disease (14, 18).

Common mechanisms for explaining the association of anxiety and COPD include cigarette smoke exposure, physical inactivity, social isolation, multiple episodes of dyspnea and chronic hypoxia, all contributing to the appearance of depressive symptoms in patients with chronic lung disease (5).

A multicentre prospective study followed 416 patients hospitalized for the acute exacerbation of COPD and discovered that anxiety was more common in women than in men (47% vs. 34%) and current smokers had a higher prevalence of both depression and anxiety than non-smokers patients (19). An article published

in 2004, showed possible explanatory models for a common pathophysiology between COPD and anxiety, including the hyperventilation model and cognitive-behavioral model (20) and in 2005, a Slovenian study revealed a direct connection between the perception of dyspnea and anxiety or depression (21).

Anxiety and depression are also important factors in disease-specific health-related quality of life and hospitalization rates, exacerbations of the pulmonary disease being the main cause for hospitalization in patients with COPD (3, 4). The presence of anxiety and/or major depressive disorder is an important risk factor for the exacerbation of symptoms and re-hospitalization within a 12-month period in patients with chronic lung disease and poor health-related quality of life (3, 22). Also, many studies identify that depression and anxiety in COPD patients lead to a lower quality of life, poorer health status and decreased adherence to treatment. Several studies have shown improvement in psychiatric symptoms after following the pulmonary rehabilitation program (23).

Epidemiological studies often show conflicting results regarding the prevalence of psychiatric disorders in COPD patients, these differences being dues to the variables assessment instruments.

CLINICAL ASSESSMENT OF PSYCHIATRIC DISORDERS IN COPD

An appropriate clinical approach of COPD patients has to identify the early symptoms of psychiatric disorders, as well the risk factors and the parameters associated with these symptoms.

There are several studies that revealed some risk factors for anxiety and/or depression in chronic pulmonary patients. For instance, a recent cross-sectional study including 122 patients with stable COPD reached the conclusion that increasing stage in BODE index is a predictor for anxious symptoms (24), BODE index being a multidimensional grading system, comprising the body-mass index, distance walked in 6 minutes (6-minute walk test), the degree of airflow obstruction (FEV_1), and score on MMRC dyspnea scale, predicting the mortality among patients with COPD (25). Another study including 84 patients diagnosed with COPD showed that the following parameters were generally associated with depression: GOLD stage, BODE index, the percent of the predicted value of FEV_1 and modified Medical Research Council (MMRC) dyspnea score (26).

A group of Turkish researchers conducted a recent trial and concluded that the major risk factor for anxiety and depression is dyspnea; also, the cytologic examination of bronchoalveolar lavage appeared to be an useful tool for identifying patients with psychiatric disorders because it shows the extent of the lung damage, which is the major mechanism underlying dyspnea (27).

A prospective cohort study including 1205 patients, with up to 30 years of follow-up, reached the important conclusion that a poor lung function is a risk factor for depression, particularly in patients with prevalent chronic disease (28).

These arguments derived from clinical studies and the well-known increased prevalence of anxiety and major depressive disorder in patients with chronic obstructive lung disease support the development of a screening strategy in patients with chronic respiratory disorders.

Screening for depression usually use

"PHQ-9 Depression Questionnaire", a self-report 9-item questionnaire, resulting in a score, quantifying the severity of depression. For anxiety, "Hamilton Anxiety Rating Scale" requires a short time to apply it and to analyze the answers (29). Both screening questionnaires should be applied in patients with chronic lung disease admitted in hospitals, pulmonary rehabilitation centers or in outpatients, at their regular pulmonologist visit.

This psychiatric assessment is highly recommended in COPD patients as it enables the clinician to identify patients who need undergo a more appropriate diagnostic interview and also the high-risk patients.

THERAPEUTIC APPROACH – SPECIFIC ISSUES

When anxiety and/or depression are diagnosed in the setting of a chronic obstructive pulmonary disease, there are several difficult issues regarding the therapeutic approach.

For anxiety, pharmacological treatment is one of the most convenient choices. The most common course of treatment is the use of serotonin-reuptake inhibitors (SSRI), which decreases the perception of dyspnea. Citalopram and escitalopram are preferred because they have an advantage in patients taking multiple drugs, having the lowest potential to interfere with cytochrome P450 system (30).

Benzodiazepines, such as clonazepam or alprazolam, have anxiolytic effects and they are used to treat anxiety, but they could interfere with central respiratory drive, leading to respiratory depression. Furthermore, these drugs have increased rates of withdrawal symptoms and a half or a quarter of the usual starting dose is recommended in respiratory patients (30).

Patients who do not respond to this treatment are ideal candidates for psychotherapy, delivered under multiple forms: cognitive-behavioral therapy, interpersonal therapy or problem-solving therapy (31). In patients with chronic lung disease, the addition of psychotherapy and pulmonary rehabilitation program has been demonstrated to decrease anxiety (32).

Moreover, choosing an antidepressant for patients with COPD is a very difficult matter and depends on the pattern of depression. It is very important that in patients with chronic lung disease the selected medication not to have as therapeutic effect sedation, because respiratory depression is an important potential side-effect of psychotropic medication. In this regard, it is recommended to use SSRIs or newer antidepressants, such as venlafaxine, duloxetine and mirtazapine (33).

CONCLUSION

Anxiety and depression are co morbidities often present in the clinical presentation of patients with chronic obstructive lung disease, requiring diagnosis and appropriate treatment. As there are no laboratory findings to confirm these diagnoses, increased awareness of healthcare providers and screening tools are important steps in managing psychiatric disorders in pulmonary patients.

The correlation between chronic lung disease and psychiatric disorders is very complex, including somatic, psychological and social factors. Nowadays it is possible to successfully treat psychiatric disorders associated with chronic lung disease, thus improving the quality of patient's life, lowering the costs associated with hospitalization and improving patient's role in the society.

Further studies are needed to clarify the pathogenic mechanisms underlying the association COPD – psychiatric disorders and to evaluate the effects of new treatment options so that the clinical guidelines can be renewed periodically.

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NEWS

DOES WEIGHT MISPERCEPTION HAVE AN

IMPACT ON EATING BEHAVIOR?

A recent study among spanish adolescents had shown that females tend to perceive themselves as being overweight when in fact their body mass index is between normal limits. On the other hand, the majority of people who consider that they have normal weight, but in reality they are overweight, are males. There has been spotted another difference between genders in reported physical activity. It seems that males are more physically active than females. Moreover, females often go on a diet only for aesthetic reasons. The conclusions of the study are that the misperception of weight results in eating disorders. For example, if a person considers that is overweight at a normal BMI value, will follow an unfit diet, low in vitamin, minerals and macronutrients. A desired outcome is to provide better nutritional education from a young age since eating disorders are related to low self-esteem and ill general health. (Jáuregui-Lobera I, Ezquerra-Cabrera M, Carbonero-Carreño R, Ruiz-Prieto I, Weight misperception, self-reported physical fitness, dieting and some psychological variables as risk factors for eating disorders, *Nutrients* 2013; *5*: 4486-4502).

Mihaela Constantin

EVALUATION OF STUDENT PERCEPTION OF THE LECTURE "ORAL FUNCTIONAL MEDICAL REHABILITATION: BALNEOPHYSIOTHERAPY"

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EVALUATION OF STUDENT PERCEPTION OF THE LECTURE "ORAL FUNCTIONAL MEDICAL REHABILITATION: BALNEOPHYSIOTHERAPY" (Abstract): The **aim** of the study was to assess this course as perceived by the 6th year dentistry students. **Material and methods:** Applying and validating the "Dundee Ready Education Environment Measure (DREEM)" for the first time in Romania. **Results and discussion:** The reliability of the test according to Cronbach alpha method, which measures the internal consistency of a test, was 0.87 (Cronbach alpha coefficient should be above 0.70). As to the reliability coefficients (internal consistency) for each test subscale and for the entire test, DREEM proved a very good overall reliability, with alpha = 0.89. The reliability of each DREEM subscale was also analyzed. The DREEM test results, with a global score of 149, indicate a "more positive than negative" perception (score between 101 and 150). **Conclusions:** The DREEM test showed that the assessment and treatment of pain in dental-maxillary pathology and TMJ dysfunctions through medical rehabilitation by means of balneophysiotherapy is of interest to final year dental medicine students. As a student-teacher partnership, modern education cannot ignore the practical needs of students and should add to their theoretical training with courses and practical training programs necessary in their future profession. **Keywords:** DENTAL EDUCATION, CURRICULUM, ORAL HEALTH EDUCATION, MEDICAL REHABILITATION, TEMPORO-MANDIBULAR JOINT, FUNCTIONALITY

The curriculum for the 6th (final) year students of the Iasi Faculty of Dentistry has included since 2004 the course in "Oral Functional Medical Rehabilitation: Balneophysiotherapy", aimed at helping students acquire the methodology for rehabilitation treatment of orodental and craniofacial pain, as well as temporomandibular joint (TMJ) dysfunctions (1).

The Romanian Society of Physical and Rehabilitation Medicine, second in Europe after France (2, 3), was founded in 1922 and the use of mineral waters in the preventive treatment of periodontal inflammatory conditions has been introduced in Romania since 1987, by the WHO Romania Centre for Infant Oral Rehabilitation and the Academy of Medical Sciences of Romania at Baltatesti Resort, Neamt county (2). This was achieved in correlation with WHO guidelines on oral health and prevention in dentistry (4), as well as the European direc-

tives for healthy aging and physical activity with adequate nutrition for old age (3).

The course curriculum consists of 10 2-hour lectures and 20 2-hour practical works at patient's bedside; the students fills in an observation sheet for orodental diagnosis and general medical condition based on current clinical and laboratory investigations.

MATERIAL AND METHODS

The test has 50 items and assesses the environment in which future doctors are trained (5), and also the efficiency, degree of interest and perceived importance of the course and practical works among students, using the 4-point Likert scale, with the following options: *Strongly agree, Agree, Uncertain, Disagree, Strongly Disagree*.

Of the total 185 final year dental students, 174 students, of which 23 foreign students, were asked to fill in the assessment test, an adaptation of the Dundee Ready Educational Environment Measure (DREEM) test in a paper-pencil survey, on the course and practical works in "Oral Medical Rehabilitation: Balneophysiotherapy" upon course completion. The questions targeted student perception, expectations, attitude, interest and satisfaction in the course and practical works. The test was written in its original template; written informed consent from all participants and hospital ethics committee approval in accordance with Helsinki declaration were obtained.

This is an important aspect of medical education, being rather neglected in dental educational research. The aim of the study was to fill this gap by applying and validating for the first time the DREEM test in a Romanian-speaking sample of dental medicine students (6, 7, 8, 9). Student responses were used to quantify educational effectiveness and their attitude regarding the information acquired during the course and its usefulness in their training as future dentists (6, 10, 11, 12).

RESULTS AND DISCUSSION

The questionnaire assesses 5 subscales, each with a different interpretation, as follows. The maximal score for the DREEM test is 200, indicating an ideal educational environment; minimum test score is 0. Items 4, 8, 9, 17, 28, 35, 39, 48 and 50 are negative scores; therefore, the scores for these items should be reversed in order to obtain the total score by summing up the subjects' responses to all the items of the test/ **all item responses**.

The test is divided into 5 subscales:

- *Students Perception of Learning* contains items 1, 7, 13, 16, 20, 22, 24, 25, 38, 44, 47 and 48, maximum score 48. The obtained score was 35, and the interpretation was: 25-36 "A more positive perception";
- *Students Perception of Course organizers* contains items 2, 6, 8, 9, 18, 29, 32, 37, 39, 40 and 50. The obtained score was 33, and the interpretation was: 23-33 "Moving in the right direction";
- *Students Academic Self-Perception* subscale contains items 5, 10, 21, 26, 27, 31, 41 and 45. The obtained score result was 24, and the interpretation was: 17-24 "Feeling more on the positive side";
- *Students Perceptions of Atmosphere* subscale contains items 11, 12, 17, 23, 30, 33, 34, 35, 36, 42, 43 and 49. The obtained score was 35, and the interpretation was: 25-36 "A more positive attitude";
- *Students Social Self- Perceptions* subscale contains items 3, 4, 14, 15, 19, 28 and 46. The obtained score was 18, and the interpretation was: 15-21 "Not too bad";

The authors of the test reported a good validity and reliability. We analyzed the

reliability and validity of this tool adapted for our course in a dental medicine student sample. Because the test has 5 subscales, for a good content validity, the 5 subscales have to positively correlate with each other. All Pearson *r* correlation coefficients between the 5 test subscales are important, with average and high values (tab. I).

We also analyzed the DREEM reliability using the Cronbach alpha method. A high Cronbach alpha coefficient tells us that the test has high internal consistency, i.e. the test items correlate with each other. For good reliability, Cronbach alpha coefficient should be above 0.70. A reliability coefficient between 0.60 and 0.70 tells us that the test has acceptable reliability; if the reliability coefficient is below 0.60, the test is considered to have a fairly low reliability. The authors reported for the entire test a Cronbach alpha reliability coefficient of 0.87.

TABLE I

Pearson's *r* correlation coefficients for the 5 test subscales

Variables	1	2	3	4	5
1. Students Perception of Learning	1.000	0.575	0.605	0.741	0.490
2. Students Perception of Course organizers		1.000	0.415	0.714	0.405
3. Students Academic Self-Perception			1.000	0.595	0.449
4. Students Perceptions of Atmosphere				1.000	0.535
5. Students Social Self-Perceptions					1.000

In our student sample, the test had a very good overall reliability, with alpha = 0.89. We also analyzed the reliability of each subscale; for the first 4 DREEM subscales, the Cronbach alpha reliability coefficients indicated an acceptable and good validity, whereas for the *Students Social Self- Perceptions* subscale the Cronbach alpha reliability coefficient was low. Therefore, the reliability of the fifth subscale was low, meaning that the degree of confidence in the results obtained through this subscale was low (tab. II, III).

Comparing the obtained average scores to those provided by the authors of the test, we noticed that the average total score entered the category 101-150: more positive than negative.

TABLE II

Reliability coefficients (internal consistence) for each test dimension and for the entire test

Dimensions	No. of items	Cronbach alpha coefficient
Students Perception of Learning	12	0.739
Students Perception of Course organizers	11	0.651
Students Academic Self-Perception	8	0.655
Students Perceptions of Atmosphere	12	0.730
Students Social Self-Perceptions	7	0.319
Total Score	50	0.895

TABLE III
Average scores and standard deviations for each test subscale and for the entire test

Dimensions	No. of items	Average	Standard deviation
Students Perception of Learning	12	31.38	5.52
Students Perception of Course organizers	11	28.67	5.01
Students Academic Self-Perception	8	21.81	3.82
Students Perceptions of Atmosphere	12	31.78	5.84
Students Social Self-Perceptions	7	17.81	2.98
Total Score	50	131.44	18.96

Analyzing the averages for the five DREEM subscales and comparing them to the standards provided by the author, we found that:

- The average score for the *Students Perception of Learning* subscale was interpreted as: 25-36 - a more positive perception;
- The average score for the *Students Perception of Course Organizers* subscale was interpreted as: 23-33 - moving in the right direction;
- The average score for the *Students Academic Self-Perception* subscale was interpreted as: 17-24- feeling more on the positive side;
- The average score for the *Students Perceptions of Atmosphere* subscale were interpreted as: 25-36 - a more positive attitude;
- The average score for the *Students Social Self-Perceptions* subscale was interpreted as: 15-21-not too bad.

The above data show that for both the entire test and for each test subscale students perception was positive, which means that they appreciate the course.

CONCLUSIONS

The analysis of test results demonstrates students' interest and appreciation of the knowledge and practical skills acquired during this course. The test identified the need for practical work in the general medical pathology to be illustrated by clinical cases and to be carried out individually by students in Dental Medicine. Also, the university curriculum for the final years of study should be adapted according to the changes in dental pathology, in keeping with updated information, so that to meet the four goals:

1. Tailor treatment to the individual patient, and thus to receive the best treatment;
2. Transfer research findings to the practicing dentist, because they are the main caregiver of patients with **TMJ dysfunction**;
3. Balance the judgment of best research evidence with clinical expertise in the choice of treatment;
4. Gain more information about patient preferences and values and what impact this has on treatment outcome.

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NEWS

INCREASED EXPRESSION OF ANTIMICROBIAL PEPTIDES AND TLR IN TINEA AND PITYRIASIS VERSICOLOR

A study by Brasch *et al.* investigated the role of non-cellular factors in the epidermal defence against fungi that cause superficial tinea or pityriasis versicolor, based on the observation that these pathogens are usually confined to stratum corneum. Using immunostainings in skin biopsies, researchers found an increase in expression of defensins 2 and 3 (hBD-2, hBD-3), psoriasin, RNase7, toll-like receptors 2 and 4 (TLR2, TLR4) in certain layers of lesional epidermis when compared to normal skin. In infected epidermis, defensins hBD2 and hBD3 were expressed in the stratum corneum and granulosum, while psoriasin was detected more common in the upper skin layers. However, the expression of TLR9 and dectin 2 showed no significant differences between normal and infected skin. The results of the study demonstrate the presence of antimicrobial peptides and ligands that contribute to fungal containment in tinea and pityriasis versicolor. (Brasch J, Mörig A, Neumann B, Proksch E. Expression of antimicrobial peptides and toll-like receptors is increased in tinea and pityriasis versicolor. *Mycoses.* 2013 Aug 19. doi: 10.1111/myc.12118) .

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RISK ASSESSMENT IN THE DEVELOPMENT OF ATRIAL FIBRILLATION AT PATIENTS WITH ASSOCIATE THYROID DYSFUNCTIONS

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RISK ASSESSMENT IN THE DEVELOPMENT OF ATRIAL FIBRILLATION AT PATIENTS WITH ASSOCIATE THYROID DYSFUNCTIONS (Abstract): Thyroid hormones are an important regulator of cardiac function and vascular system. Atrial fibrillation is a common cardiac arrhythmia and an important risk factor for the ischemic cerebral vascular accident and heart failure. **Objectives:** The examination of the risk to develop atrial fibrillation in relation to the whole spectrum of thyroid dysfunctions. **Material and methods:** All participants to our study were selected from among subjects with cardiovascular disorders whose hormonal thyroid status had been controlled previously and who hadn't been treated for thyroid functional disorders. Based on these criteria we defined a study lot made up of 791 subjects, 700 women and 91 men, aged between 22 to 86, with a mean age of approximately 60 years old, divided into five groups, based on the level of thyroid hormones. Once the lot was constituted, evaluations were made of the cardiovascular and thyroid condition at 6 and 12 months. **Results:** During monitoring, most patients who developed atrial fibrillation were registered in the groups with manifest clinical hyperthyroidism, 34,62% and respectively with subclinical hypothyroidism, 38,6%. The main risk factors at the patients with manifest clinical hyperthyroidism were: female gender (RR=1.97) and age above 60 (RR=1.33), as well as the presence of coronary disease in the personal pathological record (RR=3.31), HBP (RR=1.46) and cardiac frequency in excess of 80 beats/min (RR=1.38). The main risk factors that led to atrial fibrillation among the patients with subclinical hypothyroidism, were: obesity (RR=2.21), the presence in the personal record of heart disease (RR=2,0), age over 60 (RR=1.90) and female sex (RR=1.30). At the patients who had been administered beta blockers prior to admission (RR=0.99), ACEI (RR=0.85) and/or antiarrythmic drugs (RR=0.54), the medication represented a protective factor against developing atrial fibrillation. **Conclusions:** Thyroid dysfunctions are associated with an increased risk to develop atrial fibrillation, both in the case of clinically manifest and the subclinical manifest forms. These results support long term screening for thyroid dysfunctions for patients with atrial fibrillation. **Keywords:** HYPERTHYROIDISM, HYPOTHYROIDISM, ATRIAL FIBRILLATION.

Many of the symptoms and signs in the sphere of thyroid dysfunctions are due to the effects of thyroid hormones on the cardiovascular system and the hemodynam-

ic modifications that occur (1). Both hypothyroidism and hyperthyroidism trigger cardiovascular modifications which may lead to or worsen heart failure, atrial fibrillation, and pectoral angina. The fact is the more important as the prevalence of thyroid dysfunctions is estimated at 9-15% (2).

The main effects of thyroid hormones on the heart and peripheral vascular system are represented by the decrease of peripheral vascular resistance and the increase of heart frequency while at rest, the contractility of the myocardium and the increase of the blood flow. Atrial fibrillation represents the most frequent rhythm disorder incurred by the patient with hyperthyroidism, its prevalence being estimated at 2-20% of the patients (3). In addition, atrial fibrillation is responsible for most hospital admissions of the patient with hyperthyroidism.

The purpose of the present study was to evaluate the relationship between thyroid dysfunction and the risk of atrial fibrillation at patients who also associate cardiovascular pathology.

MATERIAL AND METHODS

All participants included in our study were selected from the subjects with cardiovascular disorders who had been controlled in terms of the hormonal thyroid condition in the 3rd Medical Clinic of the "St. Spiridon" University Emergency Hospital,"Grigore T. Popa" University of Medicine and Pharmacy, Iasi and who had not been treated previously for functional thyroid disorders. The study groups were constituted between 2006-2012.

We included both male and female subjects, based on the serum value of free triiodothyronine (FT3), free thyroxine (FT4) and thyroid-stimulating hormone (TSH). After the overnight fast, all patients were subjected to a complete medical evaluation, lab exams, electrocardiogram and echocardiography. Among the exclusion criteria we included: age <18, hypophysis/hypothalamic disorders, pregnancy, specific thyroid treatment received prior to the inclusion in the study. Based on these criteria we defined a study group divided into five lots, based on the level of thyroid hormones: a) 81 patients with subclinical hyperthyroidism (10,2%); b) 165 patients with hyperthyroidism (20,9%); c) 75 patients with subclinical hypothyroidism (9,5%); d) 170 patients with hypothyroidism (21,5%); e) witness - 300 patients with cardiovascular modifications, without thyroid dysfunction (37,9%).

The analysis was conducted using the package *SPSS 13.0* (SPSS Inc, Chicago, Illinois, USA).

RESULTS

At the witness group, 19.4% (58 cases) of the patients showed atrial fibrillation during monitoring, registering a slight decrease of the cases with atrial fibrillation, until the end of this study, at 18% (54 cases) (tab. I, fig. 1). The main risk factors were: female sex (RR=1.99), heart frequency over 80 beats/min (RR=1.56), the LDL-cholesterol above 130 mg/dl (RR=1.55), the presence of heart disease in the personal record (RR=2.86), HBP (RR=1.50), obesity (RR=1.54) and age over 60 (RR=1.40). The treatment with antiarrythmic drugs, administered prior to the inclusion in the study, represented a protective factor (RR=0.80) (tab. II, fig. 2).

Among the patients with subclinical hyperthyroidism, 8.6% (7 cases) showed atrial fibrillation at the beginning of the monitoring period, all with paroxysmal atrial fibrillation, while at the end of the study we registered a percentage of 9.9% of pa-

tients with atrial fibrillation as related to the entire study group (tab. I, fig. 1). The main risk factors identified were: heart frequency over 80 beats/min (RR=4.38), the presence of HBP in the antecedents (RR=4.0) and of heart disease (RR=3.60), increased values of triglycerides (RR=4.18) and age over 60 (RR=3.23). The treatment with antiarrythmic drugs (RR=0.20) and/or beta blockers (RR=0.99) represents a protective factor against the development of atrial fibrillation (tab. II, fig. 2).

TABLE I

Distribution of patients with atrial fibrillation during the monitoring period for thyroid dysfunction

Rhythm disorders	Witness		HYPER subclinical		HYPER		Hypo subclinical		Hypo	
	n	%	n	%	n	%	n	%	n	%
Atrial fibrillation (AF) - at the beginning of the study										
Total	58	19.4%	7	8.6%	57	34.6%	29	38.6%	38	22.3%
Permanent AF	29	9.7%	-	-	27	16.4%	16	21.3%	16	9.4%
Paroxysmal AF	29	9.7%	7	8.6%	30	18.2%	13	17.3%	22	12.9%
Atrial fibrillation (AF) - at the end of the study										
Total	54	18.0%	8	9.9%	43	26.1%	25	33.3%	32	18.8%
Permanent AF	31	10.3%	1	1.2%	27	16.4%	17	22.7%	18	10.6%
Paroxysmal AF	23	7.7%	7	8.6%	16	9.7%	8	10.7%	14	8.2%
p	**0,551**		**0,945**		**0,184**		**0,494**		**0,347**	

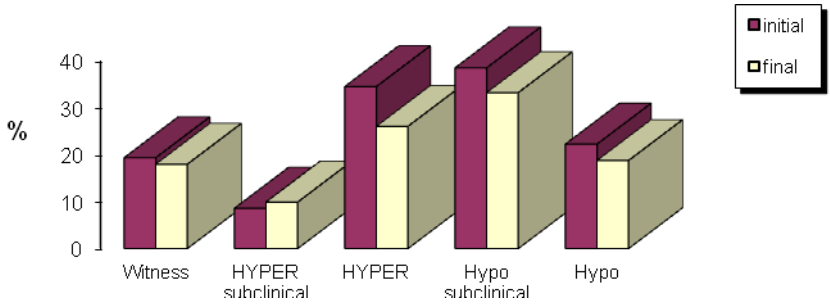

Fig. 1. Distribution of cases with atrial fibrillation depending on the moment of the study

In the group of patients with manifest clinical hyperthyroidism, 34.6 (57 cases) developed atrial fibrillation during monitoring, registering a decrease of the number to 43 cases (26.1%) until the end of the study (tab. I, fig. 1). The main risk factors were: female sex (RR=1.97) and age over 60 (RR=1.33), as well as the presence in the personal records of heart disease (RR=3.31), HBP (RR=1.46) and heart frequency of over 80 beats/min (RR=1.38). The treatment with antiarrythmic, used previously, represented a protective factor for thus study group (RR=0.54) for the development of atrial fibrillation (tab. II, fig. 2).

The main risk factors that led to atrial

fibrillation at the 38.6% (29 cases), with a slight decrease of the number of cases at 25 (33.3%) (tab. I, fig.1), among the patients with subclinical hypothyroidism, were: obesity (RR=2.21), the presence in the personal record of heart disease (RR=2,0), age over 60 (RR=1.90) and female sex (RR=1.30). At the patients who had been administered beta blockers prior to admission (RR=0.99), ACEI (RR=0.85) and/or antiarrythmic drugs (RR=0.54), the medication represented a protective factor against developing atrial fibrillation. (tab. II, fig. 2).

TABLE II

Risk factors in developing atrial fibrillation as compared on study lots

	Witness (n=300)		**Subclinical hyperthyroidism (n=81)**		**Clinical hyperthyroidism (n=165)**		**Subclinical hypothyroidism (n=75)**		**Clinical hypothyroidism (n=170)**	
	RR	**IC95%**	**RR**	**IC95%**	**RR**	**IC95%**	**RR**	**IC95%**	**RR**	**IC95%**
Female sex	1.99	1.2-3.3	1.27	0.2-9	1.97	1.4-2.7	1.30	0.4-4.3	2.73	1.6-4.6
Age≥60	1.40	0.9-2.2	3.23	0.9-12	1.33	0.9-1.9	1.90	1.0-3.5	2.40	1.3-4.5
BMI≥25 kg/m^2	1.54	1.0-2.5	1.15	0.3-5	1.15	0.7-1.8	2.21	1.4-3.5	1.24	0.7-2.3
HBP	1.50	1.0-2.3	4.00	0.5-30	1.46	1.0-2.1	1.01	0.6-1.7	2.00	1.2-3.3
DM	1.11	0.7-1.8	0.80	0.2-6	1.07	0.7-1.6	1.02	0.6-1.9	1.44	08.2.5
Heart disease	2.86	1.9-4.4	3.60	0.6-10	3.31	2.8-4.2	2.00	1.2-3.6	4.64	3.3-6.6
HF≥80beats/min	1.56	1.0-2.4	4.38	1-20	1.38	0.9-2.1	1.07	0.6-1.9	2.39	1.4-4.0
LDLc≥130mg/dl	1.55	1.0-2.4	2.82	0.6-13	1.07	0.7-1.6	1.01	0.6-1.7	2.70	1.6-4.5
Tg≥160 mg/dl	1.05	0.6-1.7	4.18	0.6-31	1.41	1.0-2.1	1.17	0.6-2.2	4.27	2.0-9.0
ACEI	1.03	0.6-1.7	1.16	0.3-5	1.01	0.8-1.5	0.85	0.3-2.2	0.84	0.4-1.8
Beta blocker	1.14	0.7-1.8	0.99	0.7-1.5	1.08	0.7-1.6	0.99	0.5-2.1	0.44	0.2-1.2
Antiarrythmic	0.80	0.6-1.0	0.20	0.4-0.9	0.54	0.3-0.8	0.54	0.3-1.0	0.60	0.4-1.0

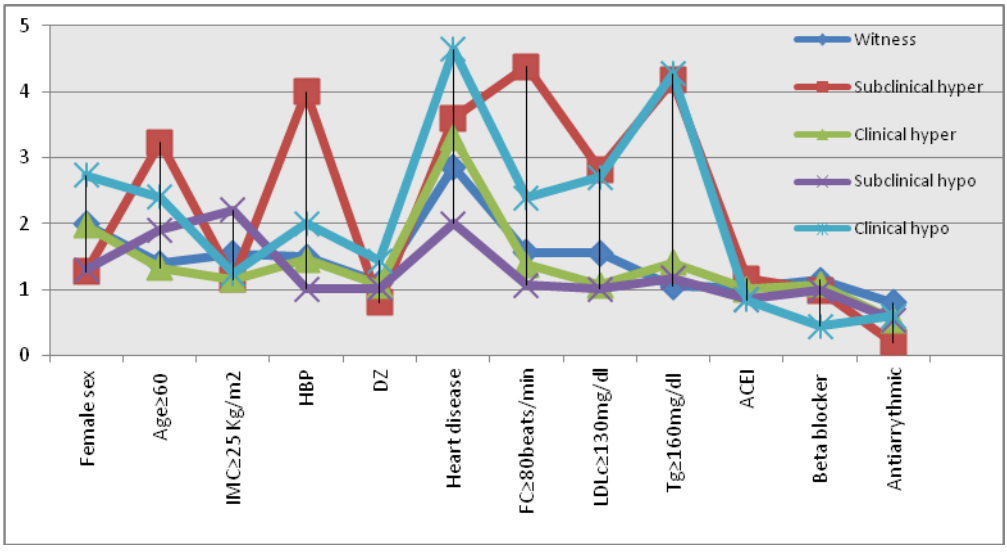

Fig. 2. Relative risk of atrial fibrillation on study lots

Among the patient with manifest clinical hypothyroidism, 22.3% (38 cases) showed atrial fibrillation during monitoring, with a slight decrease of the number to 32 cases (18.8%) (tab. I, fig. 1). The main risk factors identified were: increased levels of triglycerides (RR=4.27) and of LDL-cholesterol (RR=2.70), female sex (RR= 2.73) and age over 60 (RR=2.40), the presence of heart disease in the personal record (RR=4.64), HBP (RR=2.0) and hyperglycemia (RR=1.44), heart frequency of over 80 beats/min (RR=2.39) and obesity (RR= 1.24). ACEI (RR=0.84), antiarrythmic drugs (RR=0.60) and/or beta blockers (RR=0.44), administered prior to the inclusion in the study represent protective factors against developing atrial fibrillation in this study group (tab. II, fig. 2)

At the patients with atrial fibrillation from the lot with subclinical hyperthyroidism, data shows the efficiency of the ACEI treatment and beta blockers.

At the patients with atrial fibrillation from the lot with clinical hyperthyroidism, the most efficient treatment consisted in beta blockers and thyroid treatment (tab III).

TABLE III

Treatment of patients with thyroid dysfunction and atrial fibrillation

Atrial fibrillation (AF)	Witness (n=58)		HYPER subclinical (n=7)		HYPER (n=57)		Hypo Subclinical (n=29)		Hypo (n=38)	
	n	%	n	%	n	%	n	%	n	%
Cardiovascular treatment										
Beta-blocker	34	58,6%	5	71,4%	43	75,4%	14	48,2%	18	47,3%
ACEI	22	37,9%	3	42,8%	18	31,5%	8	27,5%	11	28,95
Calcium channel blockers	13	22,4%	1	14,2%	7	12,2%	11	37,9%	12	31,5%
Diuretic	17	29,3%	1	14,2%	7	12,2%	7	24,1%	9	23,6%
Antiplatelet	51	87,9%	5	71,4%	30	52,6%	26	89,6%	35	92,1%
Anticoagulant	26	44,8%	2	28,5%	21	36,8%	7	24,1%	22	57,8%
Antiarrhythmics	21	36,2%	2	28,5%	20	35%	6	20,6%	9	23,6%
Digoxin	7	12%	1	14,2%	5	8,7%	3	10,3%	7	18,4%
Hipolipemiant	33	56,8%	2	28,5%	21	36,8%	13	44,85	29	76,3%
Thyroidian treatment										
	-	-	4	57,1%	56	98,2%	22	75,8%	38	100%

At the patients from the lot with subclinical hypothyroidism, the treatments used for atrial fibrillation showed the efficiency of thyroid treatment accompanied by hypolipidemic treatment and antiplatelet.

At the patients with atrial fibrillation from the lot with clinical hypothyroidism, the most efficient treatment consisted in thyroid treatment associated with hypolipidemic and antiplatelet (tab III).

DISCUSSION

Atrial fibrillation represents the most common rhythm disorder incurred by the hyperthyroid patient and is responsible for most hospital admissions of the patient with hyperthyroidism. In a study that included a number of 40,628 patients with

hyperthyroidism, the prevalence of atrial fibrillation was of 8.3%, and the most important risk factors for the development of atrial fibrillation were male sex, ischemic heart disease, valvulopathy and congestive heart failure. These data are partially consistent with the results of our study, which showed as an additional risk factor old age and female sex. What is of importance is that subclinical hyperthyroidism is associated with the same risk of developing atrial fibrillation as the manifest clinical hyperthyroidism (4,5). It is also to be noticed that the prevalence of hyperthyroidism at the patients with newly developed atrial fibrillation is of 1%, which is very low (6). Nevertheless, if we take into consideration patients with atrial fibrillation of unknown causes, the prevalence of atrial fibrillation is of 13%. Thus, there raises the question, whether thyroid hormones should be dosed for all patients who show atrial fibrillation with a recent debut. Although the probability that hormonal tests be abnormal at patients with atrial fibrillation of recent debut is small, these tests are nonetheless recommended, due to the great conversion rate at sinus rhythm after the euthyroid status has been restored (7,8). In our study, the prevalence reached 34.6% at patients with hyperthyroidism.

If in the case of hyperthyroidism the most frequent arrhythmias are the atrial ones, especially atrial fibrillation, in the case of hypothyroidism the most frequent arrhythmias are of ventricular origin (8, 9, 10). The results of our study showed an increased risk to develop atrial fibrillation in the lots with manifest clinical hypothyroidism, but especially in the lot with subclinical hypothyroidism, which is inconsistent with the data in the literature, interpreted as being correlated with old age, the presence of heart disease at a large number of patients and the association of other cardiovascular factors.

The treatment of atrial fibrillation observes the three general principles: control of rhythm, control of heart frequency and prevention of thromboembolic complications. The date in our study showed the efficiency of medication, especially beta blockers, at patients with hyperthyroidism but also the efficiency of a specific thyroid treatment for both hyperthyroidism and hypothyroidism, in agreement with the literature data (7). The use of anticoagulant treatment at patients with hyperthyroidism and atrial fibrillation has been amply debated. As in the atrial fibrillation of different origins, the risks and benefits of the anticoagulant treatment need to be balanced, taking into consideration the hemorrhagic and thromboembolic complications (11). There are studies which showed that old age and not atrial fibrillation is the greatest risk factor at these patients to develop a cerebral vascular accident (12). Thus, it is recommended to apply various scores to assess the bleeding risks or thromboembolic complications and the individualized approach of each patient depending on the risk factors and associated co morbidities (11). In addition, the administration of aspirin represents a safer alternative to prevent thromboembolic complications at patients with fewer risk factors. In this light, the results of our study are correlated with what has been previously stated.

The fast diagnosis of hyperthyroidism and hypothyroidism, with the reinstallation of euthyroid status is associated with the recovery of sinus rhythm in most cases within 2-3 months (10, 13, 14). Patients older than 60 and those with atrial fibrillation of a longer history have a lower rate of

spontaneous conversion to sinus rhythm, in correlation with the data obtained in our study, both in the case of patients with hyperthyroidism and hypothyroidism. Thus, it is recommended to institute a therapeutic approach, either pharmacological or electrical, to reestablish sinus rhythm once hyperthyroidism or hypothyroidism has been corrected. It has been noticed that after the euthyroid condition and conversion to sinus rhythm have been restored, these patients remain in sinus rhythm for a longer period of time.

CONCLUSIONS

There is a direct relationship between the degree of thyroid dysfunction and the risk to develop atrial fibrillation. Our results showed that even the subclinical forms of hyperthyroidism or hypothyroidism were associated with an increased risk of atrial fibrillation. These results indicate long term screening for thyroid dysfunctions in case of patients with atrial fibrillation and the early initiation of endocrinelogical treatment.

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PHENOTYPIC VARIABILITY IN PATAU SYNDROME

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PHENOTYPIC VARIABILITY IN PATAU SYNDROME (Abstract): Patau syndrome has an incidence of 1/10.000-20.000, the clinical diagnosis being suggested by the triad cleft lip and palate, microphthalmia / anophthalmia and postaxial polydactyly. Most frequent cytogenetic abnormality is free and homogeneous trisomy 13 (80.0%), rarely being detected trisomy mosaics or Robertsonian translocations. The objective of the study was to identify phenotypic features of trisomy 13. **Material and Methods***:* The retrospective study was conducted on a trial group of 14 cases diagnosed cytogenetically with trisomy 13 between January 2000 and December 2012 at Iasi Medical Genetics Centre. **Results***:* Of the 14 cases, 3 were evaluated pathologically (two aborted foetuses and one stillborn), 8 cases were detected in the neonatal period, and 3 in infancy. Clinical diagnosis was supported by the identification of a model of abnormal development, mainly characterized by: maxillary cleft (lip and palate - 5 cases; lip - 1 case), ocular abnormalities (microphthalmia/anophthalmia - 7 cases; cyclopia - 1 case), postaxial polydactyly (7 cases), scalp defects (6 cases), congenital heart anomalies (10 cases, 6 patients with atrial septal defect), complete holoprosencephaly (4 cases), ear abnormalities (11 cases), broad nasal root (10 cases). An important issue in confirming the phenotypic variability of Patau syndrome is that the classic clinical triad was identified only in one case. **Conclusions***:* Patau syndrome is a disease with variable expression and is characterized by a pattern of abnormal prenatal development characterized by facial dysmorphia, polydactyly and severe birth defects (heart, brain) that generate an increased in utero and perinatal mortality. **Keywords***:* PATAU SYNDROME, TRISOMY 13, CLEFT LIP AND PALATE, MICROPHTHALMIA, POLYDACTYLY, CARDIAC DEFECTS.

Patau syndrome is caused by trisomy 13, the chromosomal etiology being identified by Patau*et al* (1960) (1). Trisomy of chromosome 13 has a different frequency in different stages of ontogenetic development. The chromosome 13 disomy was detected in 0.12% of sperm cells (2). Expression of severity of trisomy 13 is proven by the decrease of its incidence during pregnancy from 5.7% in products of abortion (3), to 0.004% in newborn deaths (4,5) and 0.0002% in living neonates (6).

Patau syndrome is a plurimalformative syndrome whose cardinal signs are cleft lip/palate, microphthalmia/anophthalmia and postaxial polydactyly. Other signs are

scalp defects, holoprosencephaly, congenital heart anomalies (7).

In most cases it is a free homogeneous trisomy, the extra chromosome having in 90% of cases a maternal origin (8). Maternal origin of extra chromosome 13 was detected also in 13 mosaic trisomy (9).

MATERIAL AND METHODS

Our study was a retrospective clinical and cytogenetic one performed on a group of 14 cases of Patau syndrome, cytogenetically confirmed between 2000 and 2012 at Iasi Medical Genetics Centre.

RESULTS

We identified three cases diagnosed postmortem (2 aborted and stillborn) and 11 cases diagnosed in the neonatal period. Most cases were female with sex ratio ♀: ♂ = 10:4.

The clinical elements that supported the clinical diagnosis of Patau syndrome (tab. I, fig. 1) were: abnormal ears (11 cases), cardiac abnormalities (10 cases), flattened nasal root (10 cases), ocular abnormalities - microphthalmia, anophthalmia, cyclopia - (8 cases), polydactyly (7 cases), cleft lip/palate (6 cases), scalp abnormalities (6 cases). Although the clinical diagnosis of Patau syndrome is supported by the triad cleft lip/palate, microphthalmia/anophthalmia and postaxial polydactyly, the combination of the three cardinal signs was identified only in one case, while in two cases we found no cardinal sign. The presence of two major signs was discovered in five cases, and a single sign, usually cleft lip was present in 6 cases.

TABLE I

The clinical features of patients with Patau syndrome

Clinical sign	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14
HPE	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Microphtalmia/ anophtalmia	+	-	-	+	-	+	-	+	-	+	+	-	-	+
Cleft lip/palate	-	+	+	-	-	-	+	-	-	+	+	+	-	-
Postaxial polydactyly	+	-	-	+	+	+	-	-	+	-	+	-	-	+
Cardiac malformations	-	+	+	+	+	+	+	+	-	+	+	+	-	-
Nose anomalies	-	+	-	-	+	+	+	+	-	+	+	+	+	+
Ear anomalies	+	+	-	-	+	+	+	+	-	+	+	+	+	+
Kidney malformations	-	-	-	+	-	-	-	-	-	-	-	+	-	-
Omphalocele	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Cutis aplasia	-	-	-	-	-	+	-	-	+	+	-	+	-	+
Severe deficiency of intellectual development	-	-	-	-	-	+	-	-	+	-	-	+	-	-
Age of diagnosis	nnm	a b	a b	n	n	n	n	n	n	n	n	n	n	n

Ab – abortion; nnm – stilbirth; n –neonatal

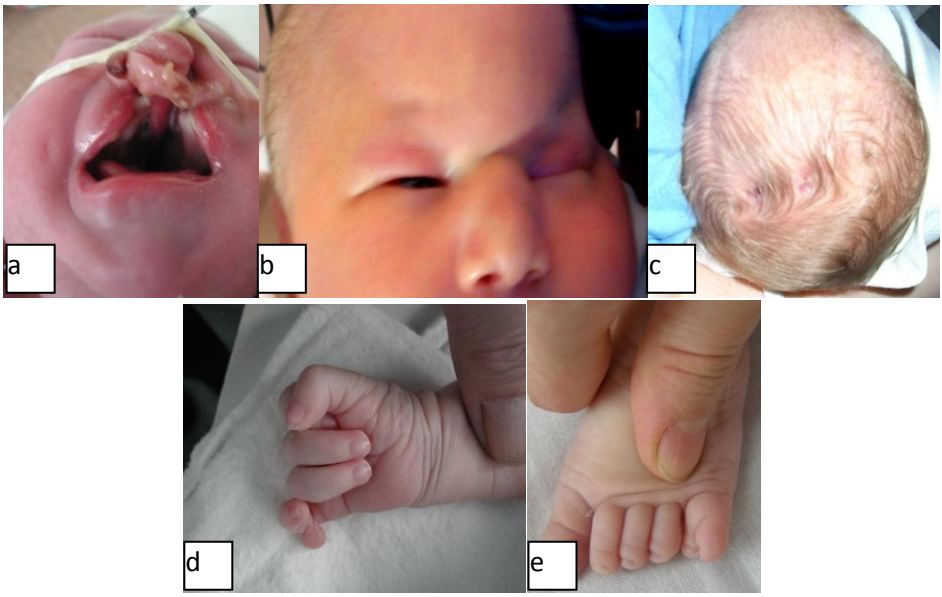

Fig. 1. Clinical signs of Patau syndrome: a. cleft lip and palate; b. anophthalmia and microphtalmia, flattened nasal root; c. scalp defect; d. hand postaxial polydactyly; d. foot postaxial polydactyly

Clinical diagnosis was confirmed by chromosomal analysis: a free homogeneous trisomy 13 - 47,XX(XY),+13 (11/14 cases), a mosaic trisomy 13 - 47,XY,+13/46,XY (1/14), a Robertsonian translocation trisomy 13/14 - 46,XX,rob(13;14)(q10; q10),+13 (1/14) and a homogeneous trisomy 13 associated with a complex abnormality 47,XX,+13/48,XX,del(2)(p11.2→pter),i(2p),+13[29]/[2] (1/14).

DISCUSSION

Patau syndrome is a rare chromosomal disorder that affects approximately 1/10.000 - 1/20.000 births. The disease is severe and in most cases produces a miscarriage or prenatal/perinatal death (7). Clinical diagnosis is based on suggestive signs and symptoms, but the disease has variable expressivity, which can sometimes complicate the diagnostic process. Using these as a starting point, we tried to identify these variable elements based on a relatively large study group - 14 cases.

We found a sex ratio ♀: ♂ = 2.5, aspect which is discordant in relation to most studies of literature. Thus, Alberman et al. (10), analyzing 985 cases of trisomy 13 are found mainly in the case of male patients, while Huether et al. (11) found a sex ratio ♀: ♂ = 0.9 (293 patients) and Bugge et al. (8) found a sex ratio ♀: ♂ = 0.73 (82 cases) (8,10,11). This discrepancy could be related to the small size of the study group, because similar results were cited by Moerman et al. (12) who found a sex ratio ♀: ♂ = 2 on a sample of 12 cases (12).

The most serious malformations of Patau syndrome are the anomalies of the central nervous system. The most severe is holoprosencephaly (HPE) a separation defect of structures from midline of cephalic extremity. It can affect the brain (one cerebral hemisphere, unique cerebral

ventricle, agenesis of the corpus callosum), ocular structures (microphthalmia/ anophthalmia or cyclopia) and frontal bud (proboscis, hypotelorism, velo-palatine median cleft). In minor forms, the only clinical manifestation is single central maxillary incisor (13). HPE is identified in 1/10.000-15.000 live newborns, 1/8.000 foetuses and 1/250 embryos, which indicates that most cases are spontaneously aborted (14, 15). Although aetiology is complex, about two fifths of the cases have a chromosomal aetiology and in 75% of them complained of a trisomy 13 (15-17). In our study, HPE was present in 4/14 cases (28.57%), in two cases being a semilobar form, and in the other two cases was an alobar form (more severe). Our data is consistent with the literature indicating that HPE in Patau syndrome is detected in 17-39% of cases (18, 19). However, pathological examinations of cases of trisomy 13 have shown a frequency of 67% for HPE aspect that could be correlated with lethality of this trisomy (20).

There are three types of HPE: alobar, lobar and semilobar. The most severe form is the alobar form, usually associated with defects of the face (cyclopia/anophthalmia and proboscis), while semilobar forms are accompanied by less severe facial changes (cleft, hypotelorism, flattening of the nose) (21). We also identified such a correlation in the two cases with alobar HPE which presents anophthalmia. However, this correlation is not always relevant, an aspect suggested by the fact that in our only case with cyclopia and proboscis, HPE has been ansemilobar one instead of alobar.

According to Cassidy HPE presence is a negative prognostic factor in trisomy 13 (7). We identified one such feature ourselves, because the four cases of HPE have been identified in the two abortions, the stillbirth and in a patient who died in the neonatal period.

Microphthalmia is a rare congenital anomaly, affecting about 14/100,000 newborns, while anophthalmia is four times more rare (3/100,000 neonates) (22). In about 16% of cases of anophthalmia/microphthalmia is a chromosomal etiology, trisomy 13 being the main abnormality. This type of congenital anomaly is present in 60-70% of cases of Patau syndrome (14, 22). In our study group these abnormalities were detected in 50% of cases, consistent with literature data point, microphthalmia being present in four cases and anophthalmia in three. Another anomaly was iris coloboma, detected in two patients, in one case being associated with microphthalmia.

Polydactyly is a relatively common congenital anomaly being detected in 1:1000 - 1:2000 births. The presence of polydactyly in Patau syndrome is one of the key elements of diagnosis, being found in 60-70% of cases (14). In our study, polydactyly was present in half of patients. In all 7 cases it was hand polydactyly, and 3 of these patients had also foot polydactyly. The bilateral form was more frequent than unilateral one (5 cases versus 2 cases) (23).

Cardiac defects are a characteristic element of trisomy 13 and were present in 80% of patients (7, 14). The most common cardiac abnormalities are atrial septal defect (ASD) and ventricular septal defect (VSD). We have identified cardiac abnormalities in 71.42% of the cases (10/14). Heart defects were isolated or associated. ASD was the most common defect (6/14). Other cardiac abnormalities were: patent ductusarteriosus (2/14), VSD (2/14), dextrocardia (2/14), transposition of great

vessels (1/14), starting point of the aorta in right ventricle (1/14), pulmonary stenosis (1/14), coarctation of the aorta (1/14). In most cases, cardiac abnormalities are not very serious and are not the cause of death. Musewe (24) indicated that only 6% of patients with trisomy 13 had a potentially lethal cardiac anomaly in the first two years of life (24). We identified a similar issue - a potentially lethal anomaly (transposition of great arteries) which is associated with VSD and DSA detected in one of the products of abortion.

Cleft lip and palate and the isolated ones are common abnormalities, detected in 1/700-1/1000 newborns. In most cases (80%) the anomaly is unilateral (usually left). On the other hand, the prevalence of cleft palate is 4/10,000 live births (14). Clefts lip and palate may be associated in 28-37% with other congenital anomalies. Cleft lip and palate is a common malformation in Patau syndrome and is found in 60-70% of cases (25, 26). The frequency of this anomaly in the studied group was 42.85%, cleft lip and palate being found in 6 patients. In four patients the cleft was bilateral and in two cases it was associated with holoprosencephaly.

Scalp defect (aplasia cutis) is a rare congenital anomaly that affects the epidermis, dermis and subcutaneous tissue, located mostly at the vertex. The aetiology may be varied; trisomy 13 being one of the most common chromosomal causes (27). Detection of the anomaly in 6 of our patients is also an argument for this statement.

Nasal abnormalities are common in Patau syndrome, the less severe defect was broad nasal root. In fact in our study this anomaly was discovered in 71.42% of cases (10/14). Serious forms of abnormalities of the nose, such as proboscis or single nostril were rare (3 cases) and were correlated with the presence of holoprosencephaly.

Ear abnormalities are a common condition in Patau syndrome, but in most cases are present only form anomalies of the external ear. We identified such a situation in 78.57% of cases (11/14). Only two patients had a unilateral external auditory canal atresia.

Severe deficiency of intellectual development was identified in all patients after the neonatal period (7). Such a deficit was found in all three infants in our study.

Clinical diagnosis of Patau syndrome should be completed with chromosome analysis, whose results allow calculation of risk of recurrence. Thus, the risk of recurrence is <1% for free trisomies. In the case of inherited Robertsonian translocation the risk is ~ 10% when translocation involved non homologous chromosomes and 100% if the involved two chromosomes 13 (28, 29). For our study, cytogenetic results showed 12 cases of free homogeneous trisomy 13, one case of mosaic trisomy 13 and 1 case of trisomy 13 by de novo Robertsonian translocation, issues consistent with the literature where the frequency of free homogeneous trisomy 13 is over 90% and a frequency of trisomy by Robertsonian translocation is 5-10% (14). Given the absence of inherited chromosomal abnormalities we have estimated that the risk of recurrence is <1% in all 14 cases.

CONCLUSIONS

Patau syndrome is a severe chromosomal condition but with a polymorphic clinical picture, which may cause difficulties in clinical diagnosis. Although, the diagnosis is confirmed by a specific clinical triad - cleft lip and palate, microphthal-

mia/anophthalmia and postaxial polydactyly – we discovered such association in only one case, while two patients had none of these clinical elements. Holoprosencephaly spectrum is the most severe, being associated with increased in utero and perinatal mortality. Knowing phenotypic variability, the detection of atypical cases is important for a correct clinical management.

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NEWS

ANTIBACTERIAL PROPERTIES OF *GARCINIA NOBILIS*

The emergence of multidrug resistance in bacteria has highlighted the need to find new bioactive compounds. A study by Fouotsa *et al.* aimed to search whether Garcinia nobilis, Oricia suaveolens and Balsamocitrus camerunensis contain constituents active against multidrug resistant bacteria. Twenty-two compounds isolated from the three medicinal plants and the crude extracts were tested against *Mycobacterium tuberculosis* and Gram-negative bacteria. MIC (minimal inhibitory concentrations) and MBC (minimal bactericidal concentrations) were determined. *G. nobilis* extract showed better anti-tuberculosis and antibacterial activities than O. suaveolens and B. camerunensis. The crude extract of G. nobilis exhibited inhibitory effects against 12 of the 14 tested bacteria. The results also indicated that some of the compounds isolated, especially 4-prenyl-2-(3,7-dimethyl-2,6-octadienyl)-1,3,5,8-tetrahydroxyxanthone, could represent natural antibacterial agents (Fouotsa H, Mbaveng AT, Mbazoa CD *et al.* Antibacterial constituents of three Cameroonian medicinal plants: Garcinia nobilis, Oricia suaveolens and Balsamocitrus camerunensis. *BMC Complement Altern Med* 2013; 13(1): 81).

Teodora Vremera

OVERWEIGHT AND OBESITY IN SCHOOL AGE CHILDREN IN WESTERN ROMANIA

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OVERWEIGHT AND OBESITY IN SCHOOL AGE CHILDREN IN WESTERN ROMANIA (Abstract): Obesity prevalence in both adults and children has reached epidemic proportions worldwide. Romania seems to follow the same trend, but studies proving this fact are scarce. Our **aim** was to determine the prevalence of overweight and obesity and to find correlations to certain health-risk behaviors in children from western Romania. **Material and methods**: In this cross-sectional study, a total of 3626 children aged 7-18 years were examined by medical students (February 2010- June 2011). Children were classified by their body mass index using International Obesity Task Force (IOTF) reference. The children were asked to complete a short questionnaire inquiring about 5 health-risk behaviors: consumption of fast-food, sweetened beverages, sweets and fruits and about physical exercise. **Results**: The overall prevalence of overweight was 18.2% (16.2% in girls and 20.7% in boys) and 7.2% for obesity (5.8% in girls and 9.0% in boys). The prevalence of both overweight and obesity was higher in boys versus girls and rural versus urban. Overweight and obesity was correlated with the consumption of sweetened beverages and sweets and also with sedentary behavior. **Conclusions**: The prevalence of overweight and obesity children is alarming, particularly in boys, higher in rural versus urban. The findings confirm the urgent need for national preventive strategy for childhood obesity and targeted interventions tailored to local circumstances. **Keywords**: OBESITY, OVERWEIGHT, SCHOOL AGE CHILDREN, ROMANIA, IOTF.

Prevalence of overweight and obesity. Prevalence surveys provide important information about the burden of disease and associated risk factors. This information is critical in order to develop effective management strategies. Obesity prevalence in both adults and children has reached epidemic proportions worldwide (1). Romania seems to follow the same trend, but prevalence studies for childhood obesity in Romania are lacking. A search on PubMed, Cochrane Library and World Health Organization reports, failed to find relevant articles regarding prevalence of overweight and obesity in Romania for school-aged children. Obesity has major implications for economical, social and personal welfare, through its direct consequences and the addition of the enormous risk for developing life-threatening diseases and psychological problems. Although there are powerful genetic factors related to the risk

of developing obesity (2), the causes of this epidemic are mainly two: a) an increase in the availability of energy dense food and drink, which promote excessive caloric consumption and b) the advances in technology that indulge a sedentary lifestyle. These issues need to be addressed particularly in the young generation (3).

Heath-risk behaviors. Childhood obesity is an important risk factor for the presence of adult overweight or obesity and the attendant risks of increased morbidity and mortality (4). The role of lifestyle choices have been highlighted in multiple international studies. Information about the interplay between specific health-risk behaviors and their combined effects on obesity are particularly scarce in relevance to Romanian population. Furthermore, Romania is experiencing a rapid health and lifestyle transition and the patterns of daily activity become more similar to those in Western countries (5).

Our aim was to determine the prevalence of overweight and obese and to examine the relationship between obesity and certain health-risk behaviors, in a sample of children in western Romania.

MATERIAL AND METHODS

Demographics. Timis is the largest county in the west part of Romania. According to the National Institute of Statistics, Timis has an area of 8,697 km^2. The young population (0 to 18 years) in Timis county was 156,678 in the 2002 census of population (results from the 2012 census of population are not available at the time of writing) (6). When the present study started, in 2010, in Timis there were 67,146 children attending school in urban setting and 33326 in rural. We randomly chose 5 urban schools (enrolling 5,676 students) and 10 rural schools (enrolling 2653 students) to collected data for this study. The response rate was 50 % urban and 38 % rural. Some students failed to be measured due to various reasons including school absenteeism and conflicting school activities/responsibilities (certain classes were not interrupted for measurement).

Prevalence of overweight and obesity. A total of 3,626 children, aged 7-18 years were examined by medical students, in the period February 2010-June 2011. 108 children were excluded from the database because they were older than 18 or younger than 7. Prior to each data collection round, all examiners were trained in taking accurate measurements, according to international measurement techniques (7). Each child underwent anthropometric assessments that were carried out during one day session. Height and body mass was obtained using calibrated scales. The subjects wore normal, light, indoor clothing without shoes. All anthropometric measurements were taken in the mornings, before lunch time. Children were asked to go to the toilet just before the measurements. The age of children was rounded down when child's age was years and 1-6 months and rounded up when the age was years and 7-12 months. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Children were considered overweight or obese based on International Obesity Taskforce (IOTF) definition (8). International cut off points for body mass index for overweight and obesity by sex between 2 and 18 years are defined by IOTF to pass through BMI of 25 and 30 kg/m^2 at age 18. Cut off points for body mass index for underweight by sex between 2 and 18 years are defined by IOTF to pass through BMI of 17 kg/m^2 at age 18 (9). Although Cole et al defined 3

grades of underweight in his paper; we will further refer as underweight, children that presented a BMI for sex and age below the cutoff of 17 at age 18.

Heath-risk behaviors.

The children were asked to complete, by themselves, a short questionnaire inquiring about 5 health-risk behaviors': consumption of fast-food and pastry, sweetened beverages, sweets and fruits and also about physical exercise. The rating scale from 1 to 5 is shown in table I. The lowest total score (=5) is related to the best practice while the highest (=25) is related to the worst behavior.

TABLE I

Rating scale for the questionnaire 5 health-risk behaviors

Question	Never	1 time /week	2 times /week	3-4 times /week	Daily
How often do you eat fast food or pastry in a week?	1	2	3	4	5
How often do you drink sweetened beverages in a week?	1	2	3	4	5
How often do you eat sweets in a week?	1	2	3	4	5
How often do you do moderate/vigorous exercise in a week?	5	4	3	2	1
How often do you eat fruits in a week?	5	4	3	2	1

Ethical considerations. Because of the non-invasive nature of the protocol consisting only of measurements that are a routine for school examinations (height, weight), we used a "passive consent" or "opt out" method. Parents were fully informed about all study procedures through a school information meeting. Parents who refused the participation communicated this tot the school principal. Prior to the measurement, the child was also asked whether s/he agrees with it. The children's names were not included in the electronic data files. Examiners ensured the basic principles of confidentiality, privacy and objectivity throughout the process. The children were measured in a private room, boys and girls separately. The approval of the University's Ethical Advisory Commission is in accordance with the Helsinki Declaration.

Statistical analysis. Descriptive statistics were run on all the variables. p values of less than 0.05 were considered statistically significant. The prevalence of overweight and obese was determined using the LMS Growth software (10). Data were analyzed using the *SPSS,* PC program (*SPSS version 19.0* - Inc., USA). Binary logistic regression was used to compute odds ratio (OR) with 95% CI for obesity associated with the different life style behaviors. In additional multivariable analyses, we examined the independent effect of each life style factor by mutual adjustment.

RESULTS

Demographics. After excluding 108

children that were younger than 7 or older than 18, a total of 3626 children, aged 7-18 years were examined. The study population consisted of: 1564 boys (43.1%) and 2062 girls (56.9%), with a male/female ratio of 1:1.31. Regarding residence, 2622 children lived in Timisoara and 1004 children in rural surroundings, with an urban/rural ratio of 2.6:1. Age and anthropometric data of the children are presented in table II.

TABLE II
Demographic and anthropometric variables of children

Parameter	Mean +/- Std. Deviation		Range	
	Girls	Boys	Girls	Boys
Age (years)	12.8 (3.17)	12.1 (2.89)	7-18	7-18
Weight (kg)	47.68 (13.91)	49.26 (17.92)	18-126.5	18.5-123
Height (cm)	153.43 (13.87)	154.55 (17.22)	107-188	108-196
BMI (kg/m^2)	19.87 (3.87)	19.9 (4.2)	11.28-45.36	8.08-40.4

Prevalence of underweight, overweight and obesity. In regards to IOTF reference, the overall prevalence for underweight was 10.34% (12.51% in girls and 7.48% in boys, p=0.05), for overweight the overall prevalence was 18.17% (16.29% in girls and 20.65% in boys p=0.2) and 7.17 % for obesity (5.77% in girls and 9.02% in boys; p=0.01) (fig. 1).

The overall prevalence in urban environment of underweight was 3.6% versus 3% in rural (p=0.3), for overweight was 18.3% versus 17.9% in rural (p=0.1), while for obesity was 6.4% in urban and 9.1% (p=0.05) in rural population (fig. 2).

The highest prevalence of overweight was found at ages 10, 11, and 12, while obesity was more prevalent in early ages 7 and 8, slowly decreasing towards 18 years. In regards to underweight our data suggest a higher prevalence at ages 17 and 18, mostly in adolescent girls (fig. 3).

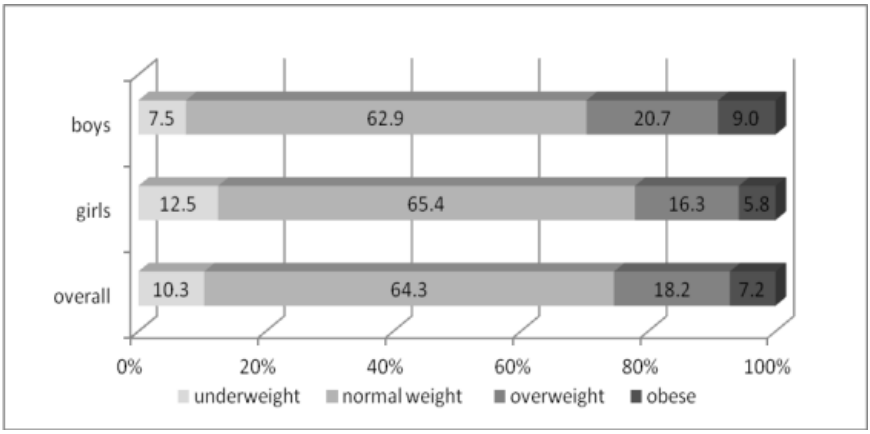

Fig. 1. Prevalence of underweight, overweight and obese in regards to IOTF reference

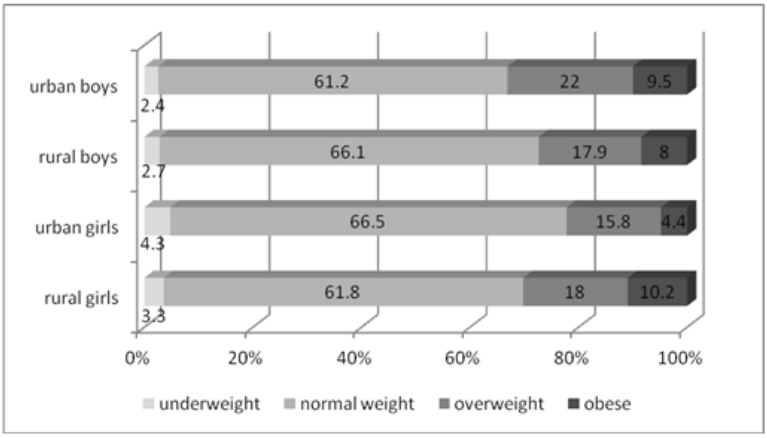

Fig. 2. Prevalence of underweight, overweight and obese in urban versus rural

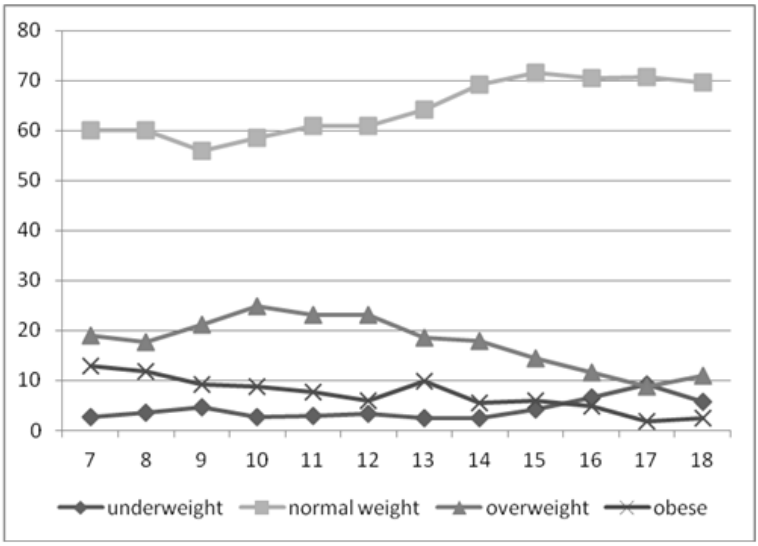

Fig. 3. Prevalence of underweight, overweight and obese in relation to age

The questionnaire was completed in full by 2393 children - 66% of children enrolled in the study, 50.6% were girls, 49.4% were boys. We did not find a strong correlation (Pearson's correlation) between BMI class and total score of the questionnaire (r_s=0.136, p=0.5), but a detailed analysis showed a stronger correlation of the two parameters that increased with age (r_s=0.358, p=0.04 for 16 to 18 years group). In scoring individual variables we observed that the strongest direct correlation was found relating BMI class to the consumption of sweets (r_s=0.536, p=0.0005) and sweetened beverages (r_s=0.243, p=0.01) and with the lowest frequency in performing physical exercise per week (r_s=0.237, p=0.0005) (fig. 4).

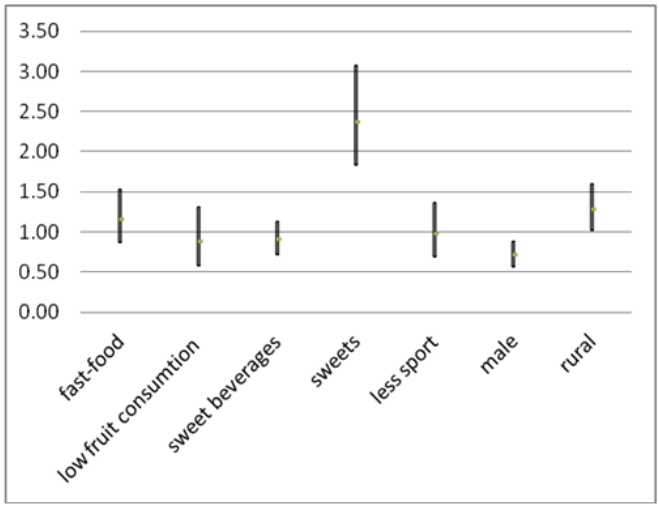

Fig. 4. Odds ratio with 95% CI for obesity associated with the different life style behaviors.

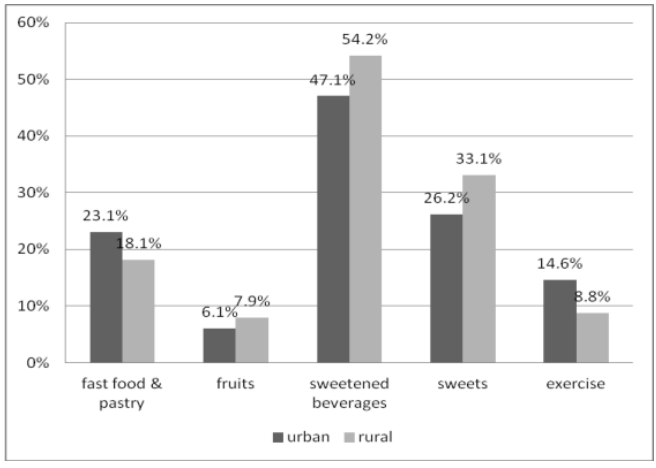

Fig. 5. The highest scores (4 and 5 = worst behavior) in urban versus rural

Further, we looked at the percentage of children who scored highest (5 on our scale) on independent variable in the questionnaire: 24% drink sweetened beverages every day, 15% eat sweets every day, while 7% eat fast food or pastry each day. Considering differences between urban and rural we found that the children who scored highest (4 and 5 = worst behavior) on the questionnaire were more prevalent in rural versus urban environment (fig. 5), but the difference was not statistically significant (p=0.83). Exception was seen in fast food and pastry consumption, which was higher for urban.

DISCUSSION

The data demonstrated a high prevalence of overweight and obesity children.

The prevalence of obesity was higher among boys versus girls and higher in rural versus urban. In Europe, for 2010, the estimates for prevalence of overweight and obesity were 38% and 10 % respectively (11). Table III shows different prevalence reports, from literature, in various countries. Even though we used the IOTF reference for BMI and some of the studies cited used different references (WHO and CDC), an indicative comparison can be made (12). Childhood obesity is becoming a global epidemic and is threatening to have reached epidemic proportions also in Romania. Alarming data generated by this study show proximate results to those from Italy.

TABLE III

Prevalence of overweight and obesity by country

Countries	Overweight %		Obesity %		Author, year, citation Growth standards
	Girls	Boys	Girls	Boys	
West Romania	16.3	20.7	5.8	9.0	Chirita Emandi A; 2012; IOTF
Greece	31.2	26.5	9.4	6.4	Tzotzas T; 2011; (13); IOTF
Canada	26.7	11.7	11.3	10.8	Twells L; 2011; (14); WHO
Italy	19.3	27.4	3.5	4.5	Cacciari E; 2006; (15); CDC
Switzerland	19.1	16.6	3.7	3.8	Zimmermann M.; 2004 ; (16); IOTF
Turkey	15.8	12.8	3.2	3.9	Turkkahraman D.2006 (17); IOTF
Eastern Mediterranean Region	1.9-21.9		7-45%		Mushtaq MU; 2011; (18); IOTF Malik M.; 2007; (19); IOTF

WHO= World Health Organization 2007;
IOTF =International Obesity Task Force 2000; CDC=Centre for Disease Control 2000

The difference in prevalence of obesity between sexes (higher in boys) may be related to maturation, growth patterns, behavioral, environmental and cultural factors (20-22), on which we have limited information relevant for the Romanian population. The increased prevalence of obesity in boys, further ads to the well known elevated short term risk for cardiovascular disease in adult men compared to women (23). The differences in prevalence of both overweight and obesity, when comparing ages of children, may be due to pubertal stage (higher prevalence in pre pubertal). Our data demonstrated higher prevalence between 8-11 years, but Tanner stage was not assessed in our study due to ethical and methodological issues. Underweight was more prevalent in girls versus boys and the prevalence increased at age 17 and 18, underlining possible cultural or peer pressure to be thin.

The higher prevalence of obesity in rural versus urban was surprising. Interestingly, children from rural environment showed that they consume more frequently sweetened beverages and sweets; and they are engaged less frequently in physical exercise. However, the difference was not statistically significant. Several studies looked at factors contributing to higher prevalence of obesity in rural areas. Weight problems in rural communities could be explained by the evolvement of farming methods, by the lack of physical activity due to socio-economic status, lower levels of medical education and interest for health

related topics, limitation of recreational facilities, less commitment time available for parents and children and high cost of activities. On the other hand, the availability of cheap, high dense energy foods could also be a cause (24, 25). Further studies are needed, in order to determine the exact causes of this issue, for Romanian socio-economic and cultural setting.

Previous researches have reported on the multivariate association of overweight with certain modifiable factors (26). We wanted to search for a correlation between BMI and some heath-risk behaviors. No significant correlation was found between obesity and the total score, but the fact that the correlations were more significant with increased age of children might raise a question about the reliability of questionnaires in young children.

Our results showed associations between obesity and consumption of sweetened beverages and sweets, in concordance with literature (27-29). It is alarming, but not surprising that in our study almost 1 in 4 children drink sweetened beverages every day. No associations between fruit consumption and obesity were observed in the current study. Other available data do not support a protective effect of fruit consumption on the risk of childhood obesity. In addition, no association between obesity and fast-food intake was proven, despite the plausible mechanisms involving increased energy uptake. We found association between reduced physical exercise and obesity, further supporting a huge number of reports (30-33). Taken together, these findings suggest that consumption of sweeten beverages, concentrated sweets and fast-food should be discouraged while physical exercise should be promoted in children in order to reduce overweight and obesity prevalence.

The gradual increase in body-weight that leads to obesity is the direct effect of a prolonged increased energy intake, which exceeds energy loss. Multiple factors can influence energy balance and, therefore, be identified as contributors to the current obesity epidemic in children, with biological, behavioral and environmental causes. Obese children are at an increased risk of developing chronic diseases, particularly diabetes mellitus and cardiovascular diseases, which can lead to the poor quality of life, an increased personal and financial burden to individuals, families, and society, and a shortened lifespan (33). Targeted interventions tailored to local circumstances are urgently needed.

It is important to take into account several limitation of the study. The geographical area for the studied population was restricted and results cannot be generalized for the country, but nevertheless, this could be the start for a national study. The age of children was rounded leading to possible minor inaccuracies regarding prevalence estimation. Dietary data in the current study were determined based on the frequency of consumption of food items without any details on the quantity consumed that could have attenuated and potentially masked certain associations.

CONCLUSIONS

The prevalence of overweight and obesity children is alarming, particularly in boys, higher in rural versus urban. Associations between obesity and consumption of sweetened beverages and sweets; and reduced physical exercise were showed. Children from rural environment showed that they consume more frequently sweetened beverages and sweets; and they are engaged less frequently in physical exer-

cise. The findings confirm the urgent need for national preventive strategy for childhood obesity and targeted interventions tailored to local circumstances.

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The authors declare no conflict of interest.

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THE PREVALENCE OF ANAEMIA IN PATIENTS WITH INFLAMMATORY BOWEL DISEASES IN NORTH-EASTERN ROMANIA

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THE PREVALENCE OF ANEMIA IN PATIENTS WITH INFLAMMATORY BOWEL DISEASES IN NORTH-EASTERN ROMANIA (Abstract): Anemia is a frequent complication of inflammatory bowel diseases (IBD). The main incriminated mechanisms are the iron deficiency and the chronic inflammation. **The aim** of this study was to determine the prevalence of anemia in IBD patients and to identify the main causes. **Material and methods:** A prospective study was carried out between April 1st 2011 and April 30th 2012 on a batch of 144 IBD patients diagnosed in the Gastroenterology and Hepatology Center of Iasi. The IBD diagnosis was based on clinical, biological, endoscopic and histological criteria. The anemia diagnosis was based on the World Health Organization criteria. **Results:** Anemia was present in 40.27 % of IBD patients. The main cause identified was mixed type anemia, followed by iron deficiency and inflammation. Iron deficiency anemia was present especially in patients with ulcerative colitis with long evolution, frequent activity spurts and extended forms of diseases; an inflammatory anemia emerged mainly in patients with extended and moderate-severe forms of the diseases. **Conclusions:** Anemia remains a frequently associated pathology to the inflammatory bowel disease that requires specific management. **Keywords:** PREVALENCE, ANEMIA, IRON DEFICIENCY, INFLAMMATORY BOWEL DISEASE.

The inflammatory bowel diseases (IBD), the Crohn disease (CD) and the ulcerative colitis (UC) are polyfactorial inflammatory conditions of the digestive tube with genetic determinism, characterized by periods of activity and remission. The evolution of both UC and CD associate a frequent but underestimated complication: anemia. Diagnosis and adequate treatment of this polyfactorial complication improves the life quality in IBD patients, decreasing hospitalization length and frequency and the need of blood transfusion (1).

Two major causes of IBD anemia have been identified in the literature (1, 2): iron deficiency and chronic inflammation. Despite this, IBD anemia is most of the times polyfactorial, often the result of inflammatory anemia (characteristic to acute spurts) associated to iron deficiency anemia. Other possible mechanisms involved are: the malabsorption of vitamin B_{12} and folic acid

met only in patients with ileal involvement or extended resections at this level, drug-induced anemia (sufasalazine, thiopurine), hemolytic anemia, myelodysplastic syndrome.

Another feature is the preponderance of this condition in young segments of the population, which involves high socio-economic costs for repeated hospitalizations and absenteeism from the work place. These are arguments for the importance of diagnosing and treating anemia in IBD (3).

The aim of this study was to determine the prevalence of anemia in IBD patients hospitalized in a third centre, and to identify the main mechanisms of anemia and the main correlations between the type of anemia and the clinical-evolutional data of the IBD.

MATERIAL AND METHODS

A prospective descriptive study was carried out between the 1st of April 2011 and the 30th of April 2012, on a batch of 144 IBD patients in the Gastroenterology and Hepatology Center of Iasi. The diagnosis of the inflammatory bowel disease was set on clinical, biological, endoscopic and histological criteria.

The demographic parameters (age, gender, type of disease, duration and extension of lesions and activity) were taken into account. In order to establish the disease activity, the CDAI score was used for the Crohn disease, while the UCDAI score was employed for the UC.

The World Health Organization (4) defines anemia as the pathologic condition when Hb decreases to < 12 g/dl in women and to <13 g/dl in men. In the classification of the type of anemia, the following parameters were monitored: the Hb value, erythrocyte indexes, serum iron level, ferritin, C reactive protein (CRP) and the erythrocyte sedimentation rate (ESR).

For the iron deficiency anemia, the inclusion criteria were: low erythrocyte indexes (MCV < 78 μc), Serum Fe < 50 mg/dl, ferritin < 30 ng/dl; for inflammatory anemia: normal erythrocyte indexes (78 μc < MCV < 94 μc), ferritin >100ng/dl, CRP> 0,5mg/dl, ESR>12 mm/1h). For the mixed anemia (inflammatory + iron deficiency) the following diagnosis criteria were considered: low or normal erythrocyte indexes, 30 ng/dl < ferritin < 100 ng/dl, Serum Fe - normal or low, CRP> 0.5 mg/dl, ESR> 12 mm/1h.

The patients showing other possible causes of anemia were excluded: genital-urinary conditions in women, gastric/ hemorrhagic duodenal ulcer, hemorrhagic hemorrhoids, tumors or anemia of other hematological causes.

The data were analyzed using the *SPSS 16.0* programme. The value of p=0.05 was taken as threshold of statistic significance.

In order to test the statistical hypotheses and to compare the groups of continuous variables and, respectively, the groups of categorical variables, the repartition Student (t) and chi-square (χ^2) were used. The univariate dispersion analysis (the ANOVA test) was also used, as well as the Levene test for the analysis of the dispersion homogeneity.

RESULTS

The study included 144 IBD patients divided into two sub-groups: sub-group A: 105 patients with UC and sub-group B: 39 patients with CD. Patients were aged between 18 and 76, the average age being 44.41 years; regarding the repartition per gender and per diagnosis: 61 were women (20 with CD, 41 with UC) and 83 men (19 with CD and 64 with UC).

Subgroup A: The extension of lesions depended on the Montreal classification; thus, of the 105 patients with UC: 21% had proctitis (E1), 50.5% left colitis (E2), and 28.5% pancolitis (E3).

As far as the disease activity at enrolment into the study is concerned: 12 patients with UC (11.4%) were in clinical and biological remission, 22 presented slight activity (21%), 32 in moderate activity (30.5%), and 39 had severe activity (37.1%).

Forty patients with UC (38.09%) met the criteria for anemia: 16 patients had iron deficiency anemia, 11- inflammatory anemia and 13 - mixed anemia (iron deficiency + inflammatory) (fig. 1).

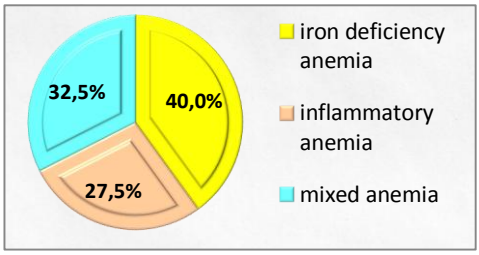

Fig. 1. Types of anemia in UC patients

Regarding the disease activity, inflammatory and mixed anemia prevailed in moderate and severe spurts. In remission, slight spurts – iron deficiency anemia prevailed (fig. 2).

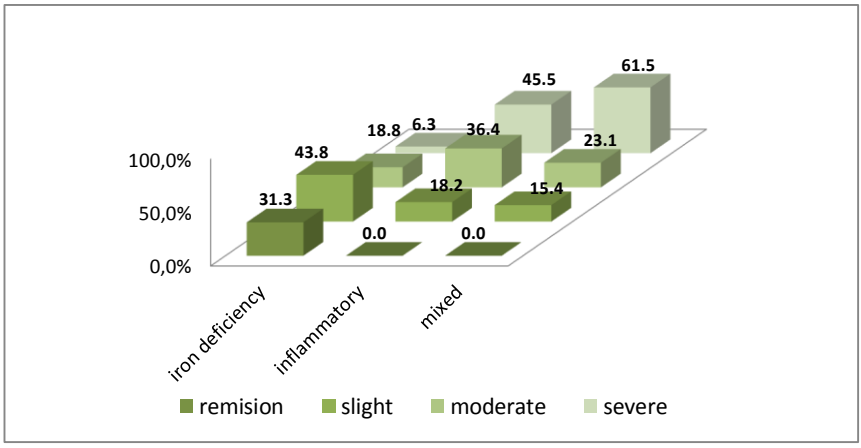

Fig. 2. Anemia type vs. UC activity form

Also, the iron deficiency anemia was found especially in patients with UC in extended forms of the disease (pancolitis, left colitis) (fig. 3) and with long evolution of the disease (5.36 years), whose history shows several activity spurts per year (3.2 spurts/ year), which led to a persistent chronic blood loss.

Subgroup B:

From the 39 patients with CD: 7.7% presented involvement of the terminal ileum (L1), 46.2% involvement of the colon (L2), 38.5% involvement of the ileum and colon (L3), 5.1% presented involvement of the upper digestive tube (L4), and 2.6% involvement of the upper digestive tube and of the terminal ileum (L1+L4).

Considering the disease activity at enrolment into the study: 12 patients (30.8%) were in clinical and biological remission, 10 presented slight activity (25.6%), 13 - moderate activity (33.3%), and 4 patients severe activity (10.3%).

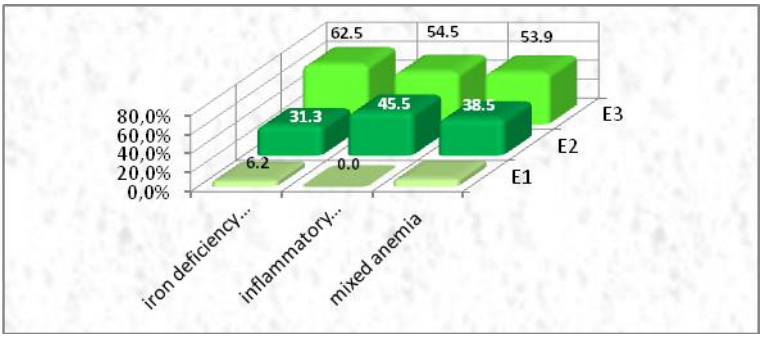

Fig. 3. Anemia type *vs.* disease localization (UC)

Eighteen (46.15%) of the 39 patients with CD had anemia: 4 patients iron deficiency anemia, 2- inflammatory anemia, 10 - mixed anemia, and 2 patients with terminal ileum resection had macrocyte anemia secondary to vitamin B_{12} and folic acid deficiency (fig. 4).

From the point of view of the disease activity, it was also noticed that in moderate and severe spurts, inflammatory and mixed anemia prevailed; in remission, slight-moderate spurts, iron deficiency anemia prevailed (fig. 5).

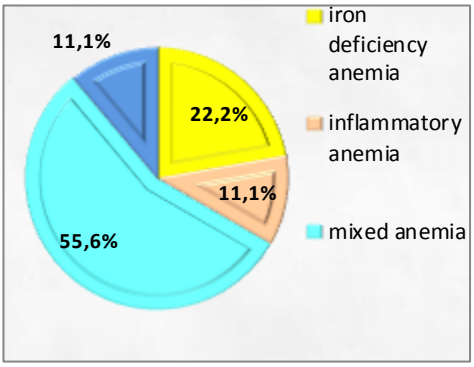

Fig. 4. Anemia types in patients with CD

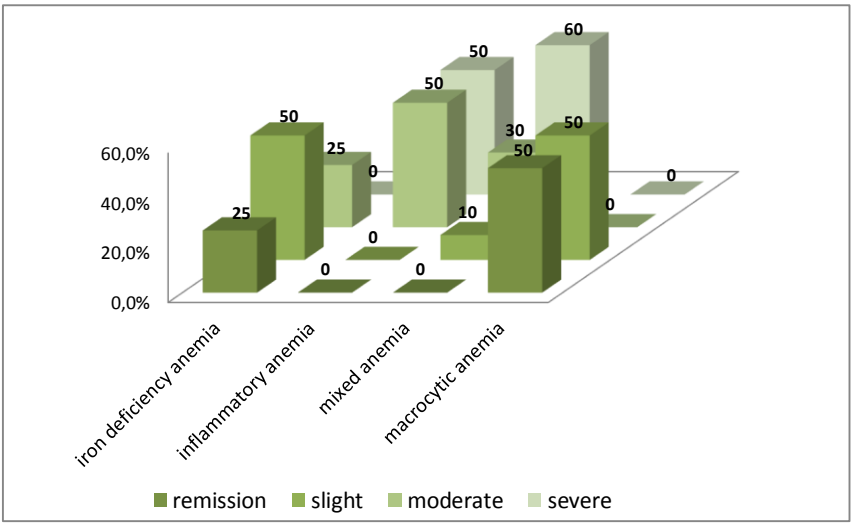

Fig. 5. Anemia type vs activity form of Crohn Disease

The comparison of the anemia cases from the two subgroups reveals the fact that anemia was more frequently found in patients with CD. Iron deficiency anemia prevailed in UC patients, while mixed anemia in patients with CD (fig. 6).

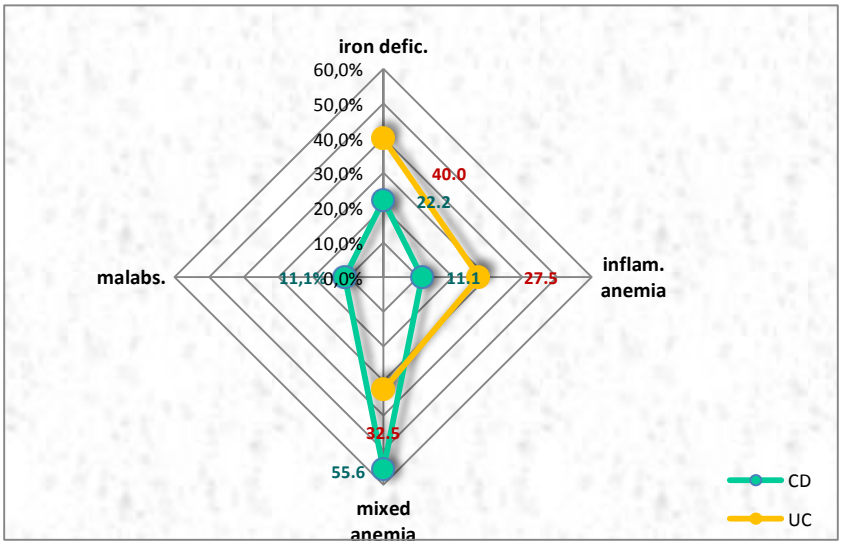

Fig. 6. Anemia type *vs.* diagnosis

TABLE I

Hematological and biochemical determinations in IBD patients with anemia

Marker	Iron deficiency anemia	Inflammatory anemia	Mixed anemia	p
Hb (g/dl)	9.81 ±1.87	11.19±1.16	10.76±2.20	**0.012**
MCV (/fl)	76.01±9.01	83.62±5.06	80.65±8.00	**0.009**
RDW (%)	15.84±2.23	14.22±2.17	14.55±1.99	**0.031**
Ferritin (ng/dl)	7.93±7.19	230.71±145.49	57.90±24.53	0.086
Serum Fe (mg/dl)	25.28±30.14	40.32±24.92	47.96±41.20	**0.001**
CRP (mg/dl)	0.34±0.148	14.39±6.04	8.34±1.28	**0.024**
ESR (mm/1h)	28.67±22.952	51.45±21.205	38.48±28.853	0.164

The analysis of the hematological and biochemical values (table 1) revealed that C reactive protein was significantly high in patients with inflammatory or mixed anemia, while RDW (red blood cell distribution) was significantly high in patients with iron deficiency anemia.

DISCUSSION

There are multiple evidences as regards the increased prevalence of anemia among patients with inflammatory bowel diseases. The iron deficiency is very frequent, in approximately 60-80% of the patients, but only one third of them develop anemia (1).

The three major causes of iron deficiency in IBD are: hemorrhage at bowel level, insufficient duodenal absorption and the diet of IBD patients, which predisposes to insufficient iron input in nutrition (5-7). Anemia

prevalence is highly variable: 6% in patients treated as outpatients and, respectively, 68% in hospitalized patients (8, 9).

Studies from the literature (10-12) establish a prevalence of anemia between 19 and 40% for the hospitalized patients, the frequent causes being iron deficiency, anemia by multiple mechanisms and inflammatory anemia. Some studies assert a higher prevalence of anemia in patients with CD compared to the patients with UC.

In the studied batch, iron deficiency was present especially in patients with ulcerative colitis with long evolution and extended forms of diseases (pancolitis, left colitis), with often activity spurts that led to a persistent chronic blood loss. Inflammatory anemia emerged mainly in patients with extended and moderate-severe forms of the diseases.

A study developed in Italy by Bergamaschi et al. (13) certifies that, at the time of diagnosis of the inflammatory bowel disease, the inflammation represents the main cause of anemia, correlated with increased markers of the inflammation. Iron deficiency anemia occurs after months or years of evolution, correlated with a persistent chronic blood loss at the intestinal level and iron malabsorption.

Another study developed in Great Britain certifies that iron absorption at the level of the digestive tract of patients with IBD in remission resembles the general population, but decreases exponentially in activity spurts (14).

Although in the literature, the first mentioned cause of anemia is iron deficiency, in our study, the main cause was mixed type anemia. At study entry, most patients had moderate and severe spurts of activity, which is explained by the profile of the hospital where the study was performed. The data show a good correlation between the severity of the activity spurts, the extension of the lesions and the presence of the inflammatory anemia and the mixed anemia.

According to the study, anemia was present in more than 40% of the patients, which overlaps with the data in the specialty literature (10-12). No significant differences were noticed in the prevalence of anemia in patients with Crohn's disease compared to UC patients (46.15% *vs.* 38.09%). In the studied batch, 2 cases of macrocyte anemia were noticed in two patients with CD and resection of terminal ileum – probably due to the deficiency of folates and vitamin B_{12} induced by the lack of absorption at the ileum level.

CONCLUSIONS

In Romania, anemia represents a pathology frequently associated to inflammatory bowel diseases and calls for an adequate diagnosis and treatment.

Mixed anemia remains the main cause of anemia among hospitalized patients, followed by inflammatory anemia and iron deficiency anemia. A good correlation is noticed between the severity of the activity spurts, the extension of the lesions and the presence of inflammatory and mixed anemia. Iron deficiency anemia is noticed especially in patients with ulcerative colitis, with extended forms of the disease and often activity spurts.

ACKNOWLEDGEMENTS

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NON-ALCOHOLIC FATTY LIVER DISEASE IS ASSOCIATED WITH CARDIOVASCULAR RISK FACTORS OF METABOLIC SYNDROME

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NON-ALCOHOLIC FATTY LIVER DISEASE IS ASSOCIATED WITH CARDIOVASCULAR RISK FACTORS OF METABOLIC SYNDROME (Abstract): **Aim**: To evaluate the prevalence of steatosis and to assess its correlations with the classical cardiovascular (CV) risk factors, components of metabolic syndrome (MS) in a rural population. **Material and Methods**: A sample of 254 subjects was enrolled in the study. Collected data included: age, gender, complete medical history, anthropometric and blood pressure (BP) measurements. The biological evaluation included metabolic and hepatic parameters. Ultrasound evaluation of steatosis relied on the criteria of the National Health and Nutrition Examination Survey (NHANES) III. **Results**: Two thirds of the study population were obese or overweight (64.96%); 32.66% had systolic BP and 27.16% diastolic BP levels higher than normal. 38% of the subjects had abnormal fasting blood glucose levels, 14.56% having glycated hemoglobin (HbA1c) values corresponding to pre-diabetes, and 9.84% to overt diabetes; 8% had low HDL-cholesterol and 14.96% high triglycerides (Tg) levels. MS was present in 50.8% of individuals. Only 10.8% of all subjects did not have an ultrasound appearance of steatosis; 28.8% had moderate and 32% severe steatosis. There were statistically significant differences in subjects with steatosis vs. subjects without steatosis with regard to body mass index (BMI), WC, presence of MS, and BP and Tg levels, but not to ALAT, ASAT and GGT values. **Conclusions:** The important prevalence of obesity, fasting hyperglycamia, steatosis and MS shows a particularly metabolic fragile population; early diagnosis and interventional strategies are mandatory. **Key words**: METABOLIC SYNDROME, STEATOSIS, CARDIOVASCULAR RISK FACTORS

Metabolic syndrome (MS) is a cluster of interrelated risk factors for CV disease, with a rising prevalence worldwide. People with any three of the following five criteria are considered to have MS: increased WC (with ethnicity specific values); high Tg (≥150 mg/dl); low HDL-cholesterol (<40 mg/dl for males and <50 mg/dl for females); elevated BP (systolic ≥130 mmHg and/or diastolic ≥85mmHg); high fasting glucose level ≥100 mg/dl (1). MS is highly prevalent in the general population (approximately 22%), with race, gender and age-related differences. It carries a high CV morbidity and mortality, particularly in the presence of diabetes, which makes early

and correct assessment mandatory (2).

Non-alcoholic fatty liver disease (NAFLD) covers a histological spectrum of diseases which include simple steatosis and non-alcoholic steatohepatitis (NASH), occurring in the absence of significant alcohol consumption or other specific causes of hepatitis. NAFLD is in fact the most common liver disease, its prevalence being estimated at 20-30% of the general population in Western countries (3, 4).

The relationship of NAFLD with MS features has been confirmed repeatedly. Abdominal obesity, hypertension, dyslipidemia (especially high Tg and low HDL-cholesterol levels), and type 2 diabetes, MS components, are associated with insulin resistance (IR), and frequently, not only that they are also associated with NAFLD, but their coexistence within the same individual increases the likelihood of more advanced forms of liver disease (2, 5, 6). Most patients with NAFLD are hyperinsulinemic and more insulin resistant compared with healthy subjects without steatosis, and there is a near universal association between NAFLD and IR, irrespective of obesity (7). There is an independent association between liver steatosis and carotid atherosclerotic plaques and endothelial dysfunction. This relationship remains statistically significant after adjustment for risk factors of MS (3, 8). NAFLD and atherosclerosis share common molecular mediators, and NAFLD itself might play an early role in the development and progression of atherosclerosis. ALAT significantly predicts the incidence of MS and type 2 diabetes, association that remains after adjustment for a large number of risk factors (including direct measurements of insulin sensitivity and insulin secretion).

All these correlations indicate that NAFLD may be the hepatic manifestation of MS (3). The presence of multiple metabolic disorders such as diabetes mellitus, obesity, dyslipidemia, and hypertension is associated with a potentially progressive, severe liver disease (3, 9).

The aim of this study was to evaluate the prevalence of steatosis in a rural community, and to assess its relationship with the classical cardio-vascular risk factors, components of MS.

MATERIAL AND METHODS

We conducted a cross-sectional study on 254 subjects, 152 women (59.84%) and 102 men (40.15%). The mean age of the study population was 55.04±16.28 years. Subjects were randomly selected from a database of 3248 people who participated in the National Health Program (2007-2008). The site of the study was Deleni, a representative rural community in Iaşi County. Subjects were convened by their general practitioners and provided written informed consent before enrolling in the study. Ethical approval was obtained from the University Ethics Committee.

The collected data included: age, gender, complete medical history, anthropometric measurements (weight, height, WC), assessment of BP, hepatic ultrasound, and biological parameters (fasting glycemia, HbA1c, total-, HDL- and LDL-cholesterol, Tg, ALAT, ASAT, GGT). Food frequency questionnaires, including alcohol consumption, were applied to all subjects (alcohol consumption was considered significant when > 20g/day.)

Body weight and height were recorded with subjects wearing light clothes and no shoes, using standard techniques and equip-

ment. We calculated BMI as weight/height^2 (kg/m^2) and used World Health Organization (WHO) criteria to define BMI categories: underweight (≤18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obesity: grade 1 - 30-34.9 kg/m^2, grade 2 - 35-39.9 kg/m^2, grade 3 ≥40 kg/m^2. WC was measured midway, between the lower rib margin and the anterior superior iliac spine, with the subject standing, at the end of a gentle expiration. Normal WC was defined as ≤80 cm in women and ≤94 cm in men. For the ultrasound evaluation of steatosis we followed the National Health and Nutrition Examination Survey (NHANES) III criteria, recording parenchymal brightness, deep beam attenuation, bright vessel walls and gallbladder walls definition; overall estimation was based on the number of ultrasound findings.

The statistically package SPSS version 16.0 for Windows (Chicago, IL, USA) was used for data analysis. A p value <0.05 was considered statistically significant. The significance of differences between continuous variables was assessed using the Student's t test and One-way ANOVA test and for categorical variables the Pearson Chi-Square test.

RESULTS

Mean BMI was 27.68±5.78 kg/m^2 (tab. I). We found that 1.96% of the study population was underweight, 33.07% normal weight, 35.83% overweight, and 29.13% obese. Two thirds of this population were obese or overweight (64.96%). As to obesity grade: 21.25% were grade 1, 5.90% - grade 2, and 1.96% - grade 3. Analyzing the WC values, it resulted that only 20.22% of men and 9.69% of women had normal WC, also meaning that the prevalence of abnormal WC values was higher in women than in men (90.27% *vs.* 79.77%, p=0.02).

TABLE I

Characteristics of the study population:

	Age (years)	Weight (kg)	Height (m)	BMI (kg/m^2)	WC men (cm)	WC women (cm)
N	254	254	254	254	89	165
Mean	55.04	72.89	1.625	27.6808	104.118	94.3636
Std. Error of Mean	1.022	0.975	0.0070	0.36279	16.6014	9.7085
Median	56.00	71.00	1.620	26.9459	103.5	95
Std. Deviation	16.288	15.540	0.1114	5.78203	16.6954	9.7380
Variance	265.291	241.503	0.012	33.432	278.738	94.8303
Minimum	21	23	0.6	16.1354	66	66
Maximum	91	126	1.9	63.8889	141	122

As to the presence of the other CV risk factors, 5.90% of the subjects already had diabetes at the time of enrolling in the study; 30.31% of subjects were known with hypertension (treated or not), with a higher prevalence in men (34.83% *vs.* 27.27% in women). Analyzing the distribution of arterial BP levels, we found that 32.66% of the subjects had systolic arterial BP and 27.16% diastolic arterial BP higher than

normal (with the preponderance of values corresponding to grade 1 hypertension – 18.11% for systolic and 19.29% for diastolic blood pressure).

With regard to the alcohol consumption, 12.5% were heavy drinkers, while 45.06% never (or almost never) drank alcohol.

The analysis of the results of biologic parameters assessment revealed that only 62% of the study population had normal fasting blood glucose levels, while the remaining 38% had levels corresponding to impaired fasting blood glucose or even diabetes. Results of HbA1c determination showed that 75.59% had normal values, 14.56% were in the range of values corresponding to pre-diabetes, and 9.84% to overt diabetes. Analyzing the lipid profile, we found that 8% of the study population presented low HDL-cholesterol, 62% high LDL-cholesterol, and 14.96% higher than normal Tg levels. As to liver tests, we noticed that 95% of the study group had normal ASAT values, while the proportions was a little lower for ALAT – 91%, and even lower for GGT – 82%. When values were abnormal, the changes were moderate, the mean values for all three markers being in the range of normal values (22.72± 14.33mg/dl for ASAT, 25.57±14.96mg/dl for ALAT and 31.66± 32.55mg/dl) (tab. II).

TABLE II

Biochemical parameters of the study population:

	Glycemia (mg/dl)	HbA1c (%)	Total cholesterol (mg/dl)	Tg (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	ALAT (u/l)	ASAT (u/l)	GGT (u/l)
Mean	112.04	5.891	179.90	109.57	46.19	111.983	20.72	25.57	31.66
Std. Error of Mean	2.030	.0565	2.407	4.659	.465	2.2817	.901	.941	2.047
Median	105.00	5.700	176.00	108	45.00	112.000	18.00	23.00	21.00
Std. Deviation	32.288	.8994	38.279	69.422	7.390	36.2204	14.334	14.968	32.559
Variance	1042.506	.809	1465.259	2403	54.607	1311.915	205.466	224.048	1060.073
Range	314	7.7	217	471	48	276.4	126	143	236
Minimum	57	4.9	93	39	24	42.8	2	3	5
Maximum	371	12.6	310	510	72	233.6	128	146	241

Using the new criteria for MS, the prevalence in the study population was very high (50.8%).

We found a significant correlation between ALAT and BMI, HDL-cholesterol, Tg, fasting blood glucose, and HbA1c, and also between WC and HDL levels. The correlation was very strong for ALAT with WC and for Tg with WC, fasting blood glucose and HbA1c.

Of the initial 254 patients, 222 underwent hepatic ultrasound for assessing the presence of steatosis. The results revealed that only 10.8% of all patients did not have an ultrasound appearance of steatosis; 28.4% had intermediate, 28.8% moderate and 32% severe steatosis. No gender or age-related differences were found.

When reported to BMI, the prevalence of steatosis was 75% in BMI normal sub-

jects, 94.3% in overweight, and 97% in obese persons, with similar proportion if WC was used as criterion (statistically significant differences, p<0.001 for BMI and p=0.032 for WC). In the non-hypertensive population 85.2% were found to have steatosis *vs.* 93.5% in the hypertensive group (p=0.048). In the presence of MS, the prevalence of steatosis was 92.4%, against 85.6% in those without MS (p>0.05). When we analyzed the data on the severity of steatosis – no/intermediate *vs.* moderate/severe steatosis, the prevalence of moderate or severe steatosis in normal weight subjects was 41.2%, increasing to 84.8% in obese and 67.8% if the MS was present (versus 54.8% in persons without MS). These are statistically significant differences - p<0.001 for BMI and WC categories and p=0.023 for MS (tab. III). The differences are not significant for the previous diagnosis of hypertension, but systolic and diastolic BP were statistically significant higher in the presence of steatosis (regardless of its severity) - p=0.003, p=0.006 respectively.

TABLE III

Prevalence of steatosis in the study population:

Steatosis															
		No/I (%)	M/S (%)			No/I (%)	M/S (%)			No/I (%)	M/S (%)			No/I (%)	M/S (%)
BMI	N	7	41.2	WC	N	79.5	35.9	HT	-	85.2	54.8	MS	-	85.6	52.9
	OW	94.3	58		AO	91.3	66.1		+	93.5	67.3		+	92.4	67.7
	Ob	97	84,8												
p		<0.001	<0.001			0.032	<0.001			0.048	0.056			0.104	0.023

I – intermediate steatosis; M – moderate steatosis; S – severe steatosis; No – normal;
OW – overweight; Ob – obese; AO – abdominal obesity; HT – hypertension (anterior diagnosis).

Analyzing the relationship between steatosis (ultrasound assessment) and biological (metabolic and hepatic) markers, we observed that ALAT, ASAT, GGT and HbA1c values did not differ in the presence of steatosis. On the other hand, BMI, WC and Tg values were significantly higher in the presence of steatosis, regardless of its severity (p<0.001). As to the severity of steatosis, age was significantly higher in the presence of moderate or severe steatosis (p<0.001).

DISCUSSION

Despite limited accuracy of NAFLD diagnosis (liver biopsy is still the gold standard), many studies suggest that 20-30% of individuals in Western countries have NAFLD. The prevalence increases from 16.5% in lean persons to 75% in obese persons (3). A study in Japanese population showed that the prevalence of NAFLD increased from 43% in individuals with impaired fasting glucose to 62% in individuals with type 2 diabetes mellitus (3).

NAFLD may represent the majority of unexplained cases of aminotrasferase elevation (10). A recent survey indicates that NAFLD may account for approximately 80% of the cases with elevated liver enzyme levels in the American population, and that 1 in 4 American adults actually

have NAFLD. Similar data have been obtained in the Japanese and Italian populations (3, 10). On the other hand, liver enzyme levels do not reliably correlate with liver histology, the full range of disease may be seen in patients who have normal transaminase levels (normal values being present in up to 78% of patients at any time) (11). In our study, we found no association between the values of liver tests and the prevalence of ultrasound steatosis. Abnormal liver enzyme levels were seen in 5% of the population for ASAT, 9% for ALAT and 12% for GGT; surprisingly, steatosis was found in 89.2% of the subjects, with 50.8% having moderate or severe forms. This observation demanded further investigation of two possibilities – either there was an error in data collection or analysing, or indeed this population had an extremely high prevalence of fatty liver. We checked for possible sources of error without finding any - for example, the subjects with known liver diseases were not enrolled in the study, and all ultrasounds were performed by the same experienced specialist. Hence, we concluded that we were faced with a population with a worrying prevalence of steatosis.

There are different suggestions regarding the level of alcohol consumption that can reliably distinguish between alcoholic fatty liver and NAFLD. Many centers accept a maximum allowable level of alcohol intake for definition of NAFLD of 140 g/week in men and 70 g/week in women (7). In our study significant alcohol consumption was recorded in 12.5% of study population, which cannot be a valid explanation for such a high prevalence of steatosis. Another possible etiologic factor is dyslipidemia and, indeed, we observed that 62% of our study population had high LDL-cholesterol level and 14.96% had hypertriglyceridemia; furthermore, subjects with steatosis had statistically significant higher Tg levels than subjects without steatosis. In addition, 14.56% of subjects had HbA1c values in the range corresponding to pre-diabetes, and 9.84% corresponding to overt diabetes, prevalence higher than any prevalence reported in the literature and than the prevalence of diabetes previously diagnosed in this population – 5.9%. All these findings suggest that the etiology of steatosis in this population is more likely metabolic.

It has been suggested that 90% of individuals with NAFLD have at least one risk factor for MS, and 33% have all the features of MS. MS is not an absolute risk indicator, because it does not include many of the factors of absolute CV risk - for example, age, sex, cigarette smoking, and LDL-cholesterol levels. Even in these conditions, it was found that patients presenting MS have twice the risk of developing CVD over the next 5 to 10 years compared to individuals without MS, and a 5-fold increase in the risk for type 2 diabetes (1).

The biological mechanisms by which NAFLD contributes to higher risk for CV disease, independent of other prognostic factors, are not fully understood. It is possible that NAFLD, in its more advanced forms, acts as a stimulus for further increased whole-body IR and dyslipidemia, leading to accelerated atherosclerosis (12). This hypothesis is also partly validated by few prospective studies demonstrating that raised liver enzymes independently predict the development of MS, and that ALAT values and ASAT/ALAT ratio might predict the onset of fasting hyperglycemia, independent of obesity (5). The presence of

multiple metabolic disorders such as diabetes, obesity, dyslipidemia, and hypertension is associated with a potentially progressive, severe liver disease (9). Hsiao et al. demonstrated that the presence of severe fatty liver correlated significantly with the prevalence and degree of hypertension, abnormal glucose and Tg metabolism (13). In our study, the percentage of subjects with steatosis, regardless of its severity, was not significantly higher in hypertensive patients, but the values of measured BP, both systolic and diastolic were significantly higher in people with steatosis.

Another surprising result regards the prevalence of the MS in our population – 50.8%, approximately twice the "admitted" prevalence of MS in general population. This observation becomes less surprising when almost two thirds of the population is overweight or obese, as shown not only by our data (64.96%), but also by a previous study, which described the preponderance of overweight and "moderate" obesity (14). The presence of MS strongly correlates with the presence of steatosis; NAFLD and MS share multiple features and generate an important CV risk, which leads to the logic deduction that they are the expression of the same dysfunction – identified as IR. As MS includes a large spectrum of metabolic disorders, it is reasonable to include NAFLD under this big "umbrella". Considering the strong clinical impact of these disorders, an early diagnosis is mandatory; the detection of steatosis should stimulate for the screening of the other MS components and *vice versa*. NAFLD should be considered part of a multi-organ system derangement in insulin sensitivity, which would explain why it is so closely linked with diabetes and MS and is an important risk factor for coronary heart disease (8).

CONCLUSIONS

In our study population we found an impressive prevalence of obesity, diabetes and pre-diabetes, and MS. In addition, 89.2% of the study population presented steatosis, 50.8% with moderate or severe steatosis. BMI, WC, BP and Tg levels were significantly higher in the presence of steatosis, regardless of its severity ($p<0.01$); between the prevalence of steatosis and the presence of MS there was a significant relationship ($p<0.05$).

Due to this cluster of metabolic and CV risk factors, we can assert that we are faced with a particularly metabolic fragile population, and that immediate dedicated interventional strategies are highly necessary for this population.

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NEWS

PARTICULAR BRAIN CENTERS ACTIVATED DURING HUMAN CONSCIOUS STATE

A study by Dresler and co. investigated which centers of the brain become active when we are aware of ourselves, the so-called state of "metaconsciousness" during lucid dreaming. The purpose of the study was to evaluate the neural correlates of lucid dreaming, using parallel EEG/fMRI recordings of night sleep in four experienced lucid dreamers. The study showed that during lucid dreaming the bilateral precuneus, cuneus, parietal lobules, and prefrontal and occipito-temporal cortices were strongly activated as compared with non-lucid REM sleep. In conclusion, lucid dreaming was associated with a reactivation of areas which are normally deactivated during REM sleep. This pattern of activity can explain the recovery of reflective cognitive capabilities that are the hallmark of lucid dreaming (Dresler M; Wehrle R; Spoormaker V et al. Neural correlates of dream lucidity obtained from contrasting lucid versus non-lucid REM sleep: a combined EEG/fMRI case study. *SLEEP* 2012; 35(7):1017–1020).

Teodora Vremera

STUDIU PROSPECTIV ASUPRA EFICIENȚEI ŞI SIGURANȚEI TRATAMENTULUI CU ADALIMUMAB ÎN POLIARTRITA REUMATOIDĂ STADIUL AVANSAT, ACTIVĂ

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PROSPECTIVE STUDY OF THE EFFICIENCY AND SAFETY OF ADALIMUMAB IN TREATMENT OF ACTIVE ESTABLISHED RHEUMATOID ARTHRITIS (Abstract): Rheumatoid arthritis (RA) is a systemic autoimmune disease characterised by synovial pannus formation leading to cartilage destruction, bone erosion, and disability. **Aim:** To evaluate the efficiency and safety of adalimumab (ADA) in association with one or more classic remissive drugs in active established RA. **Material and methods**: Prospective observational 12 months study in 33 consecutive active RA treated with ADA. Patients were assessed according to a complex protocol including both individual parameters (clinical, biological) and composite disease activity scores (DAS28, CDAI, SDAI), while response to therapy was evaluated based on EULAR and ACR response criteria. **Results**: Statistical significant improvement has been demonstrated in all patients under ADA ($p<0.05$); after 3 months of treatment 51.5% RA fulfilled ACR20, 39.3% ACR50 and 9% ACR70 criteria; after 6 months of treatment 66.6% of patients and 33.3% fulfilled ACR20 and, respectively, ACR50 criteria. Moreover, 30.3% of all patients had good response after 6 months, 66.6% moderate response and only 3.03% displayed no response in the same period. 24.2% and 45.4% of RA achieved remission (DAS28$\leq$2.6) after 9 and 12 months of ADA, while 39.3% and 21.2% had moderate activity. Rate and type of adverse events demonstrated the safety and good tolerance of ADA. **Conclusions**: Our data support the efficacy and safety of ADA in active established RA in the settings of the real-life clinical practice. **Key words**: RHEUMATOID ARTHRITIS, ADALIMUMAB, REMISSION, LOW DISEASE ACTIVITY

Poliartrita reumatoidă (PR) este o boală imuno-inflamatorie cronică, progresivă, caracterizată prin sinovită şi afectare multisistemică, cu evoluție spre distrucție articulară ireversibilă, cu dizabilitate funcțională majoră şi risc de mortalitate precoce, îndeosebi prin afectare cardio-vasculară.

Tratamentul PR a înregistrat schimbări majore ca rezultat al unor clarificări privind mecanismele patogenice şi țintele terapeutice. Astfel, este acceptat faptul că *tumor necrosis factor alpha* (TNF-α) joacă un rol major în evenimentele patogenice esențiale ale bolii, respectiv medierea sinovitei, degradarea matricei cartilaginoase şi eroziunile osoase; inhibarea efectelor sale prin

intermediul adalimumab-ului (ADA), un anticorp monoclonal uman recombinat tip IgG1, constituie una din opțiunile terapeutice moderne în PR (1,2,3). Studiile referitoare la eficiența și siguranța tratamentului cu ADA în PR au evidențiat că, ADA reduce simptomele și semnele bolii, îmbunătățește semnificativ funcția fizică și calitatea vieții, reduce și chiar inhibă distrucția articulară (4,5). Datele obținute, din medicina bazată pe dovezi, sugerează că administrarea pe termen lung a ADA este asociată cu eficiență susținută și este, în general, sigură și bine tolerată (6,7,8).

Studiul nostru a avut ca obiective evaluarea eficienței și siguranței tratamentului cu ADA în combinație cu cel puțin un remisiv clasic, în formele active de PR aflate în stadiu avansat.

MATERIAL ŞI METODE

Am efectuat un studiu prospectiv, în intervalul 2009-2010, incluzând 33 dintr-un lot de 38 pacienți cu PR activă, fără răspuns la remisivele clasice, cu indicație de terapie biologică anti-TNFα (ADA). Cazurile au fost selectate utilizând criteriile de includere stabilite în recomandările Comisiei Naționale de Reumatologie (9): diagnostic cert de PR (criteriile Colegiului American de Reumatologie 1987, revizuite); boală severă, în ciuda tratamentului administrat, cu minim 5 articulații cu sinovită activă și cel puțin două din următoarele: redoare matinală (RM)≥60 minute, viteza de sedimentare a hematiilor (VS.H) ≥ 28 mm/1h, proteina C reactivă (CRP) ≥ 20 mg/l; lipsă de răspuns după administrarea a cel puțin două remisive clasice pe un interval de minim 12 săptămâni fiecare.

Informațiile au fost obținute utilizând o fișă de evaluare care a inclus: (i) aspecte demografice, (ii) parametri clinici individuali: număr de articulații dureroase (NAD) și tumefiate (NAT), redoarea matinală, evaluarea globală a bolii de către pacient (EGP) și medic (EGM), evaluarea durerii de către pacient (EDP), (iii) parametri biologici inflamatori și imunologici (iv) parametri funcționali (*Health Assessment Questionnaire Disability Index*, HAQ-DI), precum și (v) scoruri de evaluare a activității PR: *Disease Activity Score* (DAS28), *Clinical Disease Activity Index* (CDAI) și *Simplified Disease Activity Index* (SDAI).

Răspunsul terapeutic a fost evaluat conform criteriilor EULAR și ACR, definite prin: (i) EULAR: *bun* dacă se obține îmbunătățire a DAS28 cu peste 1,2 puncte și un DAS28 final < 3,2; *moderat* pentru o îmbunătățire a DAS28 între 0,6 și 1,2 puncte și scor DAS28 în intervalul 3,2-5,1; *absent* pentru o îmbunătățire DAS28 ≤ 0.6 sau un scor DAS28 final > 5,1; (ii) ACR20, ACR50, ACR70: îmbunătățire de 20%, 50%, 70% în NAD, NAT și în cel puțin trei din următoarele: RM, EDP, EGP, EGM, HAQ-DI și VSH și/sau CRP) (10,11). Protocolul a cuprins, de asemenea, efectuarea a 5 vizite de studiu la intervale de 3 luni, cu evaluarea medicației concomitente și a efectelor adverse legate, în special, de ADA. Cercetările noastre s-au realizat în Spitalul Clinic de Recuperare Iași, cu acordul Comitetului de Etică, iar pacienții au semnat formularul de consimțământ informat înaintea începerii studiului.

REZULTATE

1. Caracteristicele lotului de pacienți. Majoritatea pacienților incluși au fost de sex feminin (87,9%), cu vârsta medie 53,64±11,9 ani și durata medie a bolii de 10,2±5,8 ani. Inițial, toți pacienții au avut o formă de boală activă (media DAS28-VSH 7,46±0,44), cu impact funcțional moderat (media HAQ-DI 2,03±0,33) (tab.I).

TABELUL I

Caracteristicile demografice şi cele legate de boală ale lotului de pacienți

vârsta (ani)	53.64±11.93 (33-71)
sex	
feminin	29 (87.9%)
masculin	4 (12.1%)
durata bolii	
≤ 5 ani	15 (45.5%)
5-10 ani	8 (24.2%)
≥ 10 ani	10 (30.3%)
stadiul bolii	
I/II	9 (27.3%)
III/IV	24 (72.7%)
remisive clasice administrate anterior	
≤ 2	6 (18.2%)
2-4	20 (60.6%)
≥ 4	7 (21.2%)
remisive clasice actuale	
MTX	5 (15.2%)
LEF	8 (24.2)
HCQ	2 (6.1%)
SSZ	2 (6.1%)
terapie combinată	16 (48.5%)

indicatori clinici	
NAD (28)	18.15±3.01 (12-23)
NAT (28)	11.67±2.52 (7-18)
RM (min)	97.58 (60-240)
EDP (0-100)	88.03±6.72 (70-100)
EGP (0-100)	88.64±5.62 (80-100)
EGM (0-100)	85.45±6.29 (70-95)
indicatori ai activității bolii	
DAS28-VSH	7.46±0.44 (6.65-8.38)
CDAI	47.19±5.59 (36.0-56.5)
SDAI	50.86±5.87 (38.2-61.1)
indicator al afectării funcționale	
HAQ (0-3)	2.03±0.33 (1.40-2.60)
markeri biologici	
VSH	68.58±29.28 (34-125)
CRP (mg/l)	36.71±19.15 (19.4-111)
markeri imunologici	
FR total (u/ml)	232.57 (5-512)
Ac anti-CCP u/ml)	114.36 (3-526)

Legendă: MTX (metotrexat); LEF (leflunomid); HCQ (hidroxicloroquine); SSZ (salazopirină); NAD (număr articulații dureroase); NAT (număr articulații tumefiate); RM (redoare matinală); EDP (evaluarea durerii pacient); EGP (evaluarea globală pacient); EGM (evaluarea globală medic); DAS28 (*Disease Activity Score 28*); CDAI (*Clinical Disease Activity Index*); SDAI (*Simplified Disease Activity Index*); HAQ (*Health Assessment Questionnaire Disability Index*); *VS*.H (viteza de sedimentare a hematiilor); CRP (proteina C reactivă); FR (factor reumatoid); Ac anti-CCP (anticorpi antipeptide ciclice citrulinate).

2. Evoluția parametrilor clinico-biologici. După trei luni, media NAD şi NAT a scăzut de la 18,15±3,01, respectiv 11,66±2,52, la 7,81±1,44, respectiv 3,84± 1,0 (p<0,001), cu rezultate similare la şase (4,51±1,67; 1,30±0,88), nouă (3,03±1,35; 0,54±0,66) şi 12 luni de tratament (2,15± 1,25; 0,30±0,63). Scădere semnificativă (p<0,001), rapidă în primele şase şi lentă în ultimele şase luni, s-a înregistrat şi pentru ceilalți parametrii individuali (fig. 1a). S-a observat o scădere precoce, semnificativă, a reactanților de fază acută, obținându-se valori apropiate de intervalul de normalitate după primele trei luni, şi anume: media VSH a scăzut de la 68,57±29,2 la 20,81± 5,19, iar media CRP a scăzut de la 36,26± 19,51 la 17,15±6,58 .

3. Evoluția indicilor de activitate şi dizabilitate a bolii. După trei luni, media DAS28-VSH a scăzut de la 7,46±0,44 la 4,70±0,30 (p<0,001), scădere ce s-a menținut şi după şase (3,61±0,40), nouă (3,11±0,51) şi 12 luni (2,80±0,55). Scădere semnificativă (p<0,001) s-a înregistrat şi pentru alți indici de activitate a bolii (CDAI, SDAI) (fig. 1b). După trei luni, media scorului HAQ-DI a scăzut cu 0,6 unități, de la 2,03±0,33 la 1,45±0,34, scădere observată şi după şase (1,13±0,37), nouă (0,95±0,42) şi 12 luni (0,83±0,46).

4. Evaluarea răspunsului terapeutic. După trei luni, 90,9% pacienți au prezentat un răspuns moderat; 3,03% unul bun, în timp ce 6% nu au înregistrat rezultate terapeutice; după şase luni, 30,3% au prezentat răspuns bun, 66,6% unul moderat şi 3% nu au avut modificări terapeutice. După nouă, respectiv 12 luni, 24,2%, respectiv 45,4% cazuri, erau în remisiune (DAS28≤2,6), iar 36,3%, respectiv 30,3%, prezentau activitate joasă (DAS28≤3,2). După trei luni, 51,5% au îndeplinit criteriul ACR20, 39,3% ACR50 şi 9%, ACR70, iar după şase luni, 66,6% au înregistrat criteriul ACR20 şi 33,3% ACR50. Pacienții care nu au avut răspuns bun sau moderat, după trei şi şase luni de tratament, au înregistrat criteriul ACR20.

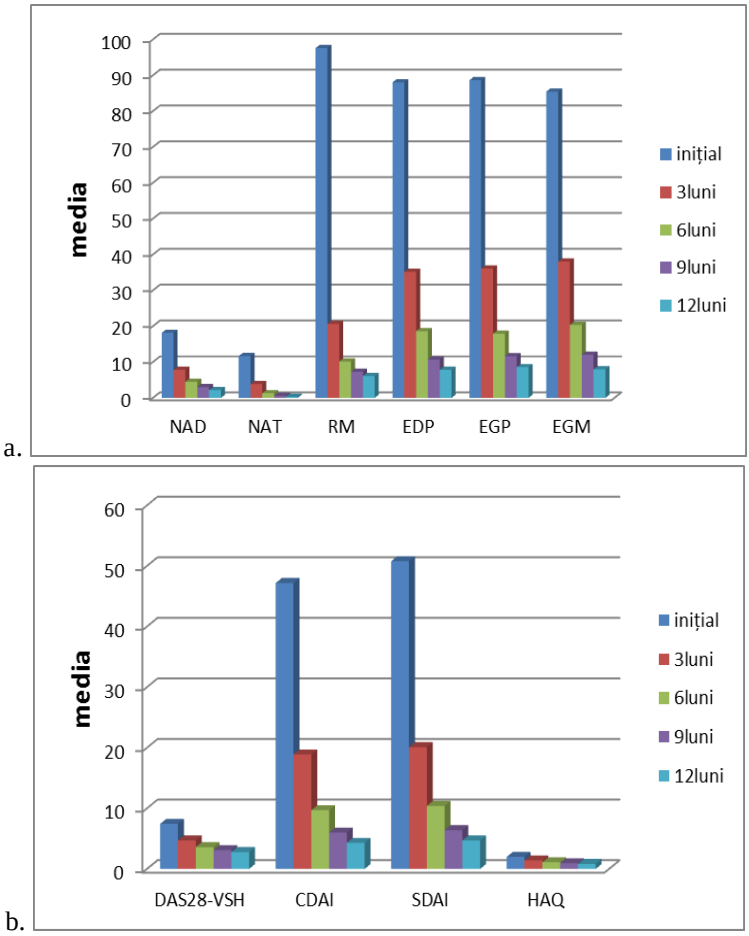

Fig. 1. Evoluția parametrilor clinici (a) şi a scorurilor de activitate a bolii (b)

5. Terapia asociată cu corticosteroizi. Din lotul de bolnavi cu PR, 33,3% au urmat tratament cu ADA asociat cu remisive clasice fără CS; 66,7% au primit CS în doză joasă (≤10mg/zi prednison). Inițial, media DAS28-VSH reflecta o boală mai activă la cei cu CS (7,36±0,21 *vs.* 7,17±0,19; p= 0,0001). În ciuda dozei de CS, diferențele în DAS28-VSH între cele două grupuri, după trei şi şase luni, au fost semnificative statistic (4,56±0.18 *vs.* 4,45±0,16; p=0,022 şi 3,46±0,28 *vs.* 3,10±0,21; *p=0,*0001), deşi comparațiile longitudinale au arătat o îmbunătățire în ambele grupuri (p<0,05). Rata celor cu răspuns bun a fost similară în cele două grupuri, după şase şi nouă luni (18,5% *vs.*11,8%; p=0,302 şi 34,7% *vs.* 25,8%; p=0,251), dar diferență semnificativă statistic (p<0,05) s-a înregistrat după 12 luni, cu un număr mai mare de pacienți cu răspuns bun la cei fără CS asociat (47,3% *vs.* 28,4%; p=0,01).

6. Evaluarea efectelor adverse. Administrarea ADA în combinație cu minim un remisiv clasic a fost bine tolerată. Nu s-au înregistrat efecte adverse grave sau severe,

ameninţătoare de viaţă; 11 pacienţi (33,3%) au prezentat efecte adverse clasificate ca reacţie uşoară la locul injecţiei. Infecţiile au survenit la 9 pacienţi (27,2%), la nivelul tractului respirator superior şi urinar inferior, fiind implicaţi *Escherichia coli*, *Proteus* şi *Klebsiella pneumoniae*. Nici un pacient nu a prezentat reactivarea tuberculozei latente, deşi unii pacienţi erau cunoscuţi cu test Quantiferon pozitiv. Pe durata studiului nu au fost înregistrate cazuri de infecţii oportuniste, neoplazie sau fenomene autoimune.

DISCUŢII

Am efectuat un studiu observaţional, prospectiv, proiectat să evalueze eficienţa şi siguranţa tratamentului cu ADA în asociere cu minim un remisiv clasic în formele active de PR aflate în stadiul avansat. Toţi parametrii clinico-biologici şi compoziţi evaluaţi au avut iniţial valori crescute, indicând o activitate crescută a PR şi impact funcţional moderat. Asocierea tratamentului cu ADA a dus la o îmbunătăţire rapidă şi susţinută în activitatea bolii şi afectarea funcţională. Astfel, s-a înregistrat o scădere precoce, semnificativă statistic, a tuturor parametrilor individuali şi compoziţi, precum şi a celor inflamatori, scădere ce s-a menţinut pe tot parcursul studiului. Îmbunătăţirea în DAS28-VSH a fost semnificativ mai mare în primele trei luni de tratament de la iniţierea terapiei comparativ cu următoarele nouă luni. După şase şi nouă luni de tratament, rata celor cu răspuns bun a fost similară în cele două grupuri (fără şi cu CS asociat), dar diferenţă semnificativă statistic s-a înregistrat după 12 luni, cu un număr mai mare de pacienţi cu răspuns bun în grupul fără CS, ceea ce arată că asocierea CS nu îmbunătăţeşte evoluţia bolii. O ameliorare importantă clinic a fost observată în primele trei luni, ceea ce vine în susţinerea datelor din literatură că, asocierea ADA la tratamentul cu remisive clasice îmbunătăţeşte capacitatea funcţională şi scade dizabilitatea. ADA asociat remisivelor clasice a fost bine tolerat, toţi pacienţii completând cele 12 luni de tratament. Majoritatea efectelor adverse raportate au fost uşoare, profilul de siguranţă al ADA fiind similar celui raportat în trialurile clinice (5,6,7,12).

CONCLUZII

Rezultatele acestui studiu demonstrează că tratamentul cu ADA în PR activă este eficient, aspect susţinut de reducerea semnificativă statistic a parametrilor clinico-biologici individuali şi compoziţi ai bolii, fiind asociată cu îmbunătăţirea funcţiei fizice şi calităţii vieţii pacienţilor. Profilul de siguranţă al ADA observat a fost comparabil cu cel din studiile anterioare. Studiul susţine utilizarea ADA în managementul pacienţilor cu forme active de PR aflate în stadiul avansat, fără răspuns adecvat la remisivele clasice, în asociere sau nu cu CS în doză joasă.

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NOUTĂȚI

INHIBITORUL DE PROTEAZĂ BOWMAN-BIRK EXTRAS DIN SEMINȚELE DE SOIA

Inhibitorii de proteaze au rol protector împotriva microorganismelor patogene. Principalii inhibitori de proteaze din semințele de soia sunt inhibitorul de tripsină Kunitz (KTI) şi inhibitorul de protează Bowman-Birk (BBI). Semințele de soia incubate în apă caldă eliberează cantități mari de proteine în mediu. Analiza electroforetică a relevat predominanța globulinei 7S, a inhibitorului de protează Bowman-Birk, inhibitorului de tripsină Kunitz, peroxidazei, α-galactozidozei. Exsudatul obținut în urma incubării semințelor de soia în apă caldă a inhibat creşterea celulelor umane ale cancerului de sân MCF-7. Deoarece semințele de soia imersate în apă caldă eliberează cantități mari de BBI enzimatic activ, metoda poate fi utilizată drept o alternativă mai simplă pentru prepararea concentratului de BBI folosit ca agent chemoprotector în cancer. (Palavalli MH, Natarajan SS, Wang TT, Krishnan HB. Imbibition of soybean seeds in warm water results in the release of copious amounts of Bowman-Birk protease inhibitor, a putative anticarcinogenic agent. *J Agric Food Chem*. 2012;60(12):3135-43.

Teodora Vremeră

A PROSPECTIVE STUDY OF PRE-ILLNESS DIET IN NEWLY DIAGNOSED PATIENTS WITH CROHN'S DISEASE

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A PROSPECTIVE STUDY OF PRE-ILLNESS DIET IN NEWLY DIAGNOSED PATIENTS WITH CROHN'S DISEASE (Abstract): **Background:** Environmental factors, including diet, seem to participate in the etiology of inflammatory bowel disease. The kind of dietetic habits before the appearance of the illness in patients with Crohn's disease (CD) has not been studied extensively. **Aim:** To prospectively assess the kind of food consumption in patients with CD exactly at the time of diagnosis and to identify dietary constituents as risk factors for development of CD. **Patients - Methods**: Twenty eight patients with a newly established diagnosis of CD (2-4 weeks), (12 men and 16 women), 30 patients with previously (between 2 – 11 years) established diagnosis of CD (14 men and 16 women) and 38 age- and sex-matched healthy controls (16 men and 22 women) were included in the study. Dietary intake was assessed by means of special questionnaire. **Results**: Comparisons between controls and newly diagnosed patients showed that increased consumption of milk and yogurt *(P=0.042),* fruits *(P=0.0001),* citrus *(P=0.0001),* vegetables *(P=0.0001),* carrots *(P=0.0001),* legumes *(P=0.036),* fish and selfish *(P=0.001),* honey *(P=0.003),* and nuts *(P=0.038),* was associated with decreased risk for CD. On the other hand, significantly increased intake of fat *(P=0.041),* olive oil *(P=0.038),* margarine *(P=0.038),* sugar *(P=0.02),* alcohol drinks *(P=0.009),* fried food *(P=0.0001),* and pasta *(P=0.0001),* was noticed on recently diagnosed patients in comparison with the healthy control group. On logistic regression analysis foods remaining statistically significant were: margarine, pasta, fried foods, fat, olives, sugar (increased risk), and yogurt, honey, fruits, nuts, fish, and citrus fruits (decreased risk). Newly diagnosed patients were significantly overweighed (64%) compared to healthy people (26%) and old patients (7%). **Conclusion**: Significant differences in many kinds of food between newly diagnosed patients with CD, patients with established CD and normal people certainly exist. Our results suggest that specific dietary patterns could be associated with higher or lower risks for CD in adults. However, whether these dietary factors are important for the development of CD or modulate the effect of other environmental factors is unknown. **Key words**: CROHN'S DISEASE, INFLAMMATORY BOWEL DISEASE, DIET, ETIOLOGY, PRE-ILLNESS DIET.

During the last years significant changes in the epidemiology of inflammatory bowel disease (IBD) were observed. The incidence of IBD and especially of Crohn's disease (CD) increased significantly in most countries. Today, in the European

countries the North to South gradient does not yet exist, while the West to East gradient is diminishing (1).

Since the increase in the incidence of CD could not be explained only by genetic or immunological factors alone, it was considered to relate with lifestyle changes. Diet, has been suggested to be one of environmental factors that interacting with genetic and immunological factors could lead to the development of CD (2).

Overall fat intake and high intakes of mono-unsaturated and poly-unsaturated fatty acids are considered to be risk factors for the development of IBD (3, 4). While clinical evidence suggests that long-chain triacylglycerols and probably ω-6 fatty acids might play a role in CD pathogenesis, polyunsaturated fatty acids (PUFAs) have emerged to modulate both inflammation and immunity processes (5) and n-3 PUFAs are examined to act as potential therapeutic agents (6). There is evidence that a-tocopherol and epigallocatechin gallate (ECGC) decrease IL-1, an important pro-inflammatory mediator crucial in IBD pathogenesis (7). Nutrigenomics is a new promising field, with a recent focus on CD related genes and nutrient interaction, such as those involving the receptor-mediated cytokine induction and autophagocytosis (8).

Other foods found in epidemiological studies to influence the expression of IBD include refined sugar, fruits, vegetables and fiber (9).

However, there is skepticism concerning the role of diet as a risk factor since it is not yet clear whether the differences in consumption are the cause or the result of the disease (10, 11). Moreover, mnemonic bias is an important problem when pre-illness diet is evaluated. Studies dealing with pre-illness diet habits of patients with CD are relatively few and their results are inconclusive (12-15).

The aim of this study was to assess the pre-illness food consumption of recently diagnosed patients with CD in comparison with healthy controls. This could indicate a possible involvement of pre-illness diet to the pathogenesis of the disease. A second comparison with the food consumption of patients with established diagnosis could possibly clear up the hypothesis that differences in consumption are more than the results of the disease.

PATIENTS AND METHODS

Population

The study was conducted at our Center for Inflammatory Bowel Disease at "Saint Panteleimon" General Hospital Nicea, Greece. The sample (non-probability convenience sampling) consisted of 96 subjects, divided into three groups. The first one consisted of 28 recently diagnosed (2-4 weeks) patients with CD (12 men and 16 women) comprising the group of "new" patients. A dietician (the first of the authors) interviewed all patients immediately after confirmation of diagnosis. The second group consisted of 30 patients with previously (between 2-11 years) established diagnosis of CD (14 men and 16 women) (group of "old" patients) and the third group consisted of 38 age and sex-matched healthy controls (16 men and 22 women).

Questionnaire

In order to collect the data a three-part questionnaire was employed. The first part included information of demographic data, height, weight and age, lifetime smoking habits, other health problems, medication and stress level (self-estimation). The main part consisted of a semi-quantitative Food

Frequency Questionnaire (FFQ) that was designed after 1) studying the relevant bibliography and consulting the original Willet's FFQ, 2) studying the methodology of former European, USA and Japan case-control studies, and 3) taking under consideration the food list applied by the EPIC study (in order to conclude regional food consumption).

The FFQ included 11 groups of food (diary products, fruit, vegetables, grains, legumes, meat, cooked meals, various food, sweets and sweeteners, fats and oils, drinks) which comprised in total 122 food items including ethnic foods and drinks. The consumption of each food item was measured by the use of an interval scale from 1 to 9, with category "1" corresponding to consumption "few times a year or at al", and category "9" corresponding to consumption "7+times a day". "Steady/ fixed quantities ("egeg one cup") or "natural" quantities ("egeg one apple") measured the size of portion.

Subjects were asked to describe the nutritional habits concerning the last two years. They also asked to describe for each food group if any changes had occurred in the past six months as a consequence of the underlying disease. Seasonal consumption of specific food items was also taken under consideration. The third part of the questionnaire included questions about food groups or specific food items that probably created troubles to the patients before the disease onset, the dietary habits changes after diagnosis, the fear of anointment of specific food items, etc.

Data collection

Data were obtained by standardized interviews lasting approximately one hour, conducted by the first of the authors. Healthy controls (except 8 of them, who were interviewed as well) completed the questionnaires after receiving them by e-mail. This group did not complete the third part of the questionnaire.

Statistical analysis

Contingency tables and chi-square tests were employed for the statistical analysis of nominal variables, and non-parametric tests (Mann-Whitney test) for the analysis of interval variables. The strength of the association between the intake of food groups and the risk of CD was verified with logistic regression analysis.

RESULTS

Foods with statistically significant difference between controls and newly diagnosed patients are shown in table I. As it can be seen in the table, statistically significantly decreased consumption of milk and yogurt, fruits, citrus, vegetables, carrots, legumes, fish and selfish, honey and nuts was noticed in the group of "new" patients with CD as compared with the group of healthy controls. Thus, patients with a recently established diagnosis of CD (group of "new" patients) described significantly less consumption of fruits *(P=0.0001)*, citrus *(P=0.0001)*, vegetables *(P=0.0001)*, carrots *(P=0.0001)*, fish and selfish *(P=0.001)*, honey (*P=0.003*), legumes *(P=0.036)*, nuts (*P=0.038*), and milk and yogurt *(P=0.042)*, in comparison with the group of healthy controls.

On the other hand, an increased consumption of fried food (P=0.0001), pasta (P=0.0001), alcohol drinks (P=0.009) sugar (P=0.02), olive oil (P=0.038), margarine (P=0.038) and fat (P=0.041), was obtained in the group of newly established diagnosis of CD ("new" patients with CD)

as compared with the group of healthy controls.

TABLE I
Foods with statistical significant differencesbetween "new" patients and healthy controls

Variable	*"New" patients*	*Healthy controls*	P value
Milk-yogurt	4.0	5.42	0.042
Fruit (total)	4.0	6.47	0.0001
Citrus fruit	1.93	4.09	0.0001
Vegetable (total)	5.36	6.68	0.0001
Carrots	1.21	3.11	0.0001
Rice	5.29	4.47	0.046
Pasta	6.5	4.74	0.0001
Legumes	2.29	3.05	0.036
Fish-shellfish	2.21	3.53	0.001
Fried food	4.00	2.21	0.0001
Sugar-sweeteners	6.00	4.16	0.002
Honey	1.36	2.95	0.003
Fats and oils	7.29	6.63	0.041
Olive oil	7.07	6.42	0.038
Margarine	3.71	2.05	0.0001
Nuts	1.21	2.21	0.005
Alcohol	5.3%	43%	0.009
Fast-food	5.3%	35.7%	0.004

Foods with statistical significant difference between "new" patients and "old patients" are shown in table 2. As the table indicates, statistically significantly decreased consumption of fish-shellfish food *(P=0.018),* and tea *(P=0.026)* and significantly increased consumption of fried food *(P=0.002),* margarine *(P=0.0001)* and coffee *(P=0.018)* was noticed among newly diagnosed patients in comparison with "old" patients (table II).

TABLE II
Foods with statistical significant differences between "new" patients and "old patients"

Variable	*"New" patients*	*"Old patients"*	P value
Fish-shellfish	2.21	3.40	0.018
Fried food	4.00	2.13	0.002
Honey	1.36	2.53	0.094
Margarine	3.71	1.60	0.0001
Coffee	64.3%	13.3%	0.018
Tea	7.1%	46.7%	0.026

TABLE III
Foods with statistical significant differencesbetween "old" patients and healthy controls

Variable	*"Old" patients*	*Healthy controls*	*P value*
Milk-yogurt	2.93	5.42	0.042
Fruit (total)	4.60	6.47	0.0001
Citrus fruit	2.67	4.9	0.001
Vegetable (total)	4.73	6.68	0.0001
Carrots	1.47	3.11	0.0001
Rice	5.53	4.47	0.022
Legumes	2.47	3.05	0.027
Sugar-sweeteners	5.47	4.16	0.002
Honey	2.53	2.95	0.003
Olive oil	7.20	6.42	0.038
Nuts	1.20	2.21	0.002
Alcohol	6.7%	43%	0.009
Refreshments	13.3%	57.9%	0.028

Table III shows foods with statistically significant differences between "old" patients in comparison with healthy controls. As the table indicates patients with a recently established diagnosis of CD (group of "new" patients) described significantly less consumption of citrus fruits *(P=0.001),* fruits (total) *(P=0.0001),* vegetables *(P= 0.0001),* carrots *(P=0.0001),* honey (*P= 0.003*), legumes *(P=0.027),* nuts (*P= 0.002*), alcohol *(P=0.009)* and refreshments *(P=0.028),* in comparison with the group of healthy controls. These differences were quite similar with those found between patients with newly established disease in comparison with healthy controls. On the contrary they consumed more rice *(P=0.022),* sugar-sweeteners *(P= 0.002),* and olive oil *(P=0.038).*

On logistic regression analysis foods remaining statistically significant were: margarine, pasta, fried foods, fat, olives, and sugar (foods that increase the risk of acquiring CD), and yogurt, honey, fruits, nuts, fish, and citrus fruits (foods that decrease the risk of acquiring CD) (table IV).

TABLE IV
Foods remaining statistically significant on logistic regression analysis (increased or decreased risk): "new" patients *vs.* healthy controls.

Variable	*Significance*	*Odds Ratio*	*95% CI - Lower*	*95% CI - Upper*
Alcohol	0.025	13.493	1.388	131.195
Margarine	0.007	6.305	1.643	42.187
Pasta	0.004	5.17	1.665	16.051
Fried	0.008	3.609	1.398	9.316
Fats-oil	0.038	3.539	1.071	11.699
Olive	0.047	2.596	1.014	6.644
Sug_sw	0.039	1.561	1.023	2.382
Rice (~)	0.117	1.555	0.896	2.698
Milk_yog	0.045	0.649	0.426	0.991
Pulses (~)	0.1	0.572	0.293	1.114
Honey	0.051	0.541	0.292	1.003
Fruits	0.013	0.297	0.114	0.774
Nuts	0.019	0.264	0.860	0.807
Fish Selfish	0.009	0.151	0.037	0.623
Vegetables	0.005	0.134	0.033	0.549
Carrot	0.003	0.037	0.040	0.333
Citrus fruits	0.002	0.031	0.152	0.652

Patients with CD that belonged to the group of "new" patients, were significantly overweighed (64%) as compared to the group of healthy people (26%) and patients with previously established CD (7%) *(P<0.046).*

DISCUSSION

Dietary factors have been implicated in the etiology of IBD from many years. Most of the studies investigated the influence of different kinds of foods in patients with IBD in comparison with normal people, while some other investigated the consumption of foods in newly established disease. However, it became quite evident that methodological considerations that usually arise when studying any pre-illness

diet, relate both to the mnemonic bias that occur when patients are asked about their past diet and the possibility that the disease and its symptoms have influenced their nutritional habits.

In this study, in order to counterbalance the above mentioned parameters, the patients were interviewed within 2-4 months from the time of diagnosis. Furthermore, they were questioned about food consumption during the last two years, but before any symptoms occurred. That is why they were also asked to describe any change in food consumption during the last six months, therefore increasing the reliability of the results. The statistical analysis methods used in this study (contingency tables and chi-square tests for the nominal and Mann-Whitney test for interval variables) were the appropriate ones according to the kind and nature of the goals of the study, the information collected and the sample size. That statistical analysis in fact pointed out the differences in food consumption between the groups. Logistic regression analysis was used to estimate the strength of each observation.

The results of our study showed that when compared controls and newly diagnosed patients an increased consumption of milk and yogurt (P=0.042), fruits (P=0.0001), citrus (P=0.0001), vegetables (P=0.0001), carrots (P=0.0001), legumes (P=0.036), fish and selfish (P=0.001), honey (P=0.003), and nuts (P=0.038), was associated with decreased risk for CD. On the other hand, significantly increased intake of fat (P=0.041), olive oil (P=0.038), margarine (P=0.038), sugar (P=0.02), alcohol drinks (P=0.009), fried food (P=0.0001), and pasta (P=0.0001), was noticed on recently diagnosed patients in comparison with the healthy control group. On logistic regression analysis foods remaining statistically significant were: margarine, pasta, fried foods, fat, olives, sugar (increased risk), and yogurt, honey, fruits, nuts, fish, and citrus fruits (decreased risk).

Food consumption and dietary choices usually reflect a life-style model. High consumption of refined and fast food, rich in saturated and total fat and different kinds of additives, accompanied by a low consumption of fruit, vegetables, legumes and other "healthy" food, is quite typical for most western-type societies. In this study, the differences between health controls and newly diagnosed patients were quite well established. Furthermore, the content of food in nutrients varies among different geographic regions around the world. Different kinds of cattle or farming techniques e.g. organic or conventional farming, food process and preparation could also affect their overall nutrient content. Local or seasonal food consumption and dietary patterns vary among different populations. All the above are crucial parameters when trying to conduct any conclusion about the protective or aggravating role of food or nutrients relatively to any disease and might also explain probable disparities among studies. In this study national and seasonal consumption was taken under consideration.

Dietary risk factors previously suggested to be related (at least epidemiologically) with CD include sugar and refined carbohydrates. Comparisons between patients with CD and normal people revealed increased consumption of refined sugar among the patients either before or after diagnosis of bowel disease (16-21).

The possibility that this was the result of patients' food choices, because of the disease's symptoms, has always been a

subject of debate. In this study, however, the pattern of increased sugar consumption was recorded among newly diagnosed patients, before the symptoms occurred. An explanation could be related to the increased intestinal permeability (22) and altered immune response (23).

In recent years, special attention has been paid to the lipid components of the diet as triggers of CD (15). Increased daily intake of n-6 polyunsaturated fatty acids relatively to n-3 PUFAs, which is considered to increase the proinflammatory leukotrienes B4 and alter arachidonic metabolism was related to the increased incidence of CD in Japan5. In our study, newly diagnosed patients also used to consume more total fat and oils and olive oil than healthy controls. Olive oil is globally known for its antioxidative properties and a potential aggravating involvement in such an inflammation process might be rather worth mentioned. However, the increased fried food consumption and the decreased vegetable one (vegetables are suggested to increase olive's oil antioxidative capacity) that were also emerged in this study, as well as the n-6/n-3 ratio mechanism previously proposed, could give a possible explanation for this observation. We have also noticed a high margarine and fast-food consumption (a diet rich in total and saturated fat) in newly diagnosed patients with CD. On the contrary, fish and shellfish consumption seemed to have a protective role against CD, in part maybe because of their high content in n-3 PUFA, which are well documented for their ability to control inflammation and enhance immunity (24). However, the content in n-3 PUFA, as well as n-6 and other nutrients, usually varies among different kinds of fish and shellfish and probable among different regions around the world. This fact could explain the discrepancy observed in the study of Sakamoto et al. (15) who found a positive association between fish and shellfish consumption and development of CD. In Greece, consumption of rich in n-3 PUFA fish (such as sardines) is part of local or seasonal nutrition patterns. It is worth mentioning that a pattern of foods characterized by fish, grains, and nut consumption was inversely associated with CD in children of both genders indicating that this specific dietary pattern could be associated with lower risks for CD in children (25).

Some previous studies have identified low intake of fruit and vegetables among CD patients indicating that the consumption of citrus fruit, fruit juices and vegetables could lower the risk of the development of IBD (9, 14, 26). However, most of the studies have not investigated long-standing disease; therefore the described results could be attributed to the disease itself. In our study, increased consumption of fruit (especially citrus fruits) and vegetables (especially carrots) was lower among CD patients, indicating their possible protective role. Fruits and vegetable are rich in a variety of phytochemicals that are related to inflammation control. The consumption of vegetables and fruits was also inversely associated with CD development in children in both genders (25).

As far as fiber consumption is concerned, a previous study showed an inverse relationship between consumption of bran and onset of CD (27). However, it is difficult to determine whether the potentially protective effect of fiber is due to its action itself or to other micronutrients contained in fruit and vegetables. The utility of low

refined carbohydrate diets in the treatment of CD has previously been suggested (28), although extensive clinical trials have not confirmed the benefits of this diet (29). It is possible –at least theoretically- that short-chain fatty acids are particularly worthy of note and are generated during the colonic fermentation of dietary fiber. A quantitative short-chain fatty acids deficiency or their oxidation by colonocytes have been implicated in the physiopathology of ulcerative colitis (30) and short-chain fatty acids *in vivo* oxidation is also lower in affected patients (31).

In our study, alcohol consumption was found to be higher among newly diagnosed CD patients suggesting that it might act as a risk factor. This finding has not been previously described. However, some suggest a possible mechanism through alteration in hepatic metabolism that results in functional iron deficiency causing impaired immune function (32). Other vitamins such as riboflavin and folate deficiency may also alter immune functions and impaired control of chronic inflammation (33). Even though this study was not designed to estimate nutrient but food consumption, its results underline that there are considerable differences among newly diagnosed CD patients' and healthy controls' diet that should be further investigated.

Finally, one interesting finding of our study was related to the presence of obesity in newly diagnosed patients compared to "old" patients and normal controls. Newly diagnosed patients were significantly overweighed (64%) compared to healthy people (26%) and old patients (7%). Obesity in patients with CD has previously been considered unusual. In a recent study from Scotland the authors described a percentage of 18% of the IBD population to be obese in comparison to approximately 23% of the Scottish population on a whole (34). A further 38% of patients were over-weight, the same percentage as the general population. Finally, there were significantly more obese men and women with CD than with ulcerative colitis (34). Although obesity in Greece has increased considerably over the last years, it seems that this finding is not the result of a chance and must be further investigated.

CONCLUSIONS

Significant differences in many food items between newly diagnosed Greek patients with CD and normal people certainly exist. Our results suggest that specific dietary patterns could be associated with higher or lower risks for CD in adults. Whether these dietary factors are important for the development of CD or modulate the effect of other environmental factors is unknown. Again is unknown if dietary modulations applied before or after the establishment of diagnosis of CD could delay the appearance of CD or benefit the course of CD. Personalized diets may be especially important for patients with CD by excluding a number of food items35. The available data suggest that the changes in the composition and characteristics of the diet which typifies modern life have been accompanied by substantial changes in the epidemiology of IBD in developed counties. Taking into consideration the results of the present study we suggest that larger studies aiming to investigate the influence of diet on the pathogenesis or the activity of the disease on subjects with different genetic background could produce important results.

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EFFECTS OF SARCOPENIA AND SARCOPENIC OBESITY ON THE HEALTH STATUS OF ELDERLY POPULATION

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EFFECTS OF SARCOPENIA AND SARPOPENIC OBESITY ON THE HEALTH STATUS OF EARLY POPULATION (Abstract). Sarcopenic obesity (SO) is a category of obesity that occurs predominantly in elderly people who experience an increase in adipose tissue associated with muscle mass loss. It is associated with higher mortality rates than sarcopenia and obesity alone, thus confirming the need to redefine obesity and sarcopenia based on the new parameters-the simple determination of the body mass index becoming unsatisfactory. Quantification of obesity is done by measuring through various imaging techniques the percentage of fat mass, muscle mass/lean body tissue and bone mass or the ratio of visceral fat to thigh muscle area (determined by computed tomography). Redefinition of sarcopenia is a decrease in striated muscle mass associated with either a decrease in muscle strength (hand grip strength) or reduced physical activity ("gait speed"); when all three criteria are met, we talk of severe sarcopenia. These new parameters result from the fact that in sarcopenia there is no parallel loss of muscle mass and muscle strength, and that the alteration of muscle strength is a more important risk factor for overall mortality than the decline in muscle mass. For these reasons, quantification of the degree of sarcopenia is done by individual assessment of the three parameters: muscle mass, muscle strength and physical activity. Although currently there is no consensus on the definition of SO, all authors agree on its serious consequences on the quality of life of the elderly, favoring cardiovascular and metabolic diseases, aggravating frailty, significantly increasing the risk of falling and of developing disabilities, and finally, increase in overall mortality. Today, SO identification, prevention and treatment become priority interventions in the therapeutic plan of the elderly to ensure successful aging and maintain a quality of life corresponding to expectations. **Keywords**: VISCERAL OBESITY, SARCOPENIA, SARCOPENIC OBESITY, ELDERLY.

Sarcopenia is the loss of muscle mass and strength that occurs with aging. There is a progressive decrease in muscle mass of approximately 8% per decade between 40 and 70 years, and doubles after the age of 80 years. This condition affects 30% of patients over the age of 60 and about 50% of those over 80 years (1). Along with sarcopenia, there is an increase in visceral fat, overweight and obesity being particularly common in this population segment. The association of these two entities has

led to the emergence of a new concept termed sarcopenic obesity.

Sarcopenic obesity (SO) was first defined by Baumgartner (2) in 2010 as the association between sarcopenia and obesity (as measured by Dual-energy X-ray absorptiometry). SO is considered a category of obesity that occurs predominantly in elderly patients who experience an increase in adipose tissue associated with muscle mass loss. (3). Studies show that SO is associated with higher mortality risk than sarcopenia and obesity alone. In fact, there is no unanimously accepted definition of SO because there are no unanimously accepted definitions for obesity and sarcopenia.

Defining obesity

Obesity is a major public health problem and a major risk factor for morbimortality through cardiovascular and metabolic diseases. The prevalence of obesity in the adult and elderly population has doubled since 1980 and is steadily increasing (3,4). The World Health Organization defines obesity - regardless of age - as associated with BMI values> 30 kg/m2 and WC > 102 cm for men and > 88 cm for women (central obesity). Studies conducted over the past 10 years showed that this definition should be supplemented with other parameters provided by body composition measurement techniques, most commonly used being Dual-energy X-ray absorptiometry (DXA) and bio impedance (BIA). These parameters newly included in the definition of obesity are the percentage of fat mass, muscle mass /lean body tissue and bone mass, or the ratio of visceral fat to thigh muscle area (determined by computed tomography) (5, 6).

This reassessment of the definition of obesity was necessary due to the conflicting data known as the obesity paradox. These data show that in the elderly population overweight and obesity (measured by Body Mass Index) are associated with a decrease in all-cause mortality, particularly in cardiovascular mortality. This contradicts the unanimously accepted data that obesity is a major cardiovascular risk factor with a devastating impact on mortality in the adult population. The explanation for this paradox seems to be that aging is associated, among others, with an increase in visceral fat but also with a progressive loss of muscle mass (sarcopenia). Sarcopenia is significantly associated with the preexisting cardiovascular risk factors; in addition, it is associated with important metabolic disorders, physical disability and increased mortality. The coexistence of sarcopenia with obesity in the elderly determines, on one hand, the prolongation of life through the beneficial effects of obesity and, on the other hand, severe impairment of the quality of life through the negative effects of sarcopenia (3, 6, 7, 8).

Obesity paradox is explained by the presence of SO, and to demonstrate this phenomenon it became necessary to quantify both sarcopenia and obesity; in this context, the simple definition of obesity by body mass index is unsatisfactory (7, 8).

Defining sarcopenia

The term sarcopenia was first proposed in 1989 by Irwin Rosenberg (from Greek "sarx" = meat, "penia" = loss) to describe the decrease in the muscle mass characteristic to the aging process (9).

Sarcopenia, part of the frailty syndrome in the elderly, had neither a unanimously accepted definition nor consensus diagnosis, codes or treatment guidelines. To address these shortcomings, the European Union Geriatric Medicine Society (EUGMS) decided in 2009 to create a sarcopenia working

group that would develop operational definitions and diagnostic criteria for sarcopenia to be used in clinical practice as well as in research studies (10). Other European scientific organizations (European Society of Clinical Nutrition and Metabolism (ESPEN), International Nutrition and Aging Academy (IANA) and the International Association of Gerontology and Geriatrics - Europe Region (IAGG-ER) were invited to join this group. The unanimously accepted conclusions have led to a new definition of sarcopenia, considered to be the decrease in the striated muscle mass associated with either the decrease in muscle strength (grip strength) or with reduced physical activity ("gait speed"); when all three criteria are met, we talk of severe sarcopenia (European Working Group on Sarcopenia in Older People).

Pathophysiological, sarcopenia is an inflammatory state triggered by pro-inflammatory cytokines and oxidative stress and resulting in age-related muscle mass loss (11,12) (fig 1).

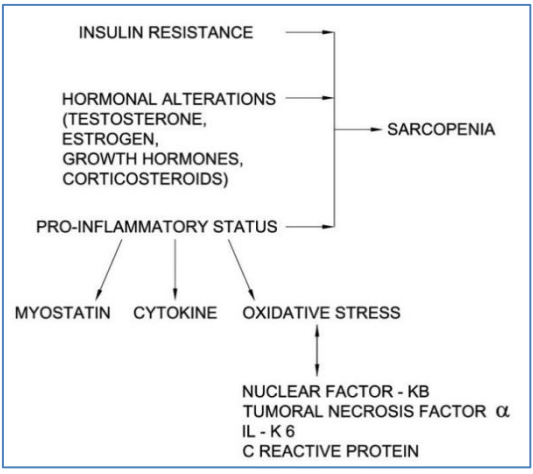

Fig. 1. Factors determining the occurrence and persistence of sarcopenia in the elderly

Sarcopenia is directly associated with insulin resistance, metabolic syndrome, and type 2 diabetes mellitus. Concurrently, insulin resistance favors muscle fiber atrophy and mitochondrial dysfunction, aggravating sarcopenia. Of the pro-inflammatory factors, a special mention is given to myostatin (growth/differentiation factor 8) that inhibits muscle cell growth and differentiation and seems to be not only a key mediator in sarcopenia but also a promising therapy (4, 13, 14).

There is a great deal of evidence that in sarcopenia there is no parallel loss of muscle mass and muscle strength (15). This finding has a special significance because the alteration in muscle strength is a more important risk factor of all-cause mortality than the decline in muscle mass (16). For these reasons, the degree of sarcopenia is quantified by individual assessment of the three parameters: muscle mass, muscle strength and physical activity.

Muscle mass is measured by several imaging techniques: computed tomography (CT), magnetic resonance imaging (MRI), dual energy X-ray absorption (DXA), bio impedance, and anthropometric measure-

ments.

Muscle strength is most often assessed by determining the hand grip strength (although for the assessment of physical function the changes occurring in the lower limbs are more relevant than those in the upper limbs), because it is a simple technique and the obtained results have a high degree of reliability (8).

Physical activity is often assessed with Short Physical Performance Battery (SPPB) based on gait speed, chair stand and balance tests, timed up and go test, 400-meter walk test, six-minute walk test (8.17).

Defining sarcopenic obesity

SO is considered as a stand-alone entity due to the accumulation of data suggesting that sarcopenia and obesity share common etiopathogenic elements and mutually potentiate one another negatively, leading to an increased risk of all-cause and particularly of cardiovascular morbimortality, increases more than the risks of obesity and sarcopenia taken separately (11, 18) (fig 2).

Increase in visceral fat is associated with an increase in inflammation markers, particularly of oxidative stress and pro-inflammatory cytokines; they will lead to worsening of sarcopenia with decrease in physical activity and energy expenditure, elements that will favor visceral fat increase, closing the circle through which obesity and sarcopenia potentiate each other negatively (19).

Obesity and sarcopenia share several common elements which cause the onset of SO (fig 3).

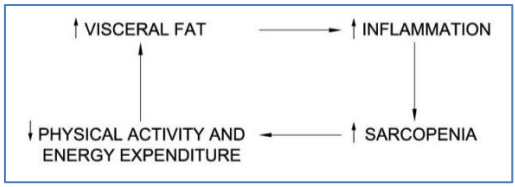

Fig. 2. Mutual negative potentiation of sarcopenia and obesity in SO.

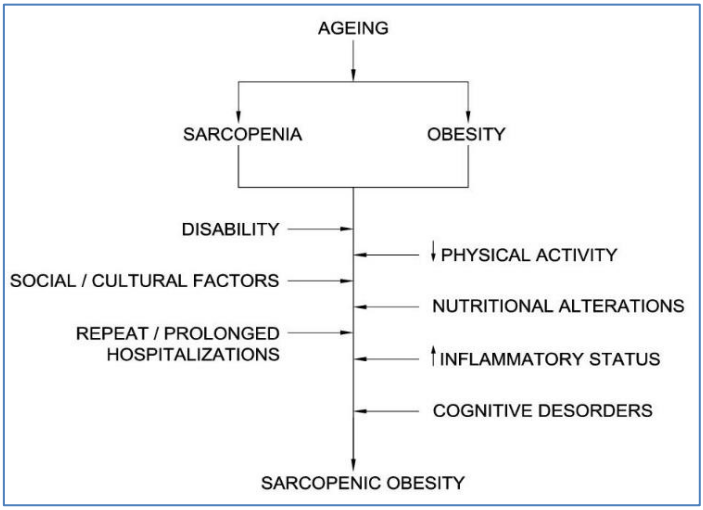

Fig. 3. Factors favoring the occurrence of Sarcopenic Obesity

Thus, sarcopenia per se is a characteristic of aging, responsible for decreased physical activity and increased inflammatory status; at the same time there is an increase in aging-related increase in adipose tissue associated with inadequate nutritional intake (low protein intake), which will further alter the balance between fat mass and muscle mass, favoring SO. Associated to these pathogenic links are a number of social/cultural factors that alter the lifestyle of the elderly, eventual disabilities caused by concomitant chronic illnesses that may be associated with repeat hospitalizations and possible cognitive disturbances, with disastrous results for lean mass - fat mass balance.

Although there is no consensus on the definition of SO, all authors agree on its serious consequences on the quality of life of the elderly, by favoring the occurrence of cardiovascular and metabolic diseases, aggravating frailty, significantly increasing the risk of falls and disabilities, finally causing an increase in all-cause mortality (19, 20, 21, 22) (fig 4).

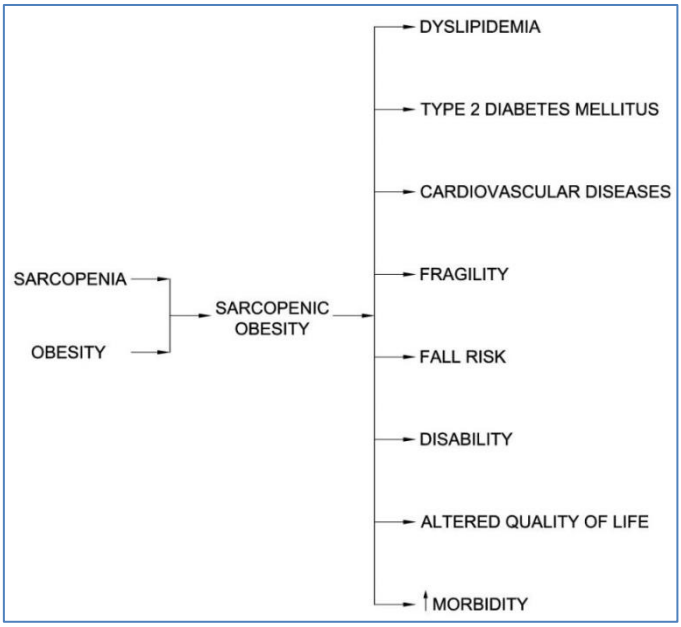

Fig. 4. Consequences of sarcopenic obesity on the state of health of the elderly population

For these reasons, prevention/improvement of SO becomes a very important task. Studies conducted in this field agree that a complex action is required both for the improvement of sarcopenia and fatty mass reduction. Maintaining/improving muscle mass and strength and maintaining adequate weight through physical activity and adequate nutrition is essential for preserving independence in old age - assessed with the help of activities of daily living (ADL) and instrumental activities of daily living (IADL) scales, especially in the elderly patient with cardiovascular and metabolic diseases (23, 24, 25). Adequate protein intake, optimally achieved by taking nutritional supplements based on essential amino acid mixtures, will result in a decrease

in insulin resistance and improvement of the degree of sarcopenia. Severe diets are not recommended, as they will not only lead to fat mass loss, but also to the "melting" of the muscle mass, with the occurrence of cachexia (26, 27, 28).

CONCLUSIONS

SO is considered as a stand-alone entity due to the accumulation of data suggesting that sarcopenia and obesity share common etiopathogenic elements and potentiate one another negatively, leading to an increase in the risk of all-cause and especially cardiovascular morbimortality higher than the risks of obesity and sarcopenia taken separately. These common elements of obesity and sarcopenia initiate a vicious circle in which the two entities potentiate one another negatively. Although there is no consensus on the definition of SO, all authors agree on its serious consequences on the quality of life of the elderly, favoring cardiovascular and metabolic diseases, aggravating frailty, significantly increasing the risk of falls and disabilities, finally causing an increase in all-cause mortality. For these reasons, prevention/improvement of SO became a very important task. Choosing the type and degree of personalized exercise should be associated with a correct protein intake, optimally achieved by the administration of nutritional supplements based on essential amino acid mixtures that will result in decreased insulin resistance and improved degree of sarcopenia degree. Selection of the therapeutic plan should be supervised by qualified personnel to avoid the severe diets that not only lower the fat mass, but also "melt" the muscle mass, leading to cachexia.

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HIPERTENSIUNEA ARTERIALĂ ESENȚIALĂ LA COPIL – FACTORI DE RISC ŞI AFECTAREA ORGANELOR ȚINTĂ

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ESSENTIAL HYPERTENSION IN CHILDREN – RISC FACTORS AND TARGET ORGAN DAMAGE (Abstract): Regarding that systemic hypertension is an important condition in childhood, we try to clarify existing data about this pathology. **Material and methods**: The aim of our study was to evaluate the incident cases of essential hypertension in children admitted during a four years period in the Children Hospital "Sf. Maria" Iaşi, Romania. The study included 60 children with essential hypertension. **Results**: 10 (16,7%) of children were in prehypertension group, 38 (63,3%) have stage 1 hypertension and 12 (20%) have stage 2 hypertension. We objectivate a different gender distribution -boy/girl: 1,5/1. The frequency of essential hypertension increased with age, the incidence was higher over 14 years. We established a statistically significant relationship between the essential hypertension body mass index and prematurity (gestation age under 37 weeks). Left ventricular hypertrophy was objectivated in 26,7% of cases. **Conclusions**: Essential hypertension in children is a pathology with increasing prevalence nowadays. This is due to synergic action of multiple risk factors involved. That is why is important to know and to prevent as much as possible this disease.
Key words: ESSENTIAL HYPERTENSION, BLOOD PRESSURE, LEFT VENTRICULAR MASS INDEX.

Hipertensiunea arterială (HTA) reprezintă cauza majoră a bolilor cardiovasculare (1). S-a demonstrat că HTA este principala cauză de mortalitate în lume şi a doua cauză de morbiditate (după prematuritate) la copil (1). Prevalența şi rata de diagnostic a HTA la copil par să fie în creştere, în principal datorită obezității infantile (3, 4). Studiul nostru urmăreşte să aducă unele clarificări privind datele demografice, factorii de risc şi afectarea organelor țintă în cazul prezenței HTA esențiale (HTAE) la copiii din Moldova, pe o perioadă de 4 ani.

MATERIAL ŞI METODE

Au fost incluse în studiu cazurile de HTAE internate, în perioada 2007 - 2010 în Spitalul de Urgențe pentru Copii „Sfânta Maria" Iaşi, provenite din județele Moldovei. Lotul de studiu a fost selectat după

măsurarea tensiunii arteriale (TA) prin metoda ascultatorie, adaptată dimensiunilor antropometrice ale copilului, pe baza înregistrării în dinamică a trei valori presionale, în trei momente diferite, care să depăşească percentilul (p) 95 pentru talie, vârstă şi sex, utilizând nomogramele propuse de ultimul ghid în domeniu (5).

Diagnosticul a fost stabilit pe criteriile specifice respectivei entități patologice, identificate prin anamneză, examen fizic şi explorări paraclinice, fiind astfel identificați 60 de copii cu HTAE. Copiii cu indicele masei corporale (IMC) mai mic decât p 85, pentru vârstă şi sex, au fost considerați normoponderali; cei cu IMC între p85 şi p95, supraponderali, iar în cazul celor cu IMC mai mare decât p95, au fost incluşi în grupul obezilor.

Evaluarea ecocardiografică a fost efectuată de acelaşi investigator folosind un ecocardiograf „Vivid 3 pro" cu un transductor de 3,5 MHz. Ecografia şi procesarea datelor s-a realizat în concordanță cu ghidurile Societății Americane de Ecocardiografie, înregistrând valorile medii din trei cicluri cardiace consecutive (6). În modul M s-au efectuat măsurătorile septului interventricular (SIV) în diastolă, a diametrului diastolic al ventriculului stâng (DdVs) şi peretelui posterior al ventriculului stâng (PpVs) în timpul diastolei. Aprecierea încadrării în intervale fiziologice sau patologice de variație a acestor parametri s-a realizat prin compararea cu valorile medii inserate în literatura de specialitate (7). Modificarea anatomică principală urmărită a fost hipertrofia ventriculară stângă (HVS), evaluată prin calcularea masei ventriculului stâng (MVS) cu ajutorul formulei lui Devereux (8). Masa calculată a ventriculului stâng a fost ulterior raportată la talia ridicată la puterea 2,7 exprimată în metri pentru a minimiza efectele vârstei, sexului şi statusului ponderal (9, 10). Astfel am obținut indicele masei ventriculului stâng (IMVS). Nu există încă un consens cu privire la valorile de referință ale IMVS. Pentru definirea HVS am luat în considerare fie o valoarea a IVMS mai mare sau egală cu p 95 pentru sex (corespunde valorilor de 36,88g/$m^{2,7}$ la fete şi 39,36 g/$m^{2,7}$ la băieți), fie o valoare mai mare sau egală cu 51 g/$m^{2,7}$ (valoare peste p 99, care se asociază cu un risc de morbiditate cardiovasculară de 4,1 ori mai mare la adulții hipertensivi) (11).

Pentru evaluarea statistică a datelor s-a utilizat programul de analiză *SPSS 17.0* (SPSS Inc., Chicago, Illinois). Semnificația statistică de prag a fost aleasă la valoarea p = 5% şi corespunzător intervalul de încredere a fost de 95%.

REZULTATE

În studiu au fost incluşi 60 de copii cu HTAE, debutul HTA la fiecare copil a fost, convențional, considerat a fi momentul diagnosticului (abordarea inițială), deoarece în practică este imposibil a depista debutul real al HTAE la copil. În studiul nostru, distribuția pe sex masculin/feminin a fost de 1,5/1. Vârsta medie a pacienților de sex feminin fost de 13,96 ± 2,36 ani, iar pentru sexul masculin a fost de 13,17 ± 2,62 ani. Evaluarea pe grupuri de vârstă şi sex a dovedit că frecvența cea mai mare de apariție a bolii a fost întâlnită la grupul de vârstă de peste 14 ani. De asemenea, s-a constatat o frecvență de 2,15 ori mai mare a HTAE la copiii din mediul urban. S-a obiectivat o diferență semnificativă statistic între statusul ponderal evaluat prin intermediul IMC la cele două sexe în favoarea băieților (p=0,016) (tab. I).

TABELUL I

Parametri analizaţi în hipertensiunea arterială esenţială la copil

Parametru	Categorii	Stadiul HTA (număr pacienţi)			total	p
		prehiper tensiune	HTA stadiul 1	HTA stadiul 2		
Vârsta (ani)	7-10	3	1	4	8	
	10-14	4	10	5	19	
	> 14	3	27	3	33	
Sex	masculin	10	15	11	36	p=0,004
	feminin	0	23	1	24	
Mediul de proveniență	urban	10	23	8	41	p = 0,310
	rural	0	15	4	19	
Indice de masă corporală (percentil)	< p85	8	25	3	36	p=0,0002
	p85 - p95	1	11	2	14	
	> p95	1	2	7	10	
Vârsta gestaţională (săptămâni)	<37	0	2	6	8	p<0,001
	37 - 42	9	33	5	47	
	>42	1	3	1	5	
Indicele de masă al ventriculului stâng (percentil)	< p95	8	28	8	44	p=0,001
	≥p95 <p99	1	5	1	7	
	>p99	1	5	3	9	

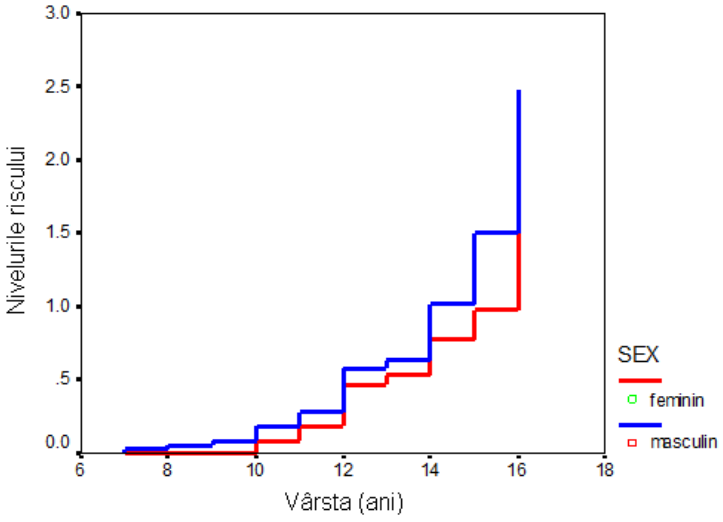

Fig. 1. Distribuţia pe sex a riscului apariţiei HTAE

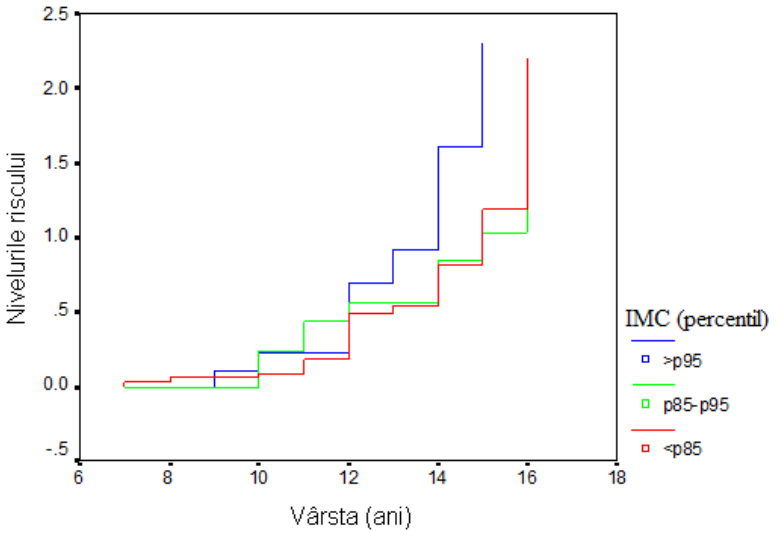

Fig. 2. Riscul apariției HTAE în relație cu valorile IMC

În relație cu stadiul HTA s-a remarcat frecvența crescută la copiii supraponderali mai frecvent asociată cu HTA stadiul 1, în timp ce la obezi s-a asociat cu HTA stadiul 2 (χ^2=17,51; GL=2; p=0,0002). Copiii cu IMC peste p 95, începând cu vârsta de 12 ani, prezintă cel mai mare risc de apariție a HTAE (fig. 2).

Analiza statistică a evidențiat faptul că vârsta gestațională sub 37 de săptămâni induce un risc crescut de apariție a HTA începând cu vârsta de 12 ani.

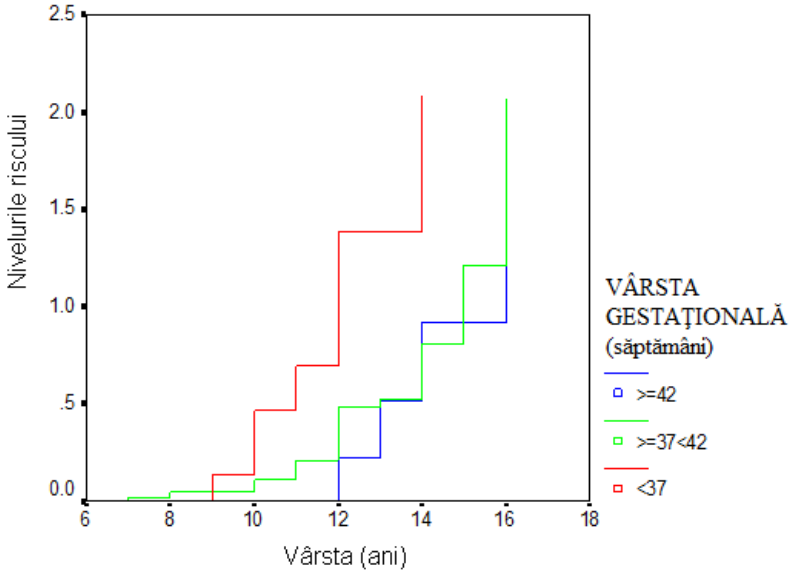

Fig. 3. Riscul apariției HTAE în relație cu vârsta gestațională

DISCUŢII

În relaţie cu stadiul HTAE lotul de studiu a fost împărţit în 3 grupuri. Astfel 10 (16,7%) copii s-au încadrat în grupul cu prehipertensiune, 38 dintre copii (63,3%) au fost incluşi în HTA stadiul 1 şi doar 12 dintre copii (20%) au aparţinut în grupul cu HTA stadiul 2. În studiul nostru s-a remarcat o creştere a frecvenţei HTA cu vârsta, cu o incidenţă mai mare la sexul masculin. Datele din literatură arată că nivelurile TA cresc odată cu vârsta la ambele sexe, iar diferenţele între acestea sunt remarcate precoce. La fete tensiunea arterială sistolică (TAS) creşte mai rapid între 6-11 ani comparativ cu perioada 12-17 ani. La băieţi se remarcă opusul acestui fenomen: o creştere mai rapidă se observă între 12-17 ani comparativ cu perioada 6-11 ani. Între 12-14 ani băieţii tind să aibă niveluri tensionale mai mari decât fetele (12). De asemenea, studiul de faţă a obiectivat o frecvenţă de 2,15 ori mai mare a HTAE la copiii din mediul urban. Acest fapt ar putea fi pus pe seama obiceiurilor alimentare nesanogene prezente frecvent în mediul urban, deşi diferenţa nu este semnificativă statistic. S-a remarcat asocierea dintre supraponderabilitate şi HTAE stadiul 1 şi dintre obezitate şi HTAE stadiul 2 ($\chi 2=17,51$; GL=2; p=0,0002). Copiii obezi prezintă cel mai mare risc de apariţie a HTA începând cu vârsta de 12 ani (fig. 2). Dacă la o determinare a prevalenţei HTAE la copil, după recomandările Task Force, aceasta era de aproximativ 1% (13), într-un studiu epidemiologie mai recent, realizat pe 5100 cazuri, prevalenta generală a HTA (după trei măsurători), a fost de 4,5% (14). Aceasta a fost explicată prin asocierea cu valorilor crescute ale IMC.

În ceea ce priveşte prematuritatea s-a remarcat un risc mai mare de apariţie a HTAE după vârsta de 12 ani, la copiii cu vârsta gestaţională mai mică de 37 de săptămâni (fig. 3). Studiile lui Barker (15) arată că asocierea independentă dintre greutatea mică la naştere şi riscul de hipertensiune şi boli cardiovasculare a fost evidenţiată la multiple populaţii din diverse zone geografice (16, 17, 18). În general copiii născuţi prematur prezintă un risc crescut de a dezvolta HTAE. Un studiu publicat în anul 2010 (19) a evidenţiat o TAS în medie cu 10 mmHg mai mare la adolescenţii şi adulţii tineri născuţi prematur comparativ cu cei născuţi la termen.

Pentru evaluarea afectării organelor ţintă secundar hipertensiunii a fost cuantificată hipertrofia ventriculară stângă prin intermediul IVMS.

26,7% dintre copiii din lotul de studiu au avut HVS, iar 15% dintre aceştia au fost diagnosticaţi cu HVS pe baza IMVS mai mare decât p 99, care se asociază cu un risc de morbiditate cardiovasculară de 4,1 ori mai mare la adulţii hipertensivi (9). Analizând valorile individuale ale IMVS, înregistrate la lotul de studiu, s-a constatat că statistic nu s-au evidenţiat diferenţe semnificative între sexe, medii de provenienţă şi grupuri de vârstă ale valorilor IMVS în contrast cu datele din literatură. Treiber et al (20) au investigat asocierea dintre IMVS şi TA la 84 de copii, cu istoric familial de HTA, demonstrând corelaţii semnificative între IMVS şi greutate, sex (mai mare la băieţi), vârsta, TAS de repaus.

CONCLUZII

În ultimii ani HTAE la copil a înregistrat o prevalenţă în creştere, urmare a acţiunii sinergice a multiplilor factori de risc implicaţi. Studiul nostru a remarcat un impact important al prematurităţii şi obezităţii asupra dezvoltării HTAE la copil. De asemenea, s-a evidenţiat o creştere a frecvenţei HTAE odată cu vârsta, atât la sexul masculin, cât şi

la feminin. Impactul HTA asupra organelor țintă, obiectivat prin IMVS, nu a fost dependent de vârstă, sex sau mediul de proveniență al copiilor incluşi în studiu.

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CONTRIBUŢII LA IMPLEMENTAREA UNUI TEST SCREENING DE APRECIERE A DEZVOLTĂRII NEUROPSIHICE LA COPIL

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CONTRIBUTIONS TO THE IMPLEMENTATION OF SCREENING TESTS FOR ASSESSING THE NEUROPSYCHIC DEVELOPMENT IN CHILDREN (Abstract): Currently, in Romania the general practitioner does not use screening tests to detect the developmental problems in children. That might be the reason why many cases are diagnosed too late. **Aim**: To evaluate the impact and the usefulness of a screening tool for neurodevelopmental disorders in primary care. **Material and methods**: This trans-sequential study was carried out a on a series of 324 children under the age of 5 in the interval January 2007–December 2010. The neurodevelopmental status of the children was assessed by two methods: routine clinical assessment, and ASQ screening tools. **Results**: Compared to routine assessment, by which 33 children (10,2%) with development delays have been detected, the use of parent-addressed questionnaires raised the detection percentage to 12% (6 more children). The use of parent-addressed questionnaires also improved the doctor-patient communication, increased the degree of parental involvement in their child's development stimulation, especially with regard to the malfunctions in verbal and cognitive behavior. **Conclusions**: The use of validated screening tests is a goal for improving the primary care practice. **Key words**: NEUROPSYCHIC DEVELOPMENT, SCREENING TEST, CHILD.

Medicul de familie, aflat în prima linie a sistemului medical, monitorizează dezvoltarea copilului prin evaluări periodice la diferite etape de vârstă. El este cel mai aproape de problemele familiei, poate sesiza şi corecta la timp carenţele nutriţionale şi de îngrijire, apreciază calitatea mediului familial, modul în care părinţii interacţionează cu copiii, condiţii care sunt esenţiale pentru dezvoltarea armonioasă, atât fizică, cât şi neuropsihică a acestora. În practica curentă, se efectuează o evaluare uzuală, nestandardizată a achiziţiilor psihomotorii ale copilului; depistarea întârzierilor în dezvoltare ţine atât de competenţa şi experienţa examinatorului, cât mai ales de timpul acordat consultaţiilor preventive. A depista cât mai precoce o tulburare în dezvoltarea neuropsihică a unui copil este de o importanţă majoră, deoarece aceasta este reversibilă până la un anumit nivel, recuperarea fiind posibilă doar dacă se corectează factorii stresanţi implicaţi. De aceea momentul intervenţiei este foarte important,

fiind condiţionat de precocitatea diagnosticului.

Studii de prevalenţă efectuate în Marea Britanie la nivelul anului 2005 de către Rydz et al. (1) arată o frecvenţă totală a dizabilităţilor în dezvoltarea copilului în primii 18 ani de viaţă, ce variază între 11,8 – 16,8%, cu impact negativ major asupra individului, a familiei şi a societăţii în ansamblu. Aceeaşi autori afirmă că, deşi există programe naţionale de supraveghere a dezvoltării neuropsihice a copilului, mai puţin de jumătate dintre pediatri utilizează testele screening, motiv pentru care doar 45 - 55% dintre copiii cu dizabilităţi (retard mental, disfuncţii ale limbajului etc.) sunt depistaţi până la vârsta şcolară, iar gradul de evidenţiere al tulburărilor de dezvoltare la copil, doar pe baza judecăţii clinice uzuale, nu depăşeşte 30%.

În Statele Unite, aproximativ 1 din 8 copii (12,5%) au dizabilităţi în dezvoltarea neuropsihică, tulburările din spectrul autist fiind diagnosticate la 1 copil din 110 (0,9%), cu o medie de 4 ani ½ a vârstei copilului în momentul diagnosticului (2).

În România, autismul - tulburare severă de dezvoltare de natură neurobiologică, rămâne o mare necunoscută. Cele mai multe dintre cazuri sunt diagnosticate târziu, după vârsta de 3 ani, deoarece nu există un sistem naţional de depistare timpurie, bazat pe verificarea indicatorilor de dezvoltare normală (motorie, cognitivă, emoţională), care să permită o orientare rapidă a medicului de familie spre o posibilă disfuncţie neuropsihică a copilului (www.autism.ro). În iulie 2010, Parlamentul României a adoptat legea autismului care prevede depistarea precoce a tulburărilor pervazive de dezvoltare prin aplicarea la nivelul practicii primare a unor chestionare tip screening, adresate tuturor copiilor cu vârste cuprinse între 1 şi 3 ani.

Studiul urmăreşte elaborarea unei metodologii pentru aprecierea dezvoltării neuropsihice a copilului la nivelul medicinii primare, care să permită intervenţia precoce şi creşterea interesului părinţilor pentru acest domeniu al dezvoltării copilului lor.

MATERIAL ŞI METODĂ

Studiul a fost efectuat în perioada ianuarie 2007 – decembrie 2010, utilizând un design mixt (studiu trans-secvenţial), pe un lot de 324 copii sub vârsta de 5 ani care au fost supravegheaţi longitudinal cu evaluare la diferite etape de vârstă. Subiecţii au fost selectaţi dintr-un grup de 425 de copii din Iaşi, născuţi în perioada octombrie 2004 – septembrie 2009, aflaţi în evidenţa cabinetului de medicină de familie, după următoarele criterii de excludere: copii cu malformaţii congenitale invalidante, cu encefalopatie hipoxic ischemică la naştere, hipotiroidism congenital, prematuri sub vârsta gestaţională de 35 săptămâni. Pentru toţi cei incluşi în lotul de studiu am solicitat şi am obţinut de la părinţi acordul de participare şi de folosire a datelor medicale, cu asigurarea confidenţialităţii şi protecţiei identităţii subiecţilor.

Consultaţia prevenţională de rutină a constat în: efectuarea unui examen clinic general, măsurători antropometrice, consemnate pe nomograme în percentile, aprecierea nestandardizată (după reperele cunoscute) a dezvoltării neuropsihice, consiliere preventivă şi nutriţională, imunizări.

La etapele de vârstă: 15 luni, 18 luni, 30 luni şi 4 ani, am aplicat părinţilor testelele ASQ 3+, respectiv ASQ – SE (Ages & Stages Questionnaires®, Brookes Publishing) şi le-am solicitat să răspundă cât mai obiectiv la itemii acestor chestionare pentru propriul copil.

Aceste teste screening sunt utilizate pe scară largă în Statele Unite, la nivelul asistenţei medicale primare, fiind un instrument de apreciere a dezvoltării neuropsihice la copil bazat pe interviul părinţilor (3-6). Sunt alcătuite din mai multe seturi de întrebări specifice pentru diferite etape de vârstă (pot fi testaţi copii între 1 şi 66 de luni) şi permit evaluarea la nivelul comportamentului verbal, motor, cognitiv şi social-afectiv. Itemii calculaţi încadrează copiii în trei categorii: dezvoltare neuropsihică în parametrii vârstei, dezvoltare la limită şi tulburare în dezvoltare care necesită asistenţă specializată. Academia Americană de Pediatrie consideră că acest test de screening are cel mai bun raport cost–beneficiu (7) iar implicarea părinţilor în supravegherea şi stimularea dezvoltării neuropsihice a copiilor are o importanţă deosebită întrucât aceştia, bine informaţi, se dovedesc capabili să ofere cele mai bune detalii (8).

Pentru efectuarea screeningului a fost întocmit un chestionar în care s-au consemnat datele generale şi medicale legate de evoluţia copilului, particularităţi ale vieţii de familie, stilul comportamental parental, metode de stimulare a dezvoltării neuropsihice utilizate de către părinţi.

După aplicarea screeningului, rezultatele au fost discutate cu familia. În condiţiile în care punctajele obţinute au fost la limită pe unul sau mai multe domenii de comportament, am recomandat stimulare şi am recurs la repetarea testului peste un interval de 1-2 luni. În cazurile în care punctajele au fost sub limita vârstei, pe cel puţin 1 domeniu de comportament, am recomandat evaluare de specialitate (pediatru, psihiatru, neurolog, psihilog, după caz).

Este importantă examinarea copiilor în condiţii optime şi realizarea unei bune comunicări cu părinţii acestora care pot fi îngrijoraţi în legătură cu rezultatele testării. Medicul trebuie să se asigure că familia a înţeles raţiunea şi utilitatea testului şi mai ales faptul că nu este un test diagnostic.

Prelucrarea şi interpretarea datelor acumulate s-a realizat prin metode descriptive, analitice şi statistică matematică (pragul de semnificaţie 95%, testele χ^2 şi *t-Student*).

REZULTATE ŞI DISCUŢII

1. *Caracteristicile lotului de studiu.* Lotul de 324 copii a fost omogen în ceea ce priveşte mediul de proveniență urban şi distribuţia pe sexe 163 băieţi (50,30%) şi 161 fetiţe (49,7%).

Vârsta părinţilor variază pentru mame între 20 – 39 de ani şi pentru taţi între 20 – 60 ani. Vârsta medie a mamei a fost semnificativ mai mică decât a tatălui (t-Student = 7,39; GL=646; p<0,001). Din familii cu nivel de trai mediu au provenit 85,8% dintre copii iar 66,7% au ambii părinţi cu studii superioare. Numărul de copii ai unei familii a fost invers proporţional cu nivelul de trai şi cu gradul de educaţie al părinţilor. Majoritatea familiilor de intelectuali au un singur copil (χ^2=15,58; GL=2; p=0,0004) şi o preocupare mai mare a mamei pentru carieră (χ^2=16,04; GL=2; p=0,0003).

2. *Depistarea tulburărilor în dezvoltarea neuropsihică a copiilor* s-a ameliorat prin utilizarea chestionarelor individuale adresate părinţilor. Comparativ cu evaluarea uzuală, prin aplicarea screeningului ASQ am identificat încă 72 de copii cu întârzieri în dezvoltare, mai ales din rândul celor necooperanţi, la care examenul clinic a fost constant dificil. Diferenţa dintre metode a fost semnificativă statistic (χ^2= 40,77; GL=2; p<0,001) (tab. I).

Analizând întârzierile în dezvoltare pe domenii comportamentale am observat că

cele mai frecvente s-au înregistrat la nivel social-afectiv şi verbal (limbajul expresiv), urmate de cele din domeniul cognitiv. Învăţarea limbajului este un proces dependent psiho-emoţional, 21 dintre copiii care au necesitat asistenţă de specialitate pentru dificultăţile de comunicare având şi diverse tulburări în dezvoltarea social-afectivă (fig. 1).

TABELUL I

Gradul de depistare al întârzierilor în dezvoltarea neuropsihică a copiilor în funcţie de metoda de examinare utilizată

Lot 324 copii	Dezvoltare în parametrii vârstei		Dezvoltare la limită		Întârzieri în dezvoltare care necesită asistenţă de specialitate		Semnificaţie statistică
	Nr.	%	Nr.	%	Nr.	%	
Evaluarea uzuală	246	75,9	45	13,9	33	10,2	χ^2=40,77; GL=2; p<0,001
Aplicarea testelor screening	174	53,7	111	32,4	39	12	

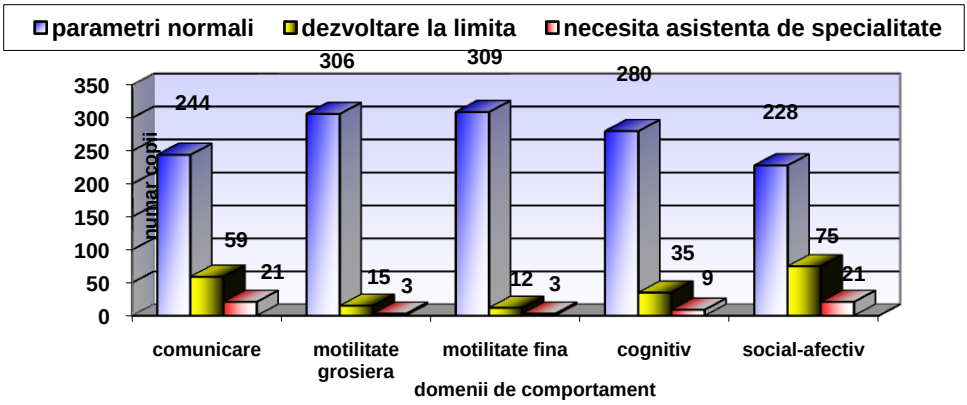

Fig. 1. Dezvoltarea neuropsihică pe domenii comportamentale a celor 324 de copii din lotul de studiu

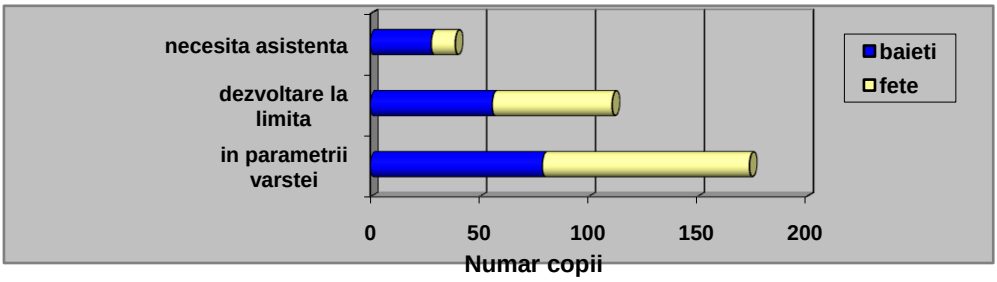

Fig. 2. Dezvoltarea neuropsihică la cei 163 de băieţi şi 161 de fete din lotul studiat

În lotul nostru, frecvenţa tulburărilor în dezvoltare care necesită asistenţă specializată, a fost de 12% (39 copii din 324), comparabilă cu 11,7% înregistrată în singurul studiu naţional efectuat în ultimii 25 de ani, proiectul CENTAUR (9). În grupul copiilor cu dizabilităţi am constatat o predominanţă a băieţilor (28 de băieţi faţă de 11 fetiţe), mai ales în ceea ce priveşte dezvoltarea achiziţiilor verbale şi social-afective (fig. 2).

Diferenţele înregistrate între sexe au fost semnificative statistic (χ^2=8,88; GL=2; p=0,012) şi concordă cu datele din literatură care arată o afectare mai crescută a băieţilor comparativ cu fetele, cu o rată de 4/1 pentru tulburările din spectrul autist şi de 7/3 sau chiar mai mare, în cazul ADHD (10).

Incidenţa tulburărilor din spectrul autist în lotul nostru a fost de 0,6% (2 băieţi din 324 copii), comparabilă cu frecvenţa actuală raportată în studiile internaţionale (0,2-0,7%) (11). Frecvenţa ADHD (Attention Deficit Hyperactivity Disorder) a fost de 0,9% (3 băieţi din 324 copii), valoare apropiată de cea raportată în studiul efectuat în 2005, de către Doina Felea şi colab. care arată o incidenţă de 1,1% a acestei afecţiuni la grupa de vârstă 0-6 ani (10). Cei cinci copii au fost suspectaţi prin evaluarea uzuală, confirmaţi prin testele screening aplicate şi sunt incluşi în prezent în programe terapeutice specifice, iar beneficiile au devenit vizibile.

Complianţa familiilor în ceea ce priveşte efectuarea consultaţiilor prevenţionale a fost foarte bună la 92% dintre copii, doar în 27 de cazuri (8%) au existat uneori dificultăţi de comunicare, reflectate şi în promptitudinea cu care aceştia au răspuns la chestionarele adresate. După aplicarea testelor screening am constatat o creştere semnificativă a ponderii întrebărilor adresate de către părinţi în legătură cu dezvoltarea neuropsihică a copiilor; practic procentul s-a dublat (de la 55,2% la 100%).

Prin testele screening am descoperit copii cu întârzieri în dezvoltare datorită stimulării deficitare şi cu tulburări de comportament determinate de o abordare educaţională neadaptată temperamentului copilului.

3. *Evaluarea şi consilierea de specialitate* a fost recomandată la 132 de copii (40,7%). Au fost evaluaţi 97 (73,5%), rezultatele testului screening aplicat s-au confirmat la 90 dintre aceştia (93%). Menţionăm că în unele cazuri, mai ales în cele problemă, părinţii au refuzat la început să accepte ideea că ar putea exista o tulburare în dezvoltarea propriului copil şi doar atunci când aceasta s-a agravat şi a devenit vizibilă pentru un nespecialist, au decis să se adreseze medicului, fiind întârziată precizarea diagnosticului şi integrarea într-un program terapeutic adecvat.

Pe durata efectuării cercetărilor s-a constatat o accesibilitate redusă a pacienţilor la testarea şi consilierea psihologică. În sistemul de asigurări de sănătate din România, serviciile menţionate nu sunt gratuite, motiv pentru care, datorită dificultăţilor financiare, puţine familii şi-au permis să utilizeze serviciile de parenting sau să apeleze la consiliere psihologică legată de dezvoltarea copilului lor. În ceea ce priveşte tratarea şi recuperarea unui copil diagnosticat cu tulburare pervazivă de dezvoltare, părinţii au posibilitatea să acceseze servicii parţial compensate. Datorită complexităţii terapiei, mama este obligată să fie tot timpul în preajma copilului, ea trebuie să renunţe la serviciu, la cariera personală, motiv pentru care veniturile familiei scad. Această realitate este confirmată şi de ultimul studiu efectuat de către Norra MacReady "Autism Often Associated With Loss of Family Income" (12), care arată că familia unui

copil autist este supusă unui dublu stres, atât emoţional cât şi financiar. Un copil cu autism necesită o terapie complexă care implică cel puţin 5 specialişti, mama este angajată în recuperarea copilului său, într-o muncă "full-time", nerecompensată financiar ci doar prin satisfacţia de a observa progresele copilului.

Luând în considerare criteriile de selecţie ale subiecţilor studiului şi faptul că majoritatea provin din familii cu condiţii de viaţă bune, numărul total al copiilor cu întârzieri în dezvoltare este ridicat, mai ales în ceea ce priveşte comportamentul verbal şi achiziţiile cognitive. Această situaţie ar putea fi legată de caracteristicile vieţii de familie din România unde, părinţii copleşiţi de obligaţiile zilnice îşi petrec din ce în ce mai puţin timp cu copiii, acordând atenţie mai mult nevoilor fiziologice ale acestora şi neglijându-le pe cele psihologice.

CONCLUZII

1. Nivelul de depistare al tulburărilor în dezvoltarea neuropsihică a copiilor a crescut prin utilizarea chestionarelor individuale adresate părinţilor, iar deficienţele identificate au fost confirmate de evaluările ulterioare de specialitate.

2. Testele screening ASQ, accesibile atât pentru părinţi cât şi pentru medic, prezintă avantajul că nu prelungesc durata consultaţiei şi asigură o deschidere spre comunicarea mai bună cu familia.

3. Experienţa dobândită prin aplicarea acestui screening de apreciere a dezvoltării neuropsihice la copil poate fi utilă pentru introducerea unor teste standardizate şi adaptate pentru a fi folosite în practica medicului de familie din România.

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SELF-NEGLECT IN THE CASE OF THE ELDERLY. WHERE ARE WE NOW?

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SELF-NEGLECT IN THE ELDERLY: WHERE ARE WE NOW? (Abstract): Self-neglect in an elderly person is a behavior that threatens his/her own health and safety. It is present when a person refuses to adequately feed, water, shelter, or clothe himself, refuses medication or medical care, and personal safety measures. **Material and method:** This is a multicentric study of self-neglect in three geriatric units from Finland, Greece and Romania The medical, social, psychological and behavioral profile analysis was based on a questionnaire; this questionnaire relied on existing studies and social, economic and medical facts in the three countries. The cognitive function, nutritional status, and the presence or absence of depression have also been assessed. **Results and discussions:** The data obtained until now support the importance of self-neglect among the elderly. The social-medical network should not only identify, diagnose, prevent, and treat the elderly affected by the phenomenon of self-neglect, but also educate the society to help them and, moreover, to prevent their marginalization. **Key words:** SELF-NEGLECT, ELDERLY, ABUSE

Demographic prognoses shows that world population is in a continuing process of ageing. In 2006, almost 500 million people worldwide were aged 65 and older. By 2030, this figure is expected to increase up to 1 billion, 1 in every 8 of the earth's inhabitants being an elderly-aged. Europe and Romania follow the same trend: in the case of the latter, the elderly population will increase by 58 million - i.e. 77% - till 2050.(1) EUROPOP2008 convergent scenario shows that in 2030 Romania will have a total population of 20 million, of which over 4 million (20%) will be elderly, with one million being of forth age. Estimates show that in 2060, Romania will have a third of its population aged over 65 (i.e. 35%) (2).

In this context, both society and medical services will increasingly confront themselves with problems and disorders that are specific to the elderly. Elderly self-neglect is one of them, since its increasing incidence it is already considered a big concern for medical services, social services, family and community.

The phenomenon of self-neglect has been subject of documentation over centuries, but it was first officially described in 1966 as the „Senile Breakdown Syndrome" or Diogenes Syndrome. This term refers to cases of severe self-neglecting with mental

illnesses, personality disorders, being also identified in the case of the youngsters. In the late 70's the term „self-neglect" came into use. Nowadays self-neglect is recognized in various countries, including in some of the European Union, as a form of elder abuse; in the United States it is mentioned in the Older Americans Act Amendments of 1987 (3-7).

Self-neglect is a multifactorial behavioral entity through which the elderly threatens their own safety or health and it is described in a person who can't provide for his/herself adequate food, water, clothing, shelter, personal hygiene, medication, safety precautions and social needs. This behavior is considered to be caused by mental, social, economical and physical disturbances, and thus is expected to promote and exacerbate such disturbances.(8) In the United States, elder self-neglect represents half or more of all cases reported to adult protective services and their number is in constant growth.(9) Incidence rate of 23/1000 is estimated to be similar to the incidences of new onset diabetes, and cataracts in the elderly and affect 3-8% of elderly population. Despite the fact that self-neglect is a factor in the rising necessity of the elderly institutionalization, it is also a marker in the growth of the mortality rate, regardless of the cognitive function or the physical shape (10-13).

MATERIAL AND METHOD

Given the lack of substantial data regarding the phenomenon of elderly self-neglect in Europe, we initiated a comparative study of elderly people falling into the incidence of the self-neglect, in terms of medical, social, economic, psychological and behavior. This prospective study was launched in 2010 and is currently ongoing in three geriatric clinics in Finland, Greece and Romania. The enrolled patients were selected from the elderly who presented themselves in the emergency room of the "Dr. C. I. Parhon" Hospital of Iasi with various acute problems; they were brought in by family, neighbors, acquaintances or ambulance crew. The subjects showed either decompensate chronic diseases or signs of severe self-neglect: severe malnutrition, severe dehydration with electrolytes imbalance, pressure sores, and insect infestations. Patients were eligible for the study if they met at least one of following criteria:

1. Persistent neglect of self-hygiene or home environment;
2. Repeated refusal of one/all services indicated for improving quality of life;
3. Self-endangerment through manifestation of unsafe behaviors (e.g. persistent refusal to treat a wound, creating life-hazards in the home, alcohol abuse).

On the basis of the current legislation, as well as a series of data from the medical literature, and socio-economical, medical, psychological and behavioral realities from the three participating countries, we conceived a questionnaire that was conducted by every participant. We also assessed the cognitive function, the nutritional status and the presence or absence of depression.

Through the first part of the questionnaire, we collected data with regards to the **socio-demographic component** of the geriatric assessment: age, gender, educational level, income, presence or absence of familial support and living conditions (alone or in company).

The second part of the questionnaire contains data on **patients' personal hygiene**:

- general aspect and general hygiene status;
- skin aspect: clean, dirty, dry, the presence of bruises, pressure sores, insect bites:
- skin appendages aspect: nails (clean / dirty, trimmed/long) and hair (clean, dirty,

combed, pediculosis);

- oral cavity aspect including teeth;
- clothes aspect: clean/soil, torn, stains / smells of feces or urine

These data were collected upon arrival of the patients by our clinics medical personnel participating in the study, and reviewed upon subsequent evaluations during hospitalization.

As part of the comprehensive geriatric evaluation, were evaluated the following indicators: **cognition -** by performing Mini Mental State Examination (MMSE), adapting scores according to educational level, presence of motor imbalance, visual or hearing deficit; **nutritional status -** by collecting data from patients or caregivers and completing the Mini Nutritional Assessment (MNA©); and **mental status –** evaluating the presence of depression by completing the Geriatric Depression Scale (GDS-4). The medical personal enrolled in the study submitted these standardized tests. A number of elderly patients could not complete the entire battery because of inability or unwillingness.

Another part of the self-neglect evaluation is represented by the data about **living conditions and household**. Related to this aspect, our questionnaire includes a series of questions addressed to the caregiver, if applicable. In some cases these questions were answered by the ambulance crew who attended the patient to the hospital. The questions about environment conditions were developed on the basis of the following issues:

- general household condition;
- the detailed aspect of all rooms including the garden and the yard (if applicable): neat/ squalor, mess, deteriorated furniture, drapers/carpets/sheets torn, human/animal waste;
- aspect of walls, sealing, windows;
- presence or functionality of household utilities (electrical installation, sanitation, gas);
- presence / absence of insects in the house or pets' living condition (if applicable)

The evaluation continues with the **chronic medical conditions.** The data were collected directly from the patient or, when this was not possible, from caregivers, previous medical documentation or previous admissions. In the presence of co morbidities, we evaluated the medication, the compliance with the treatment and the possible need to use different types of aids (hearing, dentures, eyeglasses, walkers, wheelchairs, etc.).

The last part of the questionnaires consists in questions witch evaluate if the elderly are aware of their self-neglect state, if this bothers them and, to include all data we acquired, is aimed at identifying the main cause of the phenomenon of self-neglected on the evaluated subjects.

The study' results will be centralized and completed by the Finnish partners of the project.

RESULTS AND DISCUSSION

The partial obtained data confirm the importance of self-neglect among the elderly, especially as it accompanied by the low capacity to perform activities of daily living, this leading to a poor personal hygiene, an insufficient care of the home environment, a neglect of medical conditions and the lack of treatment for them. The problem is more important as it has been demonstrated that the self-neglect thus reported and confirmed is associated with increased mortality, especially in the first year of diagnosis. This aspect has direct implications on social and medical services which should identify and intervene at an early stage in the cases of self-neglect of the elderly.

The intervention team must be complex, multidisciplinary, specialized in elderly problems and should include geriatrics,

psychologists, nurses, family doctor, social services assistant, lawyers and especially family and community)(12-16).

The measures to be applied in order to help the elderly self-neglect should consider several ethical issues:

- the people's right to autonomy but, at the same time, the obligation of the society and the medical services to act as to ensure the patients' well-being;
- the assessment of the decisional capacity, taking into account the clinical and the legal aspects with secondary implications;
- the situation in which the self-neglected elderly, through their conduct, put themselves and the others in danger

Taking these aspects in account, the social-medical network must act not only to identify, diagnostic, prevent and treat the elderly affected by the phenomenon of self-neglect, but also to educate the society which should help them and moreover, avoid marginalizing them.

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PERITONITA BACTERIANĂ SPONTANĂ: COMPLICAŢIE SEVERĂ A CIROZEI HEPATICE – STUDIU PROSPECTIV

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SPONTANEOUS BACTERIAL PERITONITIS: A SEVERE COMPLICATION OF LIVER CIRRHOSIS (Abstract): Spontaneous bacterial peritonitis (SBP) is a frequent and severe complication of decompensated cirrhosis. SBP is defined as an infection of initially sterile ascitic fluid (AF) without a detectable, surgically treatable source of infection. **Aim:** to evaluate the prevalence, incidence, pathogens and clinical outcome of patients with liver cirrhosis and SBP. **Methods:** a prospective study evaluated 34 episodes of SBP developed in 29 patients with cirrhosis and ascites admitted in hospital. SBP was diagnosed based on a polymorphonuclear cell count in ascitic fluid of >250 cells/mm^3 in the absence of data compatible with secondary peritonitis. **Results:** The mean age of the group was 56.89 ± 8.79 years, more frequently occurred in men 21 of 29 cases (72.41%). Microorganisms were isolated in 12 episodes (35.29%): Gram-negative bacteria in 8 (66.67%) and Gram-positive in 4 (33.33%). The first-line treatment failure and in-hospital mortality rates were 26.47% and 17.64%, respectively. The low in-hospital mortality seems to be related to earlier diagnosis and treatment. In the multivariate analysis, three significant independent predictive factors of in-hospital mortality were identified: a high Model of End-Stage Liver Disease (MELD) score, renal failure and SBP caused by extended-spectrum â-lactamase-producing organisms. The relapse rate of PBS was 21.73%. **Conclusions:** SBP remains to be a serious complication, especially in patients with a high MELD score. Ceftazidim represents a good choice in empirical treatment of SBP. **Key words:** LIVER CIRRHOSIS, PERITONITIS, TREATMENT, PROGNOSIS

Termenul de peritonită bacteriană spontană (PBS) a fost introdus în 1971 de către Conn şi Fessel, PBS fiind definită ca infecţia spontană a lichidului ascitic în absenţa identificării oricărei surse infecţioase intraabdominale (1).

Ciroza hepatică (CH) se caracterizează printr-o susceptibilitate crescută la infecţii datorită deficienţelor mecanismelor de apărare ale organismului. PBS este cea mai frecventă infecţie la bolnavii cirotici, urmată de infecţiile tractului urinar (20%), pneumonie (15%) şi bacteriemie (12%) (2). Recunoscută ca o complicaţie frecventă şi severă, PBS apare la 8-27% din pacienţii cirotici cu ascită spitalizaţi (3).

Supravieţuirea acestor bolnavi depinde de precocitatea diagnosticului şi tratamentului şi de instituirea profilaxiei secundare a PBS. Având în vedere că PBS poate evolua asimptomatic sau insidios, se recomandă efectuarea paracentezei diagnostice în toate cazurile de CH cu ascită şi când apar manifestări clinice sugestive precum durerile abdominale, febra, encefalopatia hepatică, agravarea disfuncţiei renale sau hepatice (4). Prezenţa în lichidul de ascită a peste 250 neutrofile/mm^3 semnifică suprainfecţia

bacteriană a acestuia (PBS).

Tratamentul antibiotic empiric trebuie început imediat ce diagnosticul a fost stabilit, înainte de a se cunoaşte agentul etiologic şi sensibilitatea *in vitro* (4). Bacteriile cel mai frecvent identificate în 65% din cazurile cu culturi pozitive sunt bacteriile Gram-negative din familia *Enterobacteriaceae*, în special *Escherichia coli* şi *Klebsiella pneumoniae*. Restul agenţilor microbieni sunt reprezentaţi de cocii Gram-pozitivi (5). Cefalosporinele de generaţia a III-a (cefotaxim, ceftriaxona, ceftazidim) şi combinaţia amoxicilină-acid clavulanic, reprezintă antibioticele de primă intenţie în tratamentul PBS.

Prognosticul pe termen lung al bolnavilor cu PBS este nefavorabil. Rata recurenţei PBS în primul an este de 40-70%, iar supravieţuirea la 1 an şi la 2 ani, după un episod de PBS este de aproximativ 30-40%, respectiv 20% şi chiar mai scăzută la pacienţii cu scor Child-Pugh peste 10 (6). Profilaxia PBS este indicată la următoarele 3 categorii de pacienţi cirotici: antecedentele de PBS, bolnavii spitalizaţi pentru hemoragie digestivă superioară şi cei cu conţinut scăzut de proteine în lichidul de ascită. Utilizarea pe termen lung a antibioticelor cu spectru larg poate determina creşterea numărului microorganismelor rezistente la antibiotice cu implicaţii considerabile asupra managementului şi tratamentului PBS, conducând la necesitatea reevaluării ghidurilor terapeutice actuale (6).

Scopul studiului prezent de a evalua trăsăturile clinice şi prognosticul bolnavilor cu ciroză hepatică complicată cu PBS internaţi în Clinica de Gastroenterologie a Spitalului Clinic Judeţean de Urgenţă „Sfântul Apostol Andrei" Galaţi.

MATERIAL ŞI METODĂ

Studiul prospectiv a inclus 122 bolnavi cu CH şi ascită internaţi în Clinica de Gastroenterologie a Spitalului Clinic Judeţean de Urgenţă „Sfântul Apostol Andrei" Galaţi în perioada 1 ianuarie 2007-31 decembrie 2007 şi monitorizaţi pe o perioadă de 36 luni. Diagnosticul CH s-a stabilit pe baza criteriilor clinice, ecografice şi histologice. Severitatea cirozei hepatice s-a apreciat folosind scorurile Child-Pugh şi MELD (model for end-stage liver disease). Scorul MELD s-a calculat utilizând formula: 9,57 x log e (creatinina, mg/dl) + 3,78 x log e (bilirubina, mg/dl) + 11,2 x log e (INR) + 6,43.

Bolnavii au fost evaluaţi anamnestic, clinic, biologic (teste standard de laborator, incluzând hemoleucograma, teste biochimice hepatice şi renale, indice de protrombină, examen de urină) şi imagistic (ecografie abdominală, radiografie toracică). Paracenteza diagnostică şi cultura lichidului de ascită s-au efectuat la internarea pacienţilor şi în cazul apariţiei în cursul spitalizării a simptomelor şi semnelor sugestive pentru PBS (febră, dureri abdominale, alterarea statusului mintal, hipotensiune arterială, deteriorarea funcţiei renale, leucocitoză) în absenţa altor factori precipitanţi identificabili. Lichidul de ascită s-a recoltat în recipiente sterile, respectând condiţiile de asepsie. În fiecare recipient cu mediu de cultură MacConkey agar s-au inoculat cel puţin 10 ml de lichid de ascită, iar bacteriile identificate prin cultură au fost testate pentru sensibilitate la antibiotice prin metoda difuziunii.

Diagnosticul PBS s-a stabilit prin evidenţierea în lichidul de ascită a peste 250 PMN / mm^3 în absenţa semnelor clinice şi radiologice de peritonită bacteriană secundară. Peritonita bacteriană secundară se defineşte prin identificarea în lichidul de ascită a două sau mai multe microorganisme sau asocierea PMN $>$ 250 /mm^3 lichid ascită cu cel puţin doi din următorii trei parametri: glucoza $<$ 50 mg/dl, proteine totale $>$ 1g/dl şi lactat dehidrogenaza (LDH) peste limita superioară a normalului a LDH seric.

Bolnavii cu PBS au primit tratament empiric cu Ceftazidim (cefalosporină de gene-

TABELUL I
Caracteristicile lotului studiat

Vârsta (ani)	56,89±8,79
Bărbaţi/Femei	21/8
Cauza cirozei, n (%)	
Consum de alcool	20 (68,96)
Virus hepatitic C	10 (34,48)
Virus hepatitic B	8 (27,58)
Clasa Child-Pugh (media ± DS)	11,68 ± 2,28
Scorul Child-Pugh, n (%)	
Clasa Child B	7 (24,14)
Clasa Child C	22 (75,86)
Scorul MELD	21,8 ± 7,4
Manifestări clinice, n (%)	
Febră	18 (52,94)
Dureri abdominale	14 (41,17)
Greaţă/vărsături	7 (20,58)
Encefalopatie hepatică	10 (29,41)
Hemoragie gastro-intestinală	6 (17,64)

raţia a III-a) intravenos, 1-2g/12 ore, minimum 5 zile, întrucât aceasta a reprezentat opţiunea terapeutică disponibilă pe perioada spitalizării, studiile indicând o eficienţă similară cu a cefotaximului. Doza antibioticului a fost ajustată conform funcţiei renale pe perioada tratamentului. În cazurile cu eşec terapeutic la antibioticul iniţial, tratamentul a fost adaptat conform antibiogramei sau empiric.

Paracenteza diagnostică s-a repetat după 48 ore de la începerea antibioterapiei pentru stabilirea eficienţei acesteia. Lipsa de răspuns la tratament s-a considerat în cazurile ce au prezentat alterarea stării generale, scăderea PMN < 25% faţă de valoarea iniţială, menţinerea culturilor pozitive după 48 ore de antibioterapie sau care au decedat înaintea repetării paracentezei. Vindecarea PBS s-a apreciat în condiţiile remiterii tuturor semnelor clinice de infecţie, scăderii PMN în lichidul de ascită < 250/ mm^3, normalizării leucocitelor în sângele periferic şi negativării culturilor din lichidul de ascită. Bolnavii care au primit chinolone p.o. mai mult de o lună înaintea episodului de PBS au fost incluşi în grupul cu antibioticoprofilaxie. Mortalitatea intraspitalicească a fost definită ca deces de orice cauză în cursul spitalizării.

Datele au fost exprimate ca medie ± deviaţie standard (DS). Pentru compararea grupurilor s-au utilizat testele *t-student* (pentru date cantitative) şi χ^2 (pentru date calitative). Prin analiză univariată au fost identificaţi factorii predictivi ai mortalităţii intraspitaliceşti. Variabilele analizate au fost vârsta, sexul, cauza cirozei, PBS, datele clinice şi de laborator efectuate (inclusiv scorurile Child-Pugh şi scorul MELD). Variabilele cu semnificaţie statistică ($p < 0,05$) la analiza univariată au fost folosite la analiza multivariată, aplicându-se analiza regresivă logistică binară „step-wise" pentru identificarea factorilor predictivi ai supravieţuirii. În scopul determinării semnificaţiei datelor la analiza multivariată s-a utilizat intervalul de încredere (IC) de 95%, valoarea $p < 0,05$ fiind considerată statistic semnificativă. Datele au fost prelucrate folosind programul *SPSS 17.0*.

REZULTATE

Studiul a inclus 34 episoade de PBS dezvoltate de 29 pacienţi (21 bărbaţi şi 8 femei) din cei 122 bolnavi cu CH şi ascită internaţi în clinică, stabilindu-se o prevalenţă a PBS de 23,77%. Caracteristicile demografice şi

clinice ale cazurilor selectate la momentul diagnosticului PBS sunt prezentate în tabelul I. Vârsta medie a fost 56,89±8,79 ani, iar sexul masculin a fost predominant (72,41%). Consumul de alcool a reprezentat cauza cea mai frecventă a CH (68,96%), urmat de infecţia cu virus hepatitic C (34,48%) şi virus hepatitic B (27,58%). Din punct de vedere al clasificării Child-Pugh, a predominat clasa Child C (75,86%), urmată de Child B (24,14%); nici un bolnav nu s-a situat în clasa Child A. Scorul MELD mediu a fost de 21,8 ± 7,4. Majoritatea cazurilor cu PBS au fost simptomatice, febra şi durerile abdominale, fiind cel mai frecvent întâlnite în 18 cazuri (52,94%), respectiv 14 cazuri (41,17%). Encefalopatia hepatică la momentul diagnosticului PBS a fost prezentă în 10 cazuri (29,41%) şi ca simptom unic al PBS în 3 cazuri (8,82%), iar insuficienţa renală în 13 (38,23%) din cazuri. Identificarea factorilor de risc pentru apariţia PBS a fost posibilă în 14 cazuri: 5 cazuri cu antecedente de PBS (4 dintre acestea având antibioticoprofilaxie pentru PBS la momentul internării), 6 cu hemoragie digestivă superioară şi 3 cu proceduri invazive (tab. I).

Cultura pozitivă din lichidul de ascită s-a înregistrat în 12 (35,29%) din cele 34 de cazuri de PBS. Bacteriile Gram-negative (BGN) au reprezentat 66,67% din totalul germenilor identificaţi (8 din 12 cazuri). *Escherichia coli* a fost cel mai frecvent întâlnită (5 cazuri, 41,66%), urmată de *Klebsiella pneumoniae* (3 cazuri, 25%). Cocii Gram-pozitivi au fost prezenţi în 4 cazuri (33,33%) (2 *Streptococcus pneumoniae*, 1 *Staphylococcus aureus* şi 1 *Enterococcus faecalis*). Comparând grupurile de PBS determinate de bacterii Gram-pozitive şi Gram-negative s-a constatat că distribuţia pacienţilor nu a fost semnificativ diferită în ceea ce priveşte scorul Child-Pugh ($p = 0,083$) şi mortalitatea intraspitalicească ($p = 0,246$), însă scorul MELD, leucocitele, PMN şi proteinele din lichidul de ascită (LA) au fost semnificativ diferite între cele 2 grupuri ($p = 0,042$, 0,026, 0,029 şi, respectiv, 0,027). În acelaşi timp, scorurile Child-Pugh şi MELD au prezentat valori mai reduse în cazurile de PBS cu bacterii Gram-pozitive (BGP) (tab II).

Testarea sensibilităţii *in vitro* a bacteriilor a arătat că 5 bacili Gram-negativi (62,5%) au fost rezistenţi la Ampicilină şi 2 (25%) la Ciprofloxacin. Rezistenţa la Ceftazidim s-a înregistrat în 4 cazuri (2 *Escherichia coli*, 1 *Klebsiella pneumoniae* şi 1 *Enterococcus faecalis*), identificate ca fiind bacterii producătoare de beta-lactamază cu spectru extins (BLSE), sensibile, însă, la Imipenem şi Meropenem.

Tratamentul empiric cu Ceftazidim s-a început în 26 cazuri (76,47%). În celelalte 8 cazuri, s-a folosit un alt antibiotic întrucât: 6 au dezvoltat PBS în pofida tratamentului cu cefalosporine iniţiat în scop profilactic înaintea procedurilor invazive sau pentru HDS), iar 2 pacienţi prezentau alergie la cefalosporine. Antibioticele de primă linie au fost administrate timp de 9±4 zile. Răspunsul la tratament s-a constatat în 19 cazuri tratate iniţial cu Ceftazidim (73,07%), în timp ce în 5 cazuri (19,23%) a fost necesară schimbarea terapiei empirice datorită scăderii cu < 25% a PMN în lichidul de ascită la paracenteza efectuată după 48 ore de la începerea antibioterapiei. În aceste cazuri, tratamentul antibiotic a fost schimbat cu Imipenem în 4 cazuri şi Ciprofloxacin într-un caz. În cele 5 cazuri cu eşec terapeutic la Ceftazidim bacteriile identificate au fost fie rezistente la Ceftazidim (4 producătoare de BLSE – 2 *Escherichia coli*, 1 *Klebsiella pneumoniae* şi 1 *Enterococcus faecalis*), fie au avut o sensibilitate redusă la Ceftazidim (*Staphylococcus aureus*). Dintre bolnavii care au primit ca tratament de primă intenţie Ceftazidim, 2 au decedat înaintea începerii antibioterapiei de linia a doua, iar doi în decurs de 72 ore după schimbarea tratamentului antibiotic.

Eşecul la tratamentul empiric s-a produs în 9 cazuri (26,47%): 7 cazuri tratate cu

TABELUL II
Caracteristici clinice şi biologice ale pacienţilor şi bacteriilor izolate

	BGP (n=4)	BGN (n=8)	Valoare p
Vârsta (ani)	56±6	55±8	0,762
Bărbaţi (%)	3 (75%)	6 (75%)	0,501
Bolnavi cu scor Child C, n (%)	3 (75%)	5 (62,5%)	0,091
Scor Child-Pugh	10,7±1,2	11,6±1,1	0,083
Scor MELD	16,3±5,1	21,1±8,4	0,042
Leucocite	10247,4±2312,8	8604,5±954,8	0,236
PMN	7880,9±1680,9	4870,9±589,9	0,112
Leucocite în LA	3853,7±1280,3	1816,7±308,6	0,026
PMN în LA	2818,4±1041,5	1265,1±216,2	0,029
Proteine în LA (g/dl)	1,43±0,35	0,87±0,41	0,027
Uree	110,8±32,7	84,7±6,4	0,341
Creatinină	1,8±0,6	1,9±0,4	0,681
Bilirubină	7,6±3,1	6,0±0,9	0,255

Ceftazidim, un caz tratat cu Ciprofloxacin şi un caz tratat cu Amoxicilină-acid clavulanic. Analiza multivariată a identificat scorul MELD la internare (p = 0,027) şi PBS produse de bacterii producătoare de BLSE (p = 0,004) ca factori predictivi independenţi pentru eşecul tratamentului antibiotic empiric. Remiterea PBS s-a obţinut în 22 din 26 cazuri tratate iniţial cu Ceftazidim (84,61%), 4 bolnavi decedând. Decesul a survenit în 2 din cele 8 cazuri care au primit ca tratament de primă linie un antibiotic diferit de Ceftazidim. Mortalitatea intraspitalicească a fost de 17,64%. Cauzele deceselor au fost reprezentate de: hemoragie digestivă superioară (3 cazuri), sindrom hepato-renal (2 cazuri) şi encefalopatie hepatică (comă) 1 caz. Pe parcursul perioadei de monitorizare recurenţa PBS s-a produs în 5 cazuri (21,73%) din cei 23 bolnavi ce au supravieţuit primului episod de PBS şi care au beneficiat de profilaxie secundară antibiotică cu chinolone.

La analiza multivariată variabilele independente în relaţie cu mortalitatea în PBS au fost insuficienţa renală (creşterea azotului ureic şi/sau creatininei serice peste 30 mg/dl, respectiv, 1,5 mg/dl) (p= 0,038), scorul MELD la internare (p = 0,017) şi PBS cu bacterii producătoare de BLSE (p = 0,026).

DISCUŢII

Scopul studiului prospectiv a fost de a evalua trăsăturile clinice actuale, răspunsul terapeutic şi prognosticul cirozelor hepatice complicate cu PBS internate în Clinica de Gastroenterologie din Spitalul Judeţean Galaţi. Aceasta analiză este justificată de modificările din ultimii ani referitoare la tipul şi etiologia infecţiilor bacteriene în ciroza hepatică (7, 8, 9). Pentru aceasta, studiul a evaluat un grup de pacienţi cu ciroză hepatică cu ascită complicată cu PBS, care au fost monitorizaţi timp de 36 luni.

Rezultatele studiului susţin recomandările ghidurilor actuale privind stabilirea diagnosticul PBS prin numărarea PMN în lichidul de ascită şi iniţierea tratamentul empiric cu cefalosporine de generaţia a III-a (în studiul prezentat Ceftazidim), avându-se

în vedere necesitatea schimbării antibioticului doar în 19,23% din cazuri. Ca urmare, Ceftazidimul poate fi considerat ca opţiune terapeutică în PBS, prezentând o eficienţă similară cu a cefotaximului - antibioticul recomandat de ghidurile actuale ca tratament de primă intenţie a PBS.

Cazurile cu eşec terapeutic la Ceftazidim datorat fie bacteriilor producătoare de BLSE (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*), fie sensibilităţii bacteriene reduse la Ceftazidim (*Staphylococcus aureus*) au avut un prognosticul rezervat, cu o rată crescută de mortalitate (50%). Aceste date indică prezenţa bacteriilor producătoare de BLSE ca fiind un factor predictiv independent pentru eşecul terapeutic şi mortalitatea intraspitalicească. Bacteriile producătoare de BLSE au fost rezistente la toate antibioticele, cu excepţia Imipenem şi Meropenem. Ca urmare, în condiţiile identificării prin cultură a bacteriilor producătoare de BLSE sau al eşecului terapeutic la administrarea de cefalosporine de generaţia a III-a, ar trebui avută în vedere înlocuirea antibioticului iniţial cu Imipenem sau Meropenem.

Diagnosticul precoce al PBS, iniţierea tratamentului antibiotic empiric şi adaptarea ulterioară a acestuia conform rezultatelor antibiogramei a permis controlul eficient al infecţiei şi reducerea mortalităţii intraspitaliceşti la 17,64%, rezultatele fiind în concordanţă cu datele din literatura de specialitate (10).

În acest studiu, prezenţa la internare a disfuncţiei renale şi scorul MELD au fost identificaţi ca factori predictivi independenţi ai mortalităţii în PBS, rezultate similare fiind raportate de alte studii (11, 12). Pe de altă parte, aceşti factori se corelează, de obicei, cu prezenţa unei insuficienţe hepatice avansate.

Datele din literatură indică o incidenţă în creştere a bacteriilor Gram-pozitive implicate în etiologia PBS de la 25% în anii 1998-1999 şi la 59% între 2000-2002 (13). În studiul prezentat proporţia cazurilor de PBS cu bacterii Gram-pozitive a fost doar 33,33%. În grupul studiat cazurile de PBS cu bacterii Gram-pozitive au prezentat valori mai reduse ale scorurilor Child-Pugh şi MELD şi concentraţii superioare ale proteinelor în lichidul de ascită, comparativ cu PBS cu bacterii Gram-negative.

Ghidurile terapeutice actuale recomandă profilaxia pe termen lung prin administrarea zilnică de Norfloxacin la pacienţii care au prezentat un episod de PBS. Însă, profilaxia cu Norfloxacin ar putea contribui la creşterea frecvenţei PBS cu bacterii rezistente la chinolone, existând studii care au raportat dezvoltarea de bacili Gram-negativi rezistenţi la chinolone în flora intestinală a bolnavilor cirotici ce au primit profilaxie pe termen lung cu Norfloxacin (14).

CONCLUZII

Peritonita bacteriană spontană este o complicaţie frecventă şi severă, ce survine în cazurile de ciroză hepatică cu ascită internate în spital.

Paracenteza diagnostică trebuie efectuată în toate cazurile de ciroză hepatică cu ascită şi când apar manifestări clinice sugestive precum durerile abdominale, febra, encefalopatia hepatică, agravarea disfuncţiei renale sau hepatice. În prezenţa a peste 250 PMN /mm^3 în lichidul de ascită se stabileşte diagnosticul PBS (cultura bacteriană pozitivă nu este absolut necesară) şi se iniţiază tratament antibiotic empiric, preferabil cu cefalosporine de generaţia a III-a (Ceftazidim în studiul prezent). În cazurile cu eşec terapeutic la Ceftazidim trebuie avută în vedere schimbarea acestuia cu Imipenem sau Meropenem.

În pofida progreselor diagnostice şi terapeutice recente, PBS încă se asociază cu o mortalitate semnificativă la bolnavii cu ciroză hepatică, în special în cazurile cu scor MELD ridicat.

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CONNECTIONS BETWEEN OBESITY AND COLORECTAL CANCER. THE ROLE OF INFLAMMATION MEDIATED BY GHRELIN AXIS IN THE ADIPOSE TISSUE

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CONNECTIONS BETWEEN OBESITY AND COLORECTAL CANCER. THE ROLE OF INFLAMMATION MEDIATED BY GHRELIN AXIS IN ADIPOSE TISSUE (Abstract): Obesity and colorectal cancer are two major global health issues. Although they seem to lack a clear connection, epidemiologic analysis found a significant association. This led to further evaluation and identification of molecular pathways that stand at the basis of these findings. One of them is chronic inflammation, a state associated with obesity and colorectal cancer. Adipose tissue from obese individuals suffers a pathogenic switch responsible for higher inflammatory capacity. This modulates the interplay between inflammation and colorectal cancer cells. Through growth factors and cytokines, adipose tissue creates a favorable microenvironment for tumor development. Our focus is on the ghrelin-growth hormone secretagogue receptor axis which promotes inflammation and has mitogenic and antiapoptotic capacity on cancer cells, acting through various mechanisms including via TNF-α which promotes tumor cell proliferation, growth and metastasis and IL-6, a molecule associated with tumor size and stage in colorectal cancer patients. **Keywords:** OBESITY, COLORECTAL CANCER, ADIPOSE TISSUE, INFLAMMATION, GHRELIN AXIS.

According to World Health Organization (WHO), overweight and obesity can be defined as an accumulation of excessive or abnormal fat which may impair an individual's health. More than 1.9 billion people over 18 years old were overweight in 2016 (1). Body mass index (BMI) is the measurement used to appreciate if an individual is overweight (BMI $\geq$ 25 kg/m^2) or obese ($\geq$ 30 kg/m^2). Excessive development of adipose tissue in the abdominal area has led to the concept of abdominal obesity which has a direct link with metabolic syndrome. Therefore, assessing obesity by measuring waist circumference or waist-to-hip ratio, instead of BMI, recognizes the role of abdominal obesity in metabolic syndrome. Besides cardiovascular and metabolic changes, obesity represents a strong connection between cancer and adipose tissue

cells, being associated with a lower prognosis (2, 3). Moreover, according to American Society of Clinical Oncology, obesity seems to overstep smoking as a risk factor for cancer mortality (4).

Obesity and colorectal cancer

Colorectal cancer (CRC) is the third leading neoplasm in United States (US) population with 97,220 new cases of colon cancer being estimated in 2018 (5). Globally, CRC is the second most common neoplasm in women and third in men. Central and Eastern European countries, including Romania, experience the highest mortality rates regardless of gender (6).

Obesity and CRC have a significant epidemiological association. In a meta-analysis conducted on 153,760 Americans, the relative risk for CRC development was 1.4 in obese men and 1.1 in obese women (7). This gender difference has also been found in other studies, as Harriss *et al.* (8) found in a meta-analysis performed on 67,361 colorectal cases, higher risk rates for colon cancer in male individuals. A possible explanation might be the differences in age at the onset of metabolic syndrome, as well as a possible estrogen protective effect due to inhibition of cell proliferation and apoptosis induction. Another reason could be different methods used for obesity assessment. When evaluated by BMI, the risk for colon cancer was increased in both genders and only in men for rectal cancer, while measurement of waist-to-hip ratio or waist circumference led to higher risk for rectal cancer in both men and women (3, 9).

Adipose tissue inflammation and colorectal cancer

Development of adipose tissue starts in utero with continuous changes throughout life. It is localized in two major areas, the subcutaneous (SAT) and visceral adipose tissue (VAT) (10). Adipocytes are the main cells within this tissue, surrounded by stromal vascular tissue. Adipose tissue can be considered a functional organ with highly metabolic activity, secreting cytokines and chemokines, which act through endocrine and paracrine pathways with impact on surrounding or distant cells, promoting inflammation. This role is mainly attributed to white adipose tissue (WAT). Excessive accumulation of WAT in the subcutaneous and visceral area, together with its abnormal functioning, has led to the concept of adiposopathy (11). It represents the interplay between adipocytes and immune system producing numerous active molecules and maintaining a state of chronic inflammation creating a favorable environment for tumor development. This might be the underlying mechanism explaining the epidemiological association between obesity and cancer (12). In a prospective study, the mortality rate in cancer patients with a BMI over 40 was 52% higher in men and 62% in women (13).

VAT seems to possess higher inflammatory capacity when compared to SAT. Firstly, this may be due to greater cell number, vascular and nerve nutrition and an increased amount of free fatty acids (14). Secondly, adipocyte hypertrophy leads to higher grades of inflammation and greater insulin resistance. Finally, expansion of adipose tissue causes oxygen deprivation among adipocytes. This triggers the hypoxia-inducible factor 1-alpha (HIF-1alpha), activation of macrophage infiltration and inflammation. After infiltrating the VAT, macrophages suffer a switch from an anti-inflammatory (M2) to a pro-inflammatory (M1) phenotype, expressing pro-inflammatory cytokines, such as tumor necrosis-α (TNF-α), interleukin-6 (IL-6),

IL-12 or IL-23, oxygen and nitrogen radicals and increased levels of superoxide anions. Moreover, preadipocytes can behave as macrophage-like cells having phagocytosis capacity and microbicidal activity. Consequently, these cells are a reliable source of growth factors and cytokines contributing to the local microenvironment for tumor growth (15). In fact, systemic and local chronic inflammation is a demonstrated risk factor for CRC in patients with inflammatory bowel disease affecting the colon. Epidemiological data shows that in this category, CRC incidence is increasing progressively, attaining 19% after 30 years evolution of disease (16). Increasing evidence shows that high circulatory levels of adipocytokines such as leptin, adiponectin, resistin, ghrelin and inflammatory cytokines provides a higher risk for CRC (17).

Focus on inflammation mediated by ghrelin axis in the adipose tissue

Ghrelin is a small peptide with a role in insulin secretion and gastric functions, stimulates adipogenesis and modulates another pituitary hormone secretion. It exerts its actions through growth hormone secretagogue receptor (GHSR) which is a receptor with two isoforms, GHSR1a and GHSR1b. GHSR1a is the predominant form, being widely distributed throughout the body. Ghrelin mRNA expression increases during fasting periods (18) which leads to ghrelin release in systemic circulation. This secretion seems to be stimulated by insulin-induced hypoglycemia, supporting the role of a mechanism regulated by plasma glucose levels (19). In obese individuals, plasma ghrelin levels seem to be lower compared with lean individuals (20). This counteracts ghrelin's anti-inflammatory effect and alters adipose tissue macrophages which express GHSR. Activation of GHRS reduces secretion of monocyte chemotactic protein-1 through aNFkB mediated mechanism necessary for monocyte activation. On the other side, ablation of GHSR promotes a macrophage shift from an M1 pro-inflammatory to an anti-inflammatory M2 phenotype in abdominal WAT suggesting that ghrelin's action on immune cells establishes a link between metabolic pathways and inflammation. These findings may be the bridge mechanism between ghrelin, obesity and inflammation with subsequent impact over malignancy.

Ghrelin seems to carry an anti- or a proapoptotic role in different cancer cells, which might be the explanation for discrepancies among study results. However, in a recent study (21), high circulating levels of ghrelin were found in patients with colon cancer in comparison with healthy subjects, being also correlated with tumor size and lower differentiation. Others have found high levels during early colon cancer stage and low levels in the late stage. High levels may be attributed to a compensatory response due to cancer weight loss and protection against cancer-induced inflammation. Administration of intraperitoneal ghrelin reduced significantly the multiplicity of colon adenocarcinomas in an AOM/DSS treatment induced carcinogenesis murine model. Moreover, it reduced the mRNA expression of pro-inflammatory cytokines within proximal colon with a decreased infiltration of neutrophils and F4/80-positive macrophages in the distal colon. Deletion ghrelin gene in mice did not decreased colon tumor formation, but larger tumors were found in $Ghrl^{-/-}$ when compared to wild type mice (22). These findings might suggest that physiological ghrelin secretion might not play the role of carcinogenesis suppressor.

Another mechanism by which ghrelin axis orchestrates carcinogenesis is through pro-inflammatory molecules. Adipocytes are a major source of TNF-α. Chronic high TNF-α expression in colorectal tissue supports tumor cell proliferation, invasion and metastasis. Subsequently, TNF-α activates Wnt, which is a procarcinogenic pathway, controlling cell proliferation, differentiation and apoptosis, being an early event in almost 90% of human CRC (23). TNF-αalso activates NF-kb, responsible for gene expression, which in turn are promoting cell proliferation, apoptosis, metastasis and angiogenesis, therefore enhancing tumor development (24). Moreover, tumor cells with TNF-α expression may escape an individual's immunological defending mechanism by inducing apoptosis in immune cells. Circulating levels of TNF-α in CRC patients can be used to appreciate the clinical outcome, with poorer prognosis when higher levels are achieved (25). Murine models of deficient TNF-α seem to be protected from chemically-induced colon cancer, with similar models for interleukin 2, 6 or 8. IL-6 binds on a soluble receptor (sIL-6R) allowing intracellular signaling mediated through glycoprotein 30 (gp30) and activation of Janus kinases (JAKs) and activator transcription 3 (STAT3) increasing the activation of several target genes. These genes have a role in tumor cell survival, proliferation, angiogenesis, apoptosis, cell adhesion, metastasis and inflammation, high serum and tumoral levels of IL-6 being found in CRC patients. It was also associated with stage, size, metastasis or survival in this category of people (26).

CONCLUSIONS

In summary, we can assert that obesity-associated inflammation represents a favorable microenvironment for tumor development. Ghrelin axis acting by protection against cancer-induced inflammation has a significant impact at molecular level in reducing proliferation and survival of neoplastic colorectal cells. This highlights the key role for ghrelin and inflammatory molecules in possible therapeutic options in obese patients with colorectal cancer.

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ROLUL VIDEOCAPSULEI ENDOSCOPICE ÎN DIAGNOSTICUL TUMORILOR DE INTESTIN SUBŢIRE – ANALIZA UNEI SERII

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CAPSULE ENDOSCOPY ROLE IN SMALL BOWEL TUMORS DIAGNOSIS (Abstract): Since the introduction of capsule endoscopy (CE), several studies suggested a higher frequency of small bowel tumors (SBTs) than previously reported. **Material and method**: We evaluated the prevalence, presentation and diagnostic work-up of SBTs în patients undergoing CE în a single referral center. During 2003 - 2009, 102 patients underwent CE. For each patient with suspected SBT at CE, with subsequent histological confirmation, we registered clinical and biological features, imaging methods performed and management. **Results**: SBTs were detected în 4.9% of patients undergoing CE. The main indication for CE was obscure gastrointestinal bleeding. All patients had undergone, before CE, at least one procedure evaluating the small bowel. All patients had iron-deficiency anemia, some presented also other gastrointestinal symptoms. The main SBT type was gastrointestinal stromal tumor. Three patients underwent single-balloon enteroscopy; surgery was performed în all patients. No retention of capsule occurred. CE could be used as first choice as diagnostic tool when SBTs are suspected. **Key words**: SMALL BOWEL TUMORS, CAPSULE ENDOSCOPY, ENTEROSCOPY

Tumorile de intestin subţire reprezintă aproximativ 3-6% din toate tumorile tubului digestiv (1); majoritatea sunt maligne, reprezentând însă doar 1,1-2,4% din tumorile maligne gastrointestinale (2). Diagnosticul tumorilor de intestin subţire rămâne dificil datorită rarităţii lor, tabloului clinic variabil şi nespecific cu care acestea se prezintă, precum şi metodelor inadecvate de investigare a intestinului subţire. Un progres incontestabil în diagnosticul tumorilor de intestin subţire îl aduce videocapsula, tehnică endoscopică revoluţionară prin acurateţe şi siguranţă. Există studii ce susţin că utilizarea acesteia în practică a crescut considerabil rata de diagnostic a tumorilor de intestin subţire, până la 2-9 % (3-7). Folosirea acesteia în cadrul unui algoritm judicios de investigare a intestinului subţire poate asigura diagnosticul şi totodată îmbunătăţi prognosticului pacienţilor cu tumori de intestin subţire.

Studiul de faţă îşi propune să prezinte cazurile de tumori de intestin subţire depistate cu ajutorul videocapsulei pe baza experienţei unui singur centru, analizând frecvenţa acestora, tipul histologic, modalitatea de prezentare clinică, demersul diagnostic complet, tratamentul şi prognosticul.

MATERIAL ŞI METODĂ

Am analizat cazurile de tumori de intestin subţire depistate cu ajutorul videocapsulei endoscopice la pacienţii investigaţi prin această metodă, pentru diverse indicaţii, în Institutul de Gastroenterologie şi Hepatologie Iaşi în perioada 2003-2009.

Au fost analizate modalitatea de prezentare clinică a acestor pacienţi, indicaţia pe baza cărora a fost efectuată investigaţia cu

videocapsula, rezultatele acesteia, investigaţiile efectuate anterior videocapsulei şi ulterior acesteia, localizarea şi tipul histologic al tumorii, conduita terapeutică şi evoluţia pacienţilor.

REZULTATE

Caracteristici generale. Dintre cele 102 investigaţii efectuate cu videocapsula endoscopică de intestin subţire în perioada 2003--2009 în Institutul de Gastroenterologie şi Hepatologie Iaşi, în cinci cazuri au fost depistate tumori de intestin subţire, ulterior confirmate histologic, reprezentând 4,9% din cazuri. Din cei cinci pacienţi, trei au fost de sex masculin, doi de sex feminin, vârsta fiind cuprinsă între 39 şi 72 de ani.

Indicaţia videocapsulei de intestin subţire. Indicaţia de efectuare a videocapsulei de intestin subţire a constituit-o la toţi cei cinci pacienţi hemoragia digestivă de cauză obscură, la trei pacienţi manifestându-se prin testul hemoragiilor oculte pozitiv şi anemie feriprivă, iar la doi pacienţi prin hemoragie digestivă manifestă; în toate cazurile etiologia a rămas neprecizată după investigaţiile endoscopice de primă linie (endoscopie digestivă superioară şi colonoscopie).

Tablou clinic asociat. Pacienţii investigaţi pentru hemoragie digestivă manifestă (melenă) de cauză obscură nu au prezentat simptome digestive asociate, ci doar simptome caracteristice sindromului anemic (astenie, vertij). În cazul pacienţilor a căror indicaţie de videocapsulă a constituit-o hemoragia digestivă ocultă, s-au înregistrat ca simptome asociate scăderea ponderală la doi pacienţi, sindrom dispeptic manifestat prin greaţă, disconfort abdominal postprandial la un pacient, precum şi tablou sugestiv de subocluzie (dureri abdominale intermitente crampiforme, vărsături alimentare, încetinirea tranzitului intestinal) la alt pacient. De altfel, motivul consultului gastroenterologic l-a constituit la această din urmă categorie de pacienţi simptomatologia digestivă, probele biologice de rutină evidenţiind sindrom anemic feripriv, cu testul hemoragiilor oculte pozitiv.

Investigaţii efectuate anterior şi ulterior videocapsulei. Toate investigaţiile cu videocapsula au fost realizate urmărind algoritmul de investigare al hemoragiei digestive oculte/obscure, toţi cei cinci pacienţii fiind investigaţi anterior videocapsulei prin endoscopie digestivă superioară şi inferioară (ambele negative), la doi din cei cinci pacienţi efectuându-se şi endoscopie digestivă superioară second-look.

Un pacient care prezenta simptomatologie digestivă manifestă ca sindrom subocluziv a efectuat înainte de videocapsulă tranzit de intestin subţire şi tomografie computerizată abdominală, fără diagnostic.

Un pacient care prezenta melene repetate a efectuat înainte de videocapsulă scintigrafie cu hematii marcate, care a evidenţiat sursă de sângerare la nivelul cadranului abdominal superior stâng.

Doi pacienţi au efectuat după videocapsulă enteroscopie care a identificat leziunea, a descris suplimentar caracteristicile macroscopice şi a stabilit localizarea, în preambulul intervenţiei chirurgicale.

Aspect endoscopic, localizare şi diagnostic histologic. Într-un caz s-a evidenţiat la videocapsulă doar sângerare activă la nivelul jejunului, fără a se identifica o leziune propriu-zisă subjacentă. La acest pacient, laparotomia exploratorie a stabilit diagnosticul de adenocarcinom avansat. La ceilalţi patru pacienţi aspectul a fost de masă (leziune protruzivă). La un pacient au fost evidenţiate două leziuni protruzive ocupând circa un sfert din lumenul intestinal ileal, una cu modificare minimă a culorii mucoasei cu aspect uşor albicios, cealaltă cu mucoasă de aspect aparent normal, ridicând în discuţie şi diferenţierea de un relief format în dinamică; în acest caz diagnosticul final histologic în urma laparoscopiei cu rezecţie de ileon a fost tumoră carcinoidă.

Fig. 1. Masă cu ulceraţie minimă duodenală

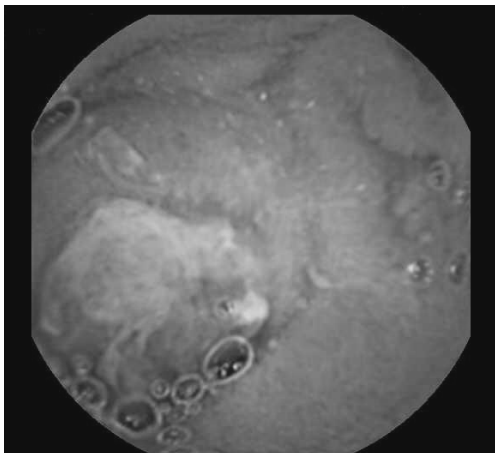

Fig. 2. Leziune ulcerată jejunală

La un pacient, masa protruzivă ocupa aproximativ o treime din lumenul duodenal şi prezenta ulceraţie minimă (fig. 1). Diagnosticul histologic al piesei operatorii a fost de tumoră stromală duodenală. La un pacient, leziunea protruzivă se prezenta pe circa o treime din circumferinţa jejunului, cu aspect modificat al culorii mucoasei (violacee); diagnosticul pe piesa operatorie a fost de tumoră stromală jejunală. La un pacient, masa protruzivă jejunală prezenta aspect ulcerat evident (fig. 2). Diagnosticul histologic pe piesa operatorie a fost de asemenea de tumoră stromală jejunală. La nici unul din aceşti patru pacienţi nu a fost evidenţiată sângerare activă din leziune, dar la cei din urmă doi pacienţi au fost vizibile cheaguri de sânge în lumenul intestinal în vecinătatea leziunii sau în aval de aceasta. Nu au fost evidenţiate stenoze.

Timpul de tranzit intestinal al videocapsulei. Timpul de tranzit intestinal a variat larg, între 2 ore şi 10 minute şi peste 8 ore.

Conduita terapeutică. Toţi pacienţii au fost referiţi serviciului de chirurgie, trei pacienţi imediat după efectuarea videocapsulei, doi pacienţi după ce a fost efectuată şi enteroscopie ulterior videocapsulei.

Un pacient a fost diagnosticat intraoperator cu adenocarcinom avansat şi nu a beneficiat de sancţiune curativă; unui pacient cu descriere intraoperatorie de stenoze ileale multiple i s-a efectuat enterectomie segmentară, efectuând apoi în urma diagnosticului histopatologic de tumoră carcinoidă chimioterapie specifică. Trei pacienţi au beneficiat de rezecţii segmentare curative pentru tumori stromale.

Evoluţie. Un pacient diagnosticat cu adenocarcinom avansat a evoluat rapid nefavorabil.

Un pacient cu tumoră carcinoidă rezecată şi chimiotratată a prezentat evoluţie favorabilă cu lipsa recidivei la patru ani după intervenţie. Trei pacienţi cu tumoră stromală jejunală benignă au evoluat favorabil postoperator.

DISCUŢII

Frecvenţa tumorilor de intestin subţire depistate a fost de 4,9 % din totalitatea investigaţiilor efectuate cu videocapsula în perioada 2003-2009 în Institutul de Gastroenterologie şi Hepatologie Iaşi, cei cinci pacienţi diagnosticaţi aparţinând unei serii de 102 pacienţi investigaţi pentru diferite indicaţii. Procentul este asemănător altor serii de dimensiuni similare comunicate până acum, respectând constatarea unui studiu multicentric european din 2008 privind corelaţia inversă a frecvenţei tumorilor de intestin subţire detectate cu numărul total

de investigaţii efectuate (8), explicaţia constând probabil în stricteţea criteriilor de investigare cu videocapsula aplicate.

În centrul nostru, videocapsula a fost administrată respectând riguros indicaţiile stabilite consensual, toate explorările fiind efectuate pentru hemoragie digestivă de cauză neprecizată, manifestă sau ocultă. La doi pacienţi, hemoragia digestivă manifestă de cauză rămasă neprecizată după endoscopie digestivă superioară şi colonoscopie a motivat administrarea videocapsulei, iar la ceilalţi trei pacienţi indicaţia a constat în depistarea biologică a anemiei feriprive cu testul hemoragiilor oculte pozitiv. Au fost evidenţiate tumori ce s-au dovedit maligne la doi pacienţi (adenocarcinom şi respectiv tumoră carcinoidă), un caz în stadiu avansat ce nu mai beneficiat de tratament curativ. Ordinea investigaţiilor a depins de simptomatologia dominantă – de exemplu s-a efectuat tomografie computerizată abdominală la pacientul cu sindrom subocluziv înaintea videocapsulei. Videocapsula a fost efectuată fie cu indicaţie *negativă* - prin lipsa de elucidare a diagnosticului după alte investigaţii, fie cu indicaţie *pozitivă* - impusă de o investigaţie anterioară cu rezultat patologic (scintigrafie cu hematii marcate).

Enteroscopia a fost efectuată în două cazuri după videocapsulă şi înaintea intervenţiei chirurgicale, aducând informaţii suplimentare privind aspectul şi localizarea tumorii. Sensibilitatea superioară a videocapsulei în detectarea leziunilor intestinului subţire faţă de alte metode a fost evidenţiată de multiple studii (9,10,11,12), studii recente sugerând eficienţa comparabilă a CE cu enteroscopia (13,14). Într-adevăr, în seria studiată, pacienţii care au efectuat enteroscopie au beneficiat de o descriere macroscopică suplimentară a leziunii, în preambulul intervenţiei chirurgicale. Timpul de tranzit intestinal a variat larg între 2 ore şi 10 minute şi peste 8 ore. Momentul depistării leziunii s-a corelat cu timpul de tranzit intestinal în sensul unui timp total de tranzit mai scurt în cazul leziunilor descrise proximal pe traiectul intestinului subţire şi al unui timp de tranzit prelungit (chiar peste durata de funcţionare a capsulei) în cazul leziunilor descrise distal, în a doua jumătate a intestinului subţire. Aceasta ar putea ridica ipoteza influenţării timpului de tranzit de către tumoră (prin stază suprajacentă sau motilitate afectată în segmentul din amonte). Desigur că observaţia necesită validare, prin studierea unui număr mai mare de cazuri.

Ca localizare a fiecărei tumori în raport cu tipul histologic, distribuţia a respectat tendinţele descrise în literatură (15) : tumora carcinoidă a avut sediu ileal, iar tumorile stromale au fost localizate jejunal. Am constatat că nu există o corelaţie între impactul vizual imediat al leziunilor şi severitatea diagnosticului. De altfel putem susţine că obiectivul capsulei nu trebuie sa fie diagnosticul cert, ci evidenţierea de indicii care impun continuarea demersului diagnostic, fie cu alte metode dedicate intestinului subţire (enteroscopie), fie laparotomie.

Nu au fost înregistrate complicaţii la nici unul dintre cazuri, videocapsula dovedindu-se o tehnică atât neinvazivă, cât şi sigură.

Toţi pacienţii au fost referiţi chirurgiei, trei pe baza rezultatului videocapsulei, doi după efectuarea în completare a enteroscopiei care a adus un plus de informaţii privind aspectul şi topografia leziunilor. Videocapsula a dictat singură indicaţia chirurgicală în cazurile în care există tablou clinic invalidant şi în cazul evidenţierii de sângerare activă fără identificare de leziune, aşadar în situaţiile când probabilitatea de a se recurge într-un final la sancţiune chirurgicală era destul de ridicată.

CONCLUZII

Procentul de tumori de intestin subţire evidenţiate la videocapsulă este mic, în contextul respectării standardului investigaţio-

nal al hemoragiei digestive obscure (oculte sau manifeste). Ar fi utile studii care să estimeze frecvenţa tumorilor de intestin subţire depistate de videocapsulă aplicată pentru alte indicaţii, pentru a stabili dacă, pentru a păstra cost-eficienţa, algoritmul trebuie păstrat sau, dimpotrivă, lărgirea indicaţiilor asigură creşterea randamentului.

Videocapsula endoscopică nu oferă diagnosticul final al tumorilor de intestin subţire, dar semnalează leziuni a căror semnificaţie ar putea fi anticipată prin analiza minuţioasă a caracteristicilor macroscopice. Ar fi necesară stabilirea în acest scop unor criterii vizuale cit mai fiabile şi de acurateţe crescută.

Videocapsula poate motiva decizia chirurgicală fie independent, fie completată de enteroscopie, în funcţie de suspiciunea clinică.

După cum am subliniat şi într-o analiză anterioară, videocapsula poate fi utilizată ca primă alegere în cazul suspiciunii de tumoră de intestin subţire, fiind urmată, dacă este necesar, de enteroscopie (cu balonaş unic sau dublu), pentru precizare histologică şi eventual tratament endoscopic (16,17).

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THE IMPACT OF FAMILIAL HYPERCHOLESTEROLEMIA ON CARDIOVASCULAR DISEASE

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THE IMPACT OF FAMILIAL HYPERCHOLESTEROLEMIA ON CARDIOVASCULAR DISEASE (Abstract): Familial hypercholesterolemia is one of the most common inherited diseases, that leads to an important cardiovascular morbidity and mortality, especially in developed countries. It is characterized by early coronary artery disease associated with cholesterol deposits that lead in time to atherosclerotic plaques, because of high levels of circulating cholesterol for a long period of time. Romania is in the group of countries at high risk for cardiovascular disease, being recommended that all internal medicine doctors and cardiologists use the SCORE Risk Chart, to calculate the cardiovascular risk for the next 10 years. The causes of hypercholesterolemia are multiple. On the one hand, the exogenous intake of dietary principles with high cholesterol content is incriminated, and on the other hand is the involvement of the genetic factor. Regarding the treatment in familial hypercholesterolemia, the most recent guidelines state that statins are the mainstay of treatment, but in addition we can also use new drugs like PCSK9 inhibitors, in combination with lifestyle changes. Through this article, we want to draw attention once again to the need for hypercholesterolemia treatment, in line with the latest recommendations of the guidelines. **Keywords**: FAMILIAL HYPERCHOLESTEROLEMIA, SCORE RISK CHART, CARDIOVASCULAR DISEASE.

Familial hypercholesterolemia is one of the most common inherited diseases, that leads to an important cardiovascular morbidity and mortality, especially in developed countries. It is characterized by early coronary artery disease associated with cholesterol deposits that lead in time to atherosclerotic plaques, because of high levels of circulating cholesterol for a long period of time. Aging and modern lifestyles have been increasingly associated with high levels of cholesterol in people all around the world, this being one of the most important cardiovascular risk factors (1). Regarding the mentioned risk, researchers described in the last dyslipidemia guideline that was published in 2016the importance of screening in all men over 40 years and in all women over 50 years or post menopause. By screening, they refer to the evaluation of all lipid fractions (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) (2).

Moreover, given the fact that Romania is in the group of countries at high risk for

cardiovascular disease, it is recommended that all internal medicine doctors and cardiologists use the SCORE Risk Chart, to calculate the cardiovascular risk for the next 10 years. Within the SCORE Risk Chart, it is taken into consideration the value of cholesterol, blood pressure, the smoker / non-smoking status, age and the patient gender. The main factor in calculating total cardiovascular risk through the SCORE Risk Chart is total cholesterol (3).

The causes of hypercholesterolemia are multiple. The exogenous intake of dietary principles with high cholesterol content and/or the involvement of the genetic factor are incriminated. Thus, we can say that hypercholesterolemia can have monogenic or multifactorial determinism. The most common monogenic form (1/200 individuals) is familial hypercholesterolemia (4, 5). The disease is transmitted predominantly autosomal dominant (new mutations are extremely rare) and is caused by mutations in LDLR genes (60-80% of cases), APOB (1-5% of cases) and PCSK9 (0-3% of cases), in 20-40% of cases the defect remaining unknown.

Differential diagnosis includes hypercholesterolemia secondary to obesity, diabetes, hypothyroidism, drugs or kidney disease, where the distribution of cases in the family is non-mandala. Also included in the differential diagnosis are autosomal recessive hypercholesterolemia (LDLRAP1 gene mutations), combined familial hyperlipidemia (associated with increased value of LDL-C and triglycerides, produced by mutations in the LPL gene) and polygenic hypercholesterolemia (determined by various genetic polymorphisms and environment side effects).Genetic testing is indicated in people with a family history of hypercholesterolemia and consists of complete sequencing (including 5', 3' regions and introns) of the LDLR, APOB and PCSK9 genes (in this order). Next Generation Sequencing can check the sequence of the three genes at the same time. In negative cases, it is recommended to study polymorphisms that are associated with hypercholesterolemia (6).

In heterozygous patients that are not receiving treatment, cholesterol levels typically range between 250 and 300 mg/dL and cardiovascular events develop in men by 30–50 years and in women by 40-60 years. On the other hand, in homozygous patients, the cholesterol levels are much higher (500-1000 mg/dL), and important atherosclerotic events start from early childhood. If left untreated, homozygous patients generally die before the age of 30 years due to accelerated severe atherosclerotic cardiovascular events (1).

The latest studies show that in Europe are approximately 4.5 million people that are affected by familial hypercholesterolemia, of whom 20-25% are children and adolescents. Of those, less than 10% are diagnosed. For a better quantification of dyslipidemia, it is necessary to dose each lipid fraction: HDL-cholesterol, which has the role of cardiovascular protection, respectively LDL-cholesterol, the main factor determining the atherosclerosis process, regardless of age (7). According to the Safe heart cohort data, only 68.2% of patients with familial hypercholesterolemia younger than 18 years old are on statins, and only 41.5% of patients had LDL-C < 3.4 mmol/L(8).

Patients with familial hypercholesterolemia may show clinical manifestations, like corneal arcus, xanthelasmas and xanthomas, but they should be looked after with attention, this leading to an earlier diagnosis and

treatment. Worldwide, familial hypercholesterolemia is underdiagnosed and undertreated, generally being detected late in adults, in part because existing diagnostic criteria are complex and not widely used outside of specialty lipid clinics (9, 10).

There are described 3 different criteria for familial hypercholesterolemia diagnosis. First, there is Simon Broome criteria that includes familial history, clinical signs, cholesterol values and genetic testing (tab. I). The advantage of this criteria is that it is very easy to remember and its applicability in a day-to-day clinic is easy and effective. The disadvantage would be that Simon-Broome criteria fails to differentiate between classical familial hypercholesterolemia due to LDLR mutations and the other genetic origins of familial hypercholesterolemia such as ApoB-100 and PCSK9 (11).

TABLE I

Simon Broome familial hypercholesterolemia register diagnostic criteria for familial hypercholesterolemia (adapted after Singh, 2015)

Criteria	Description
1	Total cholesterol concentration above 7.5 mmol/L (290 mg/dL) in adults or a total cholesterol concentration above 6.7 mmol/L (260 mg/dL) in children aged less than 16 years, or low-density lipoprotein (LDL) cholesterol concentration above 4.9 mmol/L (190 mg/dL) in adults or above 4.0 mmol/L (155 mg/dL) in children
2	Tendinous xanthoma in the patient or a first-degree relative
3	DNA-based evidence of mutation in the LDLR, Apo B-100, or PCSK9 mutation
4	Family history of myocardial infarction before age 50 years in a second-degree relative or before age 60 years in a first-degree relative
5	Family history of raised total cholesterol concentration above 7.5 mmol/L (290 mg/dL) in a first- or second-degree relative or greater than 6.7 mmol/L (260 mg/dL) in child or sibling aged younger than 16 years

"Definite" FH diagnosis requires either criteria 1 and 2 or criteria 3.
"Probable" FH diagnosis requires either criteria 1 and 4 or criteria 1 and 5.

The second criteria is Dutch Lipid Clinic Network diagnostic criteria. They are similar to the Simon Broome register criteria but require that at least one clinical criterion be met besides molecular DNA diagnosis for FH to be diagnosed (tab. II). The most important advantage of this criteria is that it addresses the molecular defect that leads to FH and this in turn will help to provide the diagnosed FH patient with a better therapeutic strategy/treatment. The disadvantages would be the lack of versatility and the fact that is very expensive (12).

The third is US MedPed program diagnostic criteria, that have total cholesterol cutoffs derived from mathematical models constructed using published cholesterol levels for familial hypercholesterolemia individuals in the USA and Japan. The cutoffs consider degree of relatedness (individuals with a relative with familial hypercholesterolemia have a higher probability of having the disease) and age (as cholesterol levels increase with age) (11, 12) (tab. III).

Reducing cholesterol levels is essential and can be achieved by lifestyle changes and by using pharmacological agents such as statins or newer therapy such as inhibitors of proprotein convertase subtilisin-kexin type 9 (13). In addition, many studies

suggest that for a better lowering of the LDL-cholesterol, a decrease in triglyceride and lipoprotein (a) would reduce the risk of ischemic cardiopathy (14).

TABLE II

Dutch Lipid Clinic Network diagnostic criteria for familial hypercholesterolemia (adapted after Singh, 2015)

Criteria	Points
Family history	
First-degree relative with known premature (men <55 years; women <60 years) coronary and vascular disease, or first-degree relative with known LDL-C above the 95th percentile	1
First-degree relative with tendinous xanthomata and/or arcus corneal is, or children aged less than 18 years with LDL-C above the 95th percentile	2
Clinical history	
Patient with premature (men <55 years; women <60 years) cerebral or peripheral vascular disease	1
Patient with premature (men <55 years; women <60 years) coronary artery disease	2
Physical exam	
Arcus corneal is prior to age 45 years	4
Tendinous xanthoma	6
Cholesterol levels mmol/L(mg/dL)	
LDL-C 4.0–4.9 (155–193)	1
LDL-C 5.0–6.4 (193–251)	3
LDL-C 6.5–8.4 (251–329)	5
LDL-C ≥8.5 (329)	8
DNA tests	
Functional mutation in the LDLR gene	8

"Definite" FH diagnosis requires more than 8 points.
"Probable" FH diagnosis requires 6–8 points.
"Possible" FH diagnosis requires 3–5 points.

TABLE III

US MedPed program diagnostic criteria for probable heterozygous familial hypercholesterolemia (adapted after Al-Rasadi, 2014)

Age (years)	Total cholesterol cut points mmol/L(mg/dL)			
	First-degree relative with FH	Second-degree relative with FH	Third-degree relative with FH	General population
<20	5.7 (220)	5.9 (230)	6.2 (240)	7 (270)
20–39	6.2 (240)	6.5 (250)	6.7 (260)	7.5 (290)
30–39	7 (270)	7.2 (280)	7.5 (290)	8.8 (340)
≥40	7.5 (290)	7.8 (300)	8 (310)	9.3 (360)

Regarding the treatment in familial hypercholesterolemia, the most recent guidelines state that statins are the mainstay of treatment, in combination with lifestyle changes. In a cohort study realized on 2,146 patients diagnosed with familial hypercholesterolemia but without coronary heart disease, after a follow-up of 8.5 years, the treatment with statins reduced the risk for coronary heart disease by 76%.

According to the guidelines, if the patient does not reach the LDL-cholesterol targets with the highest recommended or tolerable dose of statin, it should be considered the combination with ezetimibe. If the patients still do not reach the LDL-cholesterol targets after ezetimibe and statins, bile acid sequestrants can be considered (15).

Monoclonal antibodies against proprotein convertase subtilisin-kexin type 9 (PCSK9) are proteases that are synthesized in the liver and small intestine. PCSK9 is secreted from liver cells, binds to the LDL receptor and is subsequently internalized together with the LDL receptor and targets the receptor for lysosomal degradation. Therefore, the inhibition of PCSK9 determines an increased expression of LDL receptors on hepatocytes and decreased LDL-C levels in the circulation. This leads to the fact that patients with the loss of function mutations in the PCSK9 gene have very reduce LDL-C level, whereas the gain of function mutations cause familial hypercholesterolemia. Commercially available are two monoclonal antibodies that inhibit PCSK9, alirocumab and evolocumab (15).

CONCLUSIONS

In our study we considered the importance of the implication of non-modifiable risk factors (age, gender, genetic factors) in the development of cardiovascular disease, being required a more stringent control of the modifiable factors (blood pressure, blood glucose, cholesterol, stress). Through this article, we want to draw attention once again to the need for hypercholesterolemia treatment, in line with the latest recommendations of the guidelines.

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NEWS

A NEW HOPE FOR AN OLD HEART? CAN INTERFERON-FREE THERAPY ALSO REVERSE THE HCV-INDUCED CARDIOMYOPATHY IN PATIENTS WITH END-STAGE HEART FAILURE?

Several studies have previously confirmed an association between hepatitis C virus (HCV) infection and cardiomyopathy, but none could really establish a pathogenic relationship. These issues have arisen because the classic, interferon-based therapy for HCV infection has severe cardiac side-effects, thus limiting its use in patients with advanced heart failure. The aim of this prospective large-scale study was to assess the benefits of using interferon free regimens in HCV-infected patients with non-valvular, non-ischemic cardiomyopathy, the standard treatment (consisting of ombitasvir, paritaprevir, dasubavir and paritaprevir) being well-tolerated, free of cardiac side-effects. The authors were encouraged to conduct a large study because they observed that this treatment was very effective in a patient with rapidly progressive heart failure (NYHA III/IV class), who was already listed for heart transplantation. In order to have an acceptable hepatic functional status prior to the transplant, the patient received the interferon-free regimen and, surprisingly, after her complete HCV clearance, her cardiac function improved significantly to NYHA II class, heart transplant being no longer considered. The conclusion was that HCV was etiopathogenetically involved in myocardial dysfunction for at least two reasons: the interferon-free therapy does not eliminate any other cardiotropic virus (e.g. Coxsackie B3), nor does it have „per se" any antifibrotic or cardiac antiremodelation action. These promising findings need to be further confirmed on larger patient cohorts in this ongoing study. Poller W, Haghikia A, Kasner M *et al*. Cardiovascular Involvement in Chronic Hepatitis C Virus Infections - Insight from Novel Antiviral Therapies. *J Clin Transl Hepatol* 2018; 6(2): 161-167.

INDICATIONS AND CONTRAINDICATIONS IN TRAUMATIC PATHOLOGY IN PATIENTS WITH DEMENTIA

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INDICATIONS AND CONTRAINDICATIONS IN TRAUMATIC PATHOLOGY IN PATIENTS WITH DEMENTIA (Abstract): The incidence of patients with dementia has increased significantly in the last decades, with the increase in life expectancy and longevity; it is a condition age-specific and doubles with every 5 years of increase in age and it is expected to reach 65,7 million in 2030 and 115.4 million by 2050. In cases of dementia, it is contraindicated any stressful emotional or physical situation that could precipitate symptoms. In the case of trauma, there is a complex process that disturbs the homeostasis of the whole organism. It is known that the elderly has an increased risk of delirium (which is basically a separate transient illness, not a worsening of dementia, so it is reversible) during the postoperative period, and the more patients with dementia who can develop both delirium and postoperative cognitive dysfunction. An important step in general anesthesia is the anesthetic preconditioning in which the brain is stimulated by small lesions that do not produce histological changes but are sufficient to produce a biomolecular stress that will initiate protection against further severe aggressions. In conclusion, patients with dementia have a high mortality rate and therefore require careful supervision and immediate care when first suspicions arise. Delirium should be treated as a deadly emergency. They also need palliative therapies for both pain and support to provide a more familiar environment to reduce depression and anxiety. **Keywords:** TRAUMATIC PATHOLOGY DEMENTIA, ELDERLY.

The incidence of patients with dementia has increased significantly in the last decades, with the increase in life expectancy and longevity (1); it is a condition age-specific and doubles with every 5 years of increase in age (2) and it is expected to reach 65.7 million in 2030 and 115.4 million by 2050 (3, 4).

Dementia is a neurological disorder specific to the elderly and is generally recognized by a combination of symptoms such as personality disorders, memory loss and intellectual regression, transformations that have a major impact on everyday life, personal independence and inter-human relationships (5, 6, 7). Therefore, the scien-

tific world has shown interest into dementia, and over the last two decades many details have been revealed about dementia neurobiology in terms of molecular mechanism, bringing attention to unprecedented insight into this incurable disease (1).

As we age, the brain changes its' structure and chemistry (8, 9, 10). In dementia was seen that cells lose the ability to communicate with each other and eventually die (10).

If dementia occurs as a reaction to certain medications or because of a vitamin deficiency, treatment can considerably improve the patient's condition, or even cure the condition, but unfortunately, the most common forms of dementia are degenerative, especially Alzheimer's disease. But there are other types of dementia, such as vascular dementia, dementia with Lewy bodies, frontotemporal dementia, mixed dementia, Huntington's disease, Creutzfeldt-Jacob disease and pugilistic dementia (a variant of chronic traumatic encephalopathy), that differentiate both etiologically and symptomatically.

However, some of these diseases (Alzheimer's dementia, frontotemporal dementia, dementia with Lewy bodies) share a common feature: **excessive buildup of proteins in the brain** which become toxic and harm both the brain and nerve cells (10). So, the main symptoms are memory loss, disability to carry out daily tasks, personality and behavior problems such as loss of interest in daily activity, loss of confidence, sleep disorders. As the disease progresses, the brain's ability to adapt to changes and absorb certain drugs is also reduced.

In cases of dementia, it is contraindicated any stressful emotional or physical situation that could precipitate symptoms. In the case of trauma, there is a complex process that disturbs the homeostasis of the whole organism.

Generally, stress determine a hyperactivity of the hypothalamic pituitary adrenal (HPA) cortical axis with an excessive production of glucocorticoid stress hormone (cortisol). The three most important regulators of the HPA axis are the *amygdala* (has excitatory influence – recognize threat and coordinate the fear response), the *hippocampus* (inhibitory – explore the environment under safe conditions) and *prefrontal cortex* (inhibitory). Some studies showed that chronic elevated levels of cortisol lead to brain atrophy (11). Similarly, in dementia are observed the atrophy in hippocampus, inferior parietal and posterior cingulate cortex (specific for Alzheimer's disease) and thalamus and striatum (specific for frontotemporal dementia) which indicate the progression of these pathologies (12). Knowing these specific regions, we can use trial targeted treatments in the future to slow the progression of atrophy (12). We therefore deduce that it is important to limit stress factors on the patient with dementia because it decompensates the patient very quickly, accentuates atrophy, causes the appearance of delirium, which if not properly treated or unnoticed in time can lead to an exitus.

It is known that the elderly has an increased risk of delirium (which is basically a separate transient illness, not a worsening of dementia, so it is reversible (8)) during the postoperative period, and the more patients with dementia who can develop both delirium and postoperative cognitive dysfunction. It is estimated that 90% of patients with dementia develop delirium after important surgeries like hip surgery or radial head fractures Mason III (although

bipolar polyethylene prosthesis offers an easier implantation) (13).

So, it is best to be aware of the patient's condition in the trauma and orthopedics unit of one acute hospital (14). This will guide the choice of anesthesia (general or regional) and drugs' administration because some anesthetic drugs might impair the memory and cognitive function and also allow to monitor and treat as aggressively as possible to limit poor cognitive and functional impairment (15, 16).

Anesthetic medication has multiple effects:

- isoflurane induces diffuse neuroapoptosis;
- thiopental, midazolam and protoxide amplify this effect;
- sevoflurane inhibits neutrophil apoptosis;
- some neuromuscular blockers and some local anesthetics induce apoptosis;
- propofol delays synaptic axonal development (17).

An important step in general anesthesia is the **anesthetic preconditioning** in which the brain is stimulated by small lesions that do not produce histological changes but are sufficient to produce a biomolecular stress that will initiate protection against further severe aggressions.

Generally, surgical procedures lead to inflammation and immunological responses which can affect the brain due to the fact that blood flow to the brain can be impaired because of changing fluid levels or blood loss. Moreover, some surgical procedures might increase the risks of small strokes (15).

Patients with dementia also present an increased risk of other complications that are difficult to diagnose in initial stage including pneumonia, septicemia, stroke, pressure ulcers, acute renal failure requiring dialysis, urinary tract infection, lung failure requiring intubation, falls and incontinence (2, 5, 12, 18). All of these are also associated with an increase in the number of hospitalization days, higher costs of medical care and a high mortality rate (2, 5, 16).

It is estimated that mortality (5, 19, 20, 21) in first 7 days after surgery reach 14%, compared to non-dementia patients (6%) (5). Dementia it is also associated with high rate of morbidity and mortality which reach to 92% in two years, compared with 7% at those without dementia (2, 10).

DISCUSSION

As we have said so far, patients with dementia have an increased risk of severe complications. Therefore, in these patients it is recommended first, conservative treatment instead of the surgical procedure is recommended. Unfortunately, in most cases surgery is unavoidable, and it is crucial to prevent as much as possible any complications.

Demented patients do not require a specific medication before anesthesia, but sometimes it is necessary to use anxiolytics or short-acting benzodiazepines to prevent or reduce some symptoms like anxiety, irritability and agitation, specific to dementia (2).

The surgical team should be knowledgeable about the patients' illness, the medication they are taking, the associated comorbidities and how any previous anesthetics have affected them because elderly, but especially demented patients have an increased sensitivity to the drugs of anesthesia. So, the anesthetist will titrate and administer smaller doses to also prevent anesthetic-induced circulatory depression (2). In

some cases, regional anesthesia is a better choice, although there are not specific studies to prove a significant advantage of this anesthesia due to the fact that most studies do not include this category of patients.

On the other hand, it is very important to treat pain properly because demented patients are less likely to report sensations of pain, but pain may precipitate the condition of the patient with dementia and cause depression and delirium (2, 8). At the same time, delirium might suggest an infection or pain (8).

Finally, patients with dementia should completely eliminate specifying factors such as smoking and alcohol (cause permanent brain damage) which can precipitate the symptoms, although it is well known that small quantities of wine have positive influence in bones consolidation (22).

CONCLUSIONS

Patients with dementia have a high mortality rate and therefore require careful supervision and immediate care when first suspicions arise. Delirium should be treated as a deadly emergency. They also need palliative therapies for both pain and support to provide a more familiar environment to reduce depression and anxiety.

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NEWS

CONTRAST ENHANCED ULTRASONOGRAPHY: ADVANTAGES AND CONTRAINDICATIONS

Contrast agents for ultrasonography (US) were first introduced in 1996 and have been used mainly for echocardiography, Doppler US and abdominal US. Contrast-enhanced US (CEUS) has several advantages over the contrast-enhanced computed tomography (CECT) or contrast-enhanced magnetic resonance imaging (CEMR), such as no radiation, no harmful effects to the kidney or thyroid, easy accessibility and comparable comfort during use, but it has not been widely used in abdominal applications. In the case of first-generation US contrast agents, early breakdown of microbubbles limits the contrast effect during continuous scanning. With the development of second-generation US contrast agents in 2001 and the advances of the contrast-specific mode with a low-mechanical index (MI) technique in US machines, a real-time evaluation has become possible, resulting in an increasing interest in US contrast agents and CEUS. A US contrast agent is excreted via the lung after the destruction of the microbubbles; hence, it is not nephrotoxic. Further, it does not contain iodine, thereby not having any effect on the thyroid functions. Although the US contrast agent is very safe, it can be regarded as a foreign material by the immune system; therefore, a hypersensitivity reaction is possible. The incidence of a severe hypersensitivity reaction was reported in about 0.002%. The overall reported incidence of the hypersensitivity reaction was less than that occurring with the use of an iodine contrast agent in CT and was similar to that of the use of a gadolinium chelate contrast agent in MRI. Contraindications of US contrast agents are: a recent acute coronary syndrome, right-to left shunts, severe pulmonary hypertension, uncontrolled systemic hypertension and adult respiratory distress syndrome. Recently, the use of one US contrast agent in echocardiography has been temporarily suspended because of possible severe hypertension, bradycardia, cardiac arrest, and acute myocardial infarction and for another contrast agent in patients with egg allergies. Because the safety of some contrast agents has not been evaluated in pregnant women, in women who are breast-feeding, or in patients who are younger than 18 years of age using them should be avoided in these patients.

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CONTROLUL TERAPEUTIC AL HIPERTENSIUNII ARTERIALE LA DOMICILIU

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HOME CONTROL OF HIGH BLOOD PRESSURE (Abstract): Control of blood pressure is far from satisfactory, reaching ~ 13% in Romania according the SEPHAR study. **Aim**: Assessment of blood pressure control in elderly by blood pressure monitoring at home. **Material and method**: 64 hypertensive patients (36 women and 28 men) with mean age of 76.3 years which blood pressure has been normalized by admitting in hospital were followed for a period of three months, by self-measuring blood pressure at home (twice a week). **Results**: In the first week after hospital discharge the mean systolic pressure was 158± 17.06 and after adjustment of treatment was 136.76± 11.36 mmHg. To the end of the study blood pressure was controlled at a rate of 81.25 %. **Conclusions**: To increase the percentage of therapeutic control of blood pressure at home, small changes in drug dosage are needed. **Key words**: BLOOD PRESSURE CONTROL, ADJUSTING TREATMENT, HOME CONTROLED BLOOD PRESSURE

Controlul valorilor tensionale este departe de a fi satisfăcător, atingând în România ~ 13% după rezultatele studiului SEPHAR dar şi în ţările cele mai dezvoltate doar un procent de maximum 27% dintre pacienţii hipertensivi trataţi sunt controlaţi terapeutic. In practica medicală, tratamentul hipertensiunii arteriale (HTA) este orientat frecvent doar de valorile presiunii arteriale pe care pacientul le prezintă în timpul spitalizării. Din punct de vedere fiziopatologic, există o serie de mecanisme diverse, adesea intricate, implicate în realizarea unor valori presionale crescute la domiciliu, care determină creşterea debitului cardiac sau a rezistenţei vasculare periferice. Consecinţele sunt rezistenţa la tratament, apariţia reacţiilor adverse de tip fatigabilitate, cefalee, tulburări digestive, inapetenţa etc., manifestări ce duc la scăderea complianţei pacientului la tratamentul recomandat.

OBIECTIVE

Aprecierea controlului tensiunii arteriale (TA) la vârstnicii hipertensivi prin monitorizarea clinică a valorilor tensiunii arteriale la domiciliu.

MATERIAL ŞI METODĂ

Lotul de studiu a fost format din 64 pacienţi hipertensivi vârstnici 36 femei (56,25%) şi 28 bărbaţi (43,75 %) cu vârsta medie de 78,3 ani la care au fost normalizate valorile tensiunii arteriale prin internare în spital. Toţi pacienţii erau sub trata-

ment antihipertensiv şi cu valori ale tensiunii arteriale controlate (TA < 140/90 mmHg) la externare. Fiecare pacient a fost urmărit pe o perioadă de trei luni, prin măsurarea tensiunii arteriale de două ori/ săptămână dimineaţa şi seara şi de fiecare data când au apărut diferite simptomatologii (epigastralgii, cefalee, dureri reumatice, etc.). Determinarea TA s-a făcut după 5 minute de repaus în decubit dorsal sau poziţie şezândă, cu braţul la nivelul inimii, precum şi în ortostatism imediat şi la 2 minute după schimbarea poziţiei. Pacienţilor li s-a recomandat să nu consume cafea în ora precedentă examinării, să nu fumeze cu minimum 15 minute înaintea măsurătorii, să nu consume stimulente adrenergice exogene. Iniţial s-a determinat TA la ambele braţe, dacă TA era diferită la cele două braţe s-a folosit braţul cu TA cea mai mare. Ţinta pentru fiecare pacient a fost menţinerea valorilor tensiunii arteriale < 140/90 mmHg, prin modularea terapiei curente, sau prin adăugarea de noi agenţi antihipertensivi. Datele au fost procesate folosind programul *Microsoft Excel*.

REZULTATE

Vârsta medie a pacienţilor a fost de 78,3 ani, din care 36 femei (56,25%) şi 28 bărbaţi (43,75%), diagnosticaţi hipertensivi cu o vechime medie de 15,7 ± 13,5 ani, variind de la 1 an la peste 40 ani (fig. 1), având factori de risc asociaţi pentru sexul feminin: colesterol 37.2%; obezitate 24,5%; diabet zaharat 13,8%; antecedente heredo-colaterale 11,7%, iar la sexul masculin: colesterol 14,9%; obezitate 7,4%; diabet zaharat 3,7%; consum de alcool 78%; fumat 8,5%.

Valorile tensionale ale pacienţilor incluşi în studiu au fost sub 140/90 mmHg la externare, valori care au crescut în prima săptămână de la externare. Valoarea medie a tensiunii arteriale în prima săptămână de la externare a fost TAs (tensiune arterială sistolică) medie = 158,55 ± 17,06 mmHg iar TAd (tensiune arterială diastolică) medie = 81,57 ± 03 mmHg, valori care au scăzut în a doua săptămână dar fără a ajunge la valoarea ţinta de TA < 140/90 mmHg, media TAs = 153,20 ± 17 mmHg, iar media TAd = 80,05 ± 10 mmHg (tab. I). S-a semnalat un singur caz de puseu hipertensiv TA = 230/120 mmHg a doua zi de la externare şi două cazuri în care pacienţii au întrerupt intermitent medicaţia antihipertensivă.

Din punct de vedere statistic se evidenţiază diferenţe semnificative între valorile medii ale TAs şi TAd între cele 3 momente ale măsurătorii, cele mai mari diferenţe înregistrându-se între momentele externării şi prima săptămână, şi între prima săptămâna şi la 3 luni.

La includerea în studiu 38 pacienţi reprezentând 59,37 % primeau monoterapie, 19 pacienţi (29,69) biterapie iar 7 (10,94 %)

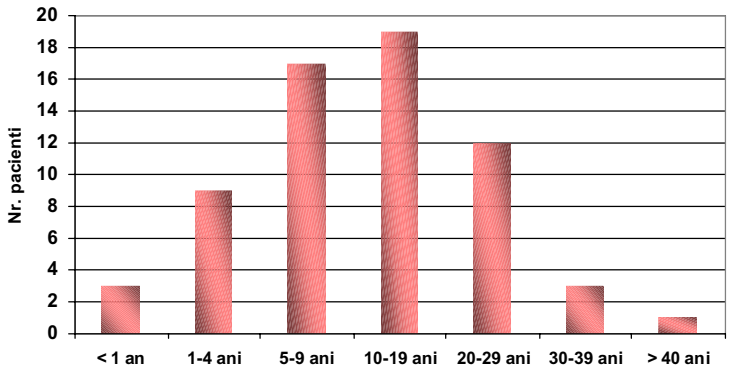

Fig. 1. Vechimea HTA

TABELUL I
Valorile TA pe parcursul studiului

	Externare	Prima săptămână	A doua săptămâna	Trei luni
TAs clinic	129,32±08	158,55±17,06	153,20±17	136,76±11,36
TAd clinic	74,76±16	81,57 ± 0,3	80,05±10	75,26±23

TABELUL II
Număr de pacienţi sub diferite scheme terapeutice

	Iniţial	Tratament ajustat
1 agent antihipertensiv	38	29
2 agenţi antihipertensivi	19	23
3 agenţi antihipertensivi	7	8
4 agenţi antihipertensivi	-	4

TABELUL III
Scheme terapeutice după clasele de medicamente antihipertensive

Monoterapie 29 pacienţi(45,31%)	IEC	22 pacienţi (34,37%)
	BB	5 pacienţi (7,81%)
	BCC	2 pacient (3,13%)
Biterapie 23 pacienţi (35,94%)	IEC+D	16 pacienţi (25%)
	IEC+BB	2 pacienţi(3,13%)
	BB+D	2 pacienţi (3,13%)
	BB+BCC	3 pacienţi (4,68%)
Tripla asociere 8 pacienţi (12,5%)	IEC+D+BB	5 pacienţi (7,81%)
	IEC+BCC+D	1 pacient (1,56%)
	BB+BCC+D	2 pacienţi (3,13%)
Cvadrupla asociere 4 pacienţi (6,25%)	IEC+BB+BCC+D	4 pacient (6,25%)

triplă asociere de medicamente antihipertensive. Modularea tratamentului s-a făcut după a doua săptămână de monitorizare clinică la domiciliu, după ce accentul s-a pus pe tratamentul nefarmacologic prin renunţarea la fumat, reducerea greutăţii corporale, reducerea sării din alimentaţie sub 2,4 g de Na, reducerea consumului de alcool, dietă echilibrată, bogată în produse vegetale (legume, fructe) şi săracă în grăsimi animale. Totodată s-a pus accent pe necesitatea administrării zilnice şi pentru toată viaţa a tratamentului HTA şi a beneficiilor pe care pacientul le are prin scăderea riscului de a dezvolta insuficienţă cardiacă, boli coronariene, tulburări de ritm, accident vascular cerebral, maladii renale şi reducerii riscului de deces datorită acestor maladii.

Alegerea drogului antihipertensiv s-a făcut după recomandările ghidului de diagnostic şi management al HTA al Societăţii Europene de Hipertensiune si al Societăţii Europene de Cardiologie din anul 2007, iar orarul administrării a fost ales după tipul diurn, vesperal sau susţinut al HTA, iniţial prin creşterea dozei de antihipertensiv aflat în schema de tratament (pentru creşterea complianţei la tratament), 43 de cazuri. La cei la care valorile tensiunii arteriale au rămas peste 140/90 mmHg şi erau cu monoterapie s-a adăugat un diuretic (9 cazuri -20,93%), la 5 pacienţi (11,63%) s-a adăugat al treilea agent antihipertensiv, iar la 4 pacienţi s-a adăugat al patrulea agent antihipertensiv. La 3 pacienţi (9,3%) nu a fost necesară ajustarea tratamentului (tab. II).

După ajustarea tratamentului şi după clasele de medicamente pacienţii au primit următoarele scheme terapeutice exemplificate în tabelul III.

Complicaţia cea mai frecventă a fost starea de deshidratare uşoară, prin scăderea senzaţiei de sete urmată de astenie, discrete tulburări urinare, tuse foarte rară, confuzii discrete sau fenomene de sedare. Pe perioada de apariţie a diferitelor simptomatologii (epigastralgii, cefalee, dureri reumatice) valorile tensiunii arteriale au avut o uşoară creştere cuprinsă între 5 -15 mmHg, motiv pentru care am decis numai tratarea simptomatologiei apărute. Asocierea inhibitori ai enzimei de conversie (IECA) + diuretic (D) şi IECA + blocante ale canalelor de calciu (BCC) nu au prezentat reacţii adverse creând impresia că tratamentul prescris este cel mai adecvat, motivând pacienţii al urma în dozele şi orarul stabilit.

La finalul celor trei luni de monitorizare clinică la domiciliu ,valorile tensiunii arteriale au fost controlate la un procent de 81,25% din pacienţii incluşi în studiu, cu valori ale TAs medie de 136,76± 11,36 mmHg, iar TAd medie = 75,26±17 mmHg. La 4 pacienţi nu s-a ajuns la valoarea ţintă a TAs cu toate că pacienţii aveau asociate patru clase de medicamente antihipertensive şi au fost trimişi la alte specialităţi pentru investigaţii suplimentare, iar 8 pacienţi nu au tolerat valori tensionale sub 150-160 mmHg acuzând cefalee, vertij sau astenie fizică.

DISCUŢII

Dacă la începerea studiului eram convinşi că vom reuşi controlul terapeutic al HTA la domiciliu apropiat de 100 la 100 considerând că rată scăzută de control al HTA la domiciliu se datorează dozelor insuficiente de medicamente antihipertensive, nerespectarea regimului desodat sau neadministrării medicaţiei recomandate, acest lucru nu a fost realizat ceea ce înseamnă că există şi alţi factori care duc la creşterea valorilor tensionale la domiciliu sau creşterea TA reprezintă un mecanism compensator pentru asigurarea nevoilor energetice mărite ale organismului faţă de condiţiile de repaus din spital.

Existenţa pacienţilor care nu tolerează valorile TA ţintă ne sugerează că scăderea Tas la aceste valori la unii hipertensivi vârstnici poate duce la hipoperfuzia diferitelor organe mai periculoasă decât valori ale TA de 150-160 mmHg.

În ceea ce priveşte clasele de medicamente şi orarul de administrare IECA administraţi seara în priză unică au determinat o scădere tensională nocturnă mai pronunţată decât după administrarea matinală şi a modificat favorabil profilul tensional al pacienţilor ; beta-blocantele (BB) au redus TAs şi a aplatizat ritmul circadian tensional şi a frecvenţei cardiace ; BCC au redus TA pe parcursul zilei şi nopţii atât la administrarea matinală cât şi vesperală ; diureticele au scăzut eficient valorile tensionale la pacienţii cu tensiune diurnă. Monoterapia a fost tratamentul cel mai acceptat de pacienţi, asocierile de agenţi antihipertensivi erau privite ca o agravare a HTA, aspect peste care pacienţii au trecut după explicarea beneficiilor care le comportă asocierea diferitelor clase de agenţi antihipertensivi. Din punct de vedere al complianţei la tratament pentru creşterea procentului de control terapeutic al hipertensiunii arteriale în populaţie îi revine medicului de familie (prin consiliere psihologică, reţete cu preţ scăzut), pacientului şi nu în ultimul rând, familiei pacientului ţinând cont că hipertensivul vârstnic este un pacient cu nevoi speciale.

Rezultatele studiilor : ALLHAT, VALUE, ASCOT, INVEST, COOPERATE, PROGRES aduc informaţii dovedite asupra importanţei tratamentului HTA, comparând eficacitatea şi beneficiul claselor de agenţi antihipertensivi prin reducerea TA per se precum şi beneficiul pe un organ ţintă. In ceea ce priveşte numărul de agenţi antihipertensivi, monoterapia poate fi tratamentul iniţial sau pe termen lung dacă valorile TA sunt ţinute sub control şi nu apar reacţii adverse, rezul-

tate obţinute şi în studiul nostru pe 29 pacienţi (45,31%). Beneficiile terapiei combinate, în studiul nostru pe 35 pacienţi (54,69 %), au fost demonstrate de majoritatea studiilor clinice mari (ASCOT, ALLHAT, INVEST) pentru obţinerea şi menţinerea valorilor ţintă ale TA, în special la pacienţii cu risc înalt, argumente aduse de studiul VALUE.

Beneficiile tratamentului antihipertensiv asupra protecţiei cardiovasculare sunt demonstrate şi de concluziile rezultate din meta-analiza efectuată pe cele 147 de studii clinice privind HTA publicate între anii 1966 şi 2007.

CONCLUZII

Scăderea TA la domiciliu spre valorile ţintă nu este tolerată de toţi pacienţii vârstnici, posibil asocierii leziunilor aterosclerotice, impunându-se o urmărire mai îndeaproape a acestor pacienţi

Orarul de administrare a medicaţiei antihipertensive trebuie să corespundă cu tipul de HTA (matinal, vesperal sau susţinut).

Iniţierea de măsuri pentru ajustarea schemei terapeutice şi creşterea complianţei la tratament.

Urmărirea în evoluţie, sesizarea complicaţiilor ce pot apărea la pacientul hipertensiv şi adoptarea atitudinii corecte de trimitere a pacientului la medicii de alte specialităţi.

Există pacienţi hipertensivi la care s-a normalizat TA în spital, dar fără a mai atinge valorile ţintă la domiciliu în pofida ajustării dozelor sau introducerii de noi agenţi antihipertensivi.

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NOUTĂŢI

CANCERUL PANCREATIC

Articolul sintetizează progresele făcute în ultimii ani în înţelegerea mecanismelor moleculare, dar şi a diagnosticului, stadializării şi tratamentului pacienţilor cu cancer pancreatic în stadiile precoce. Cancerul pancreatic este rezultatul acumulării unor mutaţii genice successive. Acesta îşi are originea la nivelul epiteliului ductal şi evoluează din leziuni premaligne. Progresia de la o displazie epitelială minimă spre o displazie epitelială severă şi în final la cancer invaziv ar fi determinate de acumularea succesivă de mutaţii genice care includ : activarea oncogenei KRAS2, inactivarea genei supresoare de tumori *CDKN2A*, *inactivarea genelor supresoare de tumori TP53 sau DPC4*. Un studiu genetic recent pe 24 cazuri de cancere pancreatice a arătat că celulele canceroase pancreatice sunt purtatoare în medie de 63 de anomalii genetice per cancer, care pot fi grupate în 12 căi de semnalizare moleculare ; demonstrând astfel cât de eterogenă şi complexă este genetica acestui cancer. Cancerul pancreatic este caracterizat de prezenţa unei strome dense, slab vascularizate. Celulele pancreatice stelate joacă un rol important în formarea şi turnover-ul acesteia. Activate de către diverşi factori de creştere, celulele stelate secretă colagen şi alte componente ale matricei extracelulare. Celulele stromale sintetizează diverse proteine sau factori de creştere care au fost asociate cu un prognostic sumbru şi cu o rezistenţă la tratament. Acestea ar putea însă fi ţinte ale tratamentului în viitor. 1-5% din structura tumorii sunt celule cu proprietăăţi de celule stem, capabile de regenerare nelimitată. Aceste celule sunt rezistente la chimio si radioterapie ceea ce ar explica de ce acestea nu au beneficii în tratamentul bolii. O înţelegere mai bună a mecanismelor moleculare ale acestui cancer ar deschide noi posibilităţi de tratament de tipul unor molecule inhibitoare ale oncogenelor sau inhibitoare ale unor căi moleculare (RAS, Src sau MEK) anticorpi monoclonali contra unor proteine membranare de tipul mezotelinei, noi nanotehnologii sau agenţi adenovirali. Complexitatea şi eterogenitatea acestui cancer indică necesitatea unor tratamente individualizate (HidalgoM. Pancreatic cancer. *N Engl J Med* 2010, 362 (17) : 1605-1617).

Roxana Cojocariu

THE TREATMENT OF ORGAN DYSFUNCTION IN HIV-POSITIVE PATIENTS

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THE TREATMENT OF ORGAN DYSFUNCTION IN HIV-POSITIVE PATIENTS (Abstract) **Aim:** To evaluate the clinical and viro-immunological status in order to manage multiple organ disfunction of HIV-infected patients admitted to Intensive Care Unit (ICU) during active antiretroviral therapy. **Material and Methods:** Retrospective study, using medical data of HIV infected patients from the Regional HIV/AIDS Center of the "Sf. Parascheva" Infectious Diseases Clinical Hospital Iasi, admitted to the ICU, between 1st January 2016-31 December 2018. **Results:** In this study were included 21 patients with different HIV/AIDS stages and a depressed clinical status requiring evaluation and monitoring in the ICU. They were predominantly male with an average age of 34 years old. The viral-immunological status revealed the CD4 lymphocytes value less than 50 cells/mmc for most of the cases (19 patients), and a viral load significantly increased. As opportunistic infections were recorded: Tuberculosis (8 cases), *Cryptococcal* meningitis (1 case), *Mycobacterium avium* infection (1 case), *Cytomegalovirus* infection (6 cases). The most common complications were: respiratory distress syndrome and bacterial pneumonia (8 cases); and severe sepsis (4 cases), being evaluated and surveilled in ICU. Nine patients needed oral tracheal intubation and vasopressor support. All patients received antiretroviral therapy in association with specific antibiotherapy or antifungals depending on complication. Only one case had favorable evolution, the rest of the patients died. **Conclusions:** The survival rate of HIV-infected patients admitted to the ICU was extremely low even if they had critical care for severe complications and opportunistic infections. **Keywords:** HIV-PATIENTS, ORGAN DISFUNCTION, HAART, INTENSIVE CARE TREATMENT.

Human Immunodeficiency Virus (HIV) infection is an important medical problem all over the world. The latest global statistics show that in 2017 there were 36.9 million people living with HIV. From this number, 34.5 million are adults, 17.8 million young girls (over 15 years old). Worldwide, approximately 1.8 million people were newly infected, and about 1 million people died each year from AIDS-related illnesses. In December 2017, 21.7 million people living with HIV were accessing antiretroviral therapy (1).

In the United States, about one million people are infected with HIV. There are about 40,000 to 60,000 new cases of HIV infection each year (2).

In Eastern Europe and Central Asia, there were 1.8 million people living with HIV in 2017, and in the same year about 40,000 people died of AIDS-related illnesses (1).

The epidemiological profile of patients with HIV infections is shifting in different

countries. There are a significant number of homosexual males who are infected in large urban areas, but there is a growing proportion of infected patients who are women who live in smaller cities or rural areas and who have acquired their infection heterosexually or via intravenous drug abuse (2).

The general report for Romania, showed a total of 23,302 cases of HIV/AIDS in 30 June 2018, of which 15,212 are living with HIV, about 257 people newly diagnosed and 68 deaths in the last year (3).

The population of HIV-infected patients is also getting older: patients with HIV infection benefit from improved management and live longer. Individuals without HIV infection are also living longer, and are sexually active longer, extending the period of risk for acquiring HIV infection.

Opportunistic infections occur in HIV-positive individuals as their immune system weakens and the etiology of the infectious organism is affected by the immunological status of the patient. In more economically developed countries, due to the extensive use of highly active antiretroviral therapy (HAART), infections like *Cytomegalovirus* (CMV), *cryptosporidiosis* and *toxoplasmosis* predominate. However, in developing countries, infections due to *Mycobacterium tuberculosis* are more common.

But given the importance of this pathology and combining various complications, some HIV-positive patients require intensive care in some critical moments. The HIV-infected patients are admitted in Intensive Care Unit (ICU) for different reasons. Some reasons are related to clinical issues related directly to HIV infection, such as opportunistic infections. As opportunistic infections have declined, the proportion of hospitalizations due to respiratory diseases, next to hepatic, renal, and cardiovascular disease has increased (4). Several antiretroviral agents have toxicities that can present pulmonary or respiratory manifestations.

When patients receive antiretroviral therapy that contain abacavir, they can develop a hypersensitivity syndrome that is difficult to distinguish from nonspecific febrile respiratory illnesses (5, 6). When HIV-infected patients are admitted to the ICU, a major issue is whether to continue their antiretroviral therapy or stop the drugs (2).

The aim of this study was to evaluate the clinical and viro-immunological status in order to manage HIV-infected patients admitted to ICU during the era of highly active antiretroviral therapy (HAART).

MATERIAL AND METHODS

We performed a retrospective study, using data from medical records of HIV infected patients from the Regional HIV/AIDS Center of the "Sf. Parascheva" Infectious Diseases Clinical Hospital Iasi admitted to the Intensive Care Unit of the same Hospital, between 1st January 2016 - 31 December 2018.

RESULTS

Of a total of 1,544 HIV-positive registered in the Iasi HIV/AIDS Regional Center, in the studied period were included 21 patients with different HIV/AIDS stages (18 cases C3 AIDS and 3 patients with stage B2 HIV seropositive) who were hospitalized in the Regional HIV/AIDS Center of Infectious Diseases Clinical Hospital Iasi, and whose clinical status was depressed requiring evaluation and monitoring in the Intensive Care Department.

The patients were clinically, biologically, serologically, bacteriologically, viro-immunologically and imagistically evaluated.

They were predominantly male, with an average age of 34 years, from urban area (tab. I).

Most of the cases were patients from pediatric cohort. The clinical condition from

admission was severely influenced, most of them had fever, tachycardia, tachypnea, hypoxia (tab. II).

TABLE I
Patients characteristics

Patients characteristics	Number of cases
24-30 years old	13
30-40 years old	3
> 40 years old	5
Male	18
Female	3
Urban area	11
Rural area	10
C3 AIDS stage	18
B2 HIV seropositive	3

TABLE II
Patients clinical status

Clinical status	Number of cases
Fever (t > 37.8˚C)	12
Asthenia	8
Tachycardia (> 90/min)	13
Tachypnea (> 20/min)	15
Hypoxia (PaO_2/FiO_2 < 280mmHg)	16
Dry cough	9
Oliguria	5
Confusion syndrome, aphasia	5
Meningeal contracture syndrome/neck stiffness	7
Wasting syndrome	4
q SOFA (quick Sepsis Related Organ Failure Assessment) score 2-3	4

The viral-immunological status revealed a value for CD4 lymphocytes very low from 1 to 275 cells/mmc, for most of the cases (19 patients) the CD4 value was less than 50 cells/mmc (fig.1).

Also, the viral load was significantly increased, the highest one was 5 million IU/mL (fig. 2), in accordance with the HIV patient's condition.

There were cases where viral loads recorded values between 50,000 copies/mL and 72,000 copies/mL with CD4 T cell counts, below 100 cells/mmc, especially for *late presenters* HIV-positive patients.

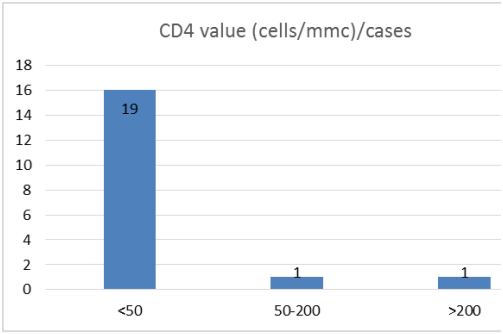

Fig. 1. Cases distribution by CD4 value (cells/mmc)

The serological tests have most frequently revealed hepatitis B virus infection and cytomegalovirus infection (Ag HBs - 6 cases, anti HCV antibodies – 2 cases, anti-*Toxoplasma gondii* (IgM/IgG) antibodies - 2 cases, anti-*Cytomegalic virus* (IgM/IgG) antibodies – 6 cases). Anti-*Treponema palladium* antibodies were positive in 4 cases.

As opportunistic infections were recorded: Tuberculosis (8 cases), *Cryptococcal* meningitis (1 case), *Mycobacterium avium* infection (1 case), *Cytomegalovirus* infection (6 cases), (fig. 3).

The blood culture did not revealed bacterial growth after 7 days of incubation. The lingual scrape revealed *Candida sp.* in most of the cases (10 cases).

Pulmonary tuberculosis was diagnosed in 8 cases by chest radiography, the sputum exam highlighted the presence of acid-alcohol resistant bacilli, and also *Quanti-Feron-TB Gold* tests were positive, followed by pneumological evaluation for mycobacterial culture and antituberculosis regimens.

There were also two cases of C3 stage AIDS disease that have associated tuberculosis with extrapulmonary localization: the lymph node and abdomen.

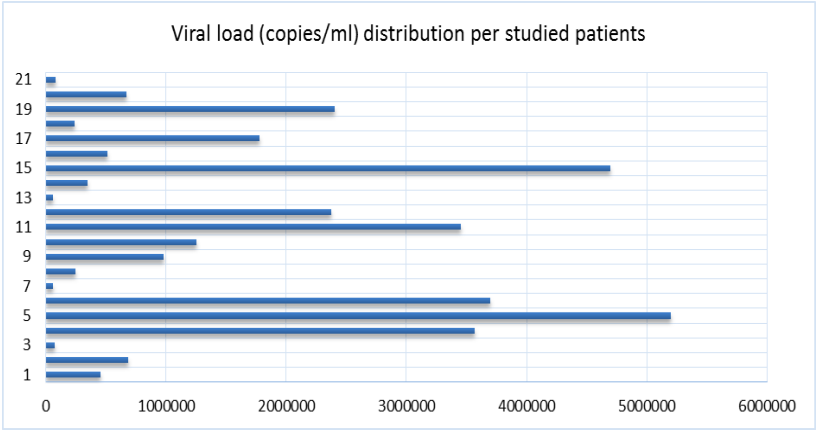

Fig. 2. Cases distribution by viral load value (IU/mL)

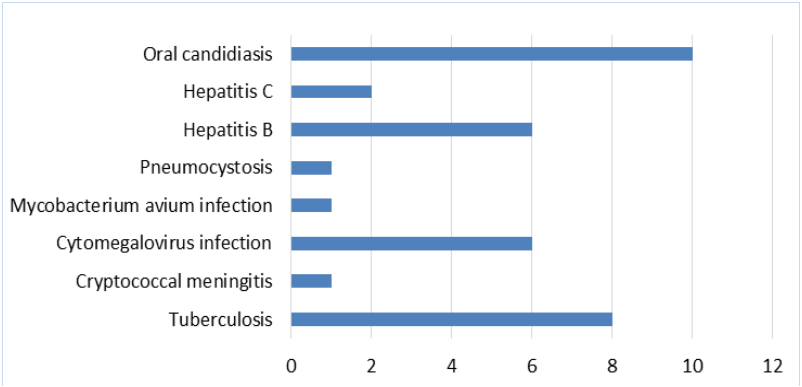

Fig. 3. Cases distribution by opportunistic infections

In one case, the diagnosis of cryptococcosis meningitis was established by examination of cerebrospinal fluid (CSF): 7 ecn/mmc, albuminorachia = 0.55g/L, glycorrhachia = 34g/L, chlororurachia = 6.65g/L, and *Cryptococcus neoformans* was identified in the fungal culture of the CSF. Also, the meningoencephalitis was recorded in 6 cases. In four cases cerebral malignant lymphoma was associated.

The most common complications were respiratory distress syndrome and bacterial pneumonia in HIV-positive patients with pulmonary tuberculosis (8 cases), and other cases had severe sepsis criteria associated with multiple organ dysfunction, with qSOFA (quick Sepsis Related Organ Failure Assessment) score: 2-3 (4 cases).

Clinical evolutive status was correlated with hepatic and renal failure, in association with dyselectrolytemia, and hypoproteinemia, subsequently patients needed assistance, evaluation and monitorization in Intensive Care Unit. Seven patients required oral tracheal intubation, 11 patients needed vasopressor support.

All patients required antiretroviral therapy in association with specific antibiotherapy or antifungals (most common was: Fluconazole, Anidulafungin, Itraconazole), depending on complication*: Candidiasis*, *Cryptococcal* meningitis, according to anti-

fungal susceptibility testing.

Also, in same cases was used nebulized polymyxin E (Colistin) for treatment of multidrug-resistant pneumonia, or in order to prevent it. Also, antituberculosis therapy was needed in treatment of pulmonary tuberculosis in 8 patients. The trimethoprim/ sulfamethoxazole was used for prophylaxis of pneumocystosis (tab. III).

The association of antibiotics was based on complications or opportunistic infections, in order to avoid drug interactions (antiretrovirals and antibiotics), as Liverpool HIV Interactions (7) require.

TABLE III
Therapy used

Antibiotic regimens used
Cephalosporins/Carbapenems + Fluoroquinolones/Aminoglycosides
Cephalosporins + Trimethoprim/sulfamethoxazole
Antituberculosis therapy

Two patients registered adverse reaction to antiretroviral therapy. The average hospitalization period was 14 days. Only one case had favorable evolution, the rest of the patients died.

DISCUSSION

In the current context of HAART, the complications that require critical care support in intensive care units (ICU), are still present, being a serious medical problem. The respiratory failure was the leading cause of ICU admissions among HIV-infected patients, followed by sepsis and neuro-oncological disease. The most affected age was the young age of patients coming from the Romanian pediatric cohort. The CD4 lymphocyte count in the patients that required admission in ICU, was very low, being associated with suppressed cellular immunity, and its association with increased mortality has also been demonstrated in prior studies (8, 9). Other studies that were conducted in European countries, also had the same causes of ICU admissions observed in our study (10). Sepsis and higher rate of mortality in the seropositive HIV patients was associated also in previous studies (8, 11-13). Co infection HIV/HCV has become a major cause of morbidity and mortality among seropositive HIV patients in HAART's era (14, 15), but in our study most of the cases were coinfection with hepatitis B virus, and in few cases hepatitis C virus was involved (15). Neurological disorders: malignant lymphoma and meningoencephalitis were noted especially on pediatric cohort like in other studies has been showed (16). The most commonly used antiretroviral regimens included rescue therapies with the administration of two protease inhibitors in combination with two nucleoside reverse transcriptase inhibitors, as previous studies described (17). The high mortality rate of HIV seropositive patients requiring monitoring in intensive care was also recorded in other previous similar studies (10, 12, 18). Our study was limited to reported cases of seropositive HIV patients in the Moldavian area.

CONCLUSIONS

The collaboration with intensive care unit was absolutely necessary in order to manage the complications and opportunistic infections in HIV-infected patients. The survival rate of HIV-infected patients admitted to the ICU was extremely low even if they had critical care for severe complications of HIV infection or for conditions related to HAART toxicity.

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BETABLOCKERS AND VARICEAL BLEEDING IN PATIENTS WITH DECOMPENSATED LIVER CIRRHOSIS

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BETABLOCKERS AND VARICEAL BLEEDING IN PATIENTS WITH DECOMPENSATED LIVER CIRRHOSIS (Abstract): It is well known that nonselective betablockers (NSBBs) reduce the risk of first bleeding and rebleeding from esophageal varices in liver cirrhosis. Recent studies suggested that in decompensated cirrhosis NSBB can be harmful and can increase mortality, so they should be used only in the "therapeutic window". This article summarizes the data regarding the role of NSBBs in the prevention of variceal bleeding, focused on decompensated liver cirrhosis and its complication. Ascites per se is not a contraindication for NSBBs, but if ascites is severe or refractory it is better to avoid high propranolol doses (>80 mg). The treatment must be discontinued when the patient with decompensated liver cirrhosis develops signs of circulatory dysfunction: arterial hypotension (systolic blood pressure <90 mmHg), renal failure (serum creatinine >1.5 mg/dL) or when Na<130 mEq/L. In spontaneous bacterial peritonitis and acute on chronic liver failure, NSBBs have favorable effects independent of decreasing the hepatic venous pressure gradient. **Keywords**: DECOMPENSATED LIVER CIRRHOSIS, NON-SELECTIVE BETABLOCKERS, BLEEDING.

Liver cirrhosis (LC) of any cause is a complex disease with high mortality. In USA it is the fifth leading cause of adult deaths and ranks eighth in economic cost among major illness; in Europe it accounts for 1.8% of all deaths (1). According to WHO there are 30 annual deaths per million population and the global average is 2.4 deaths per million (1). Romania is one of the European countries with the highest prevalence and mortality rate (36 per 100,000) (2).

At present, as the treatment of liver diseases causes substantial economic burden globally, we can rightly ask ourselves if there is a drug that is cheap, easy to use and has a large utility in chronic liver disease which could determine a decrease in the severe complications and thus to improve the quality of life and survival (3).

In 1981 Lebrec (4) demonstrated that Propranolol, a non-selective β-blocker (NSBB), reduced the risk of bleeding and rebleeding from esophageal varices in LC

and it was used until 2010 without other debates (5). We must mention that almost all studies in the literature excluded patients with advanced LC. In 2010 Serste (6) published a study which demonstrated that patients with refractory ascites on propranolol had a poorer survival rate than those who were not on NSBBs. Latest studies showed contradictory correlation between NSBBs and mortality in advanced LC (7, 8). In 2012, Krag (9) proposes "the window hypothesis" that implies the existence of a therapeutic window for NSBB treatment which closes late in the natural course of cirrhosis. Krag suggested that NSBBs are effective only within the "therapeutic window". Outside the "therapeutic window" β-blockers may be ineffective and unfortunately are potentially harmful in advanced cirrhosis.

It is well-known that portal hypertension (PH) is the initial consequence of LC and it is responsible for the majority of its complications. Patients with compensated LC can be divided into two groups based on portal pressure (PP). If PP is between 5 and 10 mmHg the patients have mild PH. When PP is >10 mm Hg the patients have clinically significant PH (CSPH) (10). CSPH is associated with an increased risk of developing varices, clinical decompensation (ascites, variceal bleeding, hepatic encephalopathy) and hepatocellular carcinoma (HCC) (11-13). CSPH is an independent predictor of decompensation in patients with compensated LC. The patients with CSPH can also be divided into two groups: with and without gastroesophageal varices (10). Management of varices and variceal hemorrhage must be considered in the context of other complications of cirrhosis. In patients with CSPH and varices one of the main goals is to prevent variceal hemorrhage and decompensation. β-blockers (propranolol, nadolol and carvedilol), decrease PH and prevent the occurrence and recurrence of variceal bleeding (10).

Currently, carvedilol, a NSBB with mild α-1 adrenergic activity, is preferred. Carvedilol is more effective in reducing hepatic venous pressure gradient (HVPG) due to α-1 blockage and by that it reduces intrahepatic resistance in decompensated LC in patients with severe dysfunction. The main adverse effect compared with propranolol is the severe decrease of mean arterial pressure which leads to poor organ perfusion in one third of patients (14).

Due to the mechanism of PH reduction, NSBBs have been proved to be effective in the prophylaxis of variceal bleeding. The AASLD guideline and Baveno VI recommend it for primary and secondary prophylaxis (15, 16).

PREVENTION OF FIRST VARICEAL HEMORRHAGE

Studies have demonstrated that NSBBs prevent first hemorrhage both in compensated and decompensated LC patients with esophageal varices (10, 17). Ponyard *et al.* (17) demonstrated that the first variceal bleeding was significantly reduced not only in patients without ascites, but also in those with ascites. In Garcia-Tsao study (10) the risk of first hemorrhage was significant lower in patients receiving NSBBs compared with those receiving placebo even if their Child-Pugh score was higher than 8 (Class C). In their study, D'Amico *et al.* (18) demonstrate that NSBBs are reliable to use for the primary prophylaxis, reducing the risk of first bleeding by 10%. However, NSBBs do not reduce the mortality risk in patients with medium-large varices when

NSBBs are used for primary prophylaxis. Opposite, when used for secondary prophylaxis they reduce the risk of rebleeding by 21% and also the mortality risk (18). Gluud and Krag (19) demonstrate that, for medium and large varices, NSBB prophylaxis is as efficient as esophageal variceal band ligation (EVL).

In patient with large varices NSBBs are also effective. Villanueva *et al*. (20) demonstrated that the reduction of HVPG >10 mmHg improves the outcome. The authors reported a significant reduction of first bleeding and mortality by using NSBBs in patients with large varices (20). More than that, Hernandez-Gea *et al*. (21) demonstrated that the responders (a reduction in HVPG of ≥10 mmHg) had a lower probability of developing ascites. Finally, for clinical practice purpose, we must know that β-blockers can be successfully used in prevention of the first variceal hemorrhage. The following categories of patients can benefit from this prevention: patients with high risk of hemorrhage: medium/large varices, any size varices with red wall marks and any size varices in Child C patients. In patients with or without ascites, β-blockers are the first line of treatment for preventing the first hemorrhage episode; if the patient is β-blocker intolerant, band ligation is recommended, according to local expertise and patient preference (21).

PREVENTION OF REBLEEDING IN LIVER CIRRHOSIS

In a systematic review, Puente (22) demonstrated that the addition of drug therapy with β-blockers ± isosorbide mononitrate to EVL significantly improves the efficacy of EVL, achieving a significant reduction of rebleeding risk. The beneficial effect of combination therapy was observed on both variceal rebleeding and overall rebleeding.

A number of studies have demonstrated that reducing portal pressure with NSBBs to <12 mmHg or ≥ 20% from baseline value decreases rebleeding risk in less than 10% of the cases (23). In the β-blocker-responsive patients (decrease of HVPG <12 mmHg) bleeding episodes and death rate are significantly lower. Besides that, some studies have demonstrated that reaching these targets are associated with a significant decrease in the risk of developing other PH-related complications such as ascites or hepatorenal syndrome and significantly improves survival (24). Garcia-Tsao also demonstrated that the combination NSBB + ligation is associated with lower rebleeding rates (15). For clinical practice we must know that for prevention of rebleeding – ascites is not a contraindication (15, 23). First line therapy for all patients is combination of NSBB (propranolol or nadolol) + EVL, NSBB being the key element of this combination, acting in a physiological manner (15). Carvedilol is not recommended is secondary prophylaxis. If variceal bleeding occurs while on adequate NSBB dose the patient is considered no responder and should be evaluated for trans jugular intrahepatic portosystemic shunt (TIPS), surgery or transplantation (15, 16).

HOW TO USE BETABLOCKERS IN PATIENTS WITH ASCITES?

Ascites per se is not a contraindication for NSBBs. If ascites is severe or refractory it is better to avoid high dose propranolol (>80 mg) and the use of carvedilol, because it can determine sever decrease of mean arterial pressure and poor organ perfusion (24).

The manner in which β-blockers are

administered in patients with ascites is also important. In these patients low-dose (≤80 mg/day) propranolol administrated step by step is preferable. All these measures improve tolerance and reduce side effects until maximum tolerated dose is reached. For example, propranolol is thus administered: first week: propranolol 20 mg in the morning and in the evening; second week: 20 mg in the morning and 40 mg in the evening, third week: 20 mg in the morning and evening; fourth week (if is well tolerated): 40 mg in the morning, 20-40 mg at lunchtime, 40 mg in the evening (15, 25).

The treatment must be stopped when the patient with decompensated LC develops signs of circulatory dysfunction: arterial hypotension (systolic blood pressure < 90 mmHg), renal failure (serum creatinine >1.5 mg/dL) or when Na< 130 mEq/L (15, 16, 26). Moreover, when hepatorenal syndrome or hemorrhagic shock occur, terlipressin or vasopressors should be used. When arterial hypotension occurs, studies (15, 23) recommend to find and treat other causes of arterial hypotension (infections), reduce the NSBB doses and then discontinue the treatment and monitor blood pressure changes. When severe hypoalbuminemia (<25 g/dL) is present, plasma expansion must be used. In primary prophylaxis of variceal bleeding, studies recommend to switch from NSBB to EBL and in secondary prophylaxis it is advisable to try to maintain low propranolol dose.

BETA-BLOCKERS AND SPONTANEOUS PERITONITIS

Thalheimeire *et al.* (26) proved that in cirrhotic patients β-blockers decrease bacterial translocation from the gut by diminishing splanchnic blood flow - secondary to reducing intestinal mucosal edema and congestion and increasing intestinal transit stimulation (β-adrenergic receptor-mediated pathway) (27). NSBB also enhances the immune system by inhibiting the stress-related cyclic adenosine monophosphate-protein kinase which has an inhibitory effect on the immune system (28) and significantly decreases the incidence of postsurgical laparoscopic infections (29).

In spontaneous bacterial peritonitis (SBP), β-blockers reduce infections and improve survival, this way preventing other complications of cirrhosis, at doses of up to 160 mg/day. If in SBP severe hypotension (systolic blood pressure < 130 mmHg) and alteration of renal function do occur, β-blockers must be administered in a lower dose or stopped. They may be reintroduced after correction of kidney and circulatory dysfunction, in order to prevent recurrent hemorrhage (28). β-blockers reduce infections and improve survival independent of the prevention of bleeding and hemodynamic response; it is still not entirely clear if NSBBs can prevent the occurrence of SBP in cirrhosis with ascites (NSBB treatment ↓ bacterial translocation) (28, 29).

NSBB AND ACUTE ON CHRONIC LIVER FAILURE

Acute on chronic liver failure (ACLF) is a complication of liver cirrhosis characterized by acute deterioration of liver function, multiple organ failure and high mortality. Its main characteristic is systemic inflammation. The severity of ACLF is an independent predictor of mortality**.** It is known that NSBBs have beneficial effects on gut motility reducing bacterial translocation, permeability and systemic inflammation. Ustundag (30) reported for the first time that ongoing treatment with NSBBs in

cirrhosis is safe and reduces mortality if patients develop ACLF, reducing inflammation. Mookerjee (31) demonstrated that NSBBs reduce short time mortality (28 days) but do not improve long time mortality**.** The administered dose was 40 mg (20-80 mg) -lower than in previous studies- and the conclusion of the study is that ongoing administration of NSBB is safe, cheap and effective in cirrhotic patients developing ACLF.

CONCLUSIONS

Revising the medical literature, combined with clinical practice, we can conclude that NSBBs remain the best prophylactic treatment for first bleeding in patients with decompensated LC. Ascites per se is not a contraindication but, if ascites is severe, avoid high dose of propranolol (>80 mg) and do not use carvedilol. For secondary prophylaxis of bleeding the standard therapy consists in a combination of a NSBB (propranolol or nadolol, not carvedilol!) and EVL. In severe circulatory dysfunction (blood pressure < 90 mmHg, creatinine > 1.5 mg/dL, Na<130 mEq/L) propranolol should be stopped. We must try to restart Propranolol with progressive doses (mainly to prevent rebleeding). Due to reduction in bacterial translocation and decreasing the systemic inflammation NSBBs have also beneficial effects in other complications of LC: SBP and ACLF.

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TUBERCULOSIS: BETWEEN THE OLD AND THE NEW

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TUBERCULOSIS: BETWEEN THE OLD AND THE NEW (Abstract): Isoniazid, rifampicin, ethambutol and pyrazinamide are administered in combination for at least 6 months in pulmonary tuberculosis (TB) -one of the first causes of death worldwide- and represent the first-line antituberculotic drugs, with different mechanisms of action, efficacy and toxicity. The susceptibility of the mycobacterial subpopulations to chemotherapy can be affected in immunocompromised patients so a systemic infection, post-primary, latent or even multidrug resistant is then activated. There is an urgent need to develop new drugs or to repurpose existing ones, that have not yet been tested for their antimycobacterial activity. Moreover, the only available antitubercular vaccine does not imply protection against adult lung active TB, the most prevalent form. Additionally, the only new antitubercular agents approved in the last years - bedaquiline and delamanid - have not provided satisfactory results in eradicating this ancient disease. Recent studies have shown surprising information regarding the use of statins in TB, indicating that they limit the mycobacterial growth by promoting autophagy and apoptosis in macrophages and by inhibiting the synthesis of cholesterol, the precursor of the virulence factors. Other new findings in TB management include the beneficial effects of vitamin D supplementation, with increased healing rates, high bacterial toxicity and the observed decrease of resistant strain selection, when using phenothiazines in coadministration with isoniazid. **Keywords:** TUBERCULOSIS, STATINS, PHENOTIAZINES, BEDAQUILINE, VITAMIN D.

Despite all the efforts to eradicate it, tuberculosis (TB) remains today one of the leading infectious causes of death and a major health issue worldwide (1). It is caused by *Mycobacterium tuberculosis*, a member of a bacterial complex which also includes other species such as *M. bovis*, *M. africanum*, *M. caprae*, *M. microti* and *M. canettii*, most of them having the ability to trigger the disease mostly in humans (2-4).

Although pulmonary TB has the highest prevalence, in adults, as well as in children, the disease can also be extra-respiratory, affecting other organs – such as skin, bones, genitourinary tract, pericardium, abdomen - including the head and neck region (face and neck lymph nodes, thyroid, larynx, pharynx, salivary glands, tongue, mandible and buc-

cal mucosa) (5-11).

According to the World Health Organization (WHO) there are more than 10 million new cases of TB every year and it represents one of the first ten leading causes of death in the world and the leading death cause in patients with human immunodeficiency virus (HIV) (12), but the global mortality rate is falling at 3% every year (13) due to the fact that TB is treated with at least 6-months regimen of four first line-drugs (isoniazid, rifampicin, ethambutol and pyrazinamide) and not by drinking water, staying isolated, outside in fresh air, as they used to treat it before the first discoveries of streptomycin in 1944 and isoniazid in 1952 (12, 14, 15).

This highly threatening TB additionally possesses serious economic risks worldwide because it affects mostly individuals between 25-45 years old, which means it affects their most productive period of life (8,13). Mycobacterium can be contracted from humans or from animal hosts and it becomes more dangerous in immunosuppressed people (14, 16). Patients with sputum smear-positive are usually diagnosticated with pulmonary TB which affects the lungs and is the most contagious form of the disease because the bacteria is expelled by coughing into air-droplets, also called Flugge's drops, but it can also affect extrapulmonary sites (2, 14).

PHARMACOTHERAPY

Once active disease is manifested, 4 *first-line drugs,* with different mechanisms of action, are administered for 2 months, as an intensive phase, and for 4 months, as a continuation phase, in new TB cases; respectively, for 8 months, as an intensive phase, and 12 months, as a continuation phase, in previously treated cases, when a first treatment failure is present (3, 17, 18). Monotherapy with isoniazid can be used, for 9 months, but only in asymptomatic patients with latent TB (3, 16, 19).

To understand how to manage TB treatment, it is important to distinguish between different populations of *M. tuberculosis*, which react to various antibiotics, because *M. tuberculosis* has a slow response towards treatment, due to its capacity to form heterogenous bacterial populations and its different mechanisms of adaptation to the environment and because M. infection presents like a polymicrobial infection (4, 18, 20, 21). Subpopulations are structured by their drug tolerance or their location and they are composed of actively growing bacteria, slow or non-replicating bacteria, intracellular bacilli present in the acidic compartments of macrophages or lesions and bacilli that persist in hypoxic areas, the last ones representing the main cause of multi-drug-resistance tuberculosis (MDR-TB) (20).

Traditional anti-TB drugs

First-line drugs are different from second-line because of their efficacy over the actively replicating bacteria, activity and toxicity: isoniazid, rifampicin, ethambutol, pyrazinamide, the first two representing the most active and the most important antimycobacterial drugs, but also the ones to which MB first gains resistance (3, 16, 20). The combination of isoniazid-rifampin will cure more than 95% cases, in 9 months, but if neither isoniazid or rifampicin can be used due to prevalence of 3% resistance to both, treatment will encounter a prolonged phase for more than 2 years, the patients risking toxicity and adherence issues (14, 16).

Isoniazid is an enzyme inhibitor, highly specific for mycobacteria, which has a

similar structure to pyridoxine (vitamin B_6) and is a prodrug that inhibits mycolic acid synthesis, by covalent binding, meaning it affects the bacillus cellular wall (12, 14, 16, 22). It has bactericidal effect in dividing cells but is bacteriostatic in resting cells (3, 14). One of the most important adverse reactions is clinical fatal hepatitis which can occur in 1% patients, especially for those aged 50 or older, treated with isoniazid (2, 14). Less common, in patients with pyridoxine deficiency, are central nervous toxicity and hematologic issues, but they can be prevented by recommending 25 mg of pyridoxine with each oral dose of isoniazid, as also for pregnant women, alcoholics and patients with poor regimen of life (1, 3, 14).

Rifampicin, an antibiotic produced by a species of *Streptomyces*, introduced in the early 1970s as one of the most active antituberculotic drugs, has a sterilizing bactericidal action over slow or non-replicating subpopulations of *M. tuberculosis*, a bacilli that adapt so well to dynamic surroundings, and has represented a success in reducing the length of treatment, from 18 months to only 6 months, which means reduced toxicity and increased adherence (3, 14, 17). Rifampicin binds to the β subunit of bacterial DNA-dependent RNA polymerase and inhibits RNA synthesis, easily penetrating phagocytic cells and engulfing the bacilli (12, 14). It is active against intracellular organisms from lung cavities and it is usually administered orally in 600 mg dosage, daily or twice a week, but it can also be administered intravenously, when it is metabolized to also active rifampin (3, 14, 23). Unlike isoniazid as enzyme inhibitor, rifampicin is a potent inductor of the cytochrome P_{450}, so other treatments in TB patients such as antiretroviral or contraceptive should be reconsidered (14, 16, 21). The half-life of rifampicin is 1 to 5 hours, which shortens after a period because of induction of hepatic enzymes (2, 14). HIV patients generally receive rifabutin in a 300 mg/day dosage, instead of rifampicin, due to its lower capacity to induce microsomal activity, but, unfortunately, rifampicin-resistant organisms are also resistant to rifabutin (8, 14, 16). Adverse reactions include skin rashes, occasionally hepatitis and liver damage, nephritis and, if administered less often than twice weekly, it may cause a flu-like syndrome characterized by sweats, chills, fever, myalgia and thrombocytopenia, so patients should be warned not to skip doses (3,14). Rifapentine is an analogue of rifampin, also a bacterial RNA polymerase inhibitor, a potent enzymatic inducer and it is indicated only in rifampin-susceptible strains during the continuation phase, after 2 months of treatment (14, 16). Rifapentine should not be used in HIV infection because it gives high relapse rate with rifampin resistant organism (14, 19). Although cross-resistance with other drugs is not usually a problem, resistance can develop rapidly from a chromosomal mutation, as well as in isoniazid case when a gene mutation is involved (2, 3). Medical products exist to prevent resistance and to increase the patient's compliance for the treatment, such as fixed-combination with rifampin 120 mg, isoniazid 50 mg and pyrazinamide 300 mg, in a classic dosage of 5 to 6 tablets per day (3,22).

Because specialists cannot count differentiated populations of *M. tuberculosis* within a given patient, a multi-drug treatment is used not only with isoniazid and rifampicin, but also with *pyrazinamide* which appears most active against organisms from caseating granulomas, in an

acidic pH, in almost dormant state, with occasional bursts of metabolic activity (3, 17). When entering the lung delimited zone of the bacillus, pyrazinamide turns into pyrazinoic acid, the real active compound, through specific mycobacterial enzymes where it inhibits bacterial fatty acid synthesis (2, 3, 14). These actions represent a specific in vivo sterilizing mechanism, pyrazinamide requiring incubation within an acidic pH, found to lack under in vitro conditions (22). It has a half-life of 11 hours, is intestinally well absorbed and widely distributed in the tissues. Pyrazinamide relies on a hepatic clearance, so hepatic adverse events such as elevations of serum transaminases can occur. Similar to other first-line drugs, pyrazinamide treatment can also be associated with nausea, fever, fatigue, dark urine and pale feces, hyperuricemia with even gouty arthritis (3,14). The hyperuricemia that occurs with pyrazinamide does not respond to usual antigout medicine, in comparison with ethambutol.

Ethambutol is a specific antituberculotic agent, always given in combination with other antituberculotic drugs, in a 50 mg/kg twice a week dosage and it inhibits mycobacterial arabinosyl transferases which are involved in the intracellular synthesis of arabino glycan, the essential component of the bacillus wall (14). Ethambutol also has a bacteriostatic effect by inhibiting the synthesis of polyamines which are necessary for the normal DNA function (3, 12), is well absorbed from the gut and its maximum peak levels are reached in 2-4 hours (2, 14). It is one of the most efficient drugs in TB meningitis, without difficulty crossing the blood barrier in meningeal inflammation (2). It accumulates in renal failure due to its unchanged form in renal excretion and it can cause retrobulbar neuritis, loss of visual acuity and red-green color blindness, if administered for more than several months. Fortunately, the visual neuritis is reversible on the rapid discontinuation of the treatment (3). This is the reason why ethambutol is contraindicated in children under 6 years old (3, 14).

Resistance and cross-resistance

WHO recommends a directly observed treatment, short-course strategy (DOTS) to control tuberculosis which encourages the supervision and the direct observation of a healthcare worker for the patients in order to maintain their adherence (13, 17). The granulomatous tissue in TB, earlier discussed, is an ordered cellular architecture, structured with epithelioid cells and fibroblasts, surrounded by lymphocytes, known as Langhan's cells, which remain stable for months or even years, until lymphocytes are requested in other immune processes (9, 20, 23, 24). Necrotic dynamic lesions are formed and when the destruction reaches the bronchi, a cavity appears and its degree determines the failure of the treatment (18, 20). As a response, this affects the susceptibility of the mycobacterial subpopulations to chemotherapy so a systemic infection, post-primary, latent or even multi-drug resistant is then activated in the immunocompromised patients with the appearance of extensively multi drug resistant tuberculosis (XDR TB), mostly present in HIV co-infected patients (8, 18, 23, 25). In 2017, 558,000 people developed TB that was resistant to rifampicin, the most effective first line drug, and of these, 82% had MDR-TB, found particularly in India, China and Russia, which represented an increase from 2016 cases (13). Even though there is no standard regimen for

MDR cases, second-line tuberculostatic treatment is used for drug-resistant TB, but it is important to mention that is critically necessary to avoid monotherapy and from the other point of view, *streptomycin, kanamycin and amikacin* as well as *fluoroquinolones* may cause fetal ototoxicity and fetal growth problems and they should not be used in pregnancy or children with TB (3,17). The inherent toxicity of second-line drugs used in MDR-TB is also demonstrated by the fact that *cycloserine* may cause paranoia, confusion, depression, photosensitivity and sun precautions should be taken, *prothionamide* may trigger arthralgia and myalgia, aminoglycosides affect the kidney and vestibular functions and in all cases liver function should be monitored (2,3,20). In XDR-TB there is an additional resistance to any fluoroquinolone and any second-line injectable treatment, and it requires lengthy use of more toxic antibiotics such as meropenem, imipenem-cilastatin, macrolides, with a yet unknown and unclear efficacy (26).

All in all, drug resistance, adverse effects, poor drug quality, malabsorption or underdosing, nonadherence represent undeniable causes for treatment failure, increasing the need to investigate biomarkers that are conclusive for predicting relapses after treatment (3, 13). Moreover, the only available antitubercular vaccine does not imply protection against adult lung active TB, the most prevalent form, so there is a rush all over the world to determine new antituberculotic mechanisms, drugs and vaccines, in order to eradicate this ancient disease (17, 23).

New anti-TB drugs and regimens

New active substances such as *bedaquiline and delamanid*, both discovered in 2013, were subjects in trials for usage in MDR-TB and have received conditional approval based on Phase IIb trial results, as WHO underscores (3,12,13,17). Actually, delamanid, an imidazooxazole derivative, was found to accelerate sputum clearance of *M. tuberculosis* by almost 50% in 2 months, by liberating toxic nitric oxide within macrophages (3). Since 2014, WHO has recommended the use of delamanid as part of antitubercular regimen in MDR-TB patients, including children aged 6-17 years, due to its safety and efficacy (13). Bedaquiline (BDQ), a diarylquinoline, is the only clinical drug approved in the last 40 years and has also a molecular mechanism by directly blocking the adenosine triphosphate synthase complex, but unfortunately, it involved higher death rates in patients, even though the treatment provided efficacy (3, 13, 22). BDQ has shown activity against non-replicating and hypoxic populations of *Mycobacterium*, a first bacteriostatic effect followed by a bactericidal effect after only 4 days of treatment which means it is possible that BDQ has additional targets unidentified yet (22, 27). Different all-oral regimens containing BDQ and delamanid for MDR-TB are involved in a global clinical development organized by WHO in 2018, which tries to combine old tuberculostatic agents with new ones, such as: BDQ-pretomanid-moxifloxacin-pyrazinamide regimen, delamanid-linezolid-levofloxacin-pyrazinamide for quinolone sensitive MDR-TB, BDQ-pretomanid-linezolid, BDQ with two optimized background regimens oral for 9 months or with oral and injectables for 6 months called the STREAM (Standardized Treatment Regimen of Anti-TB Drugs for patients with MDR-TB) trial (13, 26, 27). The STREAM trial was initiated in March 2016 and the first results are expected not

earlier than 2020, but WHO mentions different trials involving BDQ as treatment in children TB, implemented in Russia, South Africa and in the Philippines (13, 28). The mediocre outcomes in MDR-TB treatment success rates have led to a series of cohort studies over 12 years in Bangladesh, and, for example, the last cohort had a cure-rate of 87.9% using a 9-months fluoroquinolone-regimen (gatifloxacin and isoniazid given at a higher dosage) in 206 patients (28). The purpose of STREAM trial, stage 1 in Ethiopia, South Africa, Vietnam and Mongolia, is to compare the 9-month Bangladesh-regimen with a standard WHO-recommended care comprised of moxifloxacin, clofazimine, ethambutol and pyrazinamide given for 40 weeks with kanamycin, isoniazid and prothionamide given during the 4-month in the intensive phase (13, 28). In stage 2 of STREAM trial, BDQ replaces kanamycin over 9 months, and levofloxacin replaces moxifloxacin, in order to avoid cardiovascular adverse effects, but results have not been published yet (13, 28, 29). The last anti-TB drugs, mentioned by WHO, involved in phases I of clinical trials are TBA-7371, shown to be effective against resistant M. tuberculosis strains, in the USA and TBI-166, with a similar efficacy to clofazimine, in China (13, 28).

Repurposing of existing drugs

Development of new TB treatments or new antibiotics is time consuming and requires high financial resources, so the repurposing of already existing drugs represents an actual possible shortcut (30, 31). This matter would not only reduce the duration of curative treatment for TB, but the initiation of host-directed therapies would enhance the effects of standard treatment and would decrease the relapses or the number of patients with MDR-TB (30,32).

The first study on the potential effect of *statins*–3-hydroxy-3-methyl-glutaryl-CoA reductase or HMG-CoA reductase inhibitors- was performed 19 years ago when Montero *et al*. showed that fluvastatin could strengthen the host response against *M. tuberculosis* by releasing more cytokines and promoting the activation of immune responses (31, 33). Furthermore, statins increase the number of interleukins through a pleiotropic- immunomodulatory and anti-inflammatory- effect on the whole immune system, they limit the mycobacterial growth by promoting autophagy and apoptosis in macrophages and by inhibiting the synthesis of cholesterol, due to their label as lipid-lowering drugs (31, 33, 34). *M. tuberculosis* needs lipids and cholesterol as a carbon source to develop its virulence factors and its cell wall, to internalize itself in host cells, to lower its susceptibility to antibiotics, which explains why hypercholesterolemic mice infected with *M. tuberculosis* have an accentuated pulmonary pathology (33, 35). The composition in lipids of the bacillus wall determines permeability to other substances and most important, its resistance to antibiotics through its architecture formed by peptidoglycan, arabinogalactan and mycolic acids linked with those lipids (30, 32, 35). Therefore, statins augment protection against TB by lowering the infection-induced lipid accumulation in macrophages, reduce the reactivation of latent TB, attenuate cholesterol levels, prevent the lung tissue destruction, regardless of sex, age and comorbidities (36). Interactions between standard antituberculotic agents and statins have also been studied and the addition of 25 mg/kg simvastatin to rifampicin 10 mg/kg, isoniazid 10 mg/kg and

pyrazinamide 150 mg/kg given five times per week, showed an important reduction in bacillary populations even in the 56th day of treatment (30). Other studies showed that the association between rifampin and atorvastatin did not increase muscle tissue damage, nor the hepatotoxicity, even though statins have been described as generators of oxidative stress in hepatic cells (32). Yet, simvastatin shortens the 6 months antituberculotic treatment, but triggers myopathy and rhabdomyolysis faster than atorvastatin and pravastatin, which are safer to use because they have not shown interactions with rifampicin (32, 34). The statins mechanism, as inhibitors of HMG-CoA reductase, leads to new ideas due to the fact that the enzymatic inhibition down-regulates not only the biosynthesis of cholesterol, but also of lipid hormones, isoprenoids and vitamin D (18, 31).

In the last 5 years, studies have shown that *vitamin D* is involved in innate immune responses, in host defense, in the control of proliferation and necroptosis of M. macrophages (19, 21). Vitamin D treatment increases not only protein kinases leading to inhibition of proinflammatory cytokine production in monocytes and macrophages, but also calcium absorption which can activate autophagy through other kinases and phosphatases and compromise the bacillus viability (2, 21). Since ancient times, TB tends to occur during the colder season when cutaneous synthesis of vitamin D from the sun exposure is reduced and serum levels are lower, and maybe treating TB patients before the 17th century by exposing them to sun and fresh air was not a bad idea after all (17, 21). Deficiencies of vitamin D and vitamin A -lipophilic vitamins- and polymorphisms in vitamin D receptors have been associated with TB risks, because there is considerable evidence that vitamin D is essential for human macrophages to kill *M. tuberculosis* in vitro (3, 13). Patients supplemented with 0.25 mg daily of vitamin D reduced the time for sputum smear conversion from positive to negative, but while other studies have proven to be disappointing, vitamin D supplementation seems to protect against infection with *M. tuberculosis* (21, 24, 25). The antimycobacterial action of statins may be a faster and more efficient way to provide an entirely new class of drugs to treat TB and may also be a very important association with the standard regimen against MDR-TB or even XDR-TB (32). Additional preclinical studies for both statins and vitamin D are required to identify further possible interactions, the safest dosage and the optimal way to administer, as prophylaxis or not (19, 31).

Another surprisingly available class of nonantibiotic drugs, discovered since 1883, has shown antituberculotic activity for more than decades, but has only been intensively studied in the past years (37,38). What is special about *phenothiazines* is that they can suffer core structure modifications in a possible manner, by remodeling N-alkylamine side chains with N-alkylsulphonates, that does not affect their ability to inhibit *M. tuberculosis*, but only their ability to influence the nervous system activity (37). Non-neuroleptic phenothiazines down-regulate calcium and potassium intracellular transport as calmodulin inhibitors, promote acidification of the phagocytic cells, therefore stimulating macrophages to kill the bacillus (37, 38). Thioridazine, especially in its (-) enantiomer form with no central nervous activity and no cardiac side effects, is able to inhibit the efflux pump in *M. tuberculosis*, to

modify the transport of various substances through the membrane, to increase the susceptibility to fluoroquinolones and to reduce resistance to isoniazid (38, 39). A promising cure in Argentina, of 10 patients from 12, with MDR-TB and XDR-TB, generated by the association of 75 mg of thioridazine with linezolid and moxifloxacin, makes phenothiazines suitable potential candidates for MDR-TB treatments (38, 40). Another study determined a human dosage of 25 mg/kg of thioridazine, associated with isoniazid and rifampicin, to be well-tolerated and to have antimycobacterial therapeutic efficacy and it also appeared that thioridazine targeted both replicating and nonreplicating bacilli in the murine model of TB (41). Furthermore, although thioridazine was ineffective as single antituberculotic therapy, in coadministration with isoniazid, it prevented the selection of resistant strains, through different pathways, mainly because it blocked the calcium binding to vital mycobacterial proteins and produced cell wall damage (39,40). They are not only antimycobacterial, antibacterial or antifungal, but also antihistaminic, analgesic, antimalarial, antiviral, anticancer and anti-inflammatory, phenothiazines being capable of activating biological systems, interaction with cellular DNA, influence over cellular neurotransmitters with a lipophilic character, so chlorpromazine, methdilazine, thioridazine and trifluoperazine are worthy of further investigations in latent TB, MDR-TB and XDR-TB cases (38, 39, 41).

CONCLUSIONS

Our study highlighted that the patient's immune status and social condition represent important factors which influence the susceptibility to infection with *M. tuberculosis* and the TB severity, one of the world's leading causes of death. In order to eradicate a bacilli that has a greater life experience than the worldwide human population it is important to gather all information regarding old and standard treatments, to repurpose drugs to reduce costs, to grow efficacy of antituberculotic agents, to define biomarkers and to understand the local survival mechanisms of the internal mycobacterial cells. For every molecule our body uses to escape from infection, Mycobacteria fights back with more molecules, so further biochemical studies should investigate not only the patient's status, but also the "social condition" of *Mycobacterium tuberculosis*.

CONFLICT OF INTERESTS

There are no conflicts of interest. All authors made equal contribution to the paper, to that of first authors, and thus share main authorship.

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CONSIDERAŢII PRIVIND UNELE EFECTE ALE ALCOOLULUI ASUPRA METABOLISMULUI LIPIDIC. STUDIU PROSPECTIV PE 200 DE PACIENŢI ÎN PERIOADA 2006-2008

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SOME CARDIOVASCULAR EFFECTS OF ALCOHOL. PROSPECTIVE STUDY ON 200 PATIENTS BETWEEN 2006-2008 (Abstract) : Several studies have confirmed that drinking a moderate amount of alcohol (ethanol) decreases the risk of heart disease, heart attack, and stroke. There are several mechanisms of health benefits of alcohol, which include anti-inflammatory, antioxidant and anti-blood clotting effects. The aim of the study is to underline the effects of alcohol consumption over cholesterol blood levels. **Material and methods** : The study was conducted on 200 individuals from urban and rural areas of Iaşi county (100 being alcohol consumers), during the 2006-2008. **Results** : The cholesterol blood levels are greater than the normal accepted values, both in men and women (urban and rural), drinkers or non-drinkers. **Conclusion** : The cholesterol levels are higher in drinkers, women and rural population included in our study. We did not do a quantification of other factors which may increase the cholesterol blood levels, such as diet, body mass index and physical activities. **Key words** : DRINKERS, ALCOHOL BENEFITS, CHOLESTEROL BLOOD LEVELS

NEUTROPHIL-LYMPHOCYTE RATIO IN DIFFERENTIAL DIAGNOSIS BETWEEN TUBERCULOSIS AND MALIGNANT PLEURAL EFFUSIONS

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NEUTROPHIL-LYMPHOCYTE RATIO IN DIFFERENTIAL DIAGNOSIS BETWEEN TUBERCULOSIS AND MALIGNANT PLEURAL EFFUSIONS (Abstract): Pleural effusion (PE) is a common clinical problem, etiologically, difficult to diagnose, requiring invasive procedures. During last years, serum neutrophil-lymphocyte ratio (NLR) has been investigated as a new inflammatory marker. The **aim** was to evaluate serum NLR in differential diagnosis between tuberculous (TB) and malignant pleural effusions (MPEs). **Material and methods**. A retrospective study included adults, diagnosed with PE and hospitalized in Constanta Pulmonology Clinic, between March 2017 and September 2018. Biomarkers were collected from serum and pleural fluid. Final diagnosis of malignancy was assessed by pleural fluid cytology and/or pleural biopsy. The evidence of Mycobacterium tuberculosis in smears or culture from pleural fluid or caseous granulomatous specific lesions in pleural biopsy samples were considered suggestive for TB etiology. **Results:** 463 cases were divided into 110 MPEs and 353 TBPEs. NLR increased in both categories of patients, mainly in malignancy group (p=0.001). **Conclusions:** serum NLR seems to be useful in evaluating the etiology of malignant or TB pleural effusions. **Keywords:** PLEURAL EFFUSIONS, TUBERCULOSIS, CANCER, NEUTROPHIL-LYMPHOYTE RATIO.

Pleural effusion (PE) is a frequent chest disease, with difficult etiologic diagnosis, requiring invasive procedures (1). In Romania, tuberculosis (TB) and malignancy are the most common causes of exudative PEs (2). Detection of *Mycobacterium tuberculosis* in pleural fluid has low sensitivity due to paucibacillary nature of TB. The evidence of caseous granuloma in pleural samples, obtained by invasive procedure like pleural biopsy, is representing gold standard of TBPE diagnosis (2, 3).

Malignant pleural effusions (MPEs) are diagnosed by revealing tumor cells in pleural fluid or tissue, by biopsy. Both malignant pleural mesothelioma and paraneoplastic effusions represent advanced forms of cancer, sometimes difficult to differentiate (4-7). Analysis of pleural lactate dehydrogenase (LDH), proteins, glucose, adenosine deaminase enzyme level (ADA), and differential cell counts in pleural fluid, remains essential and challenging for PEs etiological diagnosis (2). While neutrophilic predominance in pleural fluid is a predictor for parapneumonic effusion and elevated pleural ADA for TB (2), there is no specific biomarker for MPEs. During last years, neutrophil-lymphocyte ratio (NLR) has been investigated in serum as a new inflammatory marker, associated with poor survival in many types of solid tumors, MPEs including mesothelioma (1, 3, 8) and predictive for bacteremia, discriminating TB from parapneumonic effusions (8-10). NLR is, also, correlated with worse prognosis of inflammatory or infectious diseases, including tuberculosis and pneumoconiosis (11-13). Chest precision medicine is constantly searching for genetic, epigenetic, metabolic, inflammatory, oxidative stress biomarkers (14) and more affordable biomarkers as blood eosinophils (15), serum aminotransferases (16, 17) or blood NRL in COPD patients were proposed (18).

The purpose of this study was to evaluate the utility of NLR in serum, for exudative PEs differential diagnosis between TB and malignancy.

MATERIAL AND METHODS

A retrospective study included 463 adults, hospitalized, in Constanta Pulmonology Clinic between March 2017 and September 2018, for exudative PE. Patients informed consent the procedures for PE investigation. Study was approved by the Ethics Committee of Constanta Clinical Pulmonology Hospital, Romania, and conducted in accordance with the principles of Helsinki Declaration. Serum and pleural fluid biomarkers, such as LDH, C-reactive protein (CRP), protein, glucose, ADA, differential cell counts were assessed. Patients with transudative effusions were excluded. Etiology was based on pleural fluid cytology and bacteriology, as well as histological exam of pleural biopsy samples. All cases were divided into two groups: TBPEs and MPEs. NLR score was obtained by dividing the absolute neutrophil count by the absolute lymphocyte count in the complete blood count of the serum. Statistical analysis, performed by SPSS software version 25.0, used Pearson or Spearman correlation coefficient to evaluate the linear respectively non-linear correlation between quantitative variables or categorical variables. Univariate comparisons were made with χ^2, 2-sample t-tests, Mann-Whitney test. The difference was considered significant when $p < 0.05$.

RESULTS

463 cases with exudative PEs, mainly older in MPE group (66.64 +/- 11.40 versus 45.10 +/- 18.17; p=0.000), were divided into 2 groups: TB (n=353) and MPEs (n=110) (Table I). Statistically significant differences between groups were found for white blood cells (WBC) counts (p=0.003), pleural proteins (p=0.000) and ADA (p=0.000). Because both variables had not a normal distribution, Mann Whitney U Test was applied revealing significant differences for blood NLR (p= 0.0001) and pleural LDH values (p=0.0004) among groups (tab. I). Distribution of cases by

etiology, according to NLR quartiles (q1 < 2.3; q2= 2.3-4.95; q3 > 4.95), was statistically significant (p=0.0003) (fig. 1).

Overall, both average and median values of NLR were higher in men (4.60; 3.5) versus women (4.01; 2.95) (p=0.003), especially among MPE cases (p=0.009). Significant correlations between NLR and WBC, hemoglobin, pleural LDH were found in both groups (tab. II). NLR correlated with hemoglobin (p=0.000) and pleural LDH in TB group (p=0.027). Univariate analysis showed high NLR (≥ 5), low serum albumin (<3 g/dL), high neutrophil count (≥8,000/mm^3), and low lymphocyte count (<1,000/mm^3) associated with cavitary pulmonary TB. Multivariate analysis showed that high NLR and low serum albumin were associated, also, with cavitary form of pulmonary TB.

TABLE I

Characteristics of patients with pleural effusions (PEs)

Variable	Tuberculosis PEs (n=353)	Malignant PEs (n=110)	Total (n=463)	p value
Age	45.10 (±18.17)	66.64 (±11.40)	57.24 (±18.19)	p= 0.000
ESR	55.85 (±39.16)	46.99(±29.41)	50.93	p= 0.021
Hemoglobin	12.78 (±2.15)	13.02 (±2.10)	12.91 (±2.12)	p= 0.391
Fibrinogen	701. 53 (±330.37)	649.36(±289.97)	670.55(±306.86)	p= 0.247
WBC (/mm3)	8.49 (±2.51)	9.97 (±3.80)	9.32 (±3.37)	p=0.003
Neutrophils	12.38 (±68.63)	7.24 (±3.68)	9.51 (±45.65)	p=0.387
Lymphocytes	1.97 (±1.00)	1.83 (±0.98)	1.89 (±0.99)	p=0.278
Serum NLR	4.40 (±9.75)	4.94 (±4.19)	4.70 (±7.19)	p=0.001
Pleural LDH	837.23 (±533.64)	719.00 (±716.95)	768.46 (±648.08)	p=0.0004
Pleural Proteins	4.56 (±0.79)	4.00 (±0.97)	4.24 (±0.94)	P=0.000
Pleural ADA	62.38 (±21.55)	35.57 (±45.42)	47.27(±22.64)	P= 0.000
ADA in blood	33 (±6.77)	37.55(±7.87)	35.00 (±7.48)	P= 0.135
Legend: ESR= erythrocyte sedimentation rate, WBC= white blood cell, NLR= Neutrophil- Lymphocyte Ratio, LDH= Lactate Dehydrogenase: ADA= Adenosine Deaminase				

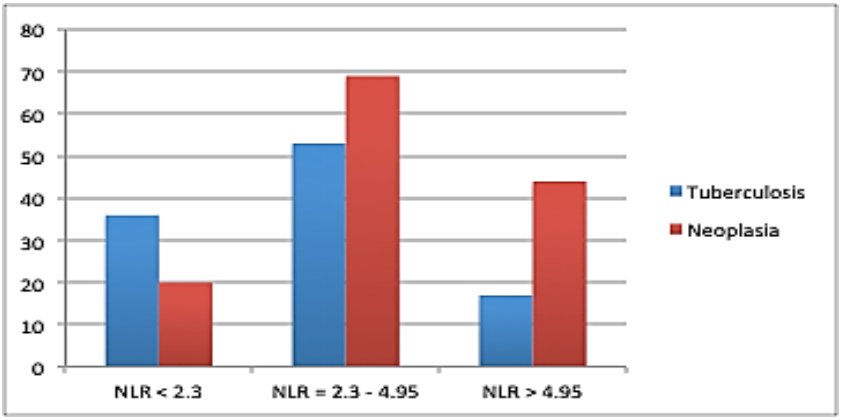

Fig 1. Distribution of cases with pleural effusions according by etiology (tuberculosis and neoplasia) patients, according to neutrophil- lymphocyte ratio (NLR) quartiles

TABLE II

Pearson correlations between serum NLR and other biomarkers

Pleural effusions (PEs)		ESR	Fibrinogen	WBC	Hb (g/dl)	Pleural LDH (U/l)	Pleural Proteins	Age	Pleural ADA	Serum ADA
Tuberculous pleurisy										
Serum NLR	Pearson Correlation	0.142	-0.257	0.273**	-.546**	.219*	0.069	-0.002	0.107	-0.214
	Sig. (2-tailed)	0.145	0.058	0.004	0.000	0.027	0.486	0.982	0.274	0.463
Malignant pleurisy										
NLR	Pearson Correlation	0.115	0.073	0.734**	-0.118	0.158	-0.150	0.156	0.051	-0.043
	Sig. (2-tailed)	0.189	0.521	0.000	0.173	0.069	0.087	0.072	0.562	0.912
All										
NLR	Pearson Correlation	0.117	-0.153	0.398**	-.368**	.160*	-0.024	0.052	0.049	0.070
	Sig. (2-tailed)	0.070	0.077	0.000	0.000	0.014	0.719	0.423	0.448	0.750

Legend: ESR= erythrocyte sedimentation rate, WBC= white blood cell, HB= hemoglobin.
NLR= Neutrophil Lymphocyte Ratio, LDH= Lactate Dehydrogenase: ADA= Adenosine Deaminase

DISCUSSION

In the last decade, NLR was proposed as a biomarker of prognosis of COPD (18), benign or malignant solid tumors (8, 19), occupational disorders (13, 20). Smoking, occupational and environmental exposure to air pollutants or known carcinogens may contribute to increased prevalence of lung cancer (21-25). PE can be the first manifestation of severe disorders, including TB and cancer. Mortality rate caused by lung cancer is high in Romania (26). Templeton *et al.*, in a recent meta-analysis of 100 studies comprising 40,559 patients with solid tumors, have found a consistent effect of elevated NLR on survival, among various disease subgroups and across cancer stages (8). The aim of this paper was to evaluate if NLR could be useful in the differential diagnosis between TB and MPEs. In our study, serum NLR had significant higher mean and median values (4.94; 3.8) in MPEs versus TB (4.40; 3.1) (p= 0.0001). NLR values range from 0.78 to 3.53 in healthy adults and cut-off value for mortality in specific diseases is between 3 and 5 (11, 19). There is a controversy on the optimal NLR cut-off values. Can NLR be an indicator of MPE as in malign nodular thyroid disease? Our study revealed high mean value (4.94) suggestive for worse prognosis. Peripheral blood lymphopenia in cancer represents a predictor of poor survival in advanced stages of cancer (27, 28). The impact of tumor growth on the host immune response is revealed by elevated NLR but cut-off is on debate (7, 29). Elevated blood NLR associates with

decreased lymphocytes and increased neutrophils, suggesting systemic inflammation and impaired cell-mediated immunity, and systemic inflammation and could be an important promoter of cancer-related cachexia (27). Inflammation plays an important role in different stages of tumor genesis (8) but the mechanisms associated with elevated NLR and poor outcomes in cancer remain unclear. TB patient identification at high risk of severe forms or evolution, reactivation or early death can be predicted by NLR (30). TB is still considered one of the oldest infectious diseases and a major cause of morbidity and mortality worldwide, and in Romania (31). High NLR may reflect relative lymphopenia. Immunosuppression related to HIV infection, biological treatments or associated comorbidities are main causes of TB infection progression to disease (32-37). Few data were published about NRL in TBPEs (1, 3). Nakao and al. reported elevated NLR in association with pulmonary cavitation (38) as our study revealed.

CONCLUSIONS

NLR is a composite marker which reflects the effects of the adaptive and innate immune responses in tuberculosis and malignant pleurisy. Because neutrophil and lymphocyte counts are nonspecific parameters, NLR seems to be a potential biomarker for differential diagnosis of TB and MPEs, but the appropriate cut-off should be further investigated.

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RISK FACTORS ASSOCIATED WITH RECURRENCE OF *CLOSTRIDIOIDES DIFFICILE* INFECTION

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RISK FACTORS ASSOCIATED WITH RECURRENCE OF CLOSTRIDIOIDES DIFFICILE INFECTION (Abstract): **Aim** To evaluate the impact of selected risk factors (social, demographic, habitual, comorbidities) on the relapses in the infection with *Clostridioides difficile*. **Material and methods:** A retrospective analysis of the hospital - based medical records of the patients with *Clostridioides difficile* infection (CDI) admitted in the Clinical Hospital of Infectious Diseases Iasi, Romania during 2017. **Results:** We retrospectively evaluated the medical data of patients with CDI admitted in the Clinical Hospital of Infectious Diseases "Sf. Parascheva" Iasi, Romania and included in our study 82 patients that had history of at least one episode of CDI. Risk factors that could be associated with young patients (under 40 years) were malnutrition. Alcohol - related liver diseases and tuberculosis were the most prominent risk factors in patients aged 40-60 years. Patients aged between 61-80 years, frequently associating chronic diseases were the most affected. **Conclusions:** Elder patients are representing an exposed group and have increased vulnerability to infection with *Clostridioides difficile*, and because of that they require a special interest for antibiotic stewardship. **Keywords**: CLOSTRIDIOIDES DIFFICILE, ELDERLY, ABUSE, RISK FACTORS, RECURRENCES.

Infectious diseases are a common cause of increased morbidity and mortality in elderly. Many factors (e.g. conditions, diseases or exposures) which increase the risk for infections may be found in older patients. The decline in immune function as result of the physiological process of immune senescence in addition to other pathological host factors (e.g. comorbidities, polypharmacy, frailty/impaired cognition, immobility) is one of the most frequent cause of the increased vulnerability of elderly to infections. (1)However, many other social factors may be involved. Some of these factors are poor understanding or acceptance of prevention, psychosocial isolation, depression, malnutrition. Among infectious diseases, *Clostridioides difficile* (formerly *Clostridium difficile)* infection (CDI) is of special interest since it is the most common infection with medical and pharmacoeconomic impact.

First identified in 1935, *Clostridium difficile* is an anaerobic Gram-positive, spore

forming rod, phylogenetically distant from the rRNA clostridial main cluster. Recently it was redefined as *Clostridioides difficile (C. difficile).* The pathogen emerged as an important pathogen in the late 1970s and currently is considered the main pathogen of healthcare-associated infectious diseases (2). Two virulent *C. difficile* strains ribotypes RT 027 and RT 078 were identified. Ribotype RT 027 has become the most important hypervirulent strain responsible for severe infections and increased mortality (3).The most important risk factors for developing CDI are antimicrobial treatment (mainly 3rd generation cephalosporins, penicillins, fluoroquinolones, lincosamines) and age. Advanced age is a noted risk factor for CDI, and commonly associated with mortality in developed countries (1). Among risk factors specific to elderly are the age-related changes in physiology, including immunosenescence and changes to the gut microbiome. Additional risk factors specific for this infection include immunodeficiency, chemotherapy, organ transplantation, inflammatory bowel disease, chronic kidney disease and exposure to individuals with *C. difficile* (3).The most difficult and increasingly common challenges in the management of CDI is the recurrent infection. Age > 65 years, severe underlying disease and continued use of antibiotics for non- CDI are considered the most preeminent risk factors for recurrences(4). The objective of this study was to examine the relationships among selected risk factors (social, demographic, habitual, comorbidities) on the recurrences of the CDI.

MATERIAL AND METHODS

This was a retrospective analysis of the hospital-based medical records of the patients with CDI admitted in the Clinic of Infectious Diseases Iaşi during 2017. *C. difficile* infection was defined by the presence of diarrhea and a positive *C. difficile* cytotoxin (toxin A and toxin B) chromatographic immunoassay. Diarrhea was defined as more than 3 loose, but also watery or unformed stools per day. The infection was classified as community-associated if the *C. difficile*-positive specimen was obtained in an outpatient without any risk factors or within 3 days after hospital admission. All other cases were considered healthcare- associated infections. Recurrence was defined as a second episode of CDI within 60 days after the first episode. We included in our analysis only cases of at least one recurrence of CDI. Patients with diarrhea and negative result for *C. difficile* toxins were excluded. The following data were collected: demographic data, social (e.g. presentation from home versus nursing facility, including nursing homes/ long-term care facilities; family caregivers) and/or habitual conditions (e.g. malnutrition, alcohol consumption).

Medical (co-morbidities-diabetes mellitus, malignancy, kidney disease, hypertension, coronary artery disease, neurological and psychiatric disorders) and medication (e.g. antibiotic use in the previous 3 months, proton pump inhibitors) were recorded and Charlson comorbidities index was used to represent the cumulative burden of comorbid illnesses (6). Results of blood tests and stool sample tests were evaluated. Age, area of provenience (rural versus urban), comorbidities, social and habitual characteristics were considered risk factors for CDI recurrences.

Results were analyzed with descriptive statistics. The relationship between risk factors and disease evolution was evaluated

using Spearman correlation test. Univariate analysis was performed using the χ^2 test to assess the differences between different age groups. Two-sided significance was assessed for all variables, applying a cut-off value of $p < 0.05$.

Statistical analysis was performed using XLSTAT version 2018 software (*Addinsoft Co*, USA). The study was approved by the Ethics Committee of the Clinic of Infectious Diseases, Iasi.

RESULTS

From a total of 247 patients with CDI hospitalized during 2017in our hospital, 82 patients had between 1 and 6 episodes of recurrences. Patients from rural areas (47.56% cases) had a higher average age (62.45 years) compared to urban ones (52.44% cases) with a mean age of 52.6 years. Some of elderly patients presented from a nursing facility; however, more than 60% of patients presented from home, but the majority of them had a history of hospitalization in the previous months. Most of the patients were aged between 71-80 years old, but the range was wide (15-88 years) (fig. 1).

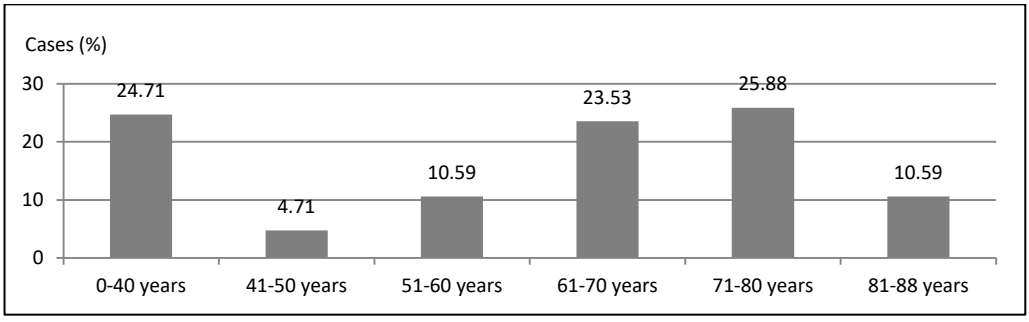

Fig. 1. Patients' age distribution

There were not significant differences gender- related; however, a slight increased incidence in females was noted (53% versus 45.79% cases) (tab. I).

TABLE I

Patients' characteristics

Age	Male (%)	Female(%)	Rural (%)	Urban (%)	Charlson comorbidity index	Mean duration of hospitalization (days)
0-40 years	42.86	57.14	42.86	57.14	1.04	9.83
41-50 years	25	75	25	75	1.36	11.27
51-60 years	22.22	77.78	55.56	44.44	3.2	12.5
61-70 years	65	35	40	60	4.68	11.23
71-80 years	40.9	59.1	54.54	45.46	5.39	10.16
81-88 years	33.33	66.67	44.44	55.56	7.1	12.63

An age-group analysis of impact of risk factors on CDI recurrences showed no significant differences between groups, except for young patients (aged below 40 years) (p=0.034). The most important underlying conditions in patients aged below 50 years were associated with a poor social status (28.57% cases). Alcohol -related liver diseases (over 50% cases) and tuberculosis (25% cases) were the most prominent risk factors in patients aged 40-60 years. In older patients, recurrence of infection was noted especially in cases of malignancies – related immunosuppression (20% cases). Malnourished patients aged between 61-80years, frequently associating chronic diseases (diabetes, malignancies, but mainly with cognitive impairment) were the most affected (fig. 2). The small sample size of patients aged over 80 years could not offer a good estimation. In 20.73% cases of the entire studied group, patients had previous antibiotherapy.

The variance analysis showed a good correlation between risk factor and age in elderly compared to young patients (fig. 3). We expected this result since the Charlson index increased by age.

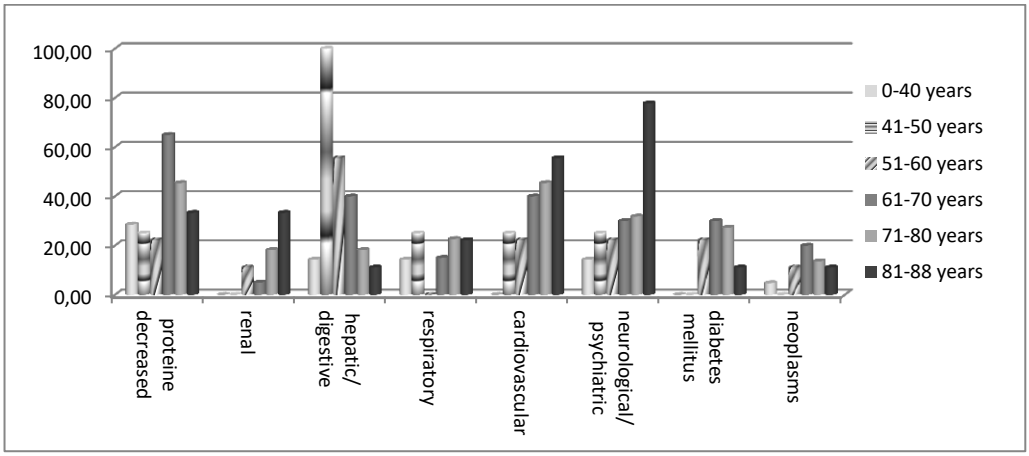

Fig. 2. Co-morbidities incidence by age groups

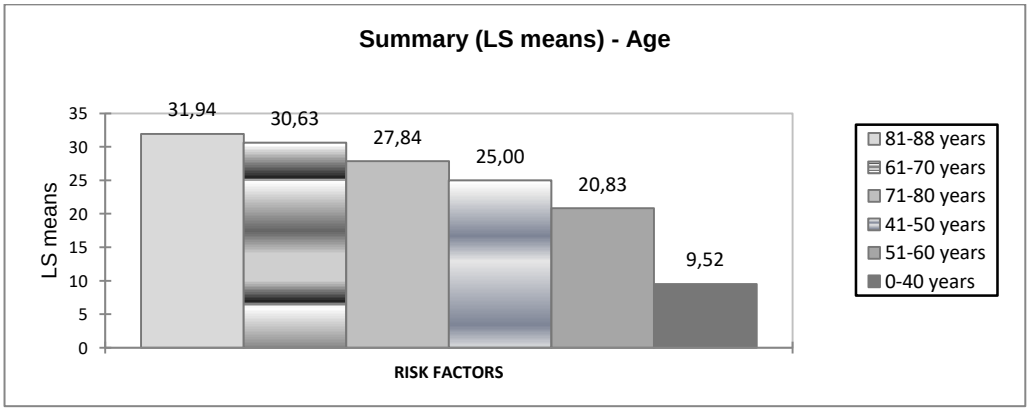

Fig. 3. Correlation between age and risk factors

Spearman correlation test identified renal diseases (renal failure, urinary catheterization) (coefficient Spearman = 0.928) and neurological and psychiatric diseases (tetraplegia /paraplegia, cognitive impairment) (coefficient Spearman = 0.943) as risk factors for CDI recurrences (tab. II).

TABLE II
Spearman correlation test results

Variables / Age	Protein decreased	Renal	Hepatic/ digestive	Respiratory	Cardio- vascular	Neurological	Diabetes melli- tus	Neoplasm
Correlation matrix (Spearman)	0.543	0.928	-0.429	0.257	0.943	0.943	0.580	0.638
Confidence intervals (95%)	[-0.540 , 0.949]	[0.280 , 0.995]	[-0.928 , 0.620]	[-0.710 , 0.888]	[0.383 , 0.996]	[0.383 , 0.996]	[-0.509, 0.955]	[-0.452, 0.964]

DISCUSSION

Clostridioides difficile has become the most common cause of health care–associated infections worldwide, and recurrence rates for CDI have been reported to vary from 5% to 50%, with an average of 20% (7). Mortality rate associated with CDI has been found to range between 5% - 34% and is doubled in intensive care unit patients (8, 9). Most of the CDI are associated with health care exposure (in hospital or even nursing home stay), but recent studies revealed that incidence of community-acquired CDI significantly increased up to 30% in recent years. In our studied cohort, up to 30% patients had CDI recurrences, as noted before in other studies (10, 11). Patients from urban areas, as well as those from rural areas, were similarly exposed to risk factors for recurrences of CDI. Our study confirms the finding in other studies that older age is a risk factor for CDI recurrences since the highest incidence was found in patients over 65 years. For every additional year of age after age 18, the risk of health care - associated CDI increased by approximately 2%; thereby, age > 65 years increases the risk for CDI 5 to 10-fold (12, 13). Significant risk factors for health care- associated CDI noted in other studies and also found in our analysis were use of antibiotics, and use of proton-pump inhibitors, chemotherapy, and hospitalization in the previous 2 months (12).As noted in previous studies, malnutrition and alcoholic and non-alcoholic cirrhosis were risk factors for CDI irrespective of age, possible due to immunosuppression induced by these conditions (14, 15). In addition, tuberculosis was found the main risk factor for recurrent CDI in 6% cases of our cohort, but tuberculosis was also identified as an independent risk factor for CDI mortality (16). Overall, patient over 65 years old, with multiple chronic disorders, functional incapacity, physical disability and

cognitive impairment were the most vulnerable for CDI recurrences. Patients with cognitive impairment and dementia are of special interest, since even with adequate food intake, these patients associate malnutrition responsible with a four - fold increase in infections (17). For elderly coming from long-term care facilities or nursing homes, not only host factors or antibiotic consumption were responsible for CDI. The primary infection and its recurrences are often facilitated by transmission of the pathogen within a closed environment, adding sometimes certain neuro-and locomotive disabilities and inability to ensure a decent living (18).

On the other hand, community-residing elderly with moderate to severe dependency and family caregivers represents a special situation (19). Elder abuse is defined a mistreatment that includes physical, sexual, psychological, emotional and financial abuse, and neglect, and violates the human rights (20). This type of abuse has significant negative impact on the quality of life with deterioration of the health status. Unfortunately, this situation is also frequent in our country. Even we could collect limited data about caregivers in our study, we have noticed that older patients with physical inability and mental impairment were often neglected, requiring prolonged hospitalization and even psychological assistance (21).

As a line of research, it could be interesting to see the role NADPH- oxidizes, that was originally identified as a component of innate host defense and is involved in many key biological function(22).

CONCLUSIONS

Older patients with risk of life-threatening infections, including *Clostridioides difficile*, require a special interest for antibiotic stewardship to reduce morbidity and mortality. In addition, since mistreatment may have serious effects on elderly's health, preventive measures must be put into place to combat neglect of caregivers against older people.

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CARDIOPULMONARY RESUSCITATION – COULD BE INAPPROPRIATE? THE EMERGENCY MEDICINE PROFESSIONALS PERCEPTION IN NORTH-EAST ROMANIA

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CARDIOPULMONARY RESUSCITATION - COULD BE INAPPROPRIATE ? THE EMERGENCY MEDICINE PROFESSIONALS PERCEPTION IN NORTH-EAST ROMANIA (Abstract): Cardiopulmonary resuscitation (CPR) is started irrespective of comorbidity or cause of cardiac arrest. We aimed to determine the prevalence of perception of inappropriate CPR of the personnel from Emergency Department (ED), the factors associated with perception and the relation to patient characteristics. **Material and methods:** A survey based on questionnaire was conducted in an ED of University Hospital, as a part of multicentric European study. Factors associated with perception of CPR and outcome were analyzed on statistic base. **Results:** Of the 54 participating clinicians, 57.4% perceived their last CPR attempt as appropriate, 38.9 % were uncertain about its appropriateness and 3.7% perceived inappropriateness. The prevalence of perception of inappropriate CPR was higher when one of this factor was present: non-shockable initial rhythm, inappropriate quality of CPR performed by witnesses before arrival, the advanced age of the patient, terminal neoplasia, low quality of life before cardiac arrest. The ED staff needs an early recognition of CPR futility, feedback, training and teamwork and performance evaluation after each CPR attempt. **Conclusions:** The perception of inappropriate CPR is present in a small percent inside ED staff. Factoring clinical judgment into the decision to (not) attempt CPR may reduce harm inflicted by excessive resuscitation attempts. **Keywords:** CPR, ED PERSONNEL, INAPPROPRIATE, SURVEY.

Cardiopulmonary resuscitation (CPR) was introduced in 60's to reverse the cardiac arrest of patients with reversible conditions such as acute myocardial infarction within the first minutes after collapse (1). Currently, most cardiac arrest patients receive CPR irrespective of their functional status and comorbidity, cause of the arrest and delay after the collapse (2, 3). Some studies have shown that over time neurologically favorable outcome after CPR has improved, but this improvement is lower for cardiac arrests with an initial non-shockable rhythm compared to an initial shockable rhythm. Data from the Cardiac Arrest Registry to Enhance Survival (CARES) from 2005 until 2012 show an increased adjusted rate of survival to hospital discharge from 2.1 to 3.9% for non-shockable rhythms and from 16.1 to 21.1% for shockable rhythms (4). A nationwide Japanese registry from 2005 until 2014 reports an increase of 1-month survival with

favorable neurological outcome from 0.8 to 1.2% for bystander-witnessed non-shockable arrests and from 10.1 to 24.9% for bystander-witnessed shockable arrests (5).

Inappropriate CPR attempts are undesirable from both the medical personnel and the scientific perspective. The patients suffering a cardiac arrest are particularly susceptible to loss of dignity and to suffer dehumanization, because they lack many typical attributes of modern human beings such as consciousness and self-determination. Furthermore, patients who are successfully resuscitated have residual cognitive and physical deficits and never recover, thus prolonging their vulnerability to disrespect, often with profound negative consequences for their relatives (10). From the logistic and financial health perspective, undifferentiated application of cardiopulmonary resuscitation may unbalance the appropriate allocation of resources.

Previous studies by Marco *et al*. found in 1995, and again in 2007, that 55% and 57% of surveyed emergency physicians, respectively, reported having attempted more than ten resuscitations that were perceived as futile in the prior three years (8). A survey found that emergency medical technicians estimated 50% of resuscitation attempts to be futile, defined as an exceptionally low likelihood of success (9). Studying clinician perceptions of inappropriate care is important since they have been associated with moral distress (11, 12) and burnout (13), which are related to self-reported suboptimal patient care practices (14, 15). The quality of future resuscitation attempts may be negatively affected if medical professionals perceive CPR attempts as inappropriate and resulting in a poor outcome.

No studies in Central and East European countries related the ED team perception of inappropriateness of CPR to actual patient outcomes. The objective of this study was to determine the prevalence of the uncertainty about CPR appropriateness and of the perception of inappropriate CPR in prehospital and emergency departments and the association with survival to hospital discharge. The secondary objectives ware to assess the association between patient and situation related factors, personnel characteristics, organizational and work-related factors, the perception of inappropriate CPR and return of spontaneous circulation (ROSC).

MATERIAL AND METHODS

This Romanian study is a part of an international multi-center cross-sectional survey conducted in 288 centers in 24 countries, the REAPPROPRIATE study, coordinate by Belgium (1). The study population consisted of ED personnel from "Sf. Spiridon" County Clinical Emergency Hospital from Iasi who are directly involved in the management of cardiac arrest in the emergency department or the prehospital setting. The data collection period was from March until November 2015 and a modified Delphi method was used to adjust a questionnaire. First version of the questionnaire was prepared by a multidisciplinary expert panel: two emergency physicians, two intensivists, two emergency nurses, one geriatrician and two clinical psychologists from Netherlands. This Dutch version had an adaptation and translation into English and after the questionnaire was translated into Romanian followed by re-translation, necessary to achieve cultural and functional equivalence (16). We designed the survey based on the questionnaire, using a 4-point rating scale, similar with Likert scale from "Fully

agree" to "Fully disagree". ED personnel, physicians and nurses, were asked about their demographic and professional background and ED working environment. Also they were asked to recall the latest cardiac arrest they encountered and to answer if they "fully agreed with starting the resuscitation" (own perception of appropriate CPR), "were unsure resuscitation should have been started" (uncertain about CPR appropriateness) or "were sure resuscitation should not have been started" (inappropriate CPR). Subsequently questions were about details of the resuscitation circumstances such as: site of arrest, patient age, gender, initial arrest rhythm, presence of a witness, ROSC, personal impression of the patient's physical condition, probable cause of arrest. All the data was included in a statistical analysis using *SPSS 25.0***.** This study was conducted in accordance with the Declaration of Helsinki. Because informed consent was not required, the study was approved by the Ethical Committee of "Grigore T. Popa" University of Medicine and Pharmacy from Iasi.

RESULTS

We sent 58 questionnaire and we received 54 completed. The latest CPR attempt encountered was reported by 54 physicians and nurses. The average age of the participants was 31+/-7.192 years, with a maximum of 53 and a minimum of 22 years. The distribution by gender was: 68.5% women and 31.5% males, similar with the gender structure inside the health system in Romania. Of the entire group, 51.8% were training doctors, 18.4% were Emergency consultants and 29.8% nurses. According to the activity field: 87% were directly involved in emergency medicine inside ED and 13% were indirect involved in emergency medicine, being radiologist. The number of working hours / week: average 40 with the limits between 30 (1.7%) to 78 (1.7%)

Years of the experience in the ED inside the population group were less than 1 year-14,8%, 1-5 years (29,7%), between 5-20 years- 53.6% and up to 25 years (1.9%).

74.1% admitted to have religious beliefs, 18.5% showed no religious beliefs while 7.4% would not answer. There is no correlation between religion and the initiation of resuscitation maneuvers or their uselessness.

Only 6.9% of respondents are involved in teaching ALS and BLS courses. The hours of BLS training attended in the last 12 months varies between 0 (for 50% of the respondents) and 48 hours (for the 5.2% of respondents).

The number of ALS course hours varies from 0 (55.2% of the respondents) to 120 hours (1.7% of the respondents).

The answers about the personal perception of work effort recognition showed that 48.1% feel appreciated, 33.3% do not feel appreciated, 9.3% do not feel appreciated at all and 9.3% feel very appreciated.

They consider themselves to be hard-working: 51.9% + 33.3%, only 5.6% and 9.3% admit not to be hard-working.

The perception about sufficient time to finish the work indicated that: 51.9% agree to have enough time, 9.3% totally agree, 27.8 do not agree with having enough time, 11.1% totally disagree.

Emergency medicine is a field with continuous learning environment. For the item "Learn new things": 59.3% total agree, 31.5% agree, 7.4% disagree and 1.9% total disagree. Also, the working environment is important for the item "Working with helpful colleagues" and the answer demonstrat-

ed: 48.1% agreement, 42.6% total agreement, 1.9% total disagreement and 7.4% disagreement. Learning from the mistakes of others: 35.2% total agreement, 57.4% agreement, 7.4% disagreement. The vast majority disagree with the difficulty of communicating with people of other culture (11.1 totally disagree and 57.4% disagree = 68.5%), 1.9% admit to have communication difficulties with people belonging to other culture.

The ED staff feel appreciated within the CPR team: 64.8% agree, 14.8% totally agree, 20.4% do not feel appreciated. Only 5.6% planned to quit the profession and 59.3% total disagree and 35.2% disagree.

When we analyzed the perception about the appropriate or inappropriate CPR, we also studied the perception of CPR futility. They could confidently express their doubts about the futility of the CPR: 11.1% total agree, 74.1% agree and 14.8% disagree.

The presence of the family during the CPR is still a subject of debate in ED and about this subject the population of the study demonstrated: agreement 11.1%, total agreement 3.7%, disagreement 53.7%, total disagreement 31.5%.

Necessity of debriefing time after CPR is consider important: total agreement 5.6%, agreement 57.4%, disagreement 31.5%, total disagreement 5.6%.

About the latest case with CPR during the recent professional life the answers demonstrated: ED personnel evaluation of the CPR opportunity: total agreement 14.8%, agreement 42.6%, disagreement 38.9%, total disagreement 3.7%.

Early recognition of CPR futility received the follow answers: 3.7% totally disagree, 7.4% disagree, 57.4% agree, 31.5% totally agree. These answers demonstrate the need to understand the CPR futility inside the ED staff.

The team leader appreciates the team effort during the latest experience in resuscitation: 35.2% totally agree, 53.7% agree, 11.1% disagree.

Regarding the perception of the ED personnel for inappropriate CPR, no correlation was found between the opportunity to start CPR and the patient particularities: the presence of the family, the presence of the DNR order, the age of the patient, the presence of dementia, the low quality of life, the initial shockable rhythm, the possibility of an organ donor, the patient age: child or adult.

There is a direct correlation of the initiation of the CPR with the site of the cardiac arrest: pre-hospital CPR is consider the right site to start CPR ($p = 0.354$), the pregnancy status of the female patient is a clear indication to start CPR ($p = 0.285$) but also the neoplasia in the terminal phase ($p = 0.410$).

We tried to explore the factors that made them feel the CPR is unnecessary and we obtained the next results:

- Inappropriate quality of CPR performed by witnesses until arrival: 38.9% very important, 37% important, 11.1% rather important, 13% unimportant
- Initial non-shockable rhythm: 22.2% very important, 42.6% important, 16.7% rather important, 18.5% unimportant
- The advanced age of the patient: 14.8% very important, 24.1% important, 22.2% rather important and 38.9% unimportant
- Patient with dementia: 5.5% very important, 20.4% important, 16.7% rather important and 57.4% unimportant
- Terminal neoplasia: 27.8% very important, 37% important, 14.8% rather important and 20.4% unimportant
- Low quality of life before CPR: 11.1% very important, 20.4% important, 20.4% rather important and 48.1% unimportant

DISCUSSION

From our knowledge, this is the first study conducted in ED in Romania, regarding the perception of futility of CPR, including emergency staff involved in cardiac arrest care, exploring potentially relevant organizational, clinical and work-related factors, as well as patient and situation-related factors, and relating these factors with patient outcome (ROSC). ED personnel was specifically asked about the latest cardiac arrest they encountered. As such the reported prevalence of perception of inappropriate CPR was based on a specific clinical event attended by the clinicians and not just on a vague general impression. Basing on our survey, we found that 57.4% of clinicians working in emergency departments and ambulance services perceived their latest CPR attempt as appropriate, and 42.6% perceived their last CPR attempt as inappropriate. Moreover, we found that the presence of objective indicators of poor prognosis only moderately increased the prevalence of perception of inappropriate CPR. In patients with non-witnessed non-shockable arrests, a group known for its highly unfavorable prognosis (4, 20) and a low rate of survival to hospital discharge, 22.2 % of ED considered it very important and 46.2% considered important the early recognition of futility of CPR. Unexpectedly, the neoplasia in terminal phase is consider an important situation of inappropriate CPR only by 64.8 % of ED personnel. These findings indicate that the majority of physicians and nurses are either unaware of the inappropriateness of their CPR attempt or only become aware of the potential consequences of their actions in extreme patient situations. The most important determinants of perception of inappropriate CPR were objective criteria such as non-shockable initial rhythm, non-witnessed arrest, older age and a poor first physical impression of the patient. This may indicate that perception of inappropriateness of appraisal and warranting team leaders to invite and consider the opinion of all experienced resuscitation team members, regardless of their role. Our results highlight the need of closed-loop systems assuring that all staff involved in resuscitation receive feedback on the results of their attempts. Debriefing and interdisciplinary ethical reflection on difficult patient cases are needed, more specifically when one team members has doubts about the appropriateness of resuscitation. More advance care planning conversations with patients and family are needed (26), based on realistic information about outcome of resuscitation thus avoiding inaccurate expectations of CPR (27). Compliance of clinicians once advance directives are established should be further improved (8, 25). This study has several limitations. First, the possibility that perceptions about appropriateness of CPR may have been affected by the resuscitation outcome. However, the questionnaire was structured in such a way that questions concerning outcome were listed at the very end. Second, data on ROSC and survival to hospital discharge were provided only for the study team and not for population included in the study.

CONCLUSIONS

Our findings suggest that emergency medicine professionals who are working on daily basis in an emergency system consider that performing the CPR maneuvers was appropriate in most of the cases but the ones who perceive CPR as inappropriate are entitled to express themselves regardless the role played inside the resuscitation team. Factoring this clinical judgment into

the decision to attempt resuscitation may reduce harm by excessive resuscitation attempts to a number of patients, facilitating DNR order in Romanian legislation for very strict defined situations and maybe a more dignified death for some people.

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NEWS

RISK OF COVID-19 AMONG FRONTLINE HEALTHCARE WORKERS

The authors performed a prospective observational study of 2135190 individuals, comprised of front-line health-care workers and the general community who were voluntary users of the COVID Symptom Study smartphone application (app). From self-reported data obtained via this app, we found that front-line health-care workers had at least a threefold increased risk of COVID-19. Compared with front-line health-care workers who reported adequate availability of PPE, those with inadequate PPE had an increase in risk. However, adequate availability of PPE did not seem to completely reduce risk among health-care workers caring for patients with COVID-19. We also found that Black, Asian, and minority ethnic health-care workers might be disproportionately affected. The results of this study could help provide greater context for previous cross-sectional reports from public health authorities suggesting 10–20% of SARS-CoV-2 infections occur among health-care workers. The results offer individual-level data additionally accounting for workplace risk factors that complement these limited reports by providing a more precise assessment of the magnitude of increased risk among health-care workers during the initial phases of the COVID-19 pandemic. Taken in the context of the requirement for testing to establish a COVID-19 diagnosis, the range of results based on either reporting a positive test for COVID-19 or symptoms predictive of COVID-19 offer several complementary estimates for risk among front-line health-care workers (Nguyen LH, Drew DA, Joshi AD, et al. Risk of COVID-19 among frontline healthcare workers and the general community: a prospective cohort study. *The Lancet* 2020; 5(9): E475-483).

DEPRESSION OF THE ELDERLY PATIENTS: FOCUS ON A SINGLE CENTER STUDY

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DEPRESSION OF THE ELDERLY PATIENTS: FOCUS ON A SINGLE CENTER STUDY (Abstract): Elderly patients may deny the existence of depressive symptoms due to the social disapproval of the diagnostic, as well as the difficulty of expressing the need for help regarding psychiatric symptoms. **The aim** of this study is to underline the importance of early diagnosis of depressive syndrome in the elderly patient and the clinical approaches. **Material and methods**: the criteria for inclusion in the study was represented by the patients over 65 years of age, diagnosed with depressive disorder, according with the criteria of ICD10, single episode or recurrent ones. **Results**: we included 77 patients and we evaluated the risk factors such as female sex, urban area of provenance, somatic comorbidities. **Conclusions**: Patients with onset of the depressive disorder over the age of 65 years old had a weaker response to treatment, an increased risk of progression to dementia and a higher rate of early mortality. In this context, the need for prophylaxis, as well as effective treatment, is crucial, using individualized therapies to the patient's needs. **Keywords**: DEPRESSION, EARLY DIAGNOSTIC, ELDERLY PATIENTS.

The World Health Organization (WHO) estimates that depression affects about 350 million people and also those in close contact with the patients. Depression is a condition that is often hidden, denied, and therefore underdiagnosed and treated (1). According to World Health Organization, in 2020 depression will be the second leading cause of disability, immediately after cardiovascular diseases. Depression is currently the fourth most common disease in the world and is one of the major human health problems (1, 2).

The Romanian League of Mental Health conducted a study related to the frequency of depression in a project carried out under the auspices of the European Union – "MINDFUL" (Mental Health Information and Determinants for European Level) in several European countries.(3) The research was conducted on a sample of about 2,400 people. Following this study, it was observed that the prevalence of major depressive episode at the time of examination was about 9%, and throughout lifetime for 21%. Depression is also twice as common in women. Romania ranks the third place in Europe in terms of the number of depressed

people, compared to the general population. Following the research carried out by the School of Public Health and Sanitary Management, the following were highlighted (2,3):

- significant increase in major depressive episode with age, from 2.1-2.6% (18-49 years) to 4.4-5.2% (over 50 years), with a growth rate of 1.2% for each age group (50-64 years and over 65 years);
- confirmation of the increased prevalence of major depressive episode during life (3.3% for all ages);
- twice as high in women (4.1% *vs.* 2.5%).

Depression in old age people is currently considered a distinct clinical entity because the clinical picture of depression have many different aspects compared to the clinical manifestation of depression in young people. The prevalence of depression is 12-45%, with an average of 20%. Depression in the elderly is considered by many practitioners to be a normal process and a consequence of aging. Old age can accentuate and at the same time suppress some symptoms of depression (4, 5). Depressive mood, the main symptom of depression, is masked in the elderly by some signs, such as: loss of appetite, sleep disorders or fatigue, which are usually not so intense in young people. The elderly present a poorer clinical picture than young patients, denying a number of important symptoms, such as feelings of guilt, suicidal ideation, and very often psychotic symptoms. Some of the most common depressive symptoms in the elderly are anxiety, somatic complains, cognitive impairment, psychomotor agitation, psychotic symptoms (6, 7).

The frequency of suicide attempts increases with age and especially male patients have an increased suicidal risk. Somatic comorbidities such as neoplasic diseases, stroke, myocardial infarction, kidney disease, but also loneliness and social isolation increase the risk of developing a specific plan for committing suicide. About two-thirds of elderly depressed patients have recurrent thoughts of death, including suicidal ideation, which are not easy to identify and 10-15% of them commit suicide (8). Psychotic symptoms in elderly patients diagnosed with depression is represented by delusional ideas with depressive content, uselessness, or guilt. Hallucinations have also been described, the most common being the auditory and visceral ones (9). Blazer identified a clinical picture specific to depression at elderly persons, characterized by depressive mood, psychomotor slowness, hypoprosexia with decreased ability to concentrate, constipation, low self-perception of health, cognitive deficit and association with a somatic disorder, picture that does not overlap with the specific diagnostic criteria in the Diagnostic and Statistical Manual or International Classification of Diseases (10, 11, 12). The clinical picture of depression in old age may be different compared to the clinical picture of depression in adulthood, due to certain factors like somatic comorbidities, anxiety, abuse of alcohol or a great consumption of medication, that may influence the clinical expression of depression and thus determined certain diagnostic difficulties (5). Also, elderly patients tend to minimize their feelings of sadness. They do not want to bother the doctor with emotional problems and instead describe an excessive concern for health or clear somatic symptoms, which can sometimes reach delusional intensities. Depressive mood varies from day to day, is often unaf-

fected by circumstances, but may still have a characteristic diurnal variation. In some cases, anxiety and psychomotor agitation may dominate the clinical picture, and mood swings may be masked by additional symptoms such as irritability, excessive alcohol consumption, histrionic behavior, exacerbation of pre-existing phobic or obsessive symptoms, or hypochondriac concerns.

Another important issue in the elderly patient is compliance with antidepressant treatment. Non-compliance with antidepressant medication in the elderly is more common than in young patients (13). It has been estimated that 70% of patients do not take between 25 and 50% of the medications prescribed to them (14). Many elderly patients with depression have substantial comorbidities, such as heart failure, diabetes, or cancer. Chronic illness is an additional risk factor for the development of depression. Optimizing medical conditions and selecting antidepressants to minimize drug interactions is relevant to managing depression. Mental disorders added to depression, such as anxiety and dementia, may have an additional effect on outcomes, but antidepressants appear to have similar efficacy in elderly and young patients (15). Although the presence of other diseases may make it difficult to diagnose depression, there is clear evidence that somatic comorbidities would substantially decrease the effectiveness of antidepressants.

MATERIAL AND METHODS

In a retrospective, descriptive study we assessed the importance of early diagnosis of depressive syndrome in elderly patients and their clinical approach, on the patients hospitalized at the "Socola" Institute of Psychiatry Iasi. One of the objectives of the study is to highlight the frequency with which depression has spread among the elderly population, analyzing data from years 2018 and the first semester of 2019. We included in the study all patients, over the age of 65 years old, which had the principal diagnostic of depressive episode, single of recurrent one, according with the criteria of diagnosis of ICD 10.

In order to obtain the information necessary for the statistical study, we used the following methods:

- clinical-anamnestic evaluation of hospitalized patients with the diagnosis of depressive disorder, over 65 years old;
- content analysis of medical documents registered in the archive.

Patients were evaluated by anamnesis, psychological evaluation, psychiatric interview, general clinical examination, paraclinical laboratory and imaging examinations.

The anamnesis and the dynamic observation of the patients during the hospitalization highlighted the dominant characteristics of the depressive syndrome, depending on the nature of the depressive episode for which they needed hospitalization, as follows: depressive mood, loss of appetite, sleeping disorders, fatigue, anxiety, somatic symptoms, cognitive impairment, psychomotor agitation, psychotic symptoms.

To make the differential diagnosis of depression with bipolar affective disorder, dementia or depressive symptoms induced by substance abuse (especially alcohol), paraclinical laboratory examinations (CBC, inflammatory syndrome, biochemistry, liver, and kidney tests) and imaging scan of the brain were performed.

For the statistical analysis of the data, we used the *Microsoft Excel* program, together with the *XLSTAT 2014* add-on for

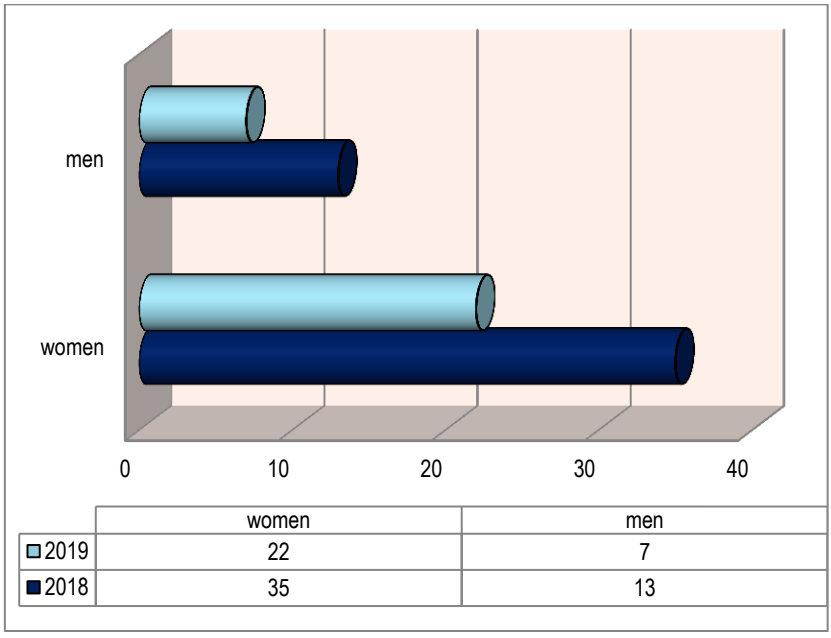
MS Excel and the IBM *SPSS Statistics 20.0* program. The information obtained was recorded in Microsoft Excel files, being then statistically processed to highlight the relationship between clinical and paraclinical data of patients.

RESULTS AND DISCUSSION

Of the 111 cases of mild, moderate, and severe depressive single episode diagnosed during the year 2018, only 28 patients were over 65 years old. Of the 94 cases of mild, moderate, and severe depressive episodes diagnosed between 1.01.2019-30.06.2019, only 12 patients are over 65 years old.

In the case of patients diagnosed with recurrent depressive disorder in 2018, of the 288 cases, only 48 patients were over 65 years of age. In the period 01.01.2019-30.06.2019, out of the 226 cases diagnosed with recurrent depressive disorder, only 29 patients are over 65 years of age. Thus, the analyzed group comprises 77 patients.

In 2018, females were 2.7 times more frequently affected compared to males, while in 2019 women were three times more frequently diagnosed with recurrent depression compare to men (fig.1).



	women	men
2019	22	7
2018	35	13

Fig. 1. Distribution of the studied group by sex

It can also be seen that aging is another risk factor, the studied group having a minimum age of 65 years, a maximum age of 81 years for 2018 and 80 years for 2019, and the average age of the first diagnostic was 69 years old in 2018, respectively 72 years old in 2019.

Analyzing the situation of the numerical distribution of patients diagnosed with depressive disorder by age categories, it can be seen that in 2018 were diagnosed 240 patients aged 18-65 years, as opposed to the 197 patients diagnosed in the first half of 2019. Within the studied group, that

of patients over 65 years of age, the situation is as follows: in 2018, 48 patients were diagnosed with depressive disorder, while in the first half of 2019, 29 patients were diagnosed (fig. 2).

Another socio-demographic risk factor for the depressive disorder analyzed was the environment of residence, being highlighted the fact that in both periods, patients coming from urban areas are more affected compared to those from rural areas (fig. 3).

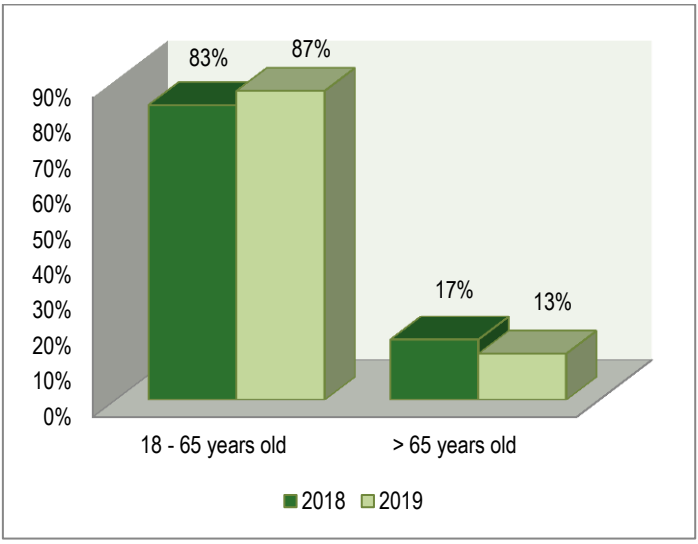


Fig. 2. Distribution of patients diagnosed with depressive disorder by the age at diagnostic

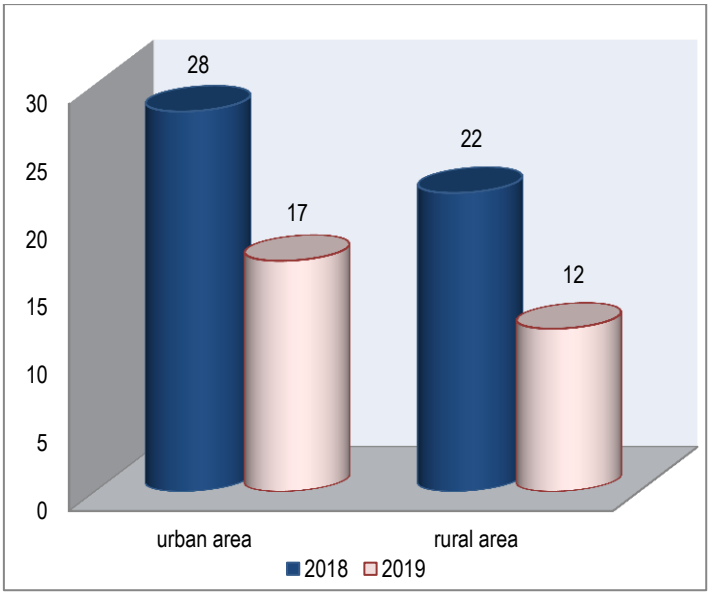
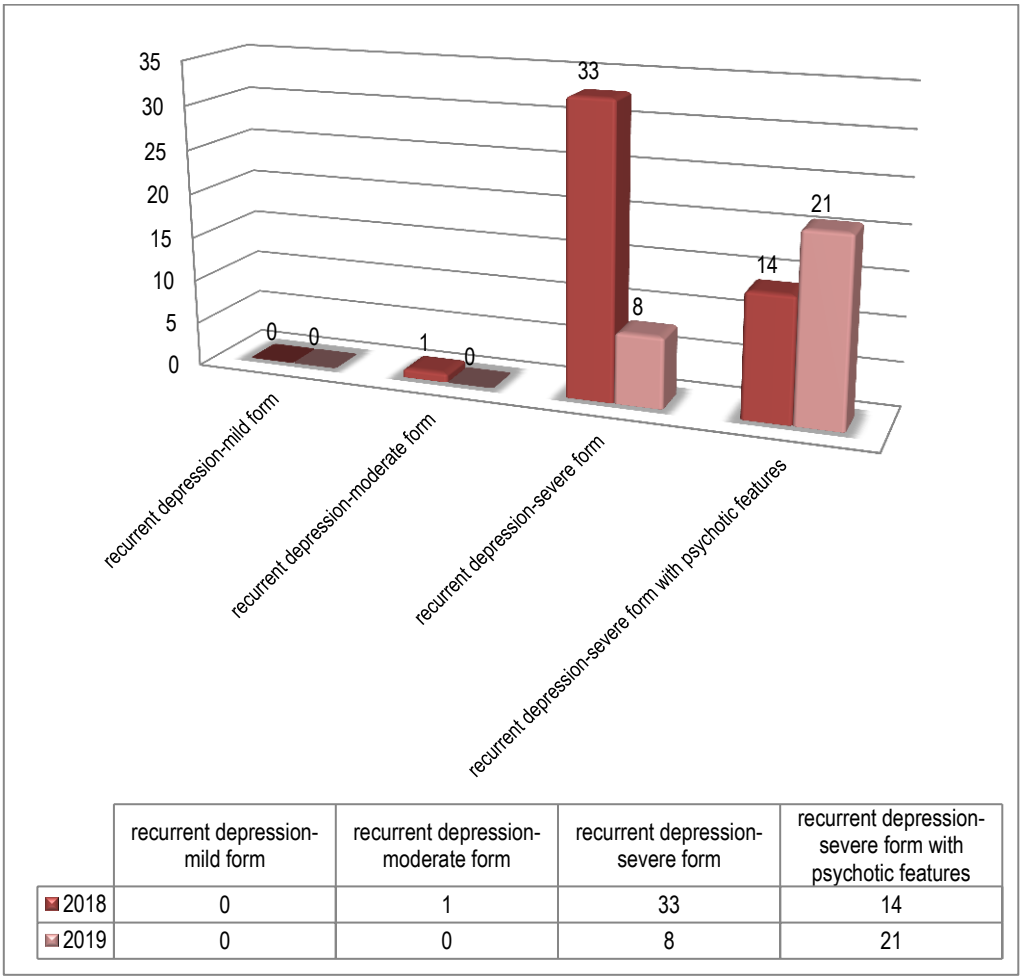


Fig. 3. Distribution of patients diagnosed with depressive disorder by area of provenance

Unfortunately, most patients diagnosed with recurrent depressive disorder during 2018 and in the first half of 2019 presented with a severe current episode with or without psychotic symptoms. In 2018, of all patients 69% were diagnosed with a severe current episode without psychotic symptoms, 29% had a current episode with psychotic symptoms and only 2% were recorded with a moderate current episode. Between 01.01.2019 and 30.06.2019 the situation is not at all better, most patients (72%) being diagnosed with a severe current episode with psychotic symptoms, while the remaining 28% were patients who presented with a severe current episode without psychotic symptoms. It can be seen that in the first half of 2019 alone, 29 patients were already registered compared to the 48 patients diagnosed in 2018, and most of them presented with the most severe form of a recurrent depressive disorder, namely episode severe with psychotic symptoms (fig. 4).



	recurrent depression-mild form	recurrent depression-moderate form	recurrent depression-severe form	recurrent depression-severe form with psychotic features
2018	0	1	33	14
2019	0	0	8	21

Fig. 4. Distribution of patients diagnosed with depressive disorder - severity form

Data on the prevalence of depression among the population over the age of 65 vary from 1-5%. The rate of depression is higher among the elderly female population, but without significant differences (16). According to data provided by a study conducted by Blazer in 2003 only in 15% of cases elderly patients show clinically significant symptoms specific for depression (7).

Cognitive decline is still the heaviest burden of the elderly, being considered by many practitioners a normal process and a consequence of aging, this contributing to an underdiagnosis and also to the delay of the administration of an adequate treatment that could minimize, in the long term, both the costs associated with late diagnosis and the complications of the pathology for this patients.

Early diagnosis and prevention of the depressive disorder are particularly important, given the fact that elderly population will triple from 1.9 billion in 2050, compared to 672 million in 2005. Increasing life expectancy to 82 years in developed countries will determine the number of the population over 85 years to reach 6.6 million in 2020, compared to 5.7 million today. As the number of patients diagnosed with mental disorders, especially mood disorders, will be 15 million by 2030, depression in old age will be one of the costliest conditions due to increased risk of suicide, somatic comorbidities, decreased functionality and the quality of life of both the patient and the family (1,15).

CONCLUSIONS

The correct diagnosis of depression subtypes is the guide in selecting and establishing the most appropriate therapeutic modalities. Depression in old age, one of the most common nosological entities, is often underdiagnosed and undertreated. Early diagnosis and prevention are particularly important. In 2030, depression in old age will be one of the costliest conditions due to increased risk of suicide, somatic comorbidities, decreased daily functionality and quality of life for both the patient and the family. As the costs are 2.3 times higher compared to the adult population, the development of a model that allows personalized treatment of depression in old age is a challenge for both clinicians and researchers (1, 15).

The burden of the depressive disorder is felt not only by the individual affected by this disease, but also by the family of the suffering person, by increasing stress and decreased quality of life, major effects on financial status, professional and socially consequences, as well as at the level of society as a whole, through a series of huge social and economic costs.

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NEWS

GASTROINTESTINAL RESPONSIVE POLYMERIC NANOPARTICLES FOR ORAL DELIVERY OF INSULIN: OPTIMIZED PREPARATION, CHARACTERIZATION AND *IN VIVO* EVALUATION

Biomacromolecules have transformed our capacity to effectively treat diseases; however, their rapid degradation and poor absorption in the gastrointestinal (GI) tract generally limit their administration to parenteral routes. An oral biologic delivery system must aid in both localization and permeation to achieve systemic drug uptake. Inspired by the leopard tortoise's ability to passively reorient, we developed an ingestible self-orienting millimeter-scale applicator (SOMA) that autonomously positions itself to engage with GI tissue. Gastrointestinal responsive polymeric nanospheres (NPs) based on hydroxypropyl methylcellulose phthalate (HPMCP) were prepared using spontaneous emulsification solvent diffusion method for improved oral administration of insulin. The NPs were prepared under optimized conditions have an encapsulation efficiency of 90% and a particle size of about 200 nm. *In vitro* drug release experiments demonstrated that the NPs exhibited a gradient release profile of loaded drug when the pH value gradually increased from 3.0 to 7.4. Enzyme resistance experiments showed that under simulated gastrointestinal conditions, the NPs protected more than 60% of the drug from being degraded by trypsin. The oral hypoglycemic experiments revealed that insulin-loaded NPs could significantly reduce blood glucose levels in diabetic rats with a relative bioavailability of 8.6%. *Ex vivo* imaging investigation of rat tissues showed that the drug-loaded NPs could promote the absorption of insulin in the ileum and colon. The work described here suggests that the gastrointestinal responsive polymeric NPs may be promising candidates for improving gastrointestinal tract delivery of hydrophilic biomacromolecules. Accordingly, the results indicated that HPMCP NPs with gastrointestinal stimuli responsiveness could be a promising candidate for oral insulin delivery. (Fang Y., Wang Q, *et al.* Gastrointestinal responsive polymeric nanoparticles for oral delivery of insulin: optimized preparation, characterization and *in vivo* evaluation, *J Pahr Sci* 2019; pii: S0022-3549(19)30253-9).

DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF NAIL PSORIASIS

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DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF NAIL PSORIASIS **(**Abstract**) Aim:** To identify the comorbidities associated with nail psoriasis and its therapeutic management. **Material and methods:** A 4-year retrospective analytical study which included 527 patients admitted to the Dermatology Department of the Iasi "Sf. Spiridon" County Clinical Emergency Hospital from Iași. **Results:** The frequency of cases without nail or arthropathic psoriasis was much higher (88.89% of cases) compared to 32.97% of cases associating arthropathic and nail psoriasis (χ^2 = 37.93, p <0.00001). Cases with nail psoriasis were significantly more commonly associated with severe forms of psoriasis with PASI scores higher than 20 (49.57% cases compared to 28.89%, Z = 3.69, p = 0.0001, and to 35.6 %, Z = 2.42, p = 0.008) and similarly, with DLQI scores higher than 20 (52.73% compared to 28.88%, Z = 4.41, p <0.00001 and to 32.54%, Z = 3.79, p = 0.00008). In patients with nail psoriasis, the highest frequency of cases with favorable outcome was obtained with biological treatment (80.85% compared to 39.86% of those with topical treatment, Z = 4.85, p <0.00001 and compared to 43.33% of those with topical and systemic immunosuppressive therapy, Z = 3.93, p = 0.00004). **Conclusions:** The presence of arthropathic psoriasis may be a risk factor for nail psoriasis, and its absence may be a protective factor against nail psoriasis. Severe forms may lead to quality of life (QOL) impairment with unfavorable, stationary and undulating course. Although nail psoriasis is usually investigated by clinical examination, the diagnosis of incipient form of disease is needed.
Keywords: NAIL PSORIASIS, PASI SCORE, DLQI (DERMATOLOGY LIFE QUALITY INDEX), ARTHROPATHY, THERAPEUTIC RESPONSE.

Psoriasis is no longer considered just a skin disease but rather a systemic chronic inflammatory disease associated with a substantial risk of increasing the morbidity rate that involves the skin, nails, joints, and other organs (1, 2). Nail disease is an important issue in dermatological practice and nail psoriasis may be present alone or associated with skin lesions. Currently, nail psoriasis associated with an inflammation of the nail bed has a prevalence ranging from 10 to 80% of psoriatic patients (3).

Psoriasis may affect the entire nail unit including the nail matrix, the nail bed

and/or the hyponychium component. Up to 30% of the psoriatic patients have arthropathic involvement (4). Symptoms of arthropathic psoriasis include swollen painful joints, causing discomfort and limiting patient mobility, usually the skin lesions precede the arthritis by 5-10 years. Over time, erosions can cause significant deformities and permanent invalidation (4). The anatomical relationship between the distal phalanx and the nail unit determines the correlation between arthropathic psoriasis and nail changes associated with systemic enthesopathy (5). Scoring systems are useful in assessing disease severity and monitoring therapy response (6). Visual examination may reveal leukonychia and loss of nail plate transparency due to involvement of mid-matrix, whereas pitting is caused by the involvement of the proximal portion of the nail matrix. Exocytosis of leukocytes beneath the nail plate leads to "oil drop" or "salmon patch" as clinical aspects, and splinter hemorrhages may appear due to capillary fragility. Another clinical form of nail psoriasis includes subungual hyperkeratosis and distal onycholysis and is caused by the parakeratosis of the distal nail bed. When the entire nail matrix is involved, the nail plate has a diffuse white appearance (3). Increased nail plate and bed thickness were reported in psoriatic patients without apparent clinical disease (7).

One of the aims of our study was to identify the clinical parameters and comorbidities correlated with nail and arthropathic disease and to evaluate their therapeutic management in psoriatic patients.

MATERIAL AND METHODS

We conducted a 4-year retrospective analytical study (2016-2019) including 527 patients diagnosed with psoriasis admitted and treated to the Dermatology Department of the Iasi "St. Spiridon" County Clinical Emergency Hospital. The study was approved by the Clinical Research Ethics Committee of the Iasi "Sf. Spiridon" County Clinical Emergency Hospital Iași and Clinical Research Ethics Committee of the Iasi "Grigore T. Popa" University of Medicine and Pharmacy. Data were extracted from the medical records in accordance with currents regulations, and security and confidentiality were warranted.

The inclusion criteria were positive clinical and/or histopathological diagnosis of psoriasis with the presence of at least one clinical form of the psoriasis disease such as plaques *vulgar psoriasis*, inverse or guttate psoriasis, palmoplantar pustular or generalized pustular psoriasis, or erythrodermic psoriasis. Special locations including the scalp, palmoplantar area, nails, genital area, large folds, and facial involvement were analyzed. Moreover, the comorbidities, such as psoriatic arthropathy including asymmetric oligoarthropathy, distal interphalangeal, mutilating arthritis, spondylitis, or rheumatoid type with distal symmetrical poly arthropathy were assessed by the rheumatologists. Exclusion criteria were uncertain clinical or histopathological psoriasis diagnosis and incomplete medical records that might affect the statistical results.

Nonparametric data from categorical variables, frequencies, and parametric data according to various scores calculated by clinicians in order to assess psoriasis severity were used. Psoriasis Area Severity Index (PASI) is an international dermatologic index used to express the severity of psoriasis which combines the severity (erythema, induration, and desquamation) and

percentage of affected area and has values with ranges from 0 to 72 (4). The Dermatology Life Quality Index (DLQI) is a questionnaire filled out by the patient and the clinician that assesses patient's QOL impairment by summing the score for each question resulting in a maximum of 30 and a minimum of 0. The higher the score, the more QOL is impaired (8). Depending on the values of this score along with DLQI score psoriasis can be classified in one of the following severity levels: mild (PASI<10, DLQI<10), moderate-severe (PASI >10) or severe psoriasis (PASI>10 and/ or DLQI >10, or when psoriasis is defined as pustular, erythrodermic or arthropathic psoriasis, or PASI <10 but with significant impairment of disease) (9).

Statistical analysis. The data were processed using version 7 of *Statistica* software. The parameters and their frequencies were analyzed and the statistical significance of differences between the mean values were assessed according to the grouping variables. The significance of the differences between the frequencies was evaluated with χ^2 *test*, appreciating that differences were as significant when the calculated value of the χ^2 test was higher than the critical value corresponding to the significance p value of 0.05. If percentages are compared, the significance of differences will be assessed using the *Z test*, considering as significant the differences located at a significance p value of 0.05 or less.

RESULTS

The study included 527 psoriatic patients with a mean age in men higher by an average of 3.06 years, the difference being statistically significant (t = 1.98, p = 0.048) (tab. I).

TABLE I

Average age difference by sex

Variable	Average		t	DF	p	N		Standard Deviation	
	Women	Men				Women	Men	Women	Men
Age	**51.11↓**	**54.17**	1.98	525	**0.048**	229	298	18.96	16.47

The frequency of the association of arthropathic psoriasis with nail psoriasis was reported for total cases of arthropathic psoriasis (tab. II).

TABLE II

Frequency of arthropathic psoriasis and nail psoriasis association

Arthropathic psoriasis	Nail psoriasis				Total		χ^2	p
	Presence		Absence					
Presence	**60**	**32.97**	122	67.03	182	100	37.93	**<0.00001**
Absence	38	11.01	**307**	**88.99**	345	100		
Total	98	18.60	429	91.40	527	100		

Since the calculated value χ^2 = 37.93 is significantly higher than the critical value at p <0.00001, the hypothesis of a significant relationship between arthropathic psoriasis and nail psoriasis is accepted, especially in the sense that the absence of arthropathic psoriasis may be a protective factor against nail psoriasis, given that

88.99% of cases without arthritic psoriasis, do not have nail psoriasis, and only 11.01% of patients with nail psoriasis had no arthropathic psoriasis.

Assessment of the relationship between nail psoriasis, PASI and DLQI severity scores (fig.1) showed that the cases with nail psoriasis were significantly more frequently associated with PASI scores higher than 20 (49.57% compared with 28.89% cases with PASI scores <10, Z = 3.69, p = 0.0001 and compared with 35.6 % with PASI scores between 10-20, Z = 2.42, p = 0.008). Similarly, cases with nail psoriasis were significantly more frequently associated with DLQI scores higher than 20 (52.73% compared to 28.88% cases with DLQI scores <10, Z = 4.41, p <0.00001 and compared to 32.54% with DLQI scores between 10-20, Z = 3.79, p = 0.00008).

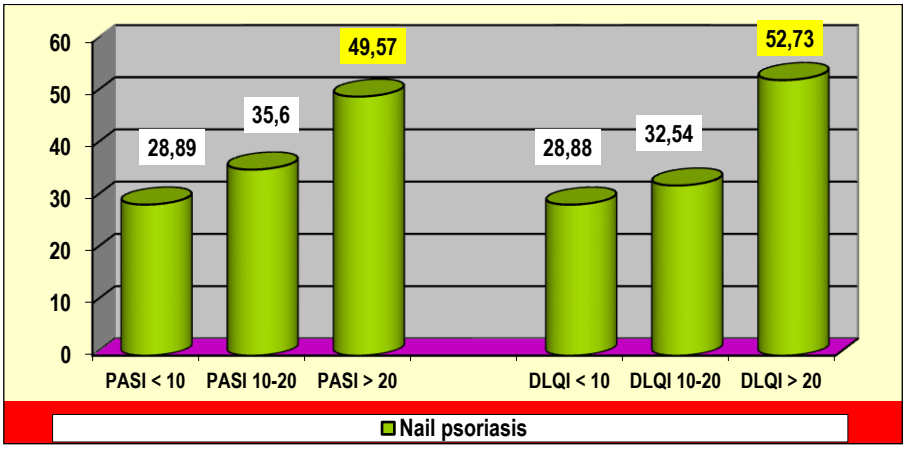

Fig. 1. Frequency differences in PASI and DLQI scores in patients with nail psoriasis

Comparison of the frequency of treatments recommended to patients with nail psoriasis highlighted the following significant differences (fig.2):

- the frequency of topical corticosteroid treatment was significantly lower compared to systemic treatments with methotrexate (34.89% compared to 50%, Z = 3.31, p = 0.005) and retinoids (34.89% compared to 58.33%, Z = 1.68, p = 0.046) and biological therapy (34.89% compared to 50.45% with biological therapy, Z = 3.04, p = 0.001)

- the frequency of topical treatment with vitamin D3 analogues was significantly lower than that of systemic treatment with methotrexate (35.62% compared to 50% with methotrexate, Z = 2.02, p = 0.02) and biological treatment (35.62% compared to 50.45% with biological treatment, Z = 1.98, p = 0.02).

The relationship between nail psoriasis and disease course was analyzed (tab. III) and by comparing the treatment response in patients with nail psoriasis we found that:

- the frequency of cases with favorable course was significantly higher after biological treatment (80.85% compared to 39.86% of those on topical corticosteroids and vitamin D3 analogues, Z = 4.85, p < 0.00001 and 43.33% of those on topical and systemic treatment with methotrexate, corticosteroids, or retinoids in pustular or erythrodermic psoriasis, Z = 3.93, p =

0.00004);

- the frequency of cases with unfavorable course was significantly higher in patients on topical treatment (13.04% compared to 4.26% of those on biological treatment, Z = 1.67, p = 0.047);

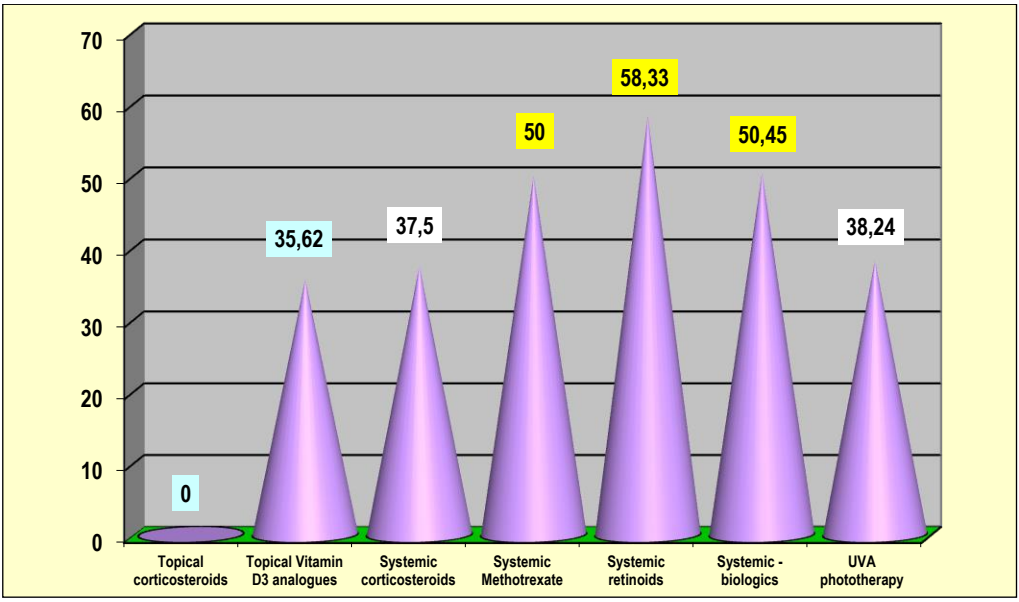

Fig. 2. Differences in the frequency of treatment in patients with nail psoriasis

TABLE III

Response to treatments administered to patients with nail psoriasis

Effect	Therapy						Significance of the difference	
	Topical		Topical and systemic		Biologic			
	N	%	N	%	N	%	Z	p
Favorable	55	**39.86↓**	26	**43.33↓**	38	**80.85**	0.46; 4.85; 3.93	0.32;**<0.00001;0.00004**
Unfavorable	18	**13.04**	5	8.33	2	**4.26↓**	0.95; 1.67; 0.84	0.17; **0.047**; 0.20
Stationary	24	**17.39**	10	**16.67**	0	**0↓↓**	0.12; 2.19; 3.06	0.45; **0.0007**; **0.001**
Undulating	41	**29.71**	19	**31.67**	7	**14.89↓**	0.28; 2.00; 2.01	0.39; **0.02**; **0.02**
Total	138	100	60	100	47	100		

- the frequency of cases with stationary course was significantly higher for topical treatment (17.39% compared to 0% of those on biological treatment, Z = 2.19, p = 0.0007) and on topical and systemic treatment (16, 67% compared to 0% of those on biological treatment, Z = 3.06, p = 0.001);

- the frequency of cases with undulating course was significantly higher with topical treatment (29.71% compared to 14.89% of those with biologic systemic treatment, Z = 2.00, p = 0.02) and topical and systemic treatment (31.67% compared to 14.89% of those with biological treatment, Z = 2.01, p = 0.02).

DISCUSSION

Current available data revealing nail in-

volvement in 10-80% of psoriatic patients (3) are confirmed by our study results; thus, of the 527 psoriatic patients 98 presented different clinical forms of onychopathy (18.6%). Other studies (10, 11, 12) estimated that up to 70-80% of patients with plaque psoriasis have nail involvement.

The prevalence of arthropathic psoriasis in patients affected by nail psoriasis ranges from 6% to 40% depending on the study design (13). Plaque psoriasis and nails psoriasis have been associated with the likelihood of developing psoriatic arthropathy. Similarly, special locations including the scalp or flexural areas have been associated with the risk of developing arthropathy with severity related to the magnitude of the cutaneous lesions. Reported data indicate that the tightest correlation with arthropathic psoriasis has been found in patients with nail disease (14). Verifying the hypothesis of a relationship between comorbidities and nail psoriasis using the χ^2 test, the results of our study revealed that there is a significant relationship between the presence of arthropathic psoriasis and nail psoriasis. The calculated value of the test $\chi^2 = 37.93$ is significant at $p < 0.00001$, and the existence of a significant relationship between arthropathic psoriasis and nail psoriasis is validated. The fact that the frequency of cases without arthropathic psoriasis and without nail psoriasis is much higher (88.89% of cases) compared to 32.97% associating arthropathic and nail psoriasis, leads to the conclusion that the absence of arthropathic psoriasis is a protective factor against nail psoriasis.

Nail involvement is associated with greater impairment of patients' QOL and also with moderate-to-severe skin disease (15). When assessing a total of 661 patients, Armesto S. *et al* (16) reported that 47.4% of patients were diagnosed with nail psoriasis and the group of patients with nail disease had more severe psoriasis (12.82 *vs.* 8.22 points) on the PASI index and included a larger percentage of patients with psoriatic arthritis (29.7% *vs.* 11.5%) (16). Our study results indicate that cases with nail psoriasis were significantly more commonly associated with severe forms of psoriasis with PASI greater than 20 (49.57% compared to 28.89%) and DLQI scores greater than 20 (52.73% compared to 28.88% cases).

Systemic therapy including phototherapy should be recommended to psoriatic patients with a moderate-to-severe form of disease defined with a body surface area (BSA) over 10%, or a PASI score>10 and/or a DLQI score >10, or when the disease is localized but presents significant functional impairment such as severe nail disease or involvement of special location such as palmoplantar area, scalp, face, or flexural areas (14, 17). Moreover, treatment recommendations (18, 19, 20) regarding nail psoriasis are related to the severity and matrix or bed involvement. Intralesional steroid injections are indicated in the case of matrix involvement alone. Topical corticosteroids alone or in combination with vitamin D3 analogues for nail psoriasis are limited to the nail bed affection, whereas systemic treatment including acitretin, methotrexate, cyclosporine or biologics may be indicated when more than 3 nails are involved according to clinical manifestation and severity scores. Although nail psoriasis is usually investigated by clinical examination, imaging techniques such as High Frequency Ultrasound or Dermoscopy are needed to diagnose incipient forms of nail psoriasis or to monitor the disease course under therapy (21, 22). In our study the frequency of topical corticosteroid treatment and similarly, the

frequency of topical treatment with vitamin D3 derivatives was significantly lower compared to systemic treatment with methotrexate, with retinoids, and biological therapy. In patients with nail psoriasis, the highest frequency of cases with favorable course resulted after biological systemic treatment whereas the unfavorable, stationary and undulating course were significantly higher following topical treatment and topical and systemic treatment with immunosuppressive therapy.

CONCLUSIONS

Based on the results of the present study, nail psoriasis is an association of an inflammation of the nail bed accompanied possibly by arthropathic psoriasis which may lead to QOL impairment in severe forms of disease. The presence of arthropathic psoriasis may be a risk factor for nail psoriasis.

The severity of nail involvement does not always parallel the intensity of cutaneous lesions, and subclinical forms of disease may be present. Therefore, non-invasive techniques such as High Frequency Ultrasound or Dermoscopy to diagnose subclinical forms of nail psoriasis progression or therapeutical response are necessary in current dermatology practice. Nail psoriasis management should be individualized, and it depends on the involvement of skin and/or joints, the number of affected nails influencing the choice of topical and/or systemic therapies.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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NEWS

THE RELATIONSHIP BETWEEN EXERCISE CAPACITY AND NEUTROPHIL/LYMPHOCYTE RATIO

Neutrophil/lymphocyte ratio (NLR) has recently been used as a potential marker to determine inflammation in cardiac and non-cardiac diseases. Functional capacity, expressed as metabolic equivalent (MET) based on treadmill test, is one of the major prognostic factors in individuals with heart failure. The NLR value, with a threshold level of 2.26, predicts a poor functional capacity with the sensitivity of 83% and specificity of 69%. Therefore, NLR was proposed as a useful tool to evaluate exercise performance of patients with heart failure. Similarly, NLR could also be used to predict functional capacity of patients before cardiac rehabilitation program. (Okan S. The relationship between exercise capacity and neutrophil/lymphocyte ratio in patients taken to cardiopulmonary rehabilitation program. *Bratisl Lek Listy* 2020; 121(3): 206-210).

STUDYING SOME POSSIBLE IRRITABLE BOWEL SYNDROME MANIFESTATIONS IN COVID-19 PATIENTS

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STUDYING SOME POSSIBLE IRRITABLE BOWEL SYNDROME MANIFESTATIONS IN COVID-19 PATIENTS (Abstract): The starting point of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic is a highly debated topic today. Despite the fact that a multitude of hypotheses have been issued in this context, this new strain beta-coronavirus is not only highly contagious, but also rapidly transmitted interpersonally through the perpetually disrupting the homeostasis. It constantly affects the lungs, and also the digestive tract according to the latest reports following its binding to the angiotensin-converting enzyme 2 (ACE2) receptors. **Material and methods:** In the present study we aimed to explain and describe some gastrointestinal deficiencies exhibited by the positive patients Therefore, the Visual Analogue Scale-IBS (VAS-IBS) questionnaire was applied to a group of positive patients (n = 22) and compared to their sex-matched controls (n = 22). The confirmation for the SARS-CoV-2 infection was carried out by specific RT-qPCR testing. **Results:** We observed that the overall scores attributed to the spectrum of Physical symptoms, Mental health and Quality of life are significantly different among the COVID-19 group dependent on sex than in the control group. However, we did not find significant differences in terms of shape and stool consistencies (as evaluated by Bristol scale). **Conclusions:** In this study, we showed that several gastrointestinal symptoms (as evaluated by VAS-IBS scale) could occur during or following SARS-CoV-2 infection. **Keywords:** COVID-19, SARS-COV-2, IRRITABLE BOWEL SYNDROME, VISUAL ANALOGUE SCALE FOR IBS, GASTROINTESTINAL MANIFESTATIONS, CLINICAL FEATURES.

Even though the human coronaviruses (HCoVs) are responsible for a low percentage in terms of annual enteric, respiratory and hepatic infections, the ongoing pandemic has placed not only Europe but the entire world in a state of emergency (1). Having as epicenter the city of Wuhan and as a consequence of the uncontrolled spread, the latest statistics issued by the World Health Organization (WHO) are showing increasing number of deceased patients.

The Severe Acute Respiratory Syndrome

Coronavirus 2 (SARS-CoV-2) is a new beta-coronavirus which is highly contagious and easily transmitted from person-to-person that has as intermediate host the Chinese rufous horseshoe bat - *Rhinolophus sinicus* (2). Compared to SARS that affected China in February 2003, its pathogenesis is quite similar to that of Middle East Respiratory Syndrome (MERS) and includes fever, dyspnea, cough, and invasive lesions on both lungs (3).

In this way, adults over the age of fifty-five and those suffering from chronic diseases are at a higher risk, which is directly reflected by the number of deaths (4-9). While most of the patients have fever and acute manifestations of the respiratory tract, several studies have also demonstrated that patients display gastrointestinal (GI) deficiencies. Diarrhea is predominant as well as the most common symptom. Indeed, the incidence varies between low, up to medium, some of them occurring before to respiratory symptoms (10).

Recently, it was observed that several antivirals used for COVID-19 treatment could lead to GI reactions similar to those observed during and following COVID-19. In this way, extensive evidence showed that hydroxychloroquine induces gastrointestinal impairments leading to diarrhea (11). Moreover, Bescos *et al.*, (12) suggested that some disinfecting agents (e.g. chlorhexidine) widely used to eradicate SARS-CoV-2 could lead to the same impairments.

On the other hand, considering that the GI symptoms occurring in irritable bowel syndrome (IBS) are strongly tied to exposure to chronic stress and elevated stress experiences such as quarantine and physical distancing consisted in a high level of stress (13,14), this study aimed to evaluate the possible occurrence of IBS symptoms in COVID-19 patients. Thus, a group of patients infected with SARS-CoV-2 and their sex-matched controls were recruited and the VAS-IBS scale was applied.

MATERIALS AND METHODS

Study participants

A total number of forty-four participants between 17-70-years old from Orygin Clinic, Iasi, Romania was enrolled in this study and subsequently divided into two equal small groups as follows: COVID-19 (male, n = 10 and female, n = 12) and their sex-matched controls (male, n = 10, and female, n = 12). All individuals were admitted into the study between June 15 to October 30. The main inclusion criteria of the participants in the study were the manifestation of the specific phenotype, with particular emphasis on the reporting of gastrointestinal ones such as fever, nausea, vomiting, and diarrhea, as well as the suspicion of infection. Were not considered eligible those with associated chronic diseases, drug or other substances usage or with an ongoing pregnancy.

Visual Analogue Scale for Irritable Bowel Syndrome

VAS-IBS scale was applied according to Bengston *et al.*, (15,16) guidelines following translation and adaptation to Romanian language and ROME IV criteria. It is a questionnaire which consist of 7 VAS scales regarding abdominal pain, diarrhea, constipation, bloating/gases, vomiting/nausea, psychological well-being and the intestinal symptoms' influence on daily life. Both groups received to fill a VAS-IBS questionnaires following a RT-qPCR detection test of SARS-CoV-2.

Ethical approval

The present study was approved by the

local committee (no 1/564/10/06/2020). Also, it was conducted according to the Helsinki Declaration of human rights and national and European regulations on Biomedical Research. Participants agreed and individually signed written informed consent for their voluntary contribution to this study.

Statistical analysis

Cumulative descriptive statistics regarding all responses were computed for each item of the VAS-ISB questionnaire with a CI of 95%. Statistical analyses were carried out using Minitab 19 software (Minitab Inc., 2019). Standard statistical analysis (One-way Single Factor ANOVA, Student's t-test) was applied, and all results were expressed as mean ± S.E.M. F values for which $p < 0.05$ were regarded as statistically significant.

RESULTS

In this study, we observed that the scores of the visual analogue scale-IBS administered to the participants were significantly higher in the COVID-19 patients, as compared to sex-matched healthy controls. More precisely, the intensity of the physical symptoms such as abdominal pain ($p < 0.001$), diarrhea ($p < 0.001$), constipation ($p < 0.001$), and bloating/gases ($p < 0.001$) was significantly elevated in the SARS-CoV-2-infected patients as showed in first figure.

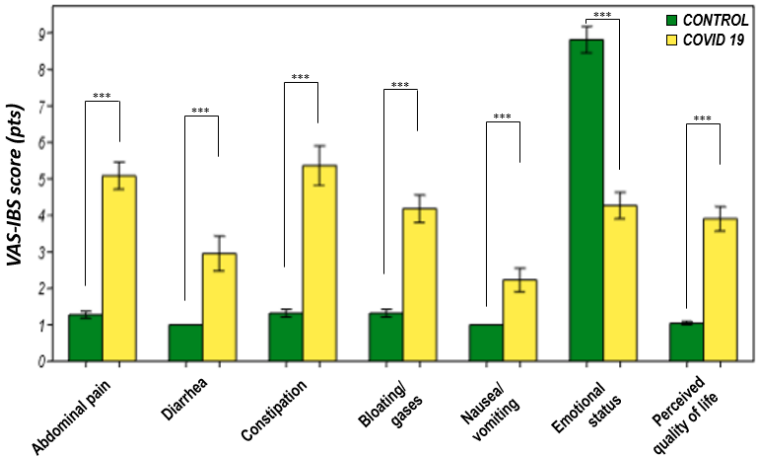

Fig. 1. VAS-IBS items scores in IBS patients and age and sex-matched healthy controls. The results are expressed as mean scores of VAS-IBS items ± SEM (n = 10 M and 12 F for each group, **p < 0.01, ***p < 0.001)

Regarding the sex differences, we observed that however no significant difference between healthy age and sex-matched controls was noted in terms of men vs women perception of abdominal pain and stool consistency, these change significantly in the COVID-19 patients. In this way, our results suggested that male COVID-19 patients are prone to exhibit constipation, while female COVID-19 reported diarrhea (fig. 2).

Furthermore, while analyzing the results stratified by sex, we observed that COVID-19 women significantly experienced frequent bloating ($p < 0.001$) and vomiting ($p < 0.01$) as compared to male COVID-19.

However, no significant differences were observed regarding the urge to defecate and the perceived sensation of incomplete defecation (fig. 3).

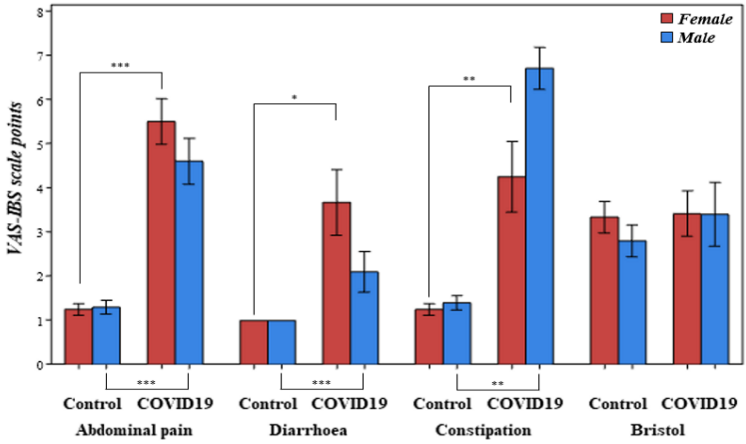

Fig. 2. The sex-stratified evaluation of abdominal pain, diarrhea and constipation severity, and Bristol score in COVID-19 patients as compared to sex-matched healthy controls. The results are expressed as mean scores of VAS-IBS items ± SEM (n = 10 M and 12 F for each group, *p <0.05, **p <0.01, ***p <0.001)

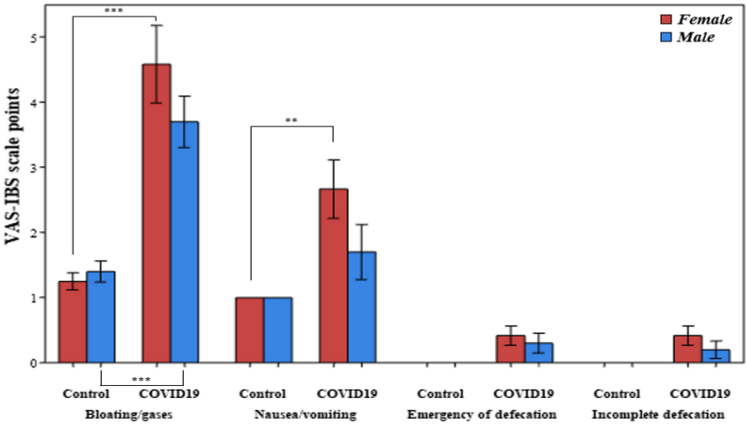

Fig. 3. The sex-stratified evaluation of bloating/ gases, nausea/ vomiting, emergency of defecation, and incomplete defecation occurrence in COVID-19 patients as compared to sex-matched healthy controls. The results are expressed as mean scores of VAS-IBS items ± SEM n = 10 M and 12 F for each group, **p <0.01, ***p <0.001)

Finally, the VAS-IBS scale also includes 2 items regarding the psychosocial and mood status in the context of the perception and changes brought by the GI symptoms burden. In the general tendency could also be seen while further stratifying the groups considering sex is required. Thus, both male and female patients stated

that their emotional status was felt lower in correlation to the perceived GI symptoms, as compared to healthy controls (p< 0.001). Similarly, both male and female patients stated that GI symptoms are affecting their quality of life in contrast to healthy sex and age-matched controls (p <0.001) (fig. 4).

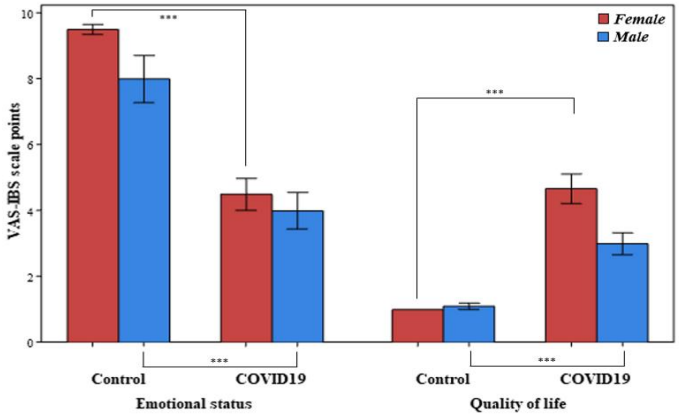

Fig. 4. The sex-stratified evaluation of the emotional status and quality of life in COVID-19 patients as compared to sex-matched healthy controls. The results are expressed as mean scores of VAS-IBS items ± SEM (n = 10 M and 12 F for each group, ***p <0.001)

Regarding the Bristol scale (p = 0.0048) (fig. 5), which is a newly added criteria for assessing the IBS subtypes, we observed that it can be seen that the frequency of diarrhea changes. More precisely, this GI disturbance is predominant in both groups-control (35%) and COVID-19 (50%). However it is hard to establish the exact cause-infection, medication or stress based on our study, given that Roque and Bouras (17) suggest in parallel that approximately 20% of the general population suffer from diarrhea.

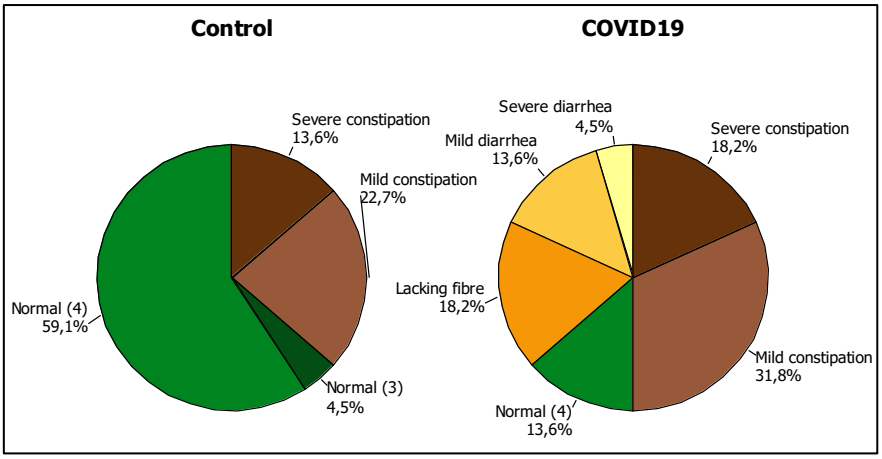

Fig. 5. Differences of the Bristol scale in terms of constipation versus diarrhea prevalence between the COVID-19 group and their sex-matched controls

The same discussion applies for the other two logical parameters, 'Incomplete defecation' (p = 0.008) and 'Emergency of defecation' (p = 0.003), considering that we observed a prevalence of 31.8% for 'Incomplete defecation' and 36.4% for 'Emergency of defecation' (figs. 6 and 7).

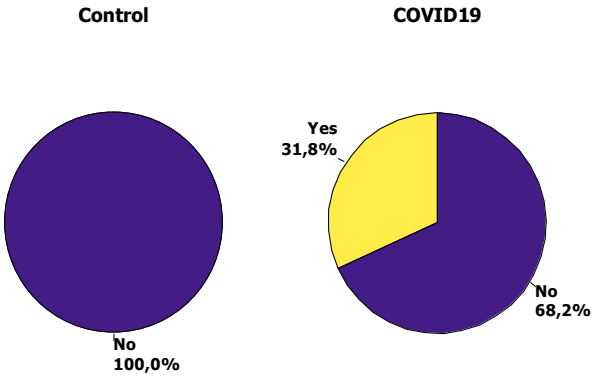

Fig. 6. The overall prevalence of 'Incomplete defecation' between the COVID-19 group and their sex- matched controls

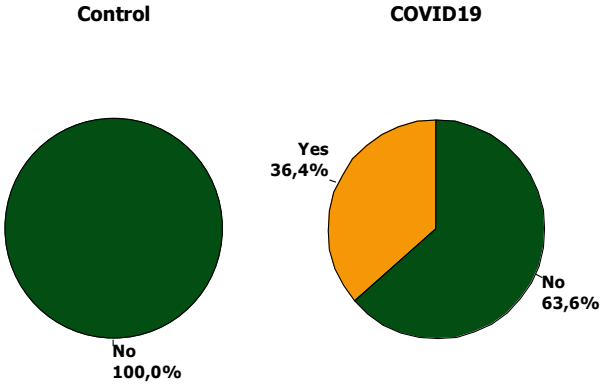

Fig. 7. The overall prevalence of 'Emergency of defecation' between the COVID-19 group and their sex-matched controls

DISCUSSION

Our present results are confirming that COVID-19 patients could exhibit GI disturbances. Moreover, we observed that these GI impairments could be evaluated by a VAS-IBS scale, thus leading to the conclusion that in this context the GI impairments could resemble the IBS gastrointestinal symptoms. Therefore, the data show that COVID-19 patients significantly frequently exhibit diarrhea, constipation, abdominal pain, bloating, and vomit-

ing. In this context, Chaoqun *et al.*, (18), Hormati *et al.*, (19), and Wang *et al.*, (20) also showed the mentioned GI impairments were present in COVID-19 patients. Congruent, they also showed that abdominal pain, vomiting, bloating, and nausea are prevalent among these individuals.

Having as purpose to determine the virological, clinical and epidemiological features of SARS-CoV-2-infected patients, Jin *et al.*, (21) enrolled in their study a cohort consisting of six hundred and fifty-one patients. From the total number, approximately 11.4% (n = 74) admitted exhibit at least one GI symptom, identical to those reported by us. Lin *et al.*, (22) revealed that 58 (61.1%) out of 95 patients had GI deficiencies, the most frequent being diarrhea. They were able to identify the niches where this virus resides within the human organism, viral signatures in stool samples being present starting from day seven, up to twelve days (23-26).

From a group of 40 patients initially included in the study, 45% of all patients did not have suffered from any gastrointestinal symptom according to the results of Effenberger *et al.*, (27) Moreover, in 55% of cases GI symptoms have ceased after only a couple of days, but in nine of them persisted for a longer time. In this context, such similar findings together with our results suggest that a correlation between SARS-CoV-2's pathogenesis and the incidence of the GI deficiencies.

Continuing with this concept, it has been hypothesized that SARS-CoV-2 can survive within its host until a certain temperature after host's death (28), and even after clearance of the respiratory tract (29). On the other hand, viral signatures can be identified after approximately four weeks since admission (25).

Particular cases have been also reported in the literature. We identified one case report in which the authors discussed the case of an 80-year-old woman previously diagnosed with left-sided ulcerative colitis (UC) that died days before the outbreak started. She developed pneumonia associated with the infection with SARS-CoV-2 and during the fourth day it passed away (30). There is certain controversy around this topic because recently was discussed about the low risk of mortality rate in patients with inflammatory bowel disease (IBD) (31). There are also strong chances for developing autoimmune disorders such as Reiter's syndrome (32), being even hypothesized that this pathogen could be associated with the Guillain-Barré syndrome (33).

Moreover, this preliminary paper of ours could open the discussion on the obvious connections that might exist between the digestive and gastrointestinal component that is associated with the neuropsychiatric one (34, 35), considering the latest expertise of our group in this area (36-40), as well as the social implications in this context (41, 42), and of course the neurological and psychiatric manifestations related to COVID-19 (43-49). In this way, our group is further working right now on establishing the interactions between digestive and neuropsychiatric manifestations, in the complex COVID-19 pathological context.

CONCLUSIONS

Based on the aforementioned, our results clearly demonstrated that VAS-IBS questionnaire is a reliable tool to measure the severity of GI deficiencies in both

healthy and infected individuals. Moreover, this could suggest some novel insights regarding the underlying mechanism between COVID-19 and gut that now are crucial.

It is possible, on one hand, that some specific symptoms of IBS may be present in patients with COVID-19 in a conjunctural manner, whether it is to think about the quality of life or the emotional status. An under-evaluated link is the psychological impact that isolation, quarantine and hospitalization have on patients diagnosed with COVID-19 and the risk towards various neurological disorders.

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CONFLICT OF INTEREST.

The authors declare that there is no conflict of interest.

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THE IMPLICATIONS OF INFLAMMATORY MARKERS AND LACTIC ACID IN ELDERLY PATIENTS WITH DEPRESSION

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THE IMPLICATIONS OF INFLAMMATORY MARKERS AND LACTIC ACID IN ELDERLY PATIENTS WITH DEPRESSION (Abstract): Depression can be defined as the nosological entity that includes both mood swings and psychotic symptoms, accompanied by emotional changes and a significant decrease in cognitive and psychomotor functions. The hypothesis of membrane theories of depression and its pathophysiology is based on the existence of a change in the structure and physiology of the cell membrane regarding receivers and loci for different substances. Tissue lesions are present in the cerebral atherosclerotic process with manifestations in the arterial endothelium whose dysfunction occurs as a result of a chronic inflammatory process caused by the atherosclerotic plaque. **Material and methods:** The study took place between January and June 2015 at the 5th Medical Clinic of the "CFR" Clinical Hospital in Iași, Romania. The study was conducted on 98 patients divided into two groups according to cardiovascular disease and the presence of depression and cognitive deficit**.** The objective of the study is the evaluation of depression and anxiety in correlation with the inflammatory profile in patients with cardiovascular disease. **Results:** Statistical analysis of intergroup differences for inflammatory markers, lactic acid and markers of subclinical atherosclerosis revealed that there are statistically significant differences between the average of said parameters between the two study groups. **Conclusions:** In the control group (patients with only cardiovascular disease) there were statistically significant correlations between the inflammatory markers, subclinical atherosclerosis, lactic acid and the assessment of depression and cognitive deficit. **Keywords:** DEPRESSION, ANXIETY, INFLAMMATORY MARKERS, LACTIC ACID, SUBCLINICAL ATHERO-SCLEROSIS.

Depression is considered an independent cardiovascular risk factor for future coronary events in healthy subjects. The most studied association between anxiety symptoms and somatic symptoms is that of cardiovascular and respiratory diseases, which can trigger states of fear, anxiety, and anxious waiting. Anxiety caused by the

presence of severe heart disease is worsened by the awareness of cardiac dysfunctions (tachycardia, extrasystoles, arrhythmias), the occurrence of retrosternal pain, breathing difficulties or fatigue (1-4).

A study carried out by the University of Iowa showed statistically significant correlations of lactic acid levels with anxiety levels. The results of the Iowa study suggest that one of the triggers for spontaneous panic attacks in patients with anxiety disorders could be increased lactic acid concentration in acid-sensitive fear circuits. Determination of lactic acid in the blood is known to play an important role in monitoring patients with tissue hypoxia (5). Clinical studies show the importance of determining blood lactate especially in patients in critical condition or at high risk of decompensation. The role of lactate in biochemistry and physiology is confirmed by clinical and experimental research. Clinically, the increase of blood level lactate indicates an exacerbation of tissue disorders and an increased risk of death. However, research has shown that the concentration of lactic acid <1 mmol/L has diagnostic significance. Lactic acid is one of the intermediates of glucose metabolism, a process that occurs in all organs and systems (6, 7). The largest production of lactate is in erythrocytes and muscle tissue, from which it enters the blood. Lactic acid is used in cardiomyocytes and hepatocytes in energy metabolism and glucose synthesis (gluconeogenesis). Sufficient perfusion of tissues with oxygen reduces glucose to CO_2 and H_2O with energy release.

Insufficient perfusion of oxygen in the tissues can greatly accelerate anaerobic processes, which are known to be accompanied by the production of a much higher amount of lactic acid, which accumulates in the tissues and blood with the development of lactic acidosis. One such disorder that the elderly is more prone to and can lead to lactic acidosis is chronic venous disease (8). The determination of this metabolic product is a necessary method of monitoring patients with hypoxia and whose clinical condition changes rapidly (9). The concentration of lactic acid, according to the data of different studies, should be in the range of1.8-2.0/1.85-2.2/2.2-2.7 mmol/L.

The increase of lactic acid production in the myocyte leads to the decrease of the intracellular pH and implicitly the inhibition of glycolysis, the use of fatty acids and the overall process of protein synthesis, which ultimately leads to the necrosis of cardiomyocytes. In ischemia, it is known that increased lactic acid levels are one of the major causes of angina (10). Another effect high lactic acid can have on the body is the influence on bone density over time and future prosthetic interventions, which may, in turn, exacerbate the overall functional status of elderly patients, thus leading to depression (11).

MATERIAL AND METHODS

The study took place between January and June 2015 at the 5th Medical Clinic of the "CFR" University Clinical Hospital in Iasi, Romania. The study was conducted on a number of 98 patients divided into two groups according to cardiovascular disease and the presence of depression and cognitive deficit.

- Group I: Normal cognition, which includes subjects with cardiovascular disease and normal psychological ageing without cognitive impairment or clinical depression - control group.
- Group II: Normal cognition, which in-

cludes subjects with cardiovascular disease and normal psychological ageing without cognitive impairment but with clinical depression.

Exclusion criteria included: history of substance use disorders, cognitive impairment, psychotic disorders, history of allergy to antihypertensive drugs, heart failure class III or IV NYHA, hepatitis B, C and/or HIV positive.

The aim of the study. To investigate the interaction of fibrinogen, C-reactive protein (CRP) as an inflammatory marker and lactic acid as a factor relating to depressive disorders. The study aims to explore whether determining the values of C-reactive protein, lactic acid and subclinical atherosclerosis have a predictive role in the occurrence of mixed depression/anxiety events in coronary patients.

The objectives of the study. Assessment of depression and anxiety in correlation with the inflammatory profile in patients with cardiovascular disease. Assessment of the clinical and biological correlations of depression-anxiety- subclinical atherosclerosis in the geriatric patient.

RESULTS

Group I

Assessment of anxiety and depression

The average value of the Hamilton scale scores for depression was 2.43 points, 85% of the patients had the values of the Hamilton scale scores for depression within the range of 0-5 points (fig. 1).

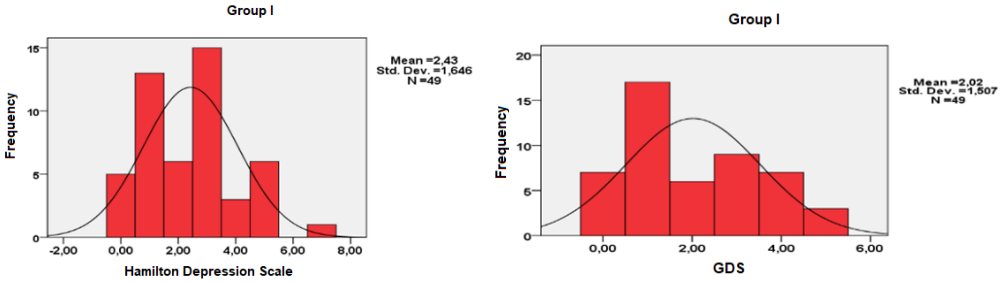

Fig. 1. Histogram of the distribution of the average values of the Hamilton scale in group I

The average value of the Geriatric Depression Scale scores was 2.02 points in group I. (fig. 1). Statistical analysis of the differences in the mean scales for assessing depression for the patients in group I by gender did not reveal statistically significant differences for both scales used (tab. I).

The average value of the Geriatric Depression Scale scores was 22.46 points, 69% of the patients had the values of the Geriatric Depression Scale scores within the range of 25-30 points (fig. 2). The average value of the Hamilton scale scores for depression was 24.06 points, 87% of the patients had the values of the Hamilton scale scores for depression within the range of 22-24 points (fig. 2).

TABLE I

Mann-Whitney test in group I

test	HDRS	GDRS
Mann-Whitney U	237,000	265,000
Wilcoxon W	562,000	590,000
Z	- 1.274	-0.707
p	0.49	-0.28

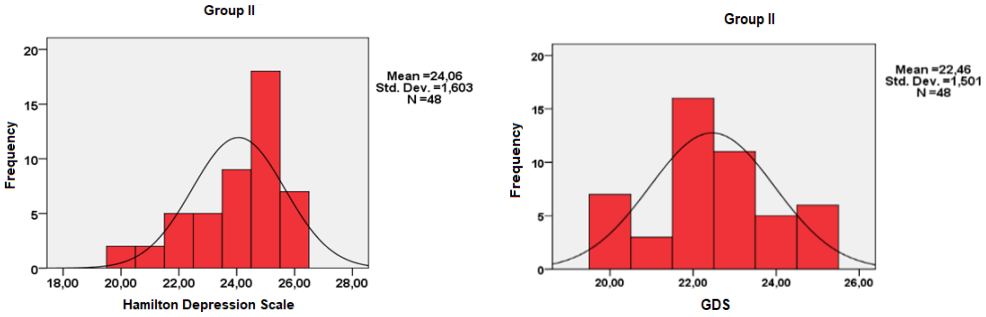

Fig. 2. Histogram of the distribution of the average values of the MMSE scores in group II.

Statistical analysis of the differences in the mean scales for the depression assessment for the patients in group II by gender did not reveal any statistically significant differences for both scales used (tab. II).

TABLE II

Mann-Whitney test in group II

test	HDRS	GDRS
Mann-Whitney U	267,500	158,500
Wilcoxon W	543,500	483,500
Z	-0.419	- 2.756
p	0.66	-0.64

Hamilton Anxiety Scale

The mean value of the Hamilton Anxiety Scale was 8.31 points, 56% of the patients had the values of the Hamilton Anxiety Scale scores in the range of 4-6 points (fig. 3).

Statistical analysis of the differences in the means of the Hamilton Anxiety Scale for the patients in Group I by gender did not show statistically significant differences (U=298.0; z=-0.041; p =0.96).

The mean value of the Hamilton Anxiety Scale scores was 18.02 points, 81% of the patients had the values of the Hamilton Anxiety Scale scores within the range of 17-20 points.

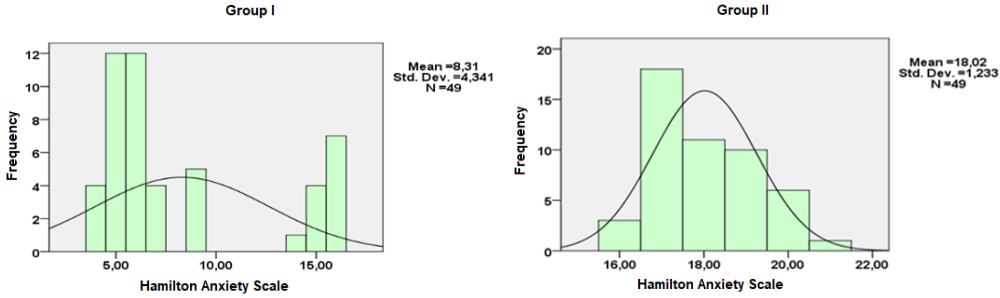

Fig. 3. Histogram of the distribution of the mean values of the Hamilton Scale for Anxiety in Group I and Group II

Statistical analysis of the differences in the means of the Hamilton Anxiety Scale for the patients in Group II by gender showed no statistically significant differences (U=287.0; z=-0.49; p =0.80).

Inflammatory markers

The mean fibrinogen value was close to 2.57 mg/dL, 59% of patients had mean fi-

brinogen values in the range of 2.5-3.0 mg/dL. The mean CRP value was 3.46 mg/dL, 89% of patients had mean CRP values in the range of 3.0-4.0 mg/dL (fig. 4).

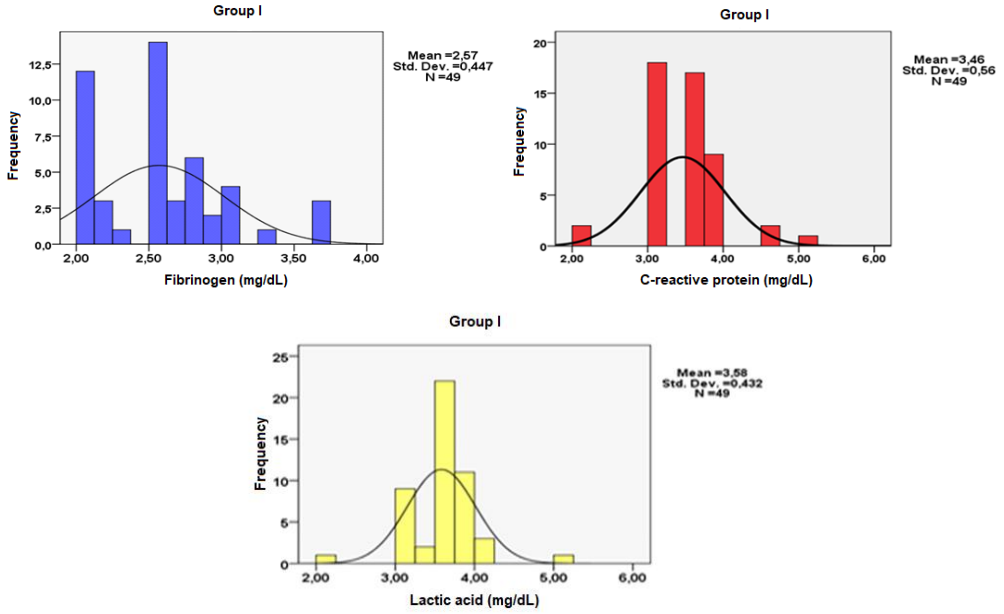

Fig. 4. Histogram of the distribution of fibrinogen means values in group I.

The mean lactic acid value was 3,68 mg/dL, 96% of patients had mean lactic acid values in the range of 3-4 mg/dL (fig. 4).

Group II

The mean fibrinogen value was 2.88 mg/dL, 67% of patients had the mean fibrinogen value in the range of 2.5-2.9 mg/dL. The mean CRP value was 3.86 mg/dL, 61% of patients had mean CRP values in the range of 3.3-3.8 mg/dL. The mean lactic acid value was 4.65 mg/dL, 69% of patients had mean lactic acid values in the range of 4.5-4.8 mg/dL (fig. 5).

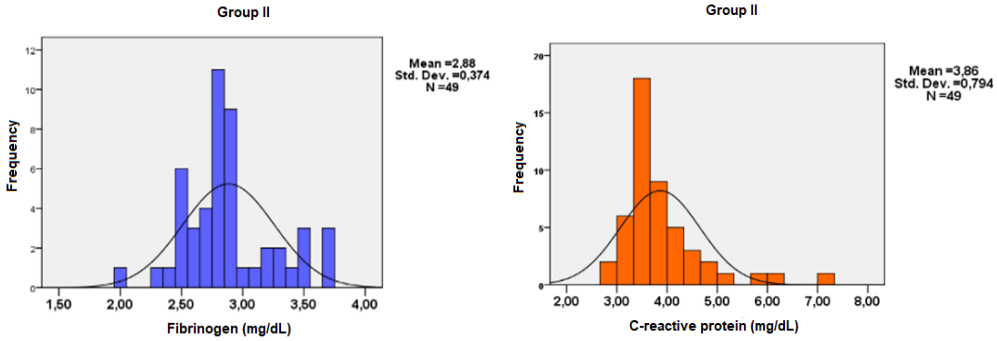

Fig. 5. Histogram of the distribution of the average fibrinogen values in group II

The markers of subclinical atherosclerosis

The literature suggests that the identification of subclinical atherosclerosis by noninvasive techniques such as ankle-brachial index (ABI), carotid intimal thickness (CIT), cardiac calcium scoring, arterial stiffness index, in asymptomatic individuals could be a useful method for both long-term predictions, where there is still no prediction tool, as well as for improving short-term prediction.

Subclinical atherosclerosis is strongly correlated with future cardiovascular events, independent of conventional cardiovascular risk factors. Its detection would be important because it would indicate an extremely aggressive intervention on risk factors, both by optimizing lifestyle and statin therapy, independent of the overall cardiovascular risk, highlighting subclinical atherosclerosis in a vascular territory being constantly accompanied by other locations.

The mean value of the ABI scores was 0.77. 94% of the patients had the ABI values in the range of 0.7-0.8 (fig. 6).

The mean value of the CIT scores was close between men (0.74±0.71 SD) and women (0.74±0.72 SD) of group I. (0.74±0.71 SD). 88% of the patients had the CIT values in the range of 0.7-0.8 (fig. 6).

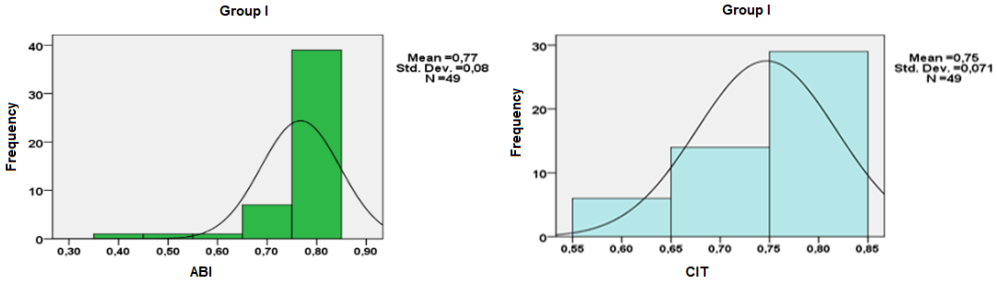

Fig. 6. Histogram of the distribution of the mean values of ABI, CIT in group I.

The mean value of the ABI scores was 0.81, 94% of the patients had the ABI values in the range of 0.7-0.8 (fig. 7).

The mean value of the CIT scores was 0.80, 88% of the patients had the CIT values in the range of 0.8-0.9 (fig. 7).

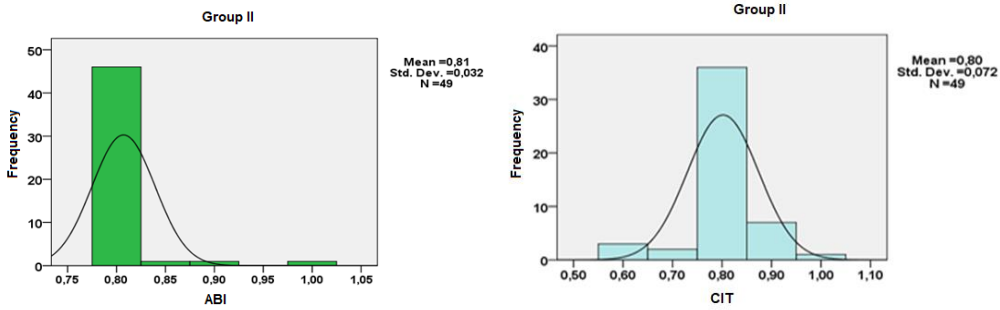

Fig. 7. Histogram of the distribution of the mean values of ABI, CIT in group II

Correlation analysis

The study of the correlations between the markers of subclinical atherosclerosis and cognitive status, depression, and anxiety in the patients of group I highlighted the following significant correlations:

- *Fibrinogen* was significantly correlated with lactic acid (p =0357. r=0.012), Hamilton Anxiety Scale (p=0.283. r=0.049), Hamilton Depression Scale (p=0.386. r=0.006), Geriatric Depression Scale (p=0.309. r=0.031), ABI (p=0.529. r ≤ 0.001), GIN (p =0.623. r ≤0.001).

- *CRP* was significantly correlated with lactic acid (p =-0.05. r ≤0.001), Hamilton Anxiety Scale (p =0.492. r ≤0.001). Hamilton Depression Scale (p =0.49. r ≤0.001).

- *Lactic acid* was significantly correlated with CRP (p =-0.5. r ≤0.001), fibrinogen (p = 0.357. r=0.012), Hamilton Depression Scale (p =0.792. r ≤0.001), Hamilton Anxiety Scale (p = 0.439. r ≤0.001), Geriatric Depression Scale (p =0.372. r=0.008), CIT (p =0.368. r=0.009).

The study of the correlations between the markers of subclinical atherosclerosis and cognitive status. depression and anxiety in the patients of group II highlighted the following significant correlations:

- *Fibrinogen* was significantly correlated with MMSE (p =0.521. r ≤0.001), Hamilton Anxiety Scale (p =0.286. r=0.046), Hamilton Depression Scale (p =0.357. r=0.012), CRP (p =-0.49. r ≤0.001)

- *CRP* was significantly correlated with lactic acid (p =-0.48. r ≤0.001), MMSE (p = 0.75. r ≤0.001), Hamilton Anxiety Scale (p =-0.349. r=0.014), Hamilton Depression Scale (p =-0.48. r ≤0.001).

- *Lactic Acid* was significantly correlated with CRP (p =-0.48. r ≤0.001), MMSE (p =-0.45. r=0.001), Hamilton Anxiety Scale (p =0.595. r ≤0.001), Hamilton Depression Scale (p =0.44. r≤0.001), Geriatric Depression Scale (p =0.296. r=0.039), CIT (p =-0.28. r=0.046).

DISCUSSION

In most degenerative processes specific to cardiovascular ageing, but also in cardiovascular diseases, adaptive structural and functional tissue changes occur at both macrovascular and microvascular levels (12). The significant increase in the carotid intimal thickness and the rarefaction of the conjunctival microcirculation (a phenomenon characterized by a decreased density of arterioles and capillaries) are examples of such changes. The carotid intimal thickness is used as a predictive index of future cardiovascular and cerebrovascular events (13). The association of the change of the carotid intimal thickness with cerebrovascular diseases, symptomatic strokes but also with cerebral lacunes was confirmed by clinical studies. An association between asymmetric cerebrovascular pathology and left cerebral pathology was also observed. High blood pressure has also been described as an independent risk factor, possibly a predictor of cerebral lacunes and cerebral white matter lesions, which in turn, are predictive factors for the development of cognitive impairments over time (14).

Oxidative stress amplifies the movement of proinflammatory cells, increases the expression of some adhesion molecules to the surface of endothelial cells and causes lipid oxidation at the blood-brain barrier (10, 15-17). Similar to our results, it was found that erythrocyte sedimentation rate and total serum proteins could be used as a predictive biomarker for high or low levels of anxiety in patients with chronic obstructive pulmonary disease, indicating that routine blood panels could serve as objective indicators of psychiatric symptoms in chronically ill patients (18).

The pathophysiology of Alzheimer's disease is described microscopically by the presence of senile plaques and neurofibrillary tangles along with the decrease in the

number of synapses and macroscopically by cerebral atrophy. Tests on oxidative damage were performed in the brains of patients with Alzheimer's disease by post-mortem harvesting. The main element of senile plaques is β-amyloid. Although this is not a defining characteristic, it is associated with Alzheimer's disease and old age. The relation of the β-amyloid with oxidative stress is controversial. Some studies give it as a source of free radicals and give it a prooxidative role (19) and as an inducer of neuronal apoptosis.

The boundaries between degenerative dementia and vascular dementia are now less clear and both types include several risk factors, clinical and/or pathological features of the same kind. Cerebrovascular disease is thought to play an important role in the presence and severity of Alzheimer's disease (9). A meta-analysis of randomized controlled research shows that cholinesterase inhibitors used in the treatment of degenerative dementia led to only minor cognitive improvements, causing uncertain clinical significance in patients with early to moderate vascular dementia.

Differences in the calibration of depression, research design and presentation of results, determined the heterogeneous nature of the findings. Risk factors for post-stroke dementia are female gender, physical disability, a history of depression and a history of mental and emotional vulnerability after stroke. The diagnosis of post-stroke depression can be masked by symptoms such as aphasia, agnosia, apraxia, and memory disorders. Although there is encouraging data on the prophylactic use of antidepressants in stroke patients, there is still insufficient evidence to implement this approach in therapeutic protocols after a stroke (1, 20).

Territorial increase of cardiac noradrenergic activity may be the condition by which desipramine (a possible noradrenaline reuptake inhibitor) leads to increased blood pressure values (1).

Another theory shows that the central serotonin system could be responsible for regulating blood pressure. Inhibition of serotonin reuptake causes a cascade of changes in central neurotransmission, accentuating the tone of the vegetative nervous system. Fluoxetine and sertraline lead to increasing and altered blood pressure in patients with orthostatic hypotension and can be used to treat recurrences of hypotension.

CONCLUSIONS

Within the control group (patients with only cardiovascular disease) there were significant statistical correlations between the inflammatory markers, subclinical atherosclerosis, lactic acid and the assessment of depression.

There were no statistically significant differences between the means of the studied parameter values based on the gender of the patient in the overall study group.

Inflammatory markers were significantly correlated with the scores obtained when assessing depression and anxiety.

Lactic acid as a substrate for anxiety has been correlated with depression.

ABI and CIT as indirect determinants of subclinical atherosclerosis were statistically significantly correlated with depression.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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SPECTRUM OF PRECIPITATING FACTORS OF HEPATIC ENCEPHALOPATHY IN PATIENTS WITH LIVER CIRRHOSIS

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SPECTRUM OF PRECIPITATING FACTORS OF HEPATIC ENCEPHALOPATHY IN PATIENTS WITH LIVER CIRRHOSIS (Abstract): Hepatic encephalopathy (HE) is one of the most frequent and debilitating complications of liver cirrhosis. Identification and correction of precipitation factors are mandatory to ensure a favorable outcome. **Material and methods:** We performed a retrospective descriptive study in cirrhotic patients with HE hospitalized during one pandemic year in a tertiary care referral center. Demographic, clinical and biological parameters, as well as the etiology of the liver cirrhosis were recorded. Precipitating factors were identified, and their prevalence was calculated, overall and according to the two main etiologies of cirrhosis. **Results:** Prevalence of HE was 71%. The most frequent overall precipitating factors were infections (43.7%), gastrointestinal bleeding (31.6%), renal failure (28.9%) and dyselectrolytemia (26.2%). According to cirrhosis etiology, significant higher frequencies of sepsis and non-adherence to therapy were found in patients with alcoholic cirrhosis compared to those with virus-related cirrhosis (6.1% *vs.* 0.8%, p=0.012, and 15.5% *vs.* 8.3%, p=0.026, respectively), while prevalence of hepatocellular carcinoma as causal factor of HE was significantly higher in virus-related cirrhotic patients compared to alcoholic cirrhosis (14.3% and 6.4%, respectively, p=0.004). Non-variceal bleeding was recorded especially in alcoholic patients. About half of patients had one single precipitating factor, nearly 40% had multiple factors, while for 11% no factor was identified. **Conclusions:** HE has high prevalence in hospitalized cirrhotic patients. The most common precipitating factors are infections, gastrointestinal bleeding, renal disfunction and electrolyte imbalances. Particular risk factors were found in relation to the etiology of cirrhosis, data that could help apply a tailored, individually-adapted preventive strategy. **Keywords:** HEPATIC ENCEPHALO-PATHY, LIVER CIRRHOSIS, PRECIPITATING FACTORS, ALCOHOLIC CRIRRHOSIS, VIRUS-RELATED CIRRHOSIS.

Hepatic encephalopathy (HE) is one of the most frequent complications of liver cirrhosis. It negatively affects patients' quality of life and has unfavorable short- and long-term impact on their clinical outcome. Moreover, because of complex therapeutic measures and frequent hospitalizations, HE may associate a significant burden in term of healthcare resources.

The frequency of HE is related to the

functional status of the liver and to the degree of portal hypertension, with direct consequence on the portosystemic shunt formation. According to the clinical presentation, HE is classified in covert HE, which includes minimal HE (detectable only by special psychometric tests) and grade 1 HE, and overt HE, which includes grade 2-4 HE according to West-Haven criteria (1). In terms of prevalence, 10-15% of patients present overt HE at the moment of the diagnosis of cirrhosis (2), and 16-21% of patients with decompensated cirrhosis associate overt HE (3). During the first 5 years after the diagnosis of cirrhosis, the risk for HE is up to 25% (4), while in evolution, up to 40% of cirrhotic patients will present with HE, mostly in a recurrent manner (5).

There are multiple well-defined precipitating factors, which can cause, by various mechanisms, episodic or recurrent episodes of HE. Identification of precipitating factor by thorough work-up is mandatory in order to apply the optimal management strategy. The recognition and, subsequently, the correction of the precipitating factor is the first step in treating HE episodes. About 90% of patients with HE requires only the correction of the precipitating factor as therapeutic approach (6), therefore identifying and controlling precipitating factors is essential.

Precipitating factors are represented mainly by infections, gastrointestinal bleeding, constipation, electrolyte disorders, renal disfunction, hepatotoxic agents, and others. Awareness and promptly recognition definitely influence favorably the individual therapeutic approach and may warrant therapeutic success. As there is a 40% cumulative risk of another recurrence during the 6 months following the previous episode of, HE (7), tight surveillance and, ideally, avoidance of precipitating factors could represent an effective prophylactic measure.

The aim of our study was to assess the prevalence of various precipitating factors for HE in patients with liver cirrhosis, and to identify particular precipitating factors according to the etiological profile of cirrhosis, data that could help apply a tailored, individually-adapted preventive strategy.

MATERIAL AND METHODS

We performed a retrospective, descriptive study, including all the patients with HE and liver cirrhosis, hospitalized during the COVID-19 pandemic year (April 1st, 2020 - March 31st, 2021) in the Institute of Gastroenterology and Hepatology, "Sf. Spiridon" County Clinical Emergency Hospital Iasi, a tertiary referral care center in North-East Romania. Among all 743 cirrhotic patients hospitalized during this period, 526 fulfilled the West Haven criteria for HE. Demographic, clinical and biological characteristics of the patients, as well as the etiology of the liver cirrhosis were recorded. Precipitating factors were identified, and their prevalence was calculated, both overall and according to the main etiologies of cirrhosis.

Statistical analysis

Analysis was performed using *SPSS version 24.0*. Categorical data was described using frequencies and percentages. Continuous data were expressed as means and standard deviation (SD). Results were considered with statistical signification for a p-value <0.05.

RESULTS

Overall prevalence of HE

Out of all 743 cirrhotic patients hospi-

talized during one pandemic year in our care center, 526 patients with HE was identified, resulting an overall HE prevalence of 71%.

Patients' characteristics

Demographic characteristics of the patients, etiology, Child-Pugh and MELD scores of liver cirrhosis, and grades of HE according to West-Haven criteria are presented in the first table. The most common causes for cirrhosis were alcohol, with almost three quarters of cases (393 patients, 74.7%) and viral, with less than a quarter (118 patients, 22.4%), while other causes were much less frequent (metabolic 1.5%, primary biliary cirrhosis 0.8%, Wilson disease 0.6%). 99% of the patients had decompensated cirrhosis, corresponding to class Child-Pugh B and C. Mean values of MELD scores were above the cut-off for liver transplantation. The most patients (40.3%) had grade 2 HE according to West-Haven criteria, followed by grade 1 (30.8%), and grade 3 (19.4%); 9.5% of patients had hepatic coma, corresponding to grade 4 HE.

TABLE I

Characteristics of the study patients

Parameters	Patients, n=526
Age, mean ± SD	57.37 ± 11.5
Gender ratio, n (%)	
Men	318 (60.5)
Women	208 (39.5)
Place of origin, n (%)	
Urban area	243 (46.2)
Rural area	283 (53.8)
Etiology of cirrhosis, n (%)	
Alcoholic	393 (74.7)
Virus-related	118 (22.4)
Hepatitis C virus infection	49 (9.3)
Hepatitis B and D virus infection	32 (6.1)
Hepatitis B virus infection	28 (5.3)
Hepatitis B and C virus infection	9 (1.7)
Nonalcoholic, nonviral	15 (2.9)
Metabolic	8 (1.5)
Primary biliary cirrhosis	4 (0.8)
Wilson disease	3 (0.6)
Child-Pugh classification	
Class (A/B/C), n (%)	5/128/393 (1/24.4/74.7)
Score, mean ± SD	10.91 ± 2.15
MELD score, mean ± SD	21.05 ± 7.95
MELD-Na score, mean ± SD	23.07 ± 8.06
West-Haven hepatic encephalopathy grading, n (%)	
1	162 (30.8)
2	212 (40.3)
3	102 (19.4)
4	50 (9.5)

Precipitating factors of HE

Type and overall frequency of each precipitating factor are presented in the second table. As nitrogen overload conditions, we recorded especially gastrointestinal bleeding (the most common type being variceal bleeding) with a prevalence of 31.6%, renal disfunction, with a prevalence of 28.9%, and constipation, prevalence 9.9%. Dyselectrolytemia, as meaningful metabolic alteration, had a prevalence of 26.2%. The systemic stress situations were dominated by infections (the highest prevalence, 43.7%), the most common being spontaneous bacterial peritonitis, urinary tract infection, *Clostridioides difficile* colitis, COVID-19, other respiratory tract infections, and cellulitis. Active alcohol consumption was recorded in 12.4% of cases. Patients' non-adherence to treatment was responsible for HE in 13.7% of cases. Other precipitating factors were hepatocellular carcinoma (HCC), TIPS and surgery. In nearly half of patients, one single factor was incriminated, while in 39.3% of cases, multiple factors were recorded. In 11.6% of cases, no precipitating factor was identified.

TABLE II

Precipitating factors of HE in the study group

Precipitating factors		Patients, n (%)
Nitrogen overload	**Gastrointestinal bleeding**	**166 (31.6)**
	Variceal bleeding	129 (23)
	Non-variceal bleeding	37 (7)
	Constipation	52 (9.9)
	Renal disfunction	**152 (28.9)**
Metabolic alteration	**Dyselectrolytemia**	**138 (26.2)**
Systemic stress	**Infections**	**230 (43.7)**
	Spontaneous bacterial peritonitis	73 (13.9)
	Urinary tract infection	41 (7.8)
	Clostridioides difficile colitis	34 (6.5)
	COVID-19	31 (5.9)
	Sepsis	25 (4.8)
	Respiratory tract infection (other than COVID-19)	21 (4)
	Cellulitis	5 (1)
	Surgery	8 (1.5)
Hepatotoxic agents	Active alcohol consumption	65 (12.4)
Others	HCC	44 (8.4)
	TIPS	4 (0.8)
Non-adherence to therapy		72 (13.7)
Number of precipitating factors		
1		258 (49.1)
≥2		207 (39.3)
Unidentified		61 (11.6)

Results of comparative analysis of precipitating factors frequencies according to main etiology of cirrhosis (alcoholic and virus-related) are presented in the Table III.

TABLE III
Comparative analysis of precipitating factors frequencies according to main etiology of cirrhosis

Precipitating factors, n (%)	Alcoholic N = 393	Viral N = 133	*P*
Gastrointestinal bleeding	124 (31.6)	42 (31.6)	0.965
Variceal bleeding	92 (23.4)	37 (27.8)	0.308
Non-variceal bleeding	32 (8.1)	5 (3.8)	0.088
Constipation	42 (10.7)	10 (7.5)	0.291
Renal failure	106 (27)	46 (34.6)	0.098
Dyselectrolytemia	103 (26.2)	35 (26.3)	0.981
Infections	181 (46.1)	49 (36.8)	0.064
Spontaneous bacterial peritonitis	58 (14.8)	15 (11.3)	0.317
Urinary tract infection	32 (8.1)	9 (6.8)	0.610
Clostridioides difficile colitis	24 (6.1)	10 (7.5)	0.568
COVID-19	20 (5.1)	11 (8.3)	0.179
Sepsis	24 (6.1)	1 (0.8)	**0.012**
Pulmonary tract infection	19 (4.8)	2 (1.5)	0.090
Cellulitis	4 (1)	1 (0.8)	0.785
Surgery	6 (1.5)	2 (1.5)	0.985
Active alcohol consumption	65 (16.5)	-	-
Non-adherence to therapy	61 (15.5)	11 (8.3)	**0.026**
HCC	25 (6.4)	19 (14.3)	**0.004**
TIPS	2 (0.5)	2 (1.5)	0.256
Number of precipitating factors			
1	189 (48.1)	69 (51.9)	0.451
≥2	160 (40.7)	47 (35.3)	0.274
Unidentified	44 (11.2)	17 (12.8)	0.622

DISCUSSION

HE is a frequent complication of liver cirrhosis, and its occurrence is usually determined by another concomitant pathological condition or acute event which act as trigger factors. Mechanisms involved are complex, hyperammonemia being the leading path towards the alteration of the brain functioning. The spectrum of the clinical manifestations of HE is heterogenous, and the severity of this complication can vary from mild, subtle symptoms to life-threatening conditions as hepatic coma. Depending on the methods of assessment and the type of study population, prevalence of HE varies. While among all cirrhotic patients, HE prevalence is usually up to 20% (3), some studies so far showed more increased prevalence in hospitalized cirrhotic patients (8). However, patients with advanced, decompensated and/or complicated cirrhosis are the most prone to

develop HE. This explains why our analysis showed a much higher prevalence (71%); during pandemic, the hospitalized population consisted almost exclusively in severe cirrhotic patients, usually associating serious specific complications. Current classification distinguish covert HE (minimal HE and grade I according to West Haven criteria) from overt HE (grades II-IV according to West Haven criteria). In our study, 162 patients (31%) had covert HE (grade I), while the majority (364 patients, 69%) presented overt HE. The majority of cirrhotic patients which present with HE manifestations need hospitalization. Treatment may involve advanced support measures, and furthermore, identification and correction of precipitating factors is mandatory. Precipitating factors are often multiple, either concomitant or consecutive to each other, because one complication of cirrhosis frequently causes another.

In our study, infections were the most frequent precipitating factors (43.7%), the commonest being spontaneous bacterial peritonitis, followed by urinary tract infections, *Clostridioides difficile* colitis, COVID-19 pneumonia, sepsis, respiratory infections (other than SARS-CoV-2) and cellulitis. Universal data so far show that infections are recognized among the main precipitating factors for both episodic and recurrent HE. There seem to be, however, certain differences according to the region reported: infections appear as the most frequent precipitating factors (responsible for up to 65% of cases of HE) in the developing countries (8, 9, 10) while studies from western world report infections with much lower frequency as precipitating factors for HE (11, 12). Adherence, good nutritional and immunologic condition and functionality of health system might explain lower frequency of infections. In relation to cirrhosis etiology, we found a significant higher frequency of sepsis as trigger factor for HE in patients with alcoholic cirrhosis compared to those with virus-related cirrhosis (6.1% and 0.8%, respectively, p=0.012). Alcoholic patients have poorer immune status and generally negative health outcome (13), which could explain why they are the most exposed to septic complications. Respiratory tract infections were found to be more frequent in alcohol-related cirrhosis, reflecting the higher risk of pulmonary complications in this group of patients, involving issues as self-neglecting or aspiration.

The second most frequent precipitating factor of HE was gastrointestinal bleeding. Nearly one third of all patients (31.6%) presented bleeding as major risk factor; among them, variceal bleeding was more prevalent, with approximately three quarters of cases, while the rest had non-variceal bleeding. Cirrhotic patients usually bleed from esophageal or gastric varices (60-65%) (14), but non-variceal sources are also recorded (mainly peptic ulcers, erosive gastritis, Mallory-Weiss syndrome) (15). In our study, non-variceal bleeding tended to be more frequent in patients with alcoholic cirrhosis compared to patients with virus-related cirrhosis. Indeed, alcohol consumption can directly cause hemorrhagic upper gastrointestinal lesions, such as acute erosive gastritis, esophageal ulcers, Mallory-Weiss syndrome, and, in the same time, it has been associated *per se* with an increased risk of upper gastrointestinal bleeding, especially secondary to peptic ulcer (16,17). While variceal bleeding is classi-

cally seen as the most dramatical and potentially lethal complication, with 6-week mortality up to 20% (18), the non-variceal bleeding in patients with cirrhosis is as well of upmost importance, since clinical outcomes are more severely influenced, in terms of morbidity and even mortality, compared to non-cirrhotic patients (19, 20). Rapid adapted treatment is mandatory in all cases of gastrointestinal bleeding, to avoid high rate of mortality.

Renal failure was the third most common precipitating factor (28.9% of all patients). There are many mechanisms of renal impairment in liver cirrhosis, leading directly to nitrogen overload and consequent brain disfunction. According to etiology of cirrhosis, renal disfunction was more frequent (34.6%) in virus-related cirrhosis (the second most common, after infections and before gastrointestinal bleeding). The spectrum of renal impairment in patients with liver cirrhosis covers several main entities, as acute kidney injury with or without features of hepatorenal syndrome, acute kidney disease and chronic kidney disease (21). In our analysis, virus-related cirrhosis was found to be more exposed to renal complications, probably because both hepatitis B virus infection and hepatitis C were reported as causes of chronic renal disease, mostly due to membranous glomerulonephritis (22), or cryoglobulinemia and focal segmental glomerulosclerosis, respectively (23).

Dyselectrolytemia was the fourth most frequent precipitating factor (26.2%), with similar prevalence in relation to the etiology of cirrhosis. Hyponatremia, hypokalemia or hyperkalemia and alkalosis are the main electrolyte imbalances that can trigger HE, independently or associated to renal disfunction (24). Diuretics and consequent hypovolemia or dehydration are risk factors for both electrolyte disorders and acute renal failure, and tight surveillance is mandatory to avoid harmful overlap.

Active alcohol consumption was unfortunately frequent as trigger factor for HE among patients with alcoholic cirrhosis (16.5%). This underlines the urge need to actively combat alcohol consumption, which is not only a main cause for chronic liver disease, but also a factor for further progression and recurrent complications. Conversely, in the viral cirrhosis group, no cases of active alcohol consumption were identified. As listing for liver transplantation requires at least 6 months abstinence, the significance of the matter is tremendous.

A particular issue appears to be non-adherence to therapy, more significant in patients with alcoholic cirrhosis compared to viral cirrhosis (15.5% and 8.3%, respectively, p=0.026). Neglecting the treatment of a potential precipitating factor or lack of proper prophylaxis measures put the patients at risk for developing HE. Again, more efforts for a better compliance seem to be needed in alcoholic patients.

An important severe, potentially fatal complication of liver cirrhosis is hepatocellular carcinoma (HCC), which develops in one third of cirrhotic patients overall during their lifetime, with annual risk of up to 8% (25). HE may be induced by hepatic failure associated to HCC. All patients with liver cirrhosis, regardless of etiology, are at risk for HCC, but hepatitis B and C virus-related cirrhosis are particularly exposed. In our analysis, prevalence of HCC as causal factor of HE was significantly higher in virus-related cirrhotic patients compared to alcoholic cirrhosis (14.3% and

6.4%, respectively, p=0.004). Surveillance is mandatory in all cirrhotic patients, to allow diagnosis in curable stages.

Seen as a relatively common precipitant factor of HE, especially for recurrent episodes (26), constipation was found to be in our study responsible for less than 10% of cases. Our study included only hospitalized patients during pandemic, while patients with non-severe forms of HE, having constipation as trigger factor, were managed as outpatients.

Surgery is a stress condition that may decompensate and induce HE (9). A few patients in our study (1.5%) had surgery as responsible factor for HE, as interventions in cirrhotic patients should be reserved only for critical and life-menacing situations, especially if already decompensated.

A less frequent but equally important causal factor for HE was trans jugular intrahepatic portosystemic shunt (TIPS). TIPS may complicate with HE in various proportions (18-50%), depending mostly on the type of stent (diameter, bare or covered) (27). In our analysis, four cirrhotic patients presented with HE as a consequence of the TIPS. Time of insertion and the type of TIPS, used as treatment of recurrent bleeding or refractory ascites, must be very well weighted, in order to assure a favorable risk-benefit ratio.

Identifying and correction of precipitating factors are essential, as in many cases, up to 90%, controlling the cause assures the effective treatment of HE episode (6). However, some cases can be challenging, when no factor is finally found or when, by contrary, multiple factors overlap. We found a relatively low proportion (11%) of patients without identifiable precipitating factor. Exhaustive work-up must be always performed in order to find the potential trigger, but clinicians must be aware of the existence of the so-called "nonprecipitated" HE, mainly caused by spontaneous portosystemic shunts (28). HE may have more than one precipitant factor, situation found in our study in a high proportion (39%), similar results being reported by other several studies (29,30,31). Independent or related to each other, various concomitant events or complications of cirrhosis can act synergically towards HE development, and their prompt recognition influence patient's outcome.

CONCLUSIONS

HE is a frequent complication of liver cirrhosis, with increased prevalence in hospitalized cirrhotic patients. Infections, gastrointestinal bleeding, renal disfunction and dyselectrolytemia are the most common overall precipitating factors, and almost 40% of patients have multiple precipitating factors. According to cirrhosis etiology, a significant higher prevalence was found for sepsis and non-adherence in alcoholic cirrhosis, while HCC was significantly more frequent as precipitating factor in virus-related cirrhosis. Non-variceal bleeding was more frequent in alcohol-induced cirrhosis. Active exhaustive search and promptly control of the precipitating factors are mandatory and being aware of the risk factors could assure early identification and treatment.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

THE RELATIONSHIP BETWEEN EXERCISE CAPACITY AND NEUTROPHIL/LYMPHOCYTE RATIO

Neutrophil/lymphocyte ratio (NLR) has recently been used as a potential marker to determine inflammation in cardiac and non-cardiac diseases. Functional capacity, expressed as MET based on treadmill test, is one of the major prognostic factors in individuals with heart failure. The NLR value with a threshold level of 2.26 predicts a poor functional capacity with the sensitivity of 83% and specificity of 69%. Therefore, NLR was proposed as a useful tool to evaluate exercise performance of patients with heart failure. Similarly, NLR could also be used to predict functional capacity of patients before cardiac rehabilitation program. (Okan S. The relationship between exercise capacity and neutrophil//lymphocyte ratio in patients taken to cardiopulmonary rehabilitation program. *Bratisl Lek Listy* 2020; 121(3): 206-210)

INFECTIVE ENDOCARDITIS ETIOLOGIC PROFILE - STILL A CLASSIC DISTRIBUTION OF MICROORGANISMS?

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INFECTIVE ENDOCARDITIS ETIOLOGIC PROFILE - STILL A CLASSIC DISTRIBUTION OF MICROOGANISMS? (Abstract): The present study analyzed etiologic profile of blood culture-positive infective endocarditis (IE) and possible differences between the main etiologies. **Material and methods:** An observational retrospective analysis was conducted in a regional hospital over 11-year period. A total of 58 consecutive patients diagnosed with infective endocarditis with positive blood cultures were included in the study. Diagnosis of IE was realized based on Duke modified criteria. All patients data was reviewed from electronic database and medical charts. Epidemiologic, clinical and laboratory parameters were analyzed. Afterwards, the main three determinant microorganisms were compared to identify differences in presentation or evolution. **Results:** Dominant etiology of IE was represented by: *Enterococcus spp.* (22.4%), *S. aureus* (18.9%), *Viridans streptococci* (17.2%), *S. gallolyticus* (10.3%) and coagulase-negative staphylococci (8.6%). Total in-hospital mortality remained high (24.1%), and no significative correlation was revealed between certain microorganism and worsening disease course (p=0.259), or in-hospital death (p=0.343). No major baseline characteristic or evolution differences were observed between the main 3 etiologies. *S. aureus* infection presented a significative association with thrombocytopenia (p=0.049) and high levels of total bilirubin (p=0.008). **Conclusions:** Infective endocarditis is still a community-acquired disease, with peculiar etiologic distribution and increased prevalence of *Enterococcus spp.* infection. In-hospital mortality of the disease remains high, independent of the etiologic profile. **Keywords:** INFECTIVE ENDOCADRITIS, ETIOLOGY, ENTEROCOCCUS SPP., EVOLUTION.

Infective endocarditis (IE) is a medical condition with high impact over mortality and morbidity due to frequent, late diagnosed, and impairing complications (1, 2). Even if the incidence of the disease is reported to be low, the diagnosis remains challenging in the context of heterogenous presentation. IE is one of the greatest "mimes" of pathology, from the well-known clinical picture of fever and new arise cardiac murmur, up to atypical presentations with the lack of fever, neuro-

logic, embolic, or systemic complications (1, 3).

Diagnostic and therapeutic options have improved over time, but the course of the disease remains unchanged. The high mortality, up to 30% of the cases, comparable with aortic dissection, makes infective endocarditis a fiercely opponent for the practitioner (4-5). It is often necessary for an "endocarditis team" to be formed, with the participation of different specialists, given the variable evolution and the need of a proper intervention to improve prognostic (1).

Nevertheless, blood cultures play a crucial role to the diagnostic of IE and to further treatment consideration. But some factors (as anterior treatment with antibiotics, or fastidious microorganisms) can lead to negative results of blood cultures (up to 50% of the cases), with subsequent difficulties in management and possible negative evolution of IE (6-7).

An epidemiological change appeared in the past 30 years, from a disease mainly affecting patients with rheumatic or congenital heart disease, determined by viridans streptococci, to one that is highly associated with degenerative heart diseases, medical interventions, often used cardiac devices or systemic procedures (dialysis, catheterization) and generated by species of staphylococci, or other aggressive microorganisms (3).

Over time, some etiologies of infective endocarditis show an increased prevalence, as it is reported for *Enterococcus spp*, a Gram-positive bacterium. The dominant species (up to 90%) is represented by *Enterococcus faecalis*. It was classical described as community acquired disease, low-symptomatic, with subacute evolution and of genito-urinary source, but nowadays it can also present as an acute, more aggressive healthcare associated infection, with antimicrobial resistance and challenges in diagnostic and treatment (8-10).

Our study aimed to assess the epidemiological profile of blood culture-positive IE in a regional hospital, to determine the distribution of microorganisms in the population and to identify differences related to etiology, data that could help in adapting therapeutic strategy, for reducing complications rate and improving disease's prognosis.

MATERIAL AND METHODS

We performed a descriptive analysis of the patients hospitalized with IE for 11-year period, from January 2007 to December 2017 in the Cardiology Department of "Sf. Spiridon" County Clinical Emergency Hospital Iasi, a regional center in North-East Romania. The study protocol was approved by the ethics committee and the hospital board. The investigation was in accordance with the Declaration of Helsinki.

From the 126 patients, 58 met the inclusion criteria for positive blood culture IE and were included in current analysis. Demographic, clinical, biological, imagistic characteristics of the patients, as well as the etiology of the infective endocarditis were recorded from electronic database or medical charts. A comparative analysis of the main 3 etiologies was performed.

Statistical analysis

Continuous variables are summarized by mean ± standard deviation (SD). Categoric variables are represented as percentages. Statistical significance was defined by a $p < 0.05$. Statistical analysis was performed using *SPSS version 24.0 software* (SPPS Inc., Chicago, IL, USA).

RESULTS

58 patients with blood culture-positive IE were included in the current analysis. Epidemiological characteristics, etiology, complications and evolution are presented in firs table.

TABLE I.

Characteristics of the study group

Parameters	**Patients, n=58**
Age, mean ± SD	58.29 ± 14.8
Gender ratio, n (%)	
Male	45 (77.5%)
Female	13 (22.4%)
Place of origin, n (%)	
Urban area	27 (46,5%)
Rural area	31 (53.4%)
Etiology of blood culture-positive IE, n (%)	
Enterococcus spp.	13 (22.4%)
Staphylococcus aureus	11 (18.9%)
Viridans streptococci	10 (17.2%)
Streptococcus gallolyticus	6 (10.3%)
Coagulase-negative staphylococci	5 (8.6%)
Neisseria sicca	2 (3.4%)
Klebsiella pneumoniae	2 (3.4%)
Pseudomonas spp.	2 (3.4%)
Group B streptococci	2 (3.4%)
Fungi	2 (3.4%)
Pasteurella pneumophilla	1 (1.7%)
Enterobacter spp.	1 (1.7%)
Streptococcus equi	1 (1.7%)
Cardiac involvement, n (%)	
Native valve	50 (86%)
Prosthetic valve	7 (12%)
Cardiac device lead	1 (1.7%)
Paravalvular complications, n (%)	9 (15.5%)
Embolic complications, n (%)	11 (18.9%)
Arrhythmias or conduction disorders, n (%)	
Atrial fibrillation	14 (24.1%)
AV blocks	4 (6.8%)
Associated diabetes mellitus, n (%)	10 (17.2%)
Cardiac predisposing condition, n (%)	27 (46.5%)
Days of hospitalization, mean ± SD	27,96 ± 15.98
In-hospital mortality, n (%)	14 (24.1%)

Abbreviations: AV= atrioventricular; IE = infective endocarditis; n = number; SD = standard deviation

No significative differences were observed between a certain microorganism and gender (p=0.116), history of predisposing cardiac condition (p=0.553), cardiac involvement (p=0.560), or paravalvular complications (p=0.947). Also, we did not

notice a significative distinction between etiologies and worsening disease (heart failure/ acute kidney failure/ hepatic dysfunction) (p=0.259), or in-hospital mortality (p=0.343).

The persistence of positive blood cultures after 10 days of antimicrobial treatment was associated with a higher in-hospital mortality (p=0.051). Other parameters for which we investigated a correlation with in-hospital mortality were cardiac involvement (p=0.484), paravalvular complications (p=0.132), patient gender (p=0.383).

Results of comparative analysis between the main 3 etiologies of IE are presented in second table II.

TABLE II.

Comparation between the main etiologies of IE

Parameter	*Enterococcus spp.* IE (n=13)	*S. aureus* IE (n=11)	Viridans streptococci IE (n=10)	p Value
Age, mean ± SD	63.38 ± 11.4	55.54 ± 18.32	63.8 ± 10.98	0.304
Male gender, n (%)	10 (76.9%)	7 (63.6%)	10 (100%)	0.116
Diabetes mellitus, n (%)	2 (15.3%)	1 (9%)	1 (10%)	0.874
Cardiac predisposing condition, n (%)	8 (61.5%)	5 (45.4%)	5 (50%)	0.553
Paravalvular complications, n(%)	2 (15.3%)	3 (27.2%)	1 (10%)	0.563
Embolic complications, n (%)	2 (18.1%)	4 (30.7%)	-	0.089
Arrhythmias or conduction disorders, n (%) atrial fibrillation AV blocks	 5 (38.4%) -	 2 (18.1%) 1 (9%)	 1 (10%) -	 0.246 0.341
Cardiac involvement, n (%) Native valve Prosthetic valve	 10 (76.9%) 3 (23%)	 9 (81.8%) 2 (18.1%)	 10 (100%) -	 0.279 0.178
Days of hospitalization, mean ± SD	31.92 ± 15.72	29.27 ± 17.71	33.3 ± 12	0.830
In-hospital mortality, n (%)	2 (15.3%)	2 (18.1%)	-	0.211
Biological markers on admission, mean ± SD WBC count CRP Hb level Platelets RDW ESR Fibrinogen Serum creatinine Total bilirubin	 12202.3 ± 5547.2 9.47 ± 5.96 10.71 ± 2.02 240076.9 ± 100614.3 16.32 ± 3.18 43.77 ± 32.49 352.4 ± 100.66 1.28 ± 2.25 0.69 ± 0.17	 10240 ± 5292.9 12.97 ± 16.13 11.17 ± 1.56 167909.1 ± 97214.66 15.95 ± 3.22 50.5 ± 41.98 472.37 ± 52.35 1.03 ± 0.38 1.57 ± 0.93	 10515 ± 2016.1 9.49 ± 5.43 9.7 ± 1.96 311000 ± 177888.2 15.93 ± 1.76 90.5 ± 20.04 569.9 ± 120.88 1.06 ± 0.3 0.59 ± 0.12	 0.544 0.828 0.204 **0.049** 0.932 **0.016** **0.0001** 0.476 **0.008**

Abbreviations: AV= atrioventricular; Bilirubin (mg/dL); CRP = C-reactive protein (mg/dL); ESR = Erythrocyte Sedimentation Rate (mm/h); Fibrinogen (mg/dL); Hb = Hemoglobin (g/L); IE = infective endocarditis; n = number; Platelet (10^6/mm^3); RDW = Red Cell Distribution Width (%); SD = standard deviation; Serum creatinine (mg/dL); WBC = White Blood Cells (10^3/mm^3)

From all cases of *Enterococcus spp.* IE, 1 strain (7.6%) had intermediary sensibility to linezolid, the other cases didn't present resistance to vancomycin, linezolid, or imipenem. 2 cases (18.1%) of *S. aureus* IE presented intermediary sensibility to linezolid according with CLSI (Clinical Laboratory Standard Institute).

DISCUSSION

Infective endocarditis etiology is highly variable depending on the geographic area, development of the region, access to medical facilities and the emergence of different microorganisms. Blood cultures remain the key diagnostic tool in etiologic identification of IE, but a significant percent of cases have negative blood cultures (up to 30-50%) (1,11). Using advanced techniques of serological or molecular diagnosis can represent tools for microorganism identification, however their applicability is relatively low in the context of local lack of availability.

Mean age of diagnosis in this study was similar to other larger data presented - the 5th and 6th decades of life (8, 11-14), but different from a study conducted in Japan, which reported older mean ages for IE (69.1 years) and the difference can be explained by different life expectancy (7). Male gender was more affected by IE, in a comparable ratio of genders (3:1) as in the literature data (8, 11-14).

33.7% of the cases in EURO-ENDO had perivalvular extension of the disease (8). The large percent compared to current study (15.5%) can be determined by the involvement of more virulent bacteria, as *S. aureus*, which are known to have a more aggressive course, with potential of generating abscesses or prothesis dehiscence (15-16).

The rate of symptomatic embolic events (18.9%) was relatively similar to EURO-ENDO (25.3%), fact that underlines the high potential for vegetation embolization, with increased morbidity. Also, IE can induce conduction disorders or arrhythmic events, and this is evidence of local extension of the disease (8,17).

In our study, the etiologic distribution of microorganism is peculiar, with the predominance of enterococci (22,4%), *S. aureus* (18,9%), and viridans streptococci (17,2%). The results are different from large cohort trials, as the ESC-EORP EURO-ENDO registry, in which staphylococci were the main microorganism identified (44.1%), followed by oral streptococci (12.3%), enterococci (15.8%), and *S. gallolyticus* (6.6%) (8,9).

The relatively low prevalence of *S. aureus*, comparative to other studies, can be a consequence of the majority of community-acquired forms of IE in the current analysis. Also, we noticed a low number of prosthetic/device related cases (13.7%), in contrast with EURO-ENDO study (40%). The later named registry included a large number of healthcare associated IE (32.9%), opposite to current study, in which all the data collected was community-acquired IE. The underlined differences create the peculiar etiologic setting, being known that healthcare associated IE is more often determined by *Staphylococcus spp.*, or gram-negative bacteria, with higher virulence and multiple drug resistance (8).

In our study *K. pneumoniae* or *Pseudomonas spp.* were isolated in small percent (3.4%), but their potential for worse evolution because of their virulence in immunocompromised host in parallel with their level of resistance in our region, especially for healthcare associated infections it must force us to keep them under observation (18-19).

Also, an intriguing aspect is represented

by the emergence of *Enterococcus spp.* as a dominant etiology for IE. The EURO-ENDO registry also presented a high percent of cases as caused by *Enterococcus spp.* (16%) (8).

A study published by Ostgaard *et al* reported the highest prevalence for *Enterococcus spp.* IE (16.7%) in patients with blood stream infections, thus recommending screening for IE by echocardiography in all patients with *Enterococcus spp.* bacteriemia. The causative mechanism of disease and the increased prevalence of IE in the case of this specific microorganism is still debatable. What is certain, it represents a main determinant of IE with a reported frequency that increases with aging and highly associated with colorectal disease (20-22).

Our comparative analysis between the main three determinants of IE did not reveal major differences regarding baseline characteristics, evolution, or in-hospital death, but slightly distinctions in biologic profile were noted. *S. aureus* virulence and possibility to induce thrombocytopenia is known (23-26). Also, we observed a statistically significative increase of total bilirubin levels for *S. aureus,* compared to enterococci or viridans streptococci. This information may help in generating a profile of IE, and possibility to adapt empiric antimicrobial treatment, until the blood culture results (27).

Reported in-hospital death of 24.1% remains high compared to other registries (1,28), as EURO-ENDO, which presented an in-hospital mortality of 17.1% (8). This may be explained by differences in time setting of the current study compared to newer analysis, which have improved diagnosis and treatment options. Also, we note the decreased access to cardiac surgery, in contrast to west-European countries or other regional centers (8). In recent years, the number of these interventions has increased in our region, but let's not forget that the risk of healthcare associated infections is also increasing, especially in parallel with antibiotic resistance (29).

Our study has several limitations. It is a single center analysis, with a relatively decreased number of patients, but adjusted to the low incidence of IE. The wide distribution over time can generate variable statistical results and differences of disease's prevalence in the context of diagnostic techniques evolution. Nevertheless, the present study confers a local view of the etiology of IE and regional characterization, making possible a better understanding of the disease course.

CONCLUSIONS

Infective endocarditis is a challenging disease, in which community-acquired form still prevails, but has a peculiar distribution for etiology and high prevalence of *Enterococcus spp.* infection. In-hospital mortality of the disease remains high, independent of the etiologic profile.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

RESIDUAL PERIODONTAL POCKET TREATMENT WITH ER: YAG LASER-ASSISTED COMPREHENSIVE PERIODONTAL POCKET THERAPY

A retrospective study realized by a Japanese group of researchers evaluated the safety and effectiveness of a novel pocket therapy using Er: YAG laser in combination with conventional mechanical scaling and root planning treatment. In the study were included forty sites in 29 elderly patients that have residual periodontal pockets of ≥ 5 mm depth, treated by curette and Er: YAG laser. After root debridement by curette, laser irradiation was performed on the root surfaces. The inflamed connective tissue on the inner gingival surface and on the bone surface/within extant bone defects was thoroughly debrided by curette and laser. In most cases, removal of the outer epithelium and coagulation of the blood clot in the pocket entrance were additionally performed with laser. The clinical parameters were evaluated before and 3, 6, and 12 months after treatment. The results showed that with Er: YAG laser-assisted pocket therapy, debridement of pockets was thoroughly, and safely performed, and favorable clinical improvements were observed in most cases. After 1-year, probing pocket depth significantly decreased from 6.4 ± 1.4 mm to 3.5 ± 1.3 mm ($p < 0.001$, 3.0 mm reduction), and clinical attachment level significantly decreased from 7.5 ± 1.6 mm to 5.2 ± 1.9 mm ($p < 0.001$, 2.3 mm gain). In conclusion, the obtained data indicate that Er: YAG laser-assisted therapy is useful for the treatment of residual pockets as a minimally invasive flapless surgery. (Aoki A, Mizutani K, Mikami R, *et al.* Residual periodontal pocket treatment with Er: YAG laser-assisted comprehensive periodontal pocket therapy: a retrospective study. *Clin Oral Investig* 2022; 26 (1): 761-771).

ETIOLOGIC SPECTRUM OF POST-OPERATIVE MENINGITIS IN NEUROSURGERY

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ETIOLOGIC SPECTRUM OF POST-OPERATIVE MENINGITIS IN NEUROSURGERY (Abstract): Postoperative meningitis is a rare neurosurgical complication with an incidence of 0.3%-1.5%. The most common pathogen in postoperative meningitis in many studies was *Staphylococci*, especially *Staphylococcus aureus*, however recent data reports increasing rates of gram-negative organisms. **Material and methods**: We performed a retrospective study including 32 patients, diagnosed with post-craniotomy meningitis, who underwent neurosurgery, hospitalized between 2016 and 2019 in "Sf. Parascheva" Clinical Hospital of Infectious Diseases from Iasi, in order to evaluate the presence of pathogens in cerebrospinal fluid samples. **Results:** The most common pathogens were *Staphyloccoccus aureus, Streptococcus pneumoniae*, followed by *Acinetobacter speciae, Pseudomonas aeruginosa, Enteroccocus faecalis* and *Escherichia coli*. Antibiotherapy was properly administrated according to the antibiogram. **Conclusions:** Early diagnosis, appropriate antibiotic treatment and an interdisciplinary approach can lead to a favorable outcome. **Keywords:** POST-OPERATIVE MENINGITIS, ETIOLOGIC AGENT. NEUROSURGICAL COMPLICATION.

Postoperative meningitis is a rare neurosurgical complication, related to intraoperative incidental durotomy, with a high mortality and morbidity rate, associated with severe neurological symptoms, prolonged hospitalization and expensive treatment (1). The incidence of meningitis after neurosurgery ranges between 0.3% and 1.5% but increases up to 22% if the patient requires shunts or devices to drain cerebrospinal fluid (CSF) or to monitor intracranial pressure (external ventricular drains or external spinal drains) (2). There is fewer data regarding the clinical course of postoperative meningitis, however the presence of consciousness disturbance, neck stiffness and fever after neurosurgery can raise suspicions of postoperative meningitis. Any delay in establishing the correct diagnosis and starting proper treatment can lead to high morbidity and mortality (3). The most common pathogen in postoperative meningitis in many studies was *Staphylococci*, especially *Staphylococcus*

aureus, however recent data reports increasing rates of gram-negative organisms (*Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae*), *Streptococcus pneumoniae, Enterococcus faecalis* and also fungal agents, such as *Candida speciae* (most frequent *Candida albicans*). Bacteria and fungi can be cultured from CSF, but in 54-70% of cases there is no bacterial/fungal growth on CSF cultures (called 'aseptic meningitis') (4). Empiric therapy for post-neurosurgical meningitis should be elected taking into consideration the most likely pathogens involved and should be based on the local resistance pattern (5).

MATERIAL AND METHODS

We performed a retrospective study, including 32 patients, diagnosed with post-craniotomy meningitis, from a total of 627 patients, who underwent neurosurgery, hospitalized between 2016 and 2019 in the "Sf. Parascheva" Clinical Hospital of Infectious Diseases, Iasi, in order to evaluate the presence of pathogens in CSF samples.

RESULTS

Our study reported 5,1% incidence of post-craniotomy meningitis. The study group consisted of 20 males (62.5%) and 12 females (37.5%), mostly aged under 60 (75%), living in rural areas (68.75%) (fig. 1). Almost 20% of patients declared a history of meningitis in their medical records.

Over 50% of patients presented underlying medical conditions, such as: cardiovascular diseases (most frequent: arterial hypertension - 50%, myocardial infarction - 31.125%), neoplasia (12.5%), diabetes mellitus (9.375%), pulmonary pathology (9.375%), liver dysfunction (9.375%), renal impairment (6.25%), and obesity (3.125%) (fig. 2).

In 50% of cases, CSF cultures identified a pathogen responsible of post-craniotomy meningitis. The most common pathogen was *Staphyloccoccus aureus* - 5 cases (25%), followed by *Streptococcus pneumoniae* - 4 cases (20%), *Acinetobacter speciae* - 4 cases (20%), *Pseudomonas aeruginosa* - 3 cases (15%) and *Escherichia coli* - 2 cases (10%), *Enterococcus faecalis* - 2 cases (10%) (fig. 3).

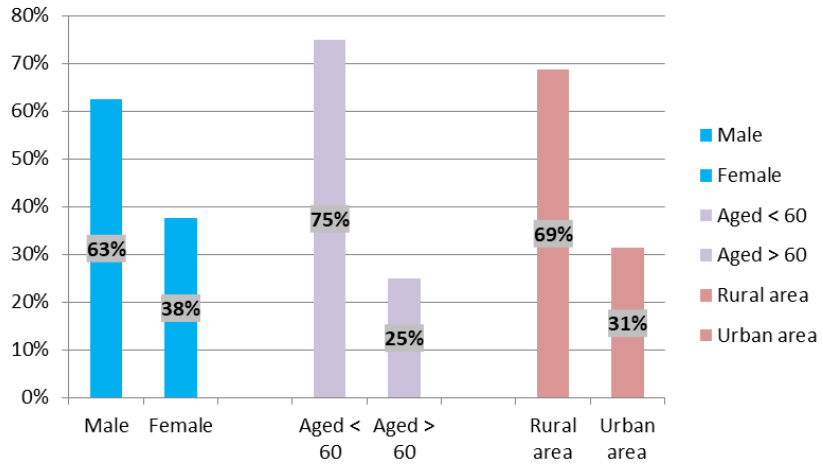

Fig. 1. Characteristics of the patients included in the study

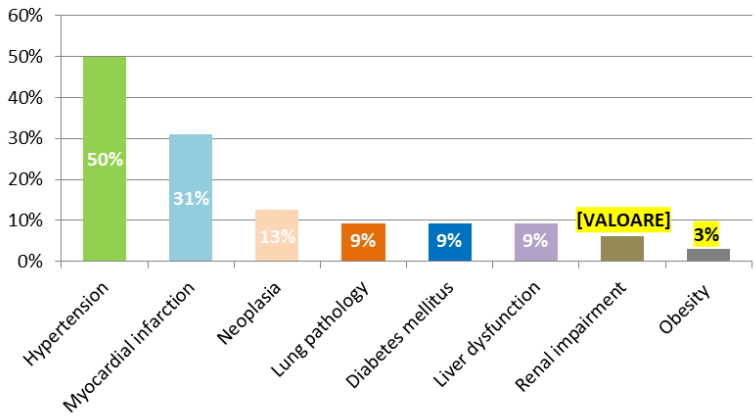

Fig. 2. Associated medical conditions in patients with post-craniotomy meningitis

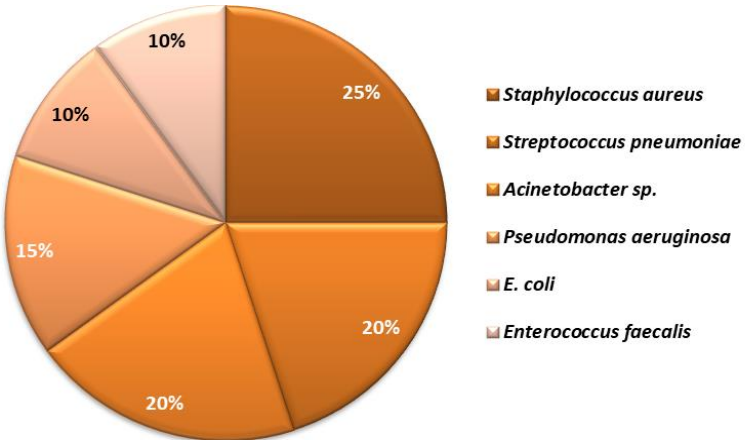

Fig. 3. Most common pathogens identified in CSF cultures

Antimicrobial susceptibility of isolates was determined using the disk diffusion method and every antibiotherapy was administered according to antibiogram. Most commonly, *Staphylococcus aureus* strains were methicillin resistant, with antimicrobial susceptible to glycopeptides (Vancomycin). All *Streptococcus pneumoniae* strains were sensitive to vancomycin, linezolid, levofloxacin, rifampicin and chloramphenicol (not used due to hematologic side effects) and resistant to penicillin. The high rate of *Acinetobacter baumannii* carbapenem-resistant has led to polymyxin B administration (intrathecal use of colistin). The treatment of *Pseudomonas aeruginosa* initially included cephalosporins (Ceftazidime), but afterwards it was switched to carbapenems (Meropenem). A switch from cephalosporins (Cefotaxime, Ceftriaxone) to carbapenems (Meropenem) was also performed in *Escherichia coli* strains. *Enterococcus faecalis* isolates were mostly treated with beta-lactam antibiotics and carbapenems, due to high incidence of vancomycin-resistance (tab. I).

TABLE I.
Etiologic agents in post-operative meningitis

Identified etiologic agent	Antimicrobial susceptibility	Number of cases	Percentage
Staphylococcus aureus	Glycopeptides (Vancomycin)	5	25%
Streptococcus pneumoniae	Glycopeptides (Vancomycin), Fluoroquinolones, Linezolid, Rifampicin	4	20%
Acinetobacter baumannii	Carbapenems (Meropenem) or Polymyxin (Colistin)	4	20%
Pseudomonas aeruginosa	Cephalosporins (Ceftazidime), Carbapenems (Meropenem)	3	15%
Escherichia coli	Cephalosporins (Cefotaxime, Ceftriaxone), Carbapenems (Meropenem)	2	10%
Enterococcus faecalis	Beta-lactam antibiotics, Carbapenems (Meropenem)	2	10%

The median period of treatment was 18 days, with favorable course in 23 cases (71.87%) and fatal evolution in 4 cases (12.5%).

DISCUSSION

Our study aimed to evaluate the presence of pathogens in CSF samples submitted to conventional culture, from patients who underwent craniotomy and had presented acute clinical manifestations compatible with meningitis, confirmed by neurologists, neurosurgeons or infectious diseases specialists. Diagnosis of meningitis was defined as a positive CSF culture, associated with meningitis signs and/or symptoms (fever, headache, neck stiffness, photophobia, neurologic symptoms) (6, 7).

It is unlikely that our results were obtained through contamination of the samples, since other postoperative patients without symptoms of meningitis were tested under the same conditions and none produced a positive CSF culture. Therefore, the etiologic agent was not identified in all 32 cases, due to the fact that patients were administered pre-operative antibiotherapy.

Blood and CSF samples were periodically tested in order to evaluate improvement or normalization of the biological parameters.

Also, daily clinical evaluations were performed on every patient by an interdisciplinary team (neurologist/neurosurgeon, infectious diseases and intensive care specialists).

CONCLUSIONS

This study confirmed that postoperative meningitis is a rare post-neurosurgical complication. A lumbar puncture should be performed for every patient with suspected meningitis, after undergoing neurosurgery, if there are no contraindications.

Early diagnosis, appropriate antibiotic/antifungal treatment and an interdisciplinary approach can lead to a favorable outcome.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

NEW INTRANASAL AND INJECTABLE GENE THERAPY FOR HEALTHY LIFE EXTENSION

As the global elderly population grows, it is socioeconomically and medically critical to provide diverse and effective means of mitigating the impact of aging on human health. Previous studies showed that the adeno-associated virus (AAV) vector induced overexpression of certain proteins, which can suppress or reverse the effects of aging in animal models. In our study, we sought to determine whether the high-capacity cytomegalovirus vector (CMV) can be an effective and safe gene delivery method for two such protective factors: telomerase reverse transcriptase (TERT) and follistatin (FST). We found that the mouse cytomegalovirus (MCMV) carrying exogenous TERT or FST (MCMVTERT or MCMVFST) extended median lifespan by 41.4% and 32.5%, respectively. We report CMV being used successfully as both an intranasal and injectable gene therapy system to extend longevity. Specifically, this treatment significantly improved glucose tolerance, physical performance, as well as preventing body mass loss and alopecia. Further, telomere shortening associated with aging was ameliorated by TERT and mitochondrial structure deterioration was halted in both treatments. Intranasal and injectable preparations performed equally well in safely and efficiently delivering gene therapy to multiple organs, with long-lasting benefits and without carcinogenicity or unwanted side effects. Translating this research to humans could have significant benefits associated with quality of life and an increased health span (Jaijyan DK , Selariu A , Cruz-Cosme R , *et al.* New intranasal and injectable gene therapy for healthy life extension. *Proc Natl Acad Sci USA* 2022: 119(20): e2121499119).

CONSIDERATIONS ON CASES OF DIFFUSE AXONAL INJURY AUTOPSIED IN THE INSTITUTE OF FORENSIC MEDICINE IASI, ROMANIA

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CONSIDERATIONS ON CASES OF DIFFUSE AXONAL INJURY AUTOPSIED IN THE INSTITUTE OF LEGAL MEDICINE IAȘI ROMANIA (Abstract) : Traumatic brain injury (TBI), mainly caused by road traffic accidents and falls, is one of the main causes of mortality. Survivors often present with debilitating motor, sensory and cognitive symptoms, leading to a reduced quality of life and a profound economic burden on society. **Material and methods**: The study was conducted by analyzing forensic cases autopsied at the Institute of Forensic Medicine Iasi, between 2015-2020, diagnosed antemortem with traumatic brain injury and *diffuse axonal injury* (DAI). The analysis includes a retrospective study of the forensic necropsy cases conducted between 2015-2020. Data were loaded and processed using statistical functions in *SPSS 18.0* at the 95% significance level. **Results:** The analysis of forensic cases autopsied at the Institute of Forensic Medicine Iasi between 2015-2020 showed a number of 7818 autopsied cases, of which 3545 were violent deaths. Of these, 567 had as cause of death craniocerebral trauma; of these only 70 cases were accompanied by the imaging diagnosis of DAI, which corresponds to 12% of all cases of craniocerebral trauma. **Conclusions**: The imaging diagnosis of DAI was refuted by pathological examination in 24 of the cases, out of a total of 70 patients with a radiological diagnosis of DAI, corresponding to 34.28% of cases. The survival rate of rural patients was significantly higher (14.27 *vs.* 7.33 days; p=0.03). Patients with DAI have a high mortality, supported by a 50% decrease after the first week in the chance of survival. **Keywords**: DIFFUSE AXONAL INJURY, TRAUMATIC BRAIN INJURY, AUTHOPSY, RESTROSPECTIVE STUDY.

Diffuse axonal injury (DAI) was first reported in the literature by Sabina J. Strich in 1956 (1). In her paper, Strich described five victims with severe dementia following traumatic brain injury who, at autopsy 5-15 months after injury, showed diffuse

degeneration of cerebral white matter. Adams and Genarelli's work explains the biomechanics of these injuries by incriminating acceleration-deceleration forces and angular acceleration (2-5). Primate studies have explained comatose states in patients with traumatic brain injury (TBI) without focal brain lesions by the detection of DAI. Specifically, axonal lesions have frequently been found at sites of predilection primarily involving the subcortical white matter of the frontal and temporal lobes, the corpus and splenium of the corpus callosum and, in the most severe cases, the rostral superior region of the brainstem (6,7). Currently, DAI is considered a common finding in patients suffering from TBI, existing in approximately half of closed TBI cases in combination with focal lesions. Isolated DAI are less common (8). However, DAI have a particularly negative impact on outcomes, as patients are frequently left with severe motor, cognitive and behavioral impairments (9). Our study aims to highlight the main necropathic features of TBI, by analyzing forensic cases autopsied at the Institute of Forensic Medicine Iasi Romania in the period 2015-2020, diagnosed antemortem, by CT examination, with DAI.

MATERIAL AND METHODS

The study was conducted by analyzing forensic cases autopsied at the Institute of Forensic Medicine Iasi. diagnosed antemortem with traumatic brain injury and DAI. The analysis includes a retrospective study of the forensic necropsy cases conducted between 2015-2020.

A study group was selected that included all cases of death due to traumatic brain injury with associated DAI. Only cases in which TBI was stated on the death certificate as the cause of death regardless of the presence of lesional associations were included in the study.

Data were loaded and processed using statistical functions in *SPSS 18.0* at the 95% significance level. Tests such as ANOVA, Skewness or Kurtosis tests ($-2 < p < 2$), Friedman test, Kruskal-Wallis test, t-Student test, F-test and multivariate analysis were used for the data presented in this study; the threshold of statistical significance was set a priori at 0.05. Data are expressed numerically or as a percentage of the whole.

Inclusion criteria: cranio-cerebral trauma with associated DAI, autopsied at the Institute of Legal Medicine Iasi Romania, between 2015-2020;

Exclusion criteria: cardiovascular disease and history of stroke, cases suffering from loss of vital functions, post-traumatic survival time of less than 20 minutes, chronic alcoholism, absence of hospitalizations after trauma, absence of associated DAI on CT examination, GSC >8, autolysis and putrefaction; this study was conducted in accordance with the standards developed by the Declaration of Helsinki, the approval of the management of the Institute of Forensic Medicine Iași, Romania (No.12/2020). Informed Consent for the autopsy is not required, the autopsy is mandatory under Romanian Law (Romanian Criminal Procedure Code Law No. 135/2010, chapter VII, article 185).

RESULTS

The analysis of forensic cases autopsied at the Institute of Forensic Medicine Iasi between 2015-2020 showed a number of 7,818 autopsied cases, of which 3,545 were violent deaths. Of these, 567 had as cause of death craniocerebral trauma; of these

only 70 cases were accompanied by the imaging diagnosis of DAI, which corresponds to 12% of all cases of cranio-cerebral trauma (figs. 1, 2).

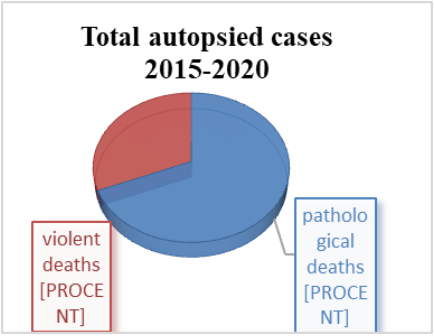

Fig. 1. Violent deaths out of all autopsied cases

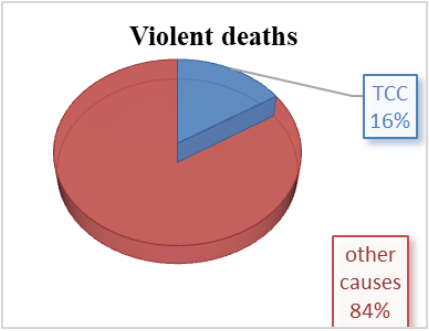

Fig. 2. Causes of death by TBI out of all violent deaths

Demographic characteristics

Gender distribution

The distribution of cases by sex showed a higher proportion of male cases (74.3%), with a M/F ratio of 2.9 : 1 (fig. 3).

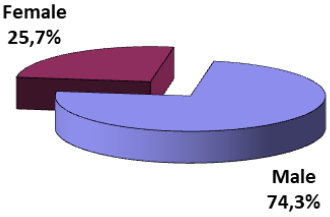

Fig. 3. Batch structure by gender

Distribution by age group

The age of patients with traumatic brain injury accompanied by DAI shows the following characteristics (tab. I, fig. 4).

The histogram of ages shows the following data: variations in the range 22-86 years; group mean 51.34 ± 15.38 years; median = 50 years; Skewness test result p = 0.224; the median value close to the mean age of the group and the Skewness test result < 2 suggest that the age range was homogeneous, so significance tests for continuous variables can be applied.

TABLE I.

Statistical indicators of age (years)

N		70
Medium		51.34
Median		50.00
Standard Deviation		15.38
Variance		29.95
Skewness Test		0.224
Std. Error of Skewness		0.287
Minim		22
Maxim		86
Percentile	25	38
	50	50
	75	62

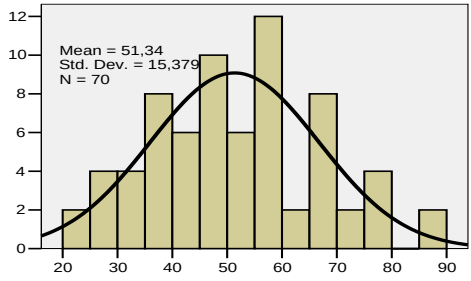

Fig. 4. Histogram of ages

The highest frequency of patients are aged over 50 years (51.4%), with no significant gender differences (53.8% of men *vs.* 66.7% of women; p=0.130) (fig. 5).

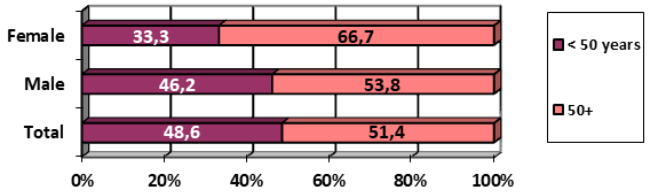

Fig. 5. Batch structure by age group and gender

Distribution by background

In the present study group, the proportion of rural patients was higher (65.7% *vs.* 34.3%), with no significant gender differences (65.4% of men *vs.* 66.7% of women; p=0.921) (fig. 6).

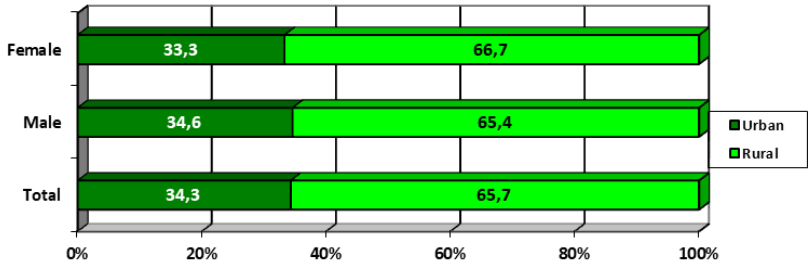

Fig. 6. Batch structure by background and gender

Clinical parameters

Mechanism of trauma production

37.1% of the patients had falls from the same level as the reason for the injury and 31.4% had road traffic accidents (fig. 7).

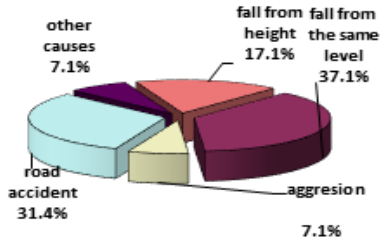

Fig. 7. Distribution of patients by mechanism of production of trauma

Depending on the presence of DAI confirmed by histopathological examination, the study group was divided into two subgroups: DAI group = 46 patients with histopathologicaly confirmed diffuse axonal lesions and non-DAI group = 24 patients with histopathologicaly confirmed diffuse axonal lesions.

The frequency of patients with confirmed diffuse axonal lesions was higher in males (73.9% *vs.* 26.1%; p=0.921), in those under 60 years of age (52.2% *vs.* 47.8%; p=0.408) and in those from rural areas (65.2% *vs.* 34.8%; p=0.903) (tab. II, fig. 8). Of the patients with DAI, 41.3% had suffered a fall from the same level and 32.6% had acquired the lesion following a road traffic accident, whereas in patients without histopathologicaly confirmed traumatic axonal lesions, 33.3% had fallen from a height (p=0.037) (tab. III, fig. 9). This contradicts data in the literature identifying road traffic accidents as the main traumatic context for the development of DAI.

TABLE II.

Group structure according to the presence of DAI

Features	Batch TAI (n=46)		Non-TAI batch (n=24)		p
	n	%	n	%	
Male	34	73.9	18	75.0	0.921
Female	12	26.1	6	25.0	
Age < 60 years	24	52.2	10	41.7	0.403
Age ≥ 60 years	22	47.8	14	58.3	
Urban	16	34.8	8	33.3	0.903
Rural	30	65.2	16	66.7	

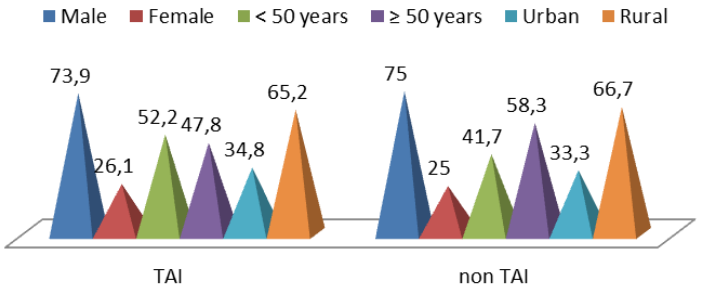

Fig. 8. Epidemiological characteristics according to the presence of DAI

TABLE III.

Correlation of DAI with the mechanism of production

Production mechanism	Batch TAI (n=46)		Non-TAI batch (n=24)	
	n	%	n	%
Fall from height	4	8.7	8	33.3
Fall from the same level	19	41.3	7	29.2
Aggresion	3	6.5	2	8.3
Road accident	15	32.6	7	29.2
Another cause	5	10.9	0	0

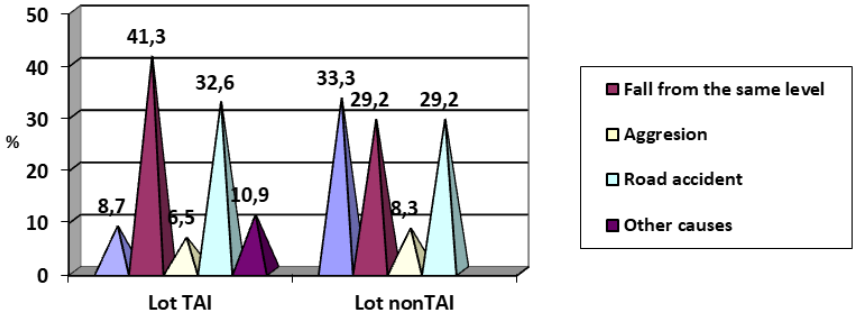

Fig. 9. Production mechanism depending on the presence of DAI

The CT result showed in those with histopathologicaly confirmed DAI, most commonly 28.6% fractures and 25.7% contusions or hematomas (fig. 10).

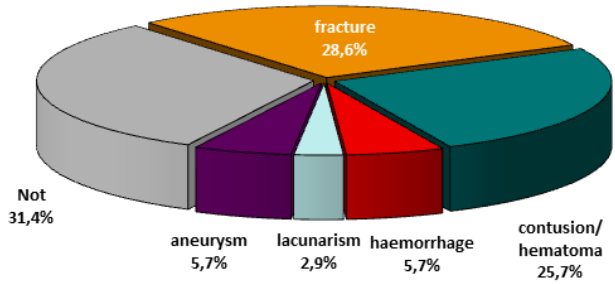

Fig. 10. Batch structure according to CT result

Survival days

The descriptive data on the number of survival days highlights the following aspects (tab. IV, fig.11):

TABLE IV.

Estimated risk of DAI based on CT result

Features	Batch TAI (n=46)	Non-TAI batch (n=24)	p	Estimated risk	
				RR	CI 95%
Fracture	12 (26.1%)	8 (33.3%)	0.527	0.88	0.59-1.32
Intracerebral contusion/ hematoma	18 (39.1%)	0 (0%)	**0.001**	1.86	1.44-2.39
Other	4 (8.6%)	2 (8.3%)	0.959	1.02	0.56-1.84

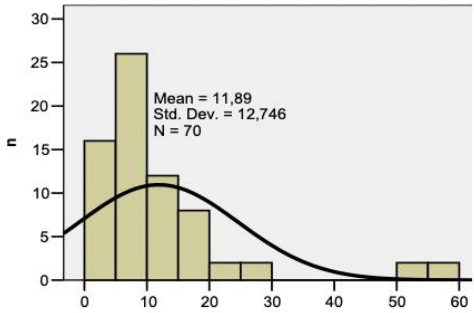

Fig. 11. Histogram of survival days

The histogram of survival day shows the following data: wide variations (72.42%) from a few hours to 60 days; group average 11.89 days ± 12.75 ; median = 8 days; Skewness test result p = 1.602; the median value at relatively close to the mean number of survival days of the group and the result of the Skewness test < 2

suggests that the series of survival days values was homogeneous, so significance tests for continuous variables can be applied; the mean number of days in hospital was slightly lower in males (11.04 *vs.* 14.33 days; p=0.349) and in those aged over 50 years (11.62 *vs.* 12.18 days; p=0.856) (figs. 12, 13 and tab. V).

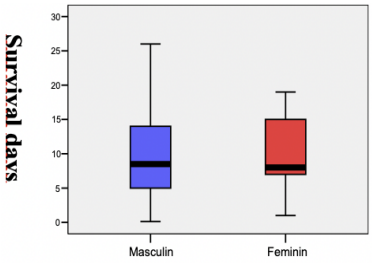

Fig. 12. Average number of survival days comparison by gender

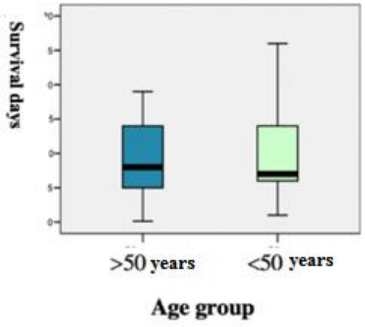

Fig. 13. Average number of survival days comparison by age group

TABLE V.

Statistical indicators for the number of survival days

N		70
Medium		11.89
Median		8
Standard Deviation		12.75
Variance		72.42
Skewness Test		1.602
Std. Error of Skewness		0.287
Minim		0
Maxim		60
Percentile	25	5
	50	8
	75	14

Of note, the mean number of survival days was significantly higher in patients from rural areas (14.27 *vs.* 7.33 days; p=0.03) (tab. VI).

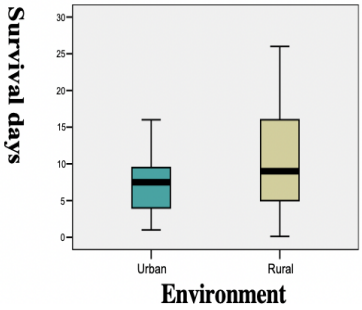

Fig. 14. Mean number of survival days compared by environment

TABLE VI.

Average number of survival days according to epidemiological characteristics

Features	Days survival ±SD	t-Student test (p)
Male	11.04 ± 10.76	0.349
Female	14.33 ± 17.42	
Age < 50 years	12.18 ± 11.73	0.856
Age ≥ 50 years	11.62 ± 11.73	
Rural	14.27 ± 14.88	**0.030**
Urban	7.33 ± 4.59	

In patients with histopathologicaly confirmed DAI, the probability of survival falls below 50% after 7 days (fig. 15), which correlates DAI with high mortality.

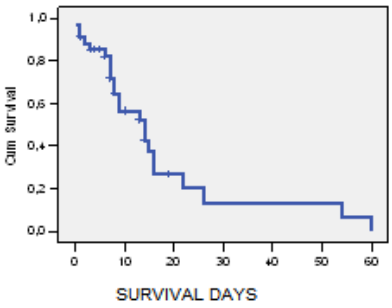

Fig. 15. Probability of survival in patients with DAI

DISCUSSION

The analysis of forensic cases autopsied at the Institute of Forensic Medicine Iasi between 2015-2020 showed a number of 7818 autopsied cases, of which 3545 were violent deaths. Of these, 567 had as cause of death craniocerebral trauma; of these only 70 were accompanied by the imaging diagnosis of DAI, which corresponds to 12% of all cases of craniocerebral trauma. An increased prevalence of traumatic brain injury was observed in males (74.3%) and in the over 50 age group (51.4%). The data obtained is consistent with the statistics in the literature in terms of gender, but inconsistent in terms of the age category most commonly affected, which states that young adults are most commonly involved. The frequency of patients with histological confirmed diffuse axonal lesions was higher in males (73.9% *vs.* 26.1%; p=0.921), those under 60 years of age (52.2% *vs.* 47.8%; p=0.408) and those from rural areas (65.2% *vs.* 34.8%; p=0.903). Skull fractures were observed in 59.4% of the cases of traumatic brain injury with associated imaging-diagnosed DAI, of which the highest incidence was identified in patients without histopathologicaly confirmed DAI (33%). In the histopathologicaly confirmed DAI group, the incidence of skull fractures was 26.1%, which confirms literature data that DAI is more common in patients with closed head injury. The most common DAI-associated injuries were concussions and intracerebral haematomas.

The mechanism of occurrence most frequently associated with DAI was falling from the same level (41.3%), which contradicts the literature, which argues that road traffic accidents are the most common mechanism involved in causing DAI. On the other hand, those involved in road traffic accidents may have had a survival time of less than 20 minutes due to associated serious injuries, with survival time of less than 20 minutes being one of the exclusion criteria in this case. In patients with histopathologicaly confirmed DAI, the probability of survival falls below 50% after 7 days. The mean number of days of survival was significantly higher in patients from rural areas (14.27 *vs.* 7.33 days; p=0.03).

CONCLUSIONS

DAI is a leading cause of mortality and permanent morbidity worldwide. The imaging diagnosis of DAI was refuted by pathological examination in 24 of the cases, out of a total of 70 patients with a radiological diagnosis of DAI, corresponding to 34.28% of cases. The certainty diagnosis for the presence of DAI remains the anatomopathological one.

The survival rate of rural patients was significantly higher (14.27 *vs.* 7.33 days;

p=0.03). Patients with DAI have a high mortality, supported by a 50% decrease after the first week in the chance of survival. The most frequently detected imaging brain lesions associated with DAI were contusions.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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AN UNANTICIPATED OBSERVATION REGARDING FGF23 DURING ONE YEAR OF GH REPLACEMENT THERAPY

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AN UNANTICIPATED OBSERVATION REGARDING FGF23 DURING ONE YEAR OF GH REPLACEMENT THERAPY (Abstract): To promote linear growth, it is essential to have normal phosphate homeostasis. Growth hormone/insulin-like growth factor (GH/IGF1) axis is a major regulator of phosphate metabolism and children with growth hormone deficiency (GHD) have increased renal phosphorus reabsorption during rhGH therapy. The novel phosphaturic fibroblast growth factor 23 (FGF23) has opposing effects, but the underlying mechanisms and interplay between both systems remain unclear. **Aim:** The main objective of this study is to investigate the possible interactions of FGF23 with the GH/IGF1 axis in the regulation of growth, calcium-phosphorus metabolism, and bone mineral density (BMD) in GHD children during the first year of treatment. **Material and methods:** Prospective, observational study of 42 non-obese prepubertal GHD children of both sexes, investigating changes in plasma FGF23, markers of mineral metabolism, and IGF-1 in the first year of rhGH therapy. **Results:** All children grew at an accelerated pace. Total BMD and total bone mineral content significantly increased after 12 months. The increase in serum phosphate was accompanied by a significant increase in plasma cFGF23 and IGF1. We found a positive association between SDS Height with IGF1 and FGF23 levels. No impact of FGF23 concentration variations on BMD was determined. **Conclusions:** FGF23 rises during rhGH therapy, an unanticipated observation given the role of FGF23 as a phosphaturic factor. GHD children have increased phosphate, associated with upregulation rather than with suppression of the phosphaturic FGF23. The association of FGF23 with height and IGF1 could indicate complex interactions between both axes in promoting linear growth. **Keywords**: GH DEFICIENCY, FGF23, BMD, IGF1.

Linear growth is influenced by numerous nutritional, metabolic, and hormonal factors, of which growth hormone (GH), calcium-phosphorus homeostasis, and bone-kidney endocrine axis mediated by the novel phosphaturic hormone fibroblast growth factor 23 (FGF23), play a critical role in appropriate bone and growth plate mineralization (1, 2, 3).

It is well acknowledged that children with GH deficiency (GHD) have smaller stature and reduced longitudinal bone growth (3, 4), with a lower bone mineral density (BMD) and delay in skeletal maturation, thus emphasizing the key role of GH/IGF1 axis in adult BMD outcomes and

the need for continuing replacement treatment during the transition from pediatric to adult age (5). The rhGH treatment determines the normalization of BMD, the most important predictor of osteoporotic fractures, with modifications in markers of bone formation and bone resorption right from the beginning of therapy (4, 5, 6).

The attainment of optimal BMD and bone growth is also influenced by the calcium-phosphorus metabolism and the more recently uncovered bone-kidney endocrine axis. In addition to the well-known paradigm of the vitamin D-parathyroid hormone endocrine system, other factors control the maintenance of phosphate homeostasis: dietary intake, intestinal absorption, chronological age (7). In addition to these dynamics, FGF23, predominantly produced by osteocytes, is a key element in regulating phosphorus metabolism with skeletal mineralization. It has been demonstrated that GHD children have increased renal phosphorus reabsorption during replacement therapy, which are accompanied by non-suppressed FGF23 levels (2, 8).

At the same time, the somatotropic axis influences calcium-phosphorus metabolism through a mechanism opposite to FGF23, causing renal phosphate reabsorption (9). Thus, GHD patients have a relative phosphorus deficiency status, and GH therapy increases renal phosphate reabsorption, but the underlying pathways remain uncertain (10). Thereby, we might be tempted to believe that a possible mechanism of GH/IGF1-induced hyperphosphatemia is through inhibition of FGF23, but current studies do not support this theory, as FGF23 levels are elevated in patients with active acromegaly (11) and GH treatment increased the levels of FGF23 in children with GHD (2, 8). Thus, the increase in serum phosphate by GH therapy seems to not be mediated through the suppression of the phosphaturic FGF23 (2). Studies reported a strong positive association of FGF23/Klotho axis with IGF-I and height that could indicate a role in linear growth through phosphate regulation (1).

There is scarce data available regarding the consequences of childhood-onset GHD on BMD. However, it has been reported that GH therapy should minimize, if not prevent the higher fracture risk associated with the lack of GH (3, 5). Moreover, although our understanding of phosphate homeostasis has been dramatically increased from previous studies, the interplay between FGF23, GH/IGF1 axis, and BMD is not fully elucidated and data in GHD children are limited. Some studies suggest that there may be a causal relationship between FGF23 levels and BMD and that a high concentration of FGF23 is associated with a low BMD in adults (12, 13, 14).

The main objective of our study is to investigate the possible interactions of FGF23 with the GH/IGF1 axis in the regulation of calcium-phosphate metabolism and BMD in GHD children during one year of GH replacement therapy.

MATERIAL AND METHODS

This was a prospective, observational study with consecutive sampling of non-obese prepubertal GHD children of both sexes. The study was carried out at the Endocrinology Clinic, "Sf. Spiridon" Emergency Hospital in Iași, Romania, following approval by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy from Iași and the written informed consent of the patients and their parents prior to participation.

A total of 42 children (14 girls, 28

boys) diagnosed with idiopathic GHD were included and started on a twelve-month course of GH therapy. The starting mean age of the children was 9.2 ± 2.6, with no significant differences between sexes. All subjects were prepubertal (Tanner Stage I) and maintained this status during the observation period. The diagnosis of GHD was established in accordance with the national GH treatment protocol and criteria (15), namely a maximum GH peak of 7 ng/mL in two GH provocation tests. Other possible etiologies for short stature were excluded (15). Magnetic resonance imaging was performed, and it showed normal hypophysis in all subjects. The exclusion criteria were pubertal onset, overweight or obese status, and concomitant existence of other metabolic disorders or diseases.

Longitudinal study. GHD children started GH replacement therapy at 0.030 mg/kg per day and upheld it for twelve months. Changes in body composition, calcium-phosphorus metabolism, and levels of serum C-terminal FGF23 were monitored thrice, first before initiating GH therapy, then at six and twelve months.

Blood sampling and biochemical analysis. At the first visit, a detailed clinical history was obtained and age, sex, weight (kg), height (cm), BMI (weight/height^2), and bone age were recorded. Morning blood samples were collected after 12 hours of fasting and then were immediately frozen at -45°C until analysis. Serum fasting IGF-I, IGF1-BP3, C-terminal FGF23, phosphorus, total calcium, magnesium, creatinine, alkaline phosphatase (ALP), 25-OH-vitamin D, parathormone and anthropometric parameters were evaluated in all patients at baseline, after 6 and 12 months of rhGH replacement therapy.

Serum C-terminal FGF23 was measured by enzyme-linked immunosorbent assay (ELISA, SEA746Hu, USCN Life Sciences, Wuhan, China; intra-assay variability of 6.96%), which measures both the active and the inactive FGF23 obtained after proteolytic cleavage of FGF23 in EDTA plasma centrifuged straight away after collection. Body composition was evaluated by total body dual-energy x-ray absorptiometry using a Hologic QDR-4500 densitometer.

Statistical analysis. Using *SPSS 24.0*, we performed a comparative study of the monitored parameters. The results are expressed as mean ± SEM (standard error of the mean) unless otherwise stated. After testing the normal distribution of data, we compared the three sets of values with the Student's t-test for paired samples and the Wilcoxon test when normal distribution was not observed. The correlations between changes in variables were evaluated using regression analysis. If statistically significant associations were identified, the regression line was constructed to estimate the intensity of these correlations. We used the standard multiple regression method to investigate the connection between several variables. To establish if the built model fits the data, we analyzed the coefficient of determination, and we considered $p<0.05$ as the threshold for statistical significance in the ANOVA test.

RESULTS

The baseline, 6- and 12-month analytic data are presented in figures 1 and 2.

All children grew at an accelerated pace, with a mean growth of ~8 cm/12 months (ΔSD height=0.57±0.24, ΔSD height velocity=1.39 ±1.53). Weight and BMI also increased steadily and significantly during the monitoring year by ~4 kg

and 0.6 units respectively (shown in Fig. 1, upper panel, ΔSD_BMI=1.88±0.43). The total body fat (BF) content and %BF registered a significant decrease throughout the study period. Instead, the lean mass increased steadily, with statistically significant differences among all three evaluations (fig. 1, lower panel). Total BMD and BMC significantly increased after 12 months, while total calcium did not show significant variations (fig. 2). The increase in serum phosphate was accompanied by a significant increase in plasma cFGF23 and IGF1 (fig. 1 middle panel and fig. 2 lower panel).

The dynamic relationship between FGF23, body composition and GH therapy

The **correlations** between body composition, calcium-phosphorus metabolism, and FGF23 levels at the three-time points are shown in tables I and II.

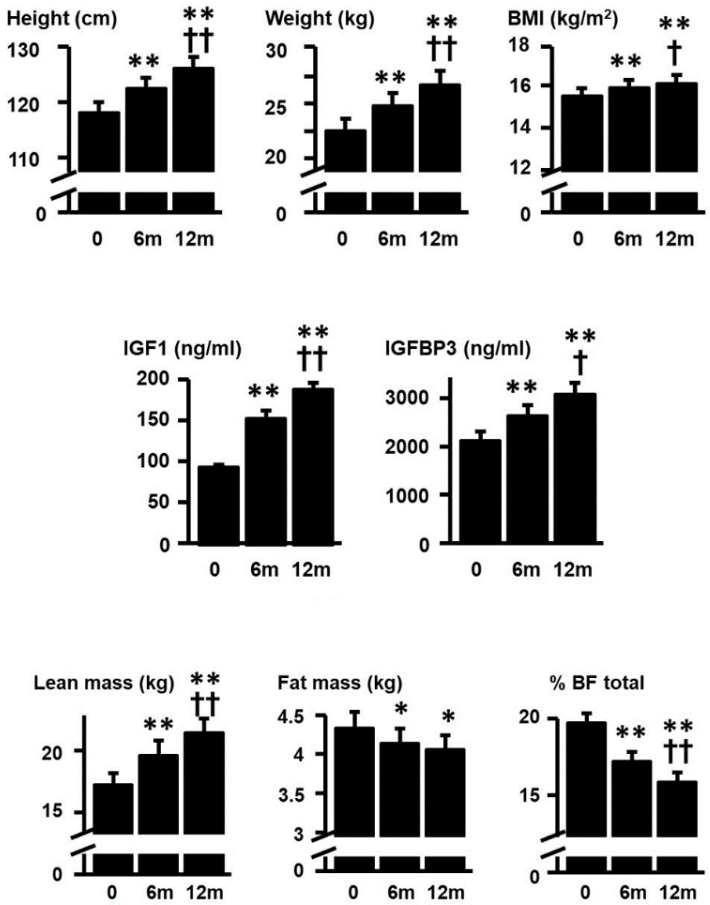
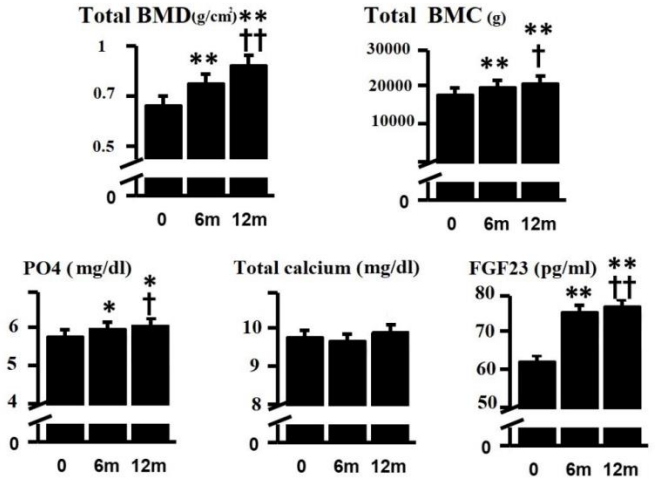


Fig. 1. The evolution of anthropometric, body composition, and somatotropic axis parameters. Asterisks - compared to mean initial value.
Daggers - compared to mean value at 6 months.



One symbol - p< 0.05. Two symbols - p < 0.01

Fig. 2. The impact of rhGH replacement therapy on calcium-phosphorous metabolism, FGF23, and BMD. Asterisks - compared to mean initial value. Daggers - compared to mean value at 6 months. One symbol - p< 0.05. Two symbols - p < 0.01.

TABLE I.

Correlations between changes in FGF23, IGF1/IFGBP3, and body composition

		Δ FGF23 (pg/mL)			Δ IGF1 (ng/ml)			ΔIGFBP3		
		Baseline	6 months	12 months	Baseline	6 months	12 months	Baseline	6 months	12 months
Height (cm)	r	-.077	.214	.328	***.677***	***.613***	***.681***	***.615***	***.583***	***.546***
	p	.629	.173	***.034***	***.000***	***.000***	***.000***	***.000***	***.000***	***.000***
SDS Height	r	-.404	-.106	.019	.409	***.563***	***.513***	***.505***	***.683***	***.646***
	p	***.008***	.503	.905	***.005***	***.000***	***.000***	***.000***	***.002***	***.000***
BMI (kg/m²)	r	-.057	.156	.216	.429	***.573***	***.516***	.453	.471	***.553***
	p	.722	.323	.169	***.005***	***.000***	***.000***	***.003***	***.002***	***.000***
ΔTotal fat (g)	r	-.208	.092	-.059	***.720***	.462	***.584***	***.609***	***.514***	.487
	p	.186	.564	.711	***.000***	***.002***	***.000***	***.000***	***.000***	***.001***
ΔTotal lean (g)	r	-.037	.209	.361	***.555***	***.668***	***.637***	***.550***	***.555***	***.588***
	p	.817	.185	***.019***	***.000***	***.000***	***.000***	***.000***	***.000***	***.000***
BMC total (g)	r	.016	.325	.193	***.546***	.434	***.520***	***.622***	***.504***	.440
	p	.922	***.036***	.221	***.000***	***.004***	***.000***	***.000***	***.001***	***.004***
Z- Score lumbar	r	-.242	-.201	-.390	-.317	-.436	-.462	-.280	-.139	-.106
	p	.123	.201	***.011***	***.041***	***.004***	***.002***	.073	.380	.503
BMD total (g/cm²)	r	.147	.450	.266	.259	***.522***	.398	.208	.250	.204
	p	.353	***.003***	.089	.098	***.000***	***.009***	.186	.110	.195
BMD lumbar (g/cm²)	r	.066	.271	.364	***.533***	***.562***	***.661***	.432	.435	***.518***
	p	.678	.082	***.018***	***.000***	***.000***	***.000***	***.004***	***.004***	***.000***

Results are expressed as coefficient r and p, with ***bold***-significant correlations;
BMI, body mass index; BMC, bone mineral content; BMD, bone mineral density.

TABLE II.

Correlations between changes in FGF23, IGF1, and calcium-phosphorus metabolism

		Δ FGF23 (pg/mL)			Δ IGF1 (ng/mL)		
		Baseline	6 months	12 months	Baseline	6 months	12 months
ΔALP (U/L)	r	-.153	.346	.322	.233	.356	.393
	p	.335	***.025***	***.038***	.335	***.024***	***0.01***
ΔPTH (pg/ml)	r	-.213	.080	.204	.238	.246	.369
	p	.175	.613	.195	.178	.523	.287
ΔPO4 (mg/dl)	r	-.151	.189	***.550***	.235	.247	***.385***
	p	.340	.229	***.000***	.239	.013	***0.05***
ΔTotal calcium (mg/dl)	r	-.035	.005	.436	.123	.389	.364
	p	.828	.974	***.002***	.687	.467	***.000***
25-(0H)- Vit D (ng/ml)	r	.197	.289	.221	-.044	***-.380***	.166
	p	.874	.542	.747	.781	***.013***	.293
IGF1 (ng/ml)	r	-.246	***.442***	.287	-	-	-
	p	.116	***.003***	.066	-	-	-
IGF1 SDS	r	-.295	.317	-.042	-	-	-
	p	.058	***.040***	.790	-	-	-

Results are expressed as coefficient r and p, with bold-significant correlations;
PTH, Parathyroid hormone; PO4, serum phosphorus; ALP, alkaline phosphatase.

GH therapy resulted in a significant improvement in the lumbar Z-score, with a significant increase in total, lumbar and neck BMD, and BMC. FGF23 values are also relatively sporadically correlated with body composition parameters. IGFBP3 is statistically significantly correlated, at all 3-time points of the assessment, with most parameters of body composition (tab. I). Serum phosphate was positively related to FGF23 (r=0.550; P<0.0001) and IGF1 (r=0.385; P<0.05). FGF23 values are proportional and positively correlated with Height (tab. I). At the 6-month evaluation, we detected moderate positive correlations of FGF23 with alkaline phosphatase, IGF1, and DS IGF1, while at the 12-month evaluation, FGF23 moderately correlated with alkaline phosphatase (tabs. I and II).

Further on, we performed dynamic correlations between variations (Δ=T12 - T0) of variables to assess the effect of GH replacement therapy compared to the effect of body composition changes upon the modifications in FGF23, after 12 months of therapy. Significant correlations were afterward introduced in regression models adjusted for age, sex, serum phosphate levels, 25 (OH) vitamin D, IGF-I, and PTH. We have included all the above parameters in the model, and we found a significant impact of IGF-I (β = .994, p = .000) and IGF-BP3 (β = -.537, p=.013) on serum FGF23. Among the tested parameters, the most important contribution to FGF23 variations at 12 months was SDS IGF1, followed by IGF1, total FAT, and IGFBP3; in addition, total BMD and BMI also have an important contribution (tab. III).

At the same time, we corroborated the level of FGF23 with the evolution of BMD and BMC. We built a multiple regression model to predict the values of total BMD and BMC at 12 months according to the values of

the following independent variables: vitamin D, PTH, FGF23, total calcium, ionic calcium, and phosphorus. The FGF23 variable, however, is not statistically significant and does not determine a significant variation of the dependent variable (tab. III).

TABLE III.

Dynamic regression models with ΔFGF23, ΔBMC as the dependent variables - adjusted for sex, BMI, IGF1, body composition

ΔFGF23 (r^2= .428)	**Beta**	**P-value**
ΔSD_IGF1	.994	***.000***
ΔSD IGFBP3	-.537	***.013***
Sex	-.286	.227
ΔBMI	.476	.074
ΔTotal fat	-.477	***.015***
ΔBMD total	-.041	.900
Δ BMC (r^2=.323)	**Beta**	**P-value**
Δ25 OH vitamin D	***.302***	***.049***
ΔPTH	.230	.182
ΔFGF23	-.127	.571
ΔTotal calcium	-.219	.225
ΔPO4	-.216	.274

Beta = standardized coefficient; with bold - significant correlations;
IGF1, Insulin-Like Growth Factor 1; SD, standard deviation;
PTH, Parathyroid hormone; PO4, serum phosphorus; BMI, body mass index;
BMC, bone mineral content; BMD, bone mineral density;

DISCUSSION

Our research is among the forefront to investigate the relationship between FGF23 and bone parameters, respectively, in GHD children. To our knowledge, the impact of FGF23 on bone mineral density has not yet been evaluated in GHD children.

In our study, we investigated the effect of GH substitution treatment on FGF23 serum levels in prepubertal GH-deficient children. We found that the increase in serum phosphate by GH therapy is accompanied by an increase in the phosphaturic FGF23 levels. Numerous studies in children and adults with GHD have presented similar results on phosphate metabolism during GH substitution therapy (9, 11, 16).

Knowledge of the disorders of phosphate homeostasis and studies of FGF23 have uncovered a bone-kidney axis and new systems of biology that govern bone mineralization, extremely important for normal linear growth.

Apart from the crucial role of promoting linear growth, GH has various metabolic effects, influencing body composition, muscle mass, adipose, and osseous tissue. Achieving and maintaining a normal peak bone mass during childhood and adolescence is important for preventing osteoporosis later in life. GH/IGF1 axis interacts with the bone-forming unit, and it has a positive effect on bone formation (3, 4, 5, 6).

FGF23, predominantly produced by osteocytes, is the principal phosphaturic factor in the bone-kidney axis, coordinating systemic phosphate homeostasis with skeletal mineralization. FGF23 is catabolized into

two active fragments, the N-terminal peptide that binds to the FGF receptor (FGFR) in the tissues and the C-terminal fragment that binds to Klotho (17). In renal proximal tubular cells, FGF23 binds to the FGFR, requiring a co-receptor, Klotho, a transmembrane protein. FGF23's primary function is to maintain normal serum phosphate concentration, promoting renal phosphate wasting and inhibition of the 1α-hydroxylase activity, downregulating the production of calcitriol (18). The net effect of both hormonal actions is to lower serum phosphate concentration. Furthermore, an intact FGF23 axis is important for normal linear growth. This becomes obvious in human genetic disorders with an excess of FGF23 (hypophosphatemic rickets) where linear growth is impaired (19).

There are only a few studies in the literature (2, 8, 11, 20) that have identified a connection between the GH/IGF1 system and the FGF23/Klotho system. Although their actions are contradictory, with opposite effects on phosphorus metabolism, both systems are crucial for linear growth and bone mineralization (21, 22). The actual interplay between them is not yet fully elucidated. The FGF23 system negatively regulates phosphate, having an overall harmful impact on bone metabolism. The FGF23/ Klotho complex blocks the Wnt bone formation signaling pathway, while FGF23 has an additive effect to that of PTH upon bone resorption (23). In this context, one may assume that GH/IGF1 axis increases serum phosphate by the suppression of the FGF23 system. However, our data, along with the results of other studies (2, 8, 11) show that the increase in serum phosphate by GH is not mediated through the suppression of the phosphaturic FGF23 system.

Our finding of a positive correlation between serum phosphate and FGF23 in children with GHD is also found in healthy children (1). This could indicate a counterregulatory mechanism between serum phosphate and FGF23, so that, when the level of phosphorus in the blood begins to increase (secondary to diet, reduced phosphate excretion by the kidneys, or in our case GH substitution treatment), FGF23 also begins to increase to maintain the PO4 levels within normal values (1). GH treatment might be the trigger factor for the elevation of FGF23 as a feedback mechanism, to maintain serum phosphate homeostasis (2). Also, the interaction between GH and FGF23 might be mediated via the activation of the renal 1-alpha-hydroxylase - upregulated by GH and downregulated by FGF23 (7, 23). Indeed, data in the literature indicate a relationship between GH/IGF-I axis with the FGF23/Klotho pathway in neonates (20), healthy patients (1), GHD children (2, 8), or patients with acromegaly (11).

In patients with acromegaly FGF23 levels are elevated and return to normal levels postoperatively (11), whereas rhGH treatment in GHD children causes a significant increase of cFGF23 (2), like our study. Therefore, the study of acromegaly patients demonstrated that the detected hyperphosphatemia is not caused by deficient FGF23 action (11). The mechanisms and pathways of interaction between these systems have not been fully elucidated so far. One could hypothesize that the effect of GH treatment on phosphate metabolism is mediated by another mechanism, such as a direct antiphosphaturic effect of the GH/IGF1 axis which is well-supported in the study of Efthymiadou A *et al*, by demonstrating a positive correlation of serum IGF1 with the ratio of the maximum rate of renal tubular reabsorption of phosphate to glomerular filtration rate (TmPO4/GFR) (2).

Our results are consistent with the ones

reported in two other previous studies. One of the reports measured only plasma cFGF23 levels (8), as we did in our series of patients. Despite the higher doses of GH used in Gardner *et al* study, the magnitude of cFGF23 rise was similar, indicating that the GH effect on cFGF23 is not dose dependent. The level of cFGF23 tended to increase, even after adjusting for age, sex, total calcium, and phosphorus (8). This is an unanticipated observation, given the role of FGF23 as phosphaturic factor. Thus, the increase in FGF23 may be the counterregulatory effect of GH therapy. Efthymiadou A *et al*, had a more intergraded approach, measuring cFGF23, iFGF23, and Klotho, but found similar results for FGF23 variations during GH treatment (2). From all three studies, we can state that the effect of GH therapy on FGF23 levels is present from the 3rd month of GH therapy (2), persisted during the 8th month (8), and lasts at least until the 12th month -as seen in our study- but is unknown how early it appears and whether is sustained as long as GH treatment lasts.

Data in the literature support this relationship of IGF1 with the FGF23/Klotho axis in healthy and in short-stature children with or without GH deficiency (24). We suggest that GH replacement therapy interacts with the FGF23/Klotho axis as a feedback mechanism for the maintenance of phosphate homeostasis.

In agreement with other authors (2), we found a positive association between SDS Height with IGF1, but more interestingly, SDS Height was also strongly associated with FGF23 levels. This aspect was also found in normal healthy children (1, 25), emphasizing the fact that phosphate homeostasis is essential for linear growth and appropriate mineralization of the growth plate; therefore, an intact FGF23 axis is important. Thus, the positive relationship we found between FGF23, height SDS, and IGF1 most likely reflects the significance of FGF23 actions on linear growth, with the rise in FGF23 rather than mirroring the GH and IGF1 effects.

The observed loss in BMD found in patients with isolated GHD as well as in those with multiple pituitary deficiencies, suggests that GHD might be the essential factor predicting low BMD (21). Previous observational studies have indicated that elevated levels of FGF23, by inhibition of calcitriol synthesis, could lead to mineralization damage and a lack of ossification in cartilage (26). In mice models, FGF23 leads to growth retardation, and overexpression of FGF23 can also inhibit osteoblast differentiation and cause matrix mineralization in cells (27). The association between FGF23 and body composition parameters, with BMD and BMC, may suggest a direct effect of the FGF23 on obtaining and maintaining an adequate BMD. Data in the literature are scarce and FGF23 has mainly been determined in conditions of bone metabolic disorders (hypophosphatemic rickets), tumor-induced osteomalacia, and in children with chronic renal failure and kidney disease, with contradictory results. Moreover, the fact that different assays are used to determine FGF23 levels (cFGF23 and / or iFGF23) could contribute to conflicting results.

In some studies, the serum level of FGF-23 was not correlated with a change in BMD in maintenance hemodialysis patients (28) or in bone diseases (30), while El-Hodhod *et al* (22) found that FGF23 contributes to low BMD in childhood inflammatory bowel disease. Wang *et al* (13) study suggests that there may be a causal relationship between FGF23 levels and BMD of the heel and femoral neck in adults. A large retrospective

study has suggested that a high concentration of FGF23 is associated with a low BMD of the hip in the elderly population (30). FGF23 was negatively and independently associated with BMD and/or BMC and contributed to up to 10% of femoral neck BMD variance in postmenopausal women in the study of Bilha S *et al.* (12). Mirza *et al.* concluded that when the serum FGF23 concentration was higher than 55.7 pg/mL, the risk of hip and non-vertebral fractures was significantly increased in elderly men (14). In our study, we found a positive correlation between FGF23 and total and lumbar BMD, but no true impact of FGF23 concentration variations on ΔBMD in multiple regression analysis. It is probably the effect of IGF1 upon linear growth that outweighs the effect of other potential regulators.

Our study is limited by the small number of patients included, the lack of urinary phosphate excretion assessment, and the period of assessment of only 12 months. However, to our knowledge, our study is the first to investigate the impact of FGF23 on BMD/BMC in GHD children during replacement rhGH therapy. Further studies are necessary to reveal the complex interplay of these factors.

CONCLUSIONS

Although our understanding of phosphate homeostasis has been dramatically increased from previous studies, the role of FGF23 during GH replacement therapy is not fully elucidated and data are limited. An intact FGF23 and GH/IGF1 axis are essential for linear growth, to achieve appropriate mineralization of the growth plate and maintained normal phosphate homeostasis. GHD children present with an increase in serum phosphate during GH therapy - not a result of the suppression, but rather with upregulation of the phosphaturic FGF23. The association of FGF23 with height SDS and IGF1 could indicate complex underlying interactions between GH/IGF1 and FGF23/Klotho axis to promote growth. This study did not find a causal relationship between FGF23 levels and BMD/BMC in GHD children; however, more studies are necessary to determine whether FGF23 rather mirrors the effects of GH/IGF1 upon calcium, phosphate, and bone homeostasis, or is truly a player in the homeostasis of growth.

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CONFLICT OF INTEREST AND FUNDING

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NEWS

LEFT ATRIAL APPENDAGE CLOSURE VERSUS NON-WARFARIN ORAL ANTICOAGULATION: WHO WILL WIN?

Atrial fibrillation (AF) is a common arrhythmia that is associated with substantial morbidity and mortality, particularly due to stroke and thromboembolism. Left atrial appendage closure (LAAC) is a non-pharmacological option for preventing cardioembolic events in patients with AF. Since treatment using non-warfarin oral anticoagulants is associated with less bleeding than warfarin, the potential benefit of LAAC relative to NOAC is unclear, prompting the PRAGUE-17 trial. Between 2015 and 2019, 201 patients were randomized per group, and followed for a median of 3.5 year in the LAAC group, and 3.5 years in the NOAC group. LAAC was non-inferior to NOAC for the primary endpoint outcome. There were also no significant differences between groups for all-stroke/TIA, clinically relevant bleeding or cardiovascular mortality. However, for non-procedural and device-related events, LAAC was associated with a significantly reduced rate of clinically-relevant bleeding. Among non-valvular patients with AF and at high risk for stroke and bleeding, the non-inferiority of LAAC to NOAC relative to the composite of cardioembolic events, CV death, significant procedure/device-related complications, or clinically-relevant bleeding, was maintained during long-term follow-up. (Osmancik P, Herman D, Neuzil P, *et al.* Left Atrial Appendage Closure versus Non-Warfarin Oral Anticoagulation in Atrial Fibrillation: 4-Year Outcomes of PRAGUE-17. *J Am Coll Cardiol* 2021; 78(19): e157 / doi: 10.1016/ j.jacc. 2021.10.023).

ASSESSING THE RESILIENCE OF HEALTHCARE PROFESSIONALS IN A ROMANIAN EMERGENCY UNIT DURING THE COVID-19 PANDEMIC

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ASSESSING THE RESILIENCE OF HEALTHCARE PROFESSIONALS IN A ROMANIAN EMERGENCY UNIT DURING THE COVID-19 PANDEMIC (Abstract): Worldwide, during COVID-19 pandemic, the healthcare professionals have been exposed to professional and existential stress. The **aims** of this study were to evaluate of the emotional impact, the degree of resilience of medical staff, immediately after exposure to the risk of COVID-19. **Material and methods**: e enrolled 64 participants (30 doctors and 34 nurses), from the Emergency Department of the "Sf. Spiridon" County Clinical Emergency Hospital from Iasi. Each participant completed a self-report 63 items questionnaire. Self-perceived stress, depression symptoms, anxiety symptoms were measured in our research. *SPSS 27.0* software was used for data analysis. **Results:** We have identified statistically significant difference between medical doctors and nursed regarding the possibility of managing the critical situation at the workplace and the difficulty understanding the situation at workplace. Sleep quality, positive emotions and life satisfaction need to be improved to increase the psychological resilience of healthcare professionals working during the COVID-19 pandemic. **Conclusions:** Doctors constitute the group with the lowest levels of psychological resilience among health care workers. This study confirms the need to improve positive emotions and weaken negative emotions of healthcare professionals. **Keywords:** RESILIENCE, EMOTIONAL WELL-BEING, SARS-CoV-2, PANDEMIC.

COVID-9 pandemic has spread worldwide and is an important global public health issue as on 3rd of February 2023, there have been 754,018,841 confirmed cases of COVID-19 in the world, including 6,817,478 deaths, reported to WHO. In Romania there have been 3,325,006 confirmed cases of COVID-19 with 67,576 deaths, reported to WHO. As of 21st of January 2023, a total of 16,102,916 vaccine doses have been administered (1).

The coronavirus SARS-CoV-2, the etiologic agent of COVID-19, is studied by many research groups, worldwide. One of the most recent papers published in Nature journal is referring to guanosine-specific

pocket in the essential NiRAN domain that could be optimized as a therapeutic target to treat new coronaviral borne diseases (2). A multidisciplinary team confirmed the short-term effectiveness of actual vaccines (the mRNA or inactivated vaccine in preventing SARS-CoV-2 Omicron BA.2 infection), and at the same time the authors underlined the need of updated studies according to fast viral evolution, to develop new vaccination strategies (3).

Medical staff, both physicians and nurses from emergency medicine have been deeply involved in the COVID-9 pandemic, in diagnosis, therapy, follow-up, and vaccination campaigns. A recent paper identified some challenges and opportunities for medical staff, recommending that learning should be used by health care professionals to promote and restore health and to improve the lives and wellbeing of individuals, families, and communities, and at the same time, the authors recommended to successfully live their own lives, to balance work and other activities, and to preserve a sense of purpose, service, and mental vitality. Maintaining the values of health-care professions is a crucial goal for health educators and for optimal management of a pandemic (4).

Using the keywords *resilience among medical staff*, we identified 161 papers published in the last year, which underlines that this topic is of high importance in medical field, from which we have selected the most representative studies. In a very recent cross-sectional study (March 2023) which involved 602 medical staff from Beijing, it was identified that resilience and self-esteem was a mediator between depression and burnout (Hao *et al.*, 2023). A 2-year observational study during COVID-19 pandemic realized in Taiwan, identified poor sleep, lack of exercise, long working hours, and being nursing staff as risk factors regarding an increase in personal burnout, and depression among health care professionals Chu *et al*., 2023). A descriptive phenomenological study published in Lancet, performed on an important number of public health workers, university staff, and hospital administrators from Philippines, identified stress and mental health exhaustion, fear of infection during service provision after the initial wave of the COVID-19 pandemic (Maravilla *et al*., 2022). Russell *et al*. conducted a study in Australia which referred to key pandemic-generated modifications to safety, clinical, workflow and practice management routines, as procedures to reduce infectious disease transmission (e.g., *staff-to-staff contact reduced greatly*), procedures for conducting consultations via phone or video, rather than face to face (e.g., *most GPs provided telehealth from the practice, but some worked from home when isolating or unwell*). The authors considered that their study was a natural experiment of the resilience of primary care in Australia (Russell *et al*., 2023).

Starting from definition of Mallak *et al*., which stated in 1998 that resilience is *the human capacity to adapt in the face of difficulty and ongoing significant life stressors* (Mallak *et al*., 1998), authors from Germany aimed to explore to what extent can critical care staff use their spirituality by themselves to reduce moral distress and strengthen their resilience. After a comprehensive analysis of 13 studies (e.g., spirituality as moral distress, spirituality as strategy, spiritual resources support, and influence of spirituality on resilience), the authors concluded that spirituality is not sufficient to develop the confi-

dence to cope with ethically critical situations, and more important is that critical care staff to be supported by their institution to reflect on their work, and to find meaning in their care. Researchers from Netherland conducted a study in an elite university hospital, analyzing how the infection prevention staff managed the scarcities emerging at the onset of the COVID-19 pandemic. The authors identified that defining safety, anticipating scarcity, and tinkering with quality could be a modality to enable a resilient response to infection prevention. Also important for these authors is updating Infection prevention guidelines for managing the emerging crisis (Bal *et al*., 2022). Similar studies have been performed in other region of the world as in Tunisia (in which the authors identified that self-reported resilience, work engagement, and burnout were the predictors of compassion satisfaction, in cross-sectional study among heath care givers caring for confirmed and suspected COVID-19 patients, through a self-administrated questionnaire) or in Saudi Arabia (in which the authors aimed to evaluate the relationship between the resilience and emotional intelligence of nurses during the COVID-19 pandemic, and they identified nationality, ward assignment, years of experience as having significant relationships with emotional intelligence). These studies concluded that there is a need for more resilience promoting interventions to be implemented for achieving psychological well-being needs of the nurses. Also, there is a need for improving emotional-intelligence skills of assisting nurses during pandemic outbreaks, and therefore emotional intelligence should be integrated into decision-making (Omri *et al*., 2022, Aljarboa *et al*., 2022). Researchers from New York evaluated mental health among Emergency Department healthcare workers in one cross-sectional study, which involved 247 health care workers. The authors found significant difference between clinical *vs.* non-clinical health care workers for psychological distress symptom burden, depression, and perceived stress. The authors considered that is necessary to maintain mental well-being of nurses and medical doctors, this being crucial for optimally functioning healthcare systems (Gustafson *et al*., 2022).

As the well-being and emotional resilience of health care professionals are essential components of continued health care services during the COVID-19 pandemic, health professionals have been observed during pandemic to have serious psychological problems, having the risk of being exposed to problems in terms of mental health (15). The well-being and emotional resilience of healthcare workers were critical components of continuing healthcare services during the COVID-19 pandemic. Thus, it is essential to anticipate the stress related to this process and to provide adequate medical assistance. Important follow-up and assessment of health workers' well-being is in terms of ensuring their successful reintegration with their colleagues.

Aims of this study were to evaluate of the emotional impact, the degree of stress of the medical staff from an Emergency Department (ED), of both doctors and medical nurses, exposed to the risk of getting infected with COVID-19, through emergency diagnosis and treatment of suspected and confirmed positive patients during 2020.

MATERIAL AND METHODS

In the period 15.11-30.11.2020, we have applied in the ED of the "Sf. Spiri-

don" County Clinical Emergency Hospital Iasi, one questionnaire based on 2 scales present in the specialized literature, described in details bellow:

a. *Brief Resilience Scale (BRS)*. The scale was developed by B. Smith *et al*. (5) to measure individual psychological resilience. It consists of six items (three reversed questions) measured on a 5-point scale (1 Never appropriate and 5 Completely appropriate). The total range of scores was between 6 and 30. Higher scores on the scale indicate a higher level of psychological resilience. The Turkish version of the scale was adapted by Dogan (2015). The adapted scale was highly sufficient in terms of CFA values (v2/ df (12.86 / 7) ¼ 1.83, NFI ¼ 0.99, CFI ¼ 0 .99, GFI ¼ 0.99, SRMR ¼ 0.03, RMSEA ¼ 0.05) and internal consistency coefficient (¼ .88).

b. *Scale related to life satisfaction (SMV)*. The SMV consists of five items measured on a 7-point Likert scale (1: Strongly disagree, 7: Strongly agree). It was developed by Diener *et al*. (1985) and adapted by de Koker (6). Higher scores on the scale indicate higher levels of life satisfaction. We have used the Romanian translated versions of above descripted scales in our 63 items self-questionnaires.

The questionnaire included 63 items and we observed that in addition to the 2 scales consecrated in the literature, the applied questionnaire also contains other relevant items for the emotional impact among the personnel in the study. The number of distributed questionnaires was 70, of which 64 were validated to be interpreted, the latter were statistically processed in this study. The survey was based on the questionnaire that was physically distributed to the staff from the emergency reception service (doctors and nurses). Participants were informed about the aims of the study and the research procedures. No reward was offered for participation. No personally identifiable information was requested.

This study had received the ethical approval of the host-hospital and the study participants have been informed and they signed the ethical consent prepared by the PI of this study.

Statistical analysis. Data processing was performed using *SPSS 27.0* package software. The method used was the *Spearman rank correlation coefficient p* (rho), which is a coefficient based on ranks, it is not influenced by the representativeness of the average, and it is generally used when the research group is small, or when at least one of the variables does not meet the administration conditions of the parametric tests. This indicator is frequently used in social and human sciences.

RESULTS

The questionnaire was applied to 64 persons, 30 doctors (M:F ratio 0.50) and 34 nurses (M:F ratio 0.4782). The age distribution was on average 38.13 years for doctors (66.67% women) and 36.47 years for nurses (67.64% women), and the average age for the whole group is 37.25 years. Out of a total of 64 people, 32.81% were infected with COVID-19, 56.25% were not infected, and 10.93% did not know if they had the virus. These data were obtained from the first 6 items of the questionnaire.

The items 7-15 were referring to a list of statements regarding concerns regarding COVID-19. The *degree of concern about COVID-19*: we note that from the category of doctors 50% are very concerned and 33.33% are concerned, and from the nurses group the results were 44.11% / 26.47% for

very concerned / concerned. Only a percentage of 3.12% is very little concerned about COVID-19. Item 10 was referring at the *virus capacity of spreading*: 50% of persons believed that the COVID-19 virus spreads a lot and only 1.56% think that it spreads very little. Item 11 asked the opinion of medical staff regarding *the access to information about the COVID-19 virus*: 56.25% consider that they have access to a lot of information, 26.6% consider that they have high access and 17.2% consider that they have medium access to information. Item 12 - *worry about contacting the virus* - 45.3% were very worried, 26.6% were worried, 14.1% average and a percentage of 6.3% were very little worried. Here we observed differences between the two categories: doctors are much more worried, as no doctor showed very little concern, while 11.8% of nurses think they are not worried about the COVID-19 virus. Item 13 – *threat*: 29.7% believed that the threat of COVID-19 influences them to be around people and 15.6% do not feel threatened. We observe differences between the two categories: doctors feel less threatened 20.6%, among doctors they consider themselves very little threatened compared to nurses, only 10% consider themselves very little threatened. 56.25% of study participants considered themselves very much threatened regarding travel plans, while only 7.81% consider themselves very little threatened. There are small differences between the two categories, 60% of doctors consider themselves threatened and 52.6% of nurses. *The influence of the threat of safety measures* was considered by 60% of the medical subjects, which believed that the infection with COVID-19 greatly influenced them in the use of safety measures, while the nurses subjects in percentage of 56.3%.

From item 16 to item 24 we applied a set of questions regarding how often the subjects were disturbed in the last 2 weeks in carrying out activities: *low interest in certain things, feeling depressed, sleep problems, feeling tired, appetite, fear of failure, concentration problems, slowness in activities or restlessness, thoughts of death or injury*. 56.66% of the doctors stated that they had low interest in carrying out the activities for only a few days *versus* the nurses which answered in percentage of 26.47%. Depression was recognized by 53.33% of the doctors, which felt depressed for a few days, and 29.41% of the nurses. We observed differences between the two categories of subjects, regarding sleeping problems: 20.58% of nurses complained of sleep problems almost every day, while 6.67% of doctors. 20.31% of all the subjects complained of feeling fatigue almost every day. 63.33% of the responding doctors do not accuse slowness or restlessness at all, 23.33% accuse a few days a week, 10% more than half of the days of the week and 3.33% almost every day. Among nurses respondents, the percentages are 61.76% not at all, 23.52% a few days, 8.82% more than half of the days of the week and 5.88% almost every day. The total percentage is 62.50% for not at all, 23.43% a few days, 9.37% more than half of the days of the week and 4.68% almost every day. When asked about the presence of thoughts related to death or injury, 90.62% of the respondents declared that they did not have such thoughts at all, and only 2.94% and 5.88% of the nurses declared that they had such thoughts in more than half of the days or almost daily. The percentage among doctors was 0% for this item.

The average response to *the fear of in-*

fection was 6.07 among doctors and 5.32 among nurses. Regarding *the stressful work environment*, the answers were approximately identical in the two categories, with an average of 6.17 for doctors and 6.06 for nurses.

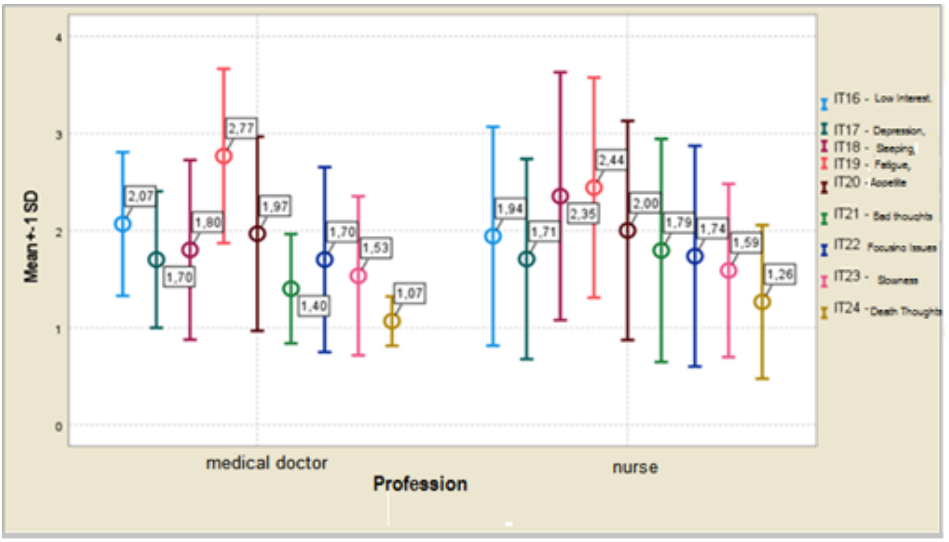

Fig. 1. Comparison between physicians and nurses regarding *low interest, depression, sleeping, fatigue, appetite, focusing issues* and *death thoughts.*

For the *presence of problems at the workplace*, the respondents declared the following related to involuntary thoughts about the workplace: 34.37% not at all, 50% moderately and only 15.63% or thinking about them extremely much. Regarding the *persistence or the feeling of being followed by problems at work*, a significant difference can be observed between doctors and nurses, 33.33% of doctors do not feel followed at all, and only 23.52% of nurses claims this thing. For the answer given to the item referring to *other things that triggered thoughts related to work*, 35.93% do not blame this fact, 50% a little and 14.06% blame this very much, the percentages are close. Feelings related to the workplace were described as extremely present by 12.50% of respondents, moderately present by 50%, with notable differences between doctors 60% and nurses 41.17% and not at all present by 37.50% of respondents. 70% of doctors and 52.94% of nurses moderately described *the need to think about problems after leaving the workplace*, 31.25% not at all and 7.81% of respondents do not think to problems at the workplace extremely much, without significant differences among the two categories of personnel. 10.93% of the respondents accused *concentration problems* extremely, 40.62% moderately, with large differences between doctors 53.33% and nurses 29.41% and not at all 48.4%, also with significant differences between doctors 36.67% and nurses 58.82%.

For the question *I totally agree with the possibility of managing the situation at work*, only 20% of the doctors answered affirmatively, unlike 44.11% of the nurses. Here a direct, statistically significant corre-

lation was also identified (p=0.047) between the professional degree and the agreement with the statement in the analyzed item. The *difficulty of understanding the situation at the workplace* reveals significant differences between the two professional categories, doctors considering the situation at the workplace more difficult to understand compared to nurses (6.7%+30% compared to 11.8%+5.9%). A statistically significant correlation (p = 0.008) was identified between occupational level and agreement with this statement in the item. Most respondents consider the *workplace structured*, respectively 26.56%, and the majority also consider the current workplace easy to influence, in a percentage of 20.31%. Many of the respondents, 17.18% mentioned total agreement and 17.18% strong agreement, consider the *work environment controllable*. Most respondents, 25%, consider *the current job attractive from a financial point of view*, and thy are neutral regarding the predictability of the current job, respectively 20.3%+21.9%+12.5%. The majority of respondents, in percentage of 57.81%, believe that they *can adapt extremely easily to the changes occurring at the workplace* and that they can quickly overcome unforeseen situations occurring at the workplace. The respondents, in an overwhelming percentage, *try to see a happy side in the event of problems*, to overcome them successfully, the percentage being 36.67%. Most respondents believe that *exposure to stress makes them stronger*, 34.37% agreeing with this statement. 43.75% of the respondents say that they recover quickly after the illness. The majority of respondents, in percentage of 53.12%, believe that they can easily overcome the obstacles in order to achieve the objectives at the workplace. Most of the surveyed staff (75%) declared *that under pressure they can stay focused and think clearly*, without identifying major differences between doctors and nurses (76.67% *vs.* 73.52%). In proportion to 75% of the medical personnel declared that it is not easy to be discouraged in the face of a failure (73.34% doctors *vs.* 76.47% nurses). Regarding the *perception of one's own person in terms of the ability to face challenges and life's difficulties*, more than half of the surveyed staff (56.3%) believe that it is a valid statement in all situations, there being no differences between doctors and nurses (56.7% *vs.* 55.9%). *Unpleasant or painful feelings such as sadness, fear and anger are easy to manage* for 84.4% of the medical staff, with a slightly higher proportion in the case of doctors (90%) compared to nurses (79.4%), the difference which is not but statistically significant. In the proportion of 59.4%, *medical personnel consider themselves less susceptible to colds, flu, and other infectious diseases*, with no differences between doctors and nurses. However, only 48.43% consider that it is unlikely that they will catch a cold, flu or other diseases when they manifest themselves in people around them (43.33% of doctor's *vs.* 52.94% of nurses, without being a statistically significant difference was identified). In accordance with the previously identified data, 75% of the surveyed staff believe that the risk of developing an infectious disease that exists in the people around them is low (80% doctors *vs.* 70.58% nurses, without identifying a statistically significant difference between them). 65.62% partially agree with the fact that they have an immune system that can protect them from most diseases that those around them have, without differences

between doctors and nurses, agreeing with the following statement regarding the possibility of doing an infectious disease to those around them (68.75% disagree). 51.56% partially agree with the fact that considering past experiences, the chances of getting sick are low even when friends are sick, without identifying differences between doctors and nurses, results found in agreement with the following statement when 46.87% declare that they do not agree at all that they have a history of susceptibility to infectious diseases.

Regarding the ways in which the surveyed medical staff believe that infections can be transmitted, the situations in which those around sneeze without covering their mouths (totally agree 71.87%), the use of used clothing (totally agree 51.56%)), touching money (totally agree 40.62%), staying around sick people (totally agree 31.25%) and less use of public telephones (totally agree 21.87%), using another person's pen (total agree 18.75%), or sharing a bottle of water with a friend (fully agree 15.62%).

DISCUSSION

This study analyzed the emotional impact, the degree of stress of the medical staff of both doctors and medical nurses, exposed to the risk of getting infected with COVID-19. In our study we have identified statistically significant difference between medical doctors and nursed for items referring to the possibility of managing the situation at the workplace (p=0.047) and to difficulty understanding the situation at workplace (p=0.008).Using the Spearman correlation coefficient, we identified that most of the correlations are positive, or directly proportional. We marked the statistically significant correlations in the first table, and we have used color codes for their intensity: yellow – weak correlations, below 0.3, orange – moderate correlations, between 0.3 – 0.5 and red – strong correlations, between 0.5 – 1.00. Item 25A (*fear of infection*) is moderately to strongly and statistically significantly correlated with the following questionnaire items: IT 13 (*threat*) – rho = 0.487, IT18 (*sleeping*) – rho = 0.406, IT 26 (*thinking*) – rho = 0.464, IT 28 (*other things*) – rho = 0.410, IT30 (*not thinking*) – rho = 0.422 and IT31 (*concentration problems*) – rho = 0.494.

The correlations recorded are directly proportional or positive, which means that the fear of infection is the greater, as the subjects indicated higher scores on the mentioned items.

Correlations were also statistically significant, directly proportional, moderate, but weaker in intensity and with items 7, 14, 19, 20, 22 and 34; the correlation with item number 43 (*I recover quickly after illness*) also belongs to the same category of intensity, but in this case the correlation changes its meaning, being inversely proportional – the subject's fear of infection is all the more pronounced the more they appreciate that the time of their recovery after an episode of illness is longer. Also, we observed statistically significant correlations, but weaker in intensity and still directly proportional, of item 25A with items 8, 16, 23, 29, 32, 57 and 58.

Item 25B (*stressful work*) is in turn statistically significantly, directly proportionally and moderately to strongly correlated with IT18 (*sleep*) – rho = 0.421, IT19 (*fatigue*) – rho = 0.445, IT20 (*appetite*) – rho = 0.418, IT26 (*thinking*) – rho = 0.441 and IT31 (*concentration problems*) – rho = 0.410 – which means that the subjects note a higher level of stress as they indicated

higher scores on the mentioned questions. There is also a moderate to strong, but inversely proportional, correlation of the stress level with question IT43 (*I recover quickly after illness*) – rho = – 0.480, respectively with high scores on this question. It can thus be stated that, when the subjects consider that it is difficult to recover from the illness, they are moderately afraid of infection, but have a higher level of stress in carrying out their professional activity. Also, the stress level of the subjects is correlated statistically significantly and directly proportionally, but moderately, with items 13 (to a lesser extent, therefore, than in the case of fear of infection), 16, 23, 32, 34 (stronger than in the case of fear of infection), 22 (similar in intensity to the case of fear of infection), and in addition with items 17, 24 and 49. We also observed a moderate correlation, statistically significant but inversely proportional, with item 42 (*stress makes me stronger*) – rho = – 0.314 – so the study subjects perceive a high level of stress at work, but do not consider the stress to make them stronger, and therefore do not motivate them positively. Some positive correlations were also observed, statistically significant but weak in intensity, with items 8, 9, 51, 55 (new correlations), 28, 30 (*much weaker than in the case of fear of infection*) and 57, 58 (*correlations similar in intensity to those in the case of the fear of infection*). In addition, the level of stress is weakly correlated, statistically significant but inversely proportional to item 41 (*I try to see humor*) – rho = -0.262.

TABLE I

Correlations between *fear of infection* and *stressful work* (items 07-63)

	ITEM 25A – Fear of infection				ITEM 25B – Stressful work			
	Spearman's correlation coefficient rho	p	C.I. 95% of rho		Spearman's correlation coefficient rho	p	C.I. 95% of rho	
			Inf. Lim.	Sup. Lim.			Inf. Lim.	Sup. Lim.
ITEM 07	.	.012	.063	.523	.204	.106	-.052	.434
ITEM 08	.261*	.038	.008	.482	.248*	.048	-.005	.471
ITEM 09	.206	.102	-.049	.436	.292*	.019	.042	.507
ITEM 10	.162	.200	-.094	.399	.150	.237	-.107	.388
ITEM 11	.130	.305	-.127	.371	.186	.141	-.070	.419
ITEM 12	.187	.140	-.069	.420	-.035	.782	-.286	.219
ITEM 13	.487**	.000	.267	.659	.375**	.002	.135	.573
ITEM 14	.321**	.010	.075	.531	.232	.065	-.022	.458
ITEM 15	.146	.251	-.111	.384	.122	.337	-.135	.364
ITEM 16	.285*	.022	.035	.502	.361**	.003	.120	.563
ITEM 17	.227	.071	-.027	.454	.374**	.002	.134	.572
ITEM 18	.406**	.001	.171	.598	.421**	.001	.188	.609
ITEM 19	.371**	.003	.130	.570	.445**	.000	.217	.627
ITEM 20	.365**	.003	.124	.566	.418**	.001	.185	.607
ITEM 21	.161	.204	-.096	.397	.200	.114	-.056	.431
ITEM 22	.365**	.003	.123	.565	.396**	.001	.159	.589
ITEM 23	.267*	.033	.015	.487	.341**	.006	.097	.547

	ITEM 25A – Fear of infection				ITEM 25B – Stressful work			
	Spearman's correlation coefficient rho	p	C.I. 95% of rho		Spearman's correlation coefficient rho	p	C.I. 95% of rho	
			Inf. Lim.	Sup. Lim.			Inf. Lim.	Sup. Lim.
ITEM 24	.151	.235	-.106	.389	.338**	.006	.093	.544
ITEM 26	.464**	.000	.240	.642	.441**	.000	.212	.624
ITEM 27	.167	.188	-.090	.402	.177	.161	-.079	.411
ITEM 28	.410**	.001	.175	.601	.269*	.031	.018	.489
ITEM 29	.281*	.024	.031	.499	.228	.070	-.027	.454
ITEM 30	.422**	.001	.189	.610	.253*	.044	.000	.475
ITEM 31	.494**	.000	.276	.664	.410**	.001	.176	.601
ITEM 32	.283*	.024	.032	.500	.371**	.003	.130	.570
ITEM 33	-.011	.932	-.263	.243	.059	.641	-.196	.308
ITEM 34	.318**	.010	.071	.528	.362**	.003	.121	.563
ITEM 35	-.074	.563	-.320	.183	-.111	.383	-.354	.146
ITEM 36	.147	.246	-.110	.385	.226	.072	-.028	.453
ITEM 37	-.065	.609	-.313	.191	-.191	.131	-.423	.065
ITEM 38	.056	.659	-.199	.305	.039	.761	-.216	.289
ITEM 39	-.059	.643	-.307	.197	-.055	.668	-.303	.201
ITEM 40	.059	.642	-.196	.307	.069	.590	-.187	.316
ITEM 41	-.145	.255	-.383	.112	-.262*	.037	-.483	-.010
ITEM 42	-.231	.066	-.457	.023	-.314*	.011	-.525	-.067
ITEM 43	-.316*	.011	-.527	-.069	-.480**	.000	-.654	-.259
ITEM 44	-.063	.622	-.311	.193	-.225	.074	-.452	.029
ITEM 45	-.122	.338	-.363	.135	-.142	.261	-.381	.114
ITEM 46	-.083	.515	-.329	.173	-.139	.273	-.379	.118
ITEM 47	-.112	.378	-.355	.145	-.174	.170	-.408	.083
ITEM 48	-.032	.804	-.282	.223	-.186	.142	-.419	.070
ITEM 49	.170	.180	-.087	.405	.337**	.007	.092	.543
ITEM 50	-.001	.994	-.254	.252	.126	.322	-.131	.367
ITEM 51	.161	.202	-.095	.398	.296*	.018	.047	.510
ITEM 52	.082	.519	-.174	.328	.040	.755	-.215	.290
ITEM 53	.029	.818	-.225	.280	.118	.352	-.139	.360
ITEM 54	-.127	.318	-.368	.130	-.167	.186	-.403	.089
ITEM 55	.154	.224	-.103	.392	.288*	.021	.038	.504
ITEM 56	-.032	.805	-.282	.223	.057	.656	-.199	.305
ITEM 57	.273*	.029	.021	.491	.253*	.044	.000	.475
ITEM 58	.296*	.018	.046	.510	.289*	.020	.040	.505
ITEM 59	.140	.269	-.117	.380	.144	.257	-.113	.383
ITEM 60	.065	.607	-.190	.313	.113	.375	-.144	.355
ITEM 61	.054	.673	-.202	.303	.030	.811	-.224	.281
ITEM 62	-.025	.843	-.276	.229	-.187	.139	-.420	.069
ITEM 63	-.032	.801	-.283	.222	.069	.586	-.187	.317

The findings of this study showed that in order to increase the psychological resilience of healthcare professionals working during the COVID-19 pandemic, there is a need to improve their sleep quality, positive emotions and life satisfaction. Levels of psychological resilience of healthcare workers in recent years have been found to be higher. Still, doctors constitute the group with the lowest levels of psychological resilience among health workers. We consider that the current study could bring some contribution to the specialized literature in this regard. Primary needs such as sleep, which are the determinants of quality of life, life satisfaction and psychological resilience should be met.

The resilience and well-being of medical staff is a subject of interest for researchers from all continents: USA (Ross *et al*., 2023), Korea (Lee *et al*., 2022), Italy (Giusti *et al*, 2022), Australia (Unjai *et al*., 2022). In these studies, the most frequent answers of medical staff were for high stress, were afraid, different levels of anxiety or depression, work overload, depressive symptoms (7–10). Researchers from Oxford, United Kingdom recognized that resilience-building programs can provide support for staff who endure highly stressful environments and can optimize resilience. In their pilot study, the authors have seen that the study participants – nurses accepted the idea of implementing web-based resilience programs, for optimizing resilience, psychological health, and communication at workplace (Henshall *et al*., 2023).

Haugland *et al.* is sustaining our findings, regarding the need for improving resilience in nurse's category and this should be an ongoing effort (Haugland *et al*, 2023).

The strength of our study is that, for our knowledge, it is one of the first studies in our country which is assessing the resilience and satisfaction with life among medical staff (doctors and nurses).

The limitation of the study is the relatively small number of participants which provided data. Another limitation is that the study participants belong from a single-based unit hospital research and the results cannot be generalized for doctors/nurses working in emergency unit in general. Also, this was a single cross-sectional study with only self-reported measurements of the participants, and improper pre-set demographic variables.

Future studies may reveal more extensive results by collecting data from a larger number of healthcare professionals. Given that the current study is an example of cross-sectional research, there is a need to conduct longitudinal studies that examine the long-term effects of the pandemic. Positive and negative emotions were found to play a significant role in the model as variables that predict the psychological resilience of health workers after the analysis. Therefore, further studies can have a better understanding of the issue by investigating the determinants of positive and negative emotions of healthcare workers during the COVID-19 pandemic. We intend that following this study, to extend the research to a larger number of personnel from the emergency services (and in other EDs in the country), as well as to the people from the ISU services, involved in emergency interventions, in order to obtain much more conclusive data regarding the stress, the behavior in general of the workers from the UPU and ISU emergency services during the COVID-19 pandemic.

CONCLUSIONS

The results of our study showed that to increase the psychological resilience of healthcare professionals working during the

COVID-19 pandemic, their sleep quality, positive emotions and life satisfaction need to be improved. Levels of psychological resilience in healthcare workers in recent years have been higher. On the other hand, higher levels of negative emotions lower the level of psychological resilience. Doctors constitute the group with the lowest levels of psychological resilience among health workers. The research results revealed a significant part of the variables that impact on the psychological resilience of healthcare workers, so that they can provide better services during COVID-19 and similar pandemics. The result of the current study showed that one of the main needs such as: sleep quality, life satisfaction and positive-negative things are part of an important prediction for the psychological resilience of medical professionals.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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THE ROLE OF MAXILLARY SINUS VOLUMETRY IN CHILDREN WITH OBSTRUCTIVE SLEEP APNEA

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THE ROLE OF MAXILLARY SINUS VOLUMETRY IN CHILDREN WITH OBSTRUCTIVE SLEEP APNEA (Abstract): **Aim**: Obstructive sleep apnea (OSA) is a common sleep disorder that can also affect children and represents a risk factor for childhood developmental problems. The aim of this study was to demonstrate if there is a correlation between the maxillary sinuses and nasal airway volumes and the presence of OSA in children. **Material and methods**: We retrospectively analyzed 59 patients from which 28 were diagnosed with OSA and 31 were healthy patients. We calculated the total nasal airway volume and maxillary sinuses volume using a open-source software for segmentation from CBCT (cone-beam computed tomography) data. **Results**: There was a significant difference regarding the BMI and the maxillary sinuses volume between the OSA patients and the control group. **Conclusions**: The maxillary sinuses reduced volume can be used as a predictor for OSA in pediatric patients with ages between 12-18 years old. **Keywords**: OBSTRUCTIVE SLEEP APNEA, CHILDREN, MAXILLARY SINUS, AIRWAY VOLUME.

Obstructive sleep apnea (OSA) is a common sleep disorder characterized by repetitive episodes of partial or complete upper airway obstruction during sleep. These episodes lead to disrupted breathing patterns, recurrent awakenings, and diminished oxygen levels in the body. OSA affects a significant portion of the population, with estimates ranging from 9% to 38% in middle-aged adults (1).

Obstructive sleep apnea is not limited to adults; it can also affect children. OSA prevalence in children varies, but estimates suggest it affects around 1-5% of children (2).

OSA in childhood is a strong risk factor for childhood developmental problems. It is closely related to neurobehavioral problems such as attention deficits, hyperactivity, learning disabilities, memory impairment, poor academic performance, and depression (3, 4) and is a risk factor for physical problems such as developmental delays (5), cardiovascular comorbidities (6), metabolic

disorders, and inflammation (7, 8).

The paranasal sinuses, including the maxillary, ethmoid, sphenoid, and frontal sinuses, are air-filled cavities within the skull bones surrounding the nasal passages. These sinuses play a role in reducing skull weight, providing structural support, and regulating air humidity and temperature (9).

Limited research has explored the correlation between pediatric OSA and paranasal sinus volume and understanding the relationship between them has clinical implications. Reduced sinus volume may impact nasal airflow and ventilation, potentially exacerbating OSA

symptoms (10). Additionally, sinus inflammation and infections may further contribute to airway obstruction in individuals with OSA (11).

The aim of this study was to reveal the associations between paranasal sinuses and nasal cavity volume and the presence of OSA in teenagers.

MATERIAL AND METHODS

Study population

This is a retrospective study of 28 patients (13 females and 15 males) aged 12-18 years old, already diagnosed with OSA based on clinical and physical examinations and polysomnography with AHI (apnea-hypopnea index ≥5), sent by the referring physician for a CBCT (cone beam computed tomography) examination at the "Medimagis Imaging Clinic" between March 2020 and December 2022.

The control group consisted of 31 subjects (13 females and 18 males) with ages between 12-18 years old and without any respiratory complaints, with a CBCT examination in the same clinic, in the same period of time.

We excluded patients with chronic or acute rhinosinusitis and allergic rhinitis, cranio-facial abnormalities or history of cranio-facial surgery.

Acquisition and Postprocessing of CBCT (cone-beam computed tomography) Data

The patients were placed in a sitting position with the Frankfurt plane parallel to the floor and the mid-sagittal plane perpendicular to the floor. The patients were told not to move, to avoid swallowing and to keep a regulated breathing interval during acquisition of images.

All included patients had CBCT examination in an interval of 1-3 months after the diagnosis and it was performed by CBCT ProMax 3D Max (Planmeca, Helsinki, Finland) with the largest field of view (FOV) available (502x502x437) with a voxel size of 0.4 mm, tube potential 90 kV, and tube current 10 mA.

Images were then exported in picture archiving and communication system (PACS) in standard DICOM format (digital imaging and communication in medicine). The data was loaded to the segmentation software, 3D Slicer, a free and open-source application for medical image manipulation (12).

The delimitations of the nasal cavity and paranasal sinuses were the following:

-a midsagittal reference plane (MSRP) was defined as a plane passing through nasion (N), anterior nasal spine (ANS), and the posterior nasal spine (PNS);

-the anterior plane was a line passing through external narines;

-the superior border was a plane passing through N, parallel to the palatal plane (that passes through ANS and PNS and is perpendicular to MSRP).

The nasal cavity and paranasal sinuses

were segmented using a threshold method that selected a range of pixels between a predefined interval related to the density of air (-1000 and -400) and the segmented volume was calculated automatically with the software's feature (fig. 1). The right and left maxillary sinuses were separated from the whole nasal airway for volume measurements (fig. 2). The process was done by radiologists.

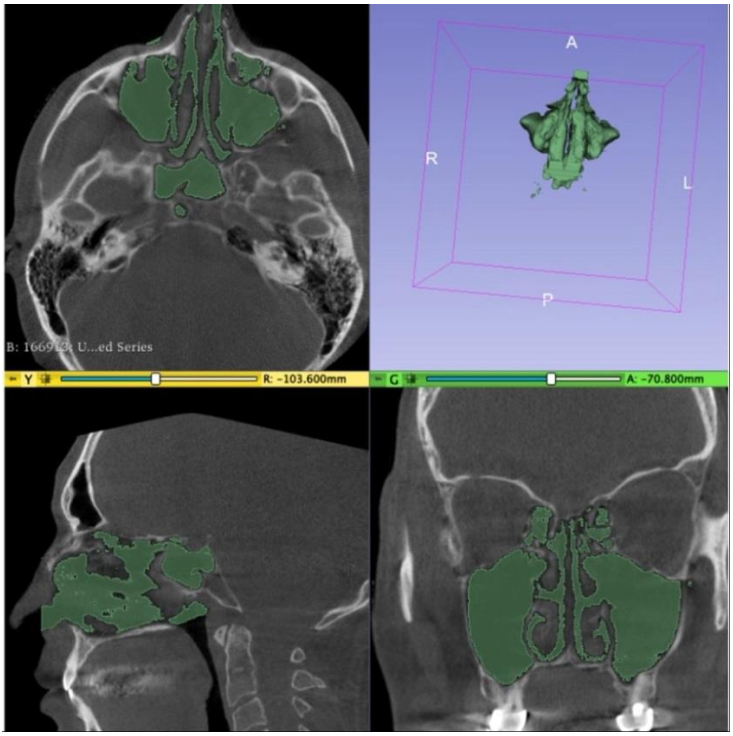

Fig. 1. Nasal airway segmentation using 3D Slicer.

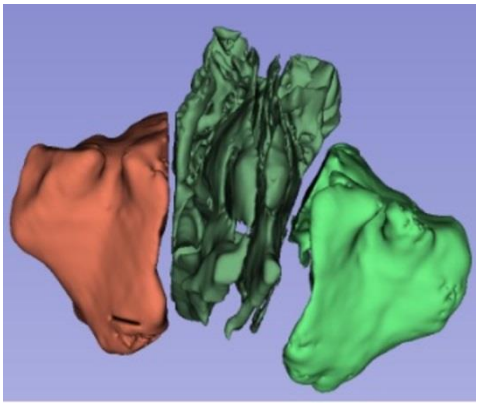

Fig. 2. Right and left maxillary sinuses separated from the rest of the nasal airway using 3D Slicer software

Statistical analysis

Statistical significance was established at a *p*-value below 0.05. Statistical analysis was performed using *SPSS* (IBM SPSS Statistics, release 24.0, Armonk, New York, NY, USA).

RESULTS

The descriptive statistics of control and OSA patients groups are displayed in Table I.

TABLE I.

Descriptive statistics of group 0 (control group) and group 1 (OSA patients).

	Age	BMI (body mass index)	Total nasal airway volume (cm3)	Maxillary sinuses volume (cm3)	Sinus/nasal airway ratio (%)
Group 0 (control)	15.06 ± 1.98	24.09 ± 3.99	84.58 ± 10.69	49.48 ± 3.54	59.57 ± 9.85
Group 1 (OSA patients)	15.39 ± 1.85	27.18 ± 1.77	82.05 ± 6.95	42.38 ± 3.29	51.83 ± 3.9
p-value	0.529	<0.001	0.268	<0.001	0.002

There was no significant differences between age or sex but there was significant differences in BMI index between the groups.

No significant difference between the volumes of total nasal airway in the two groups was found. The maxillary sinuses volume and the ratio to the total nasal airway was significantly reduced in patients with OSA compared to control group.

DISCUSSION

In this study we analyzed the correlation between the nasal airway volume, maxillary sinuses and the presence of OSA in pediatric patients above 12 years old. We found that that children with OSA had significantly smaller maxillary sinus volumes compared to the control group. Therefore, it may be said that decreased maxillary sinus volume can be a predictor of OSA development but not necessarily an exacerbating factor. Kim YJ *et al.* (13) conducted a study on 109 adult patients with OSA that underwent overnight polysomnography and CT (computed tomography) of ostiomeatal unit and found that patients with OSA had decreased ratios of maxillary sinus volume to whole nasal airway volume compared with normal subjects. Also, he didn't find any correlation between the volumes of total nasal airway or maxillary sinuses with the severity of OSA. In another study, Rodriguez *et al.* used CT and nasal fiber-optic endoscopy to assess the nasal airway volume and the anatomical changes of nasal cavity in patients with OSA and also found no association between nasal cavity volume and the severity of OSA (14).

Our study focused on pediatric patients between 12 and 18 years old because maxillary sinuses volume increase from postnatal period to adult stage but studies have shown that there isn't a significant size change after the age of 12 years. A study performed by Weiglein *et al.* on Australian cadavers' dried skulls reported that the final size of maxillary sinus is reached at 12 years of age (15). Bhushan B *et al* studied the changes of maxillary sinuses volume with age in children under 18 years old by measuring the height, weight and depth in groups 0-6 years, 6-12 years and

12-18 years using CT. The result showed that there was not a significant increase in maxillary sinus volume in patients older than 12 years old (16).

The majority of studies in the literature that measured the nasal airway volume used medical CT (13), (14), but considering we included in our study children, CBCT used as an imaging method for the nasal airway volume assessment has the advantage of a lower radiation dose and thinner slices that provide a better accuracy. CBCT is a widely used three-dimensional imaging technique and when utilizing a large field of view (FOV) protocol, it becomes a useful diagnostic tool for the upper airway assessment because it is visible in the whole CBCT volume. However, CBCT acquire images with patients in upright position unlike CT machines that require patients to be in supine position. This can be also a limitation of the study as the upright position does not simulate conditions during sleep but also an advantage considering the base of the tongue can protrude in the oropharynx airway and therefor, underestimate the volume of the airway. However, in our study we included only the nasal airway that is not influenced by the supine or upright position.

The ideal method for measuring nasal airflow remains controversial. Because the nasal airway contains not only bony structures but also dynamic ones, such as alar cartilages, turbinate, CT/CBCT images may have some limitations for airway function assessment. These aspect be considered, functional tests like nasal resistance could provide more information about nasal function.

Another aspect that must be discussed is the software used for the segmentation and volume calculation. In this study we used 3D Slicer, an open-source software (12) that has been found to be suitable for segmentation as well as in other studies (17), (18), because it is free and certified for medical use.

The effects of obesity on the development and severity of OSA are well known (19), (20). Our results supported the idea that a high BMI is correlated with the presence of OSA in children.

Nasal inflammatory conditions can also play an important role in the development of OSA (21). In our study we excluded patients with chronic or acute rhinosinusitis and allergic rhinitis in order to have a higher accuracy airway volume but there are studies that report a correlation between nasal obstruction and OSA (11), (22), (23). A survey study in Sweden has shown that respiratory conditions like rhinitis, chronic bronchitis, asthma, were significantly associated with OSA related symptoms (24). A study in Switzerland reported that perennial allergic rhinitis was found in 11% of patients with OSA and in only 2.3% of patients with chronic obstructive pulmonary disease (25). Although the potential mechanisms of the association between OSA and chronic rhinosinusitis are not completely elucidated, a study reported that some nasal inflammatory indicators (number of polymorphonuclear leukocytes, concentrations of bradykinin and the vasoactive intestinal peptide) were significantly higher in patients with OSA than in those without OSA (26). Another study found that chronic intermittent hypoxia due to OSA can determine a release of inflammatory cytokine that may contribute to mucosal and muscular inflammation of the upper airway (27).

Therefore, knowledge of other associated conditions with OSA such as nasal inflammatory diseases is important because it

can either contribute to the pathogenesis of OSA, either decrease the quality of life and the efficiency of CPAP treatment.

CONCLUSIONS

Reduced maxillary sinus volume can be a predictor for obstructive sleep apnea in pediatric patients and understanding the relationship between OSA and paranasal sinus volume has clinical implications. Further research is necessary to explore the underlying mechanisms linking OSA and paranasal sinus volume changes and to investigate potential therapeutic approaches targeting both conditions.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NONTUMORAL PORTAL VEIN THROMBOSIS IN PATIENTS WITH HEPATITIS C VIRUS AND SUSTAINED VIROLOGICAL RESPONSE - A FURTHER CHALLENGING CONSEQUENCE OF LIVER CIRRHOSIS

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NONTUMORAL PORTAL VEIN THROMBOSIS IN PATIENTS WITH HEPATITIS C VIRUS AND SUSTAINED VIROLOGICAL RESPONSE-A FURTHER CHALLENGING CONSEQUENCE OF LIVER CIRRHOSIS (Abstract): The sustained virologic response (SVR) achieved by most patients treated with direct acting antivirals (DAAs) involves multiple benefits such as regression of fibrosis and improvement in liver function. However, DAAs therapy doesn't eliminate the risk of thrombotic events. The **aim** of our study was to assess the prevalence of nontumoral portal vein thrombosis (PVT) after SVR and identification of risk factors associated with this complication. **Material and methods:** We retrospective analyzed a cohort of patients with HCV-related liver cirrhosis treated with paritaprevir/ritonavir, ombitasvir and dasabuvir (PrOD) ± ribavirin and ledipasvir/sofosbuvir (LED/SOF) ± ribavirin for 12/24 weeks, in a gastroenterology center from Romania, between October 2015 and December 2018. All patients with presumption of PVT were evaluated by abdominal ultrasound and confirmed by CT scan. **Results:** The study included 730 patients treated with DAAs, of which 35 were diagnosed with non-malignant PVT after-SVR (15 men and 20 women, mean age 57.86 ± 7.068 years), corresponding to a prevalence of 4.8%. The mean time from SVR to complication was 290.00 ± 116.639 days. Most patients with non-tumoral PVT after-SVR received LED/SOF (71.4%), while the rest received PrOD (28.6%). Twenty-four patients (68.6%) diagnosed with acute PVT and 11 patients (31.4%) with chronic PVT. During the study, an improvement in liver function was observed, with an improvement in the Child-Pugh and MELD score at the time of SVR; the evolution changes slightly at the 48-week assessment, with a slight increase in the proportion of patients with Child B and MELD ≥ 15. **Conclusions:** Occurrence of non-malignant PVT in patients with HCV-related liver cirrhosis treated with DAAs correspond to the natural evolution of the cirrhotic patient. **Keywords:** CHRONIC HEPATITIS C INFECTION, NONTUMORAL PORTAL VEIN, THROMBOSIS, SUSTAINED VIROLOGICAL RESPONSE, DIRECT-ACTING ANTIVIRALS THERAPY, LIVER CIRRHOSIS.

The sustained virologic response (SVR) achieved by most patients treated with direct-acting antivirals (DAA) involves multiple benefits such as regression of fibrosis, reduction of portal hypertension, and improvement of liver function (1,2). In

addition to the hepatic benefits of the virological cure mentioned, successful eradication of the C virus indirectly leads to the reversal of the hypercoagulable status by lowering procoagulant factors as close to normal parameters as possible, leading to a significant decrease in thrombotic complications (3, 4).

The long-term impact of sustained virological response after direct-acting antivirals on hypercoagulability associated with C viral liver cirrhosis is unknown. Up to this moment, there are few information regarding the improvement of coagulation status in patients treated with antivirals. There is a possibility that the safety profile of direct vascular antivirals may be incorrectly estimated due to the fact that there is no adequate reporting of the actual incidence of arterial and venous thrombotic events in clinical trials. However, an unexpected incidence of portal vein thrombosis (PVT) immediately after treatment with direct antivirals was recently reported (5).

Chronic viral hepatitis C (HCV) is itself associated with an increased risk of thromboembolic complications due to thrombocytopenia, low antithrombin III (ATIII) concentration, and the presence of antiphospholipid antibodies, which predisposes to arterial and venous thrombosis (6). In addition, an increase in platelet aggregation has been observed in patients with chronic HCV infection and advanced fibrosis, a phenomenon that could explain the occurrence of thrombotic events in these patients (6).

The incidence for any type of thromboembolic event reported by Enger *et al.* in a large study was 561.1 per 10,000 person/years in cirrhotic patients and 249.7 per 10,000 person/years for the control group (7). The main thrombotic complications reported in this study that could occur in a cirrhotic patient were deep vein thrombosis, portal vein thrombosis, pulmonary thromboembolism, stroke, and acute coronary syndrome, but most studies focused on non-malignant PVT, which is seen the most frequent.

A reduced number of studies have attempted to evaluate the impact of direct antiviral therapy on coagulation and the main risk factors for the development of thrombotic events in cirrhotic patients treated with direct antivirals and SVR. The majority included thrombotic events such as non-malignant PVT and the one of neoplastic origin that is most often associated with hepatocarcinoma, in which the thrombotic mechanism is different (4,5,8).

The research of Tripodi *et al.* (3) and Russo *et al.* (4), proved for the first time that DAA treatment in patients with C viral liver cirrhosis resulted in improvement of pro- and anticoagulant status in patients who have acquired SVR. It does not substantially alter their balance but makes it more stable and less likely to be disturbed, as was supposed before treatment.

The main suspected risk factor for the occurrence of thrombosis after SVR in these few cases is liver fibrosis, which remains elevated in some of the patients treated with antivirals, a fact that is indirectly reflected on the degree of portal hypertension, mechanisms responsible for venous stasis in port system level with the risk of non-tumoral portal vein (9,10,11).

Thus, this study aimed to evaluate the prevalence of non-malignant portal vein thrombosis in patients with chronic HCV infection treated with interferon-free antivirals, and whether the development of PVT de novo is a reflection of the natural history of cirrhosis in patients with long-

term viral infection or is a consequence of DAA therapy.

MATERIAL AND METHODS

We performed a retrospective study in which we included patients diagnosed with compensated and decompensated viral liver cirrhosis C, randomly selected, to receive treatment with direct antivirals (Ombitasvir/Paritaprevir/Ritonavir + Dasabuvir ± Ribavirin or Sofosbuvir/Ledipasvir ± Ribavirin) for 12/24 weeks, admitted to the Institute of Gastroenterology and Hepatology Iași, Romania, between October 2015 and December 2018. Additionally, they were considered inclusion criteria presence of non-malignant PVT. All patients were clinically and paraclinically evaluated at the time of admission; socio-demographic data, personal antecedents and symptoms were evaluated and recorded for all patients. The diagnosis of cirrhosis was based on clinical, biological and imaging data according to international guidelines. The diagnosis of viral C infection was suspected by anti-HCV Ab positivity and confirmed by HCV-RNA, the detection limit being 15 IU/mL. Sustained virologic response was defined as undetectable HCV-RNA level 12/24 weeks after completion of direct antiviral treatment.

The patients were followed for 3 years, and the data were collected as follows: at the initiation of treatment with direct antivirals (baseline), at the end of antiviral treatment (EOT), 3 months after completion of antiviral treatment (SVR12), 48 weeks after obtaining SVR12, 3 years after obtaining SVR12, on emergency admission in the event of a thrombotic complication after the period of antiviral treatment.

Blood samples for biochemical and immunological evaluations were collected at scheduled visits. At the same time, an additional amount of blood was collected to perform coagulation tests at the time of diagnosis of non-malignant PVT. Two vacutainers of whole blood containing 1/10 volume of sodium citrate as an anticoagulant were taken, each vacutainer having a capacity of 2.7 mL. Blood samples were centrifuged for 15 minutes at 2500xg/min and plasma was collected and stored in 0.5 mL devices and subsequently frozen at -80°C until examination. Processing of biological samples was performed after thawing the plasma at 37°C for 5 minutes.

Portal vein thrombosis was diagnosed using a Philips HD11XE ultrasound machine equipped with a 3.5 MHz convex probe and was confirmed by contrast-enhanced computed tomography. Ultrasound PVT was defined as the presence of hyperechoic material within the portal vein or its branches. The portal cavernoma was defined by the presence of periportal collateral circulation, with the role of short-circuiting the thrombosed area. Portal vein thrombosis was defined as partial if the thrombus occupied less than 90% of the vessel lumen and complete when the thrombus obstructed more than 90% of the vessel. Portal vein thrombosis was considered acute in the context where before the examination the patient showed no signs of PVT, and at the time of diagnosis associated symptoms such as: abdominal pain, onset or worsening of ascites, variceal bleeding, or hepatic encephalopathy.

To check the patients who were a part of our inquiry for liver fibrosis we used the FibroScan® 520 compact model (Echosens, Paris, France) equipped with the M- (normal) or XL- (obese) probe. Firstly, the inspection was performed using an M-

probe with a 3.5 MHz transducer frequency. Then, the XL probe (2.5 MHz) was used if the distance between the skin and the liver capsule was higher than 25 mm. If 10 acquisitions were made with an interquartile interval not exceeding 30%, reliable measurement was determined to have occurred. The cut-offs for liver stiffness measurements (LSM) values were: 5.6 kPa for mild fibrosis (F1), 7.1 kPa for significant fibrosis (F2), 9.5 kPa for advanced fibrosis (F3), and 12.5 kPa for cirrhosis (F4).

Statistical processing was carried out using *Microsoft Excel* and *SPSS version 28* (Inc., Chicago, IL) programs. While categorical variables were expressed as absolute values and percentages, continuous variables with normal distributions were expressed as mean SD. To compare categorical data, the Chi-square test was employed. To compare the arithmetic means of a parameter analyzed in two samples, we used the t-Student test. We employed nonparametric tests, such as the Mann-Whitney U test, and the Kolmogorov-Smirnov test to determine whether the data distributions were normal. A *p*-value of 0.05 was used to determine the statistical significance of the results. When both variables were binary, we also calculated the corresponding OR (Odds Ratio) risks - the measure of the association between the presence of a risk factor and the occurrence of an event; these risks were calculated both in absolute value and as a 95% confidence interval.

The study was carried out in accordance with the ethical principles of the Declaration of Helsinki, the protocol being explained in detail to all patients. Informed consent for participation in the study was obtained from all patients and/or their relatives.

RESULTS

General characteristics of the study population

During the study period, 730 patients with HCV liver cirrhosis were treated with DAAs therapy, of which 35 were diagnosed with non-malignant PVT, corresponding to a total incidence of 4.8%. The mean age of patients with non-malignant PVT was 57.86 ± 7.06, the female gender being prevalent (57.1%), and patients came equally from urban or rural areas (51.4% vs. 48.6%). The mean time from SVR to complication was 290.00 ± 116.639 days, with a minimum of 74 days and a maximum of 450 days. The majority of patients with post-SVR PVT received LED/SOF (71.4%), while the rest received PrOD (28.6%). The study group consisted of 24 patients (68.6%) diagnosed with acute PVT and 11 patients (31.4%) with chronic PVT; most patients diagnosed with acute PVT associated partial occlusion of the portal vein (19 patients - 79.2%) with occlusion at the level of the trunk (15 patients - 62.5%). Half of the patients treated with antivirals were included in the Child C category (45.7%), just under a third were Child B (31.4%), and almost a quarter of patients were in the Child A category (22.9%), with no statistically significant differences between MELD score values. Among patients with PVT post-SVR, 22.9% did not have esophageal varices at the time of inclusion in the treatment, the number of patients with small and large esophageal varices being almost balanced (37.1% in the first case and 40.0% in the second). All patients presented various types of comorbidities: essential hypertension - 35 patients (100%), type 2 diabetes - 24 patients (68.57%), obesity - 12 (34.3%), psychiatric - 6 (17.1%), and neoplastic antecedents - 2 (5.7%) (tab. I).

TABLE I.
Baseline characteristics of patients with PVT - post SVR

Parameters	PVT - post SVR n=35
Age (years)	57.86 ± 7.068
Sex	
male, n (%)	15 (42.9%)
female, n (%)	20 (57.1%)
Environment	
urban, n (%)	18 (51.4%)
rural, n (%)	17 (48.6%)
Treatment	
LED/SOF	25 (71.4%)
PrOD	10 (28.6%)
Type PVT	
acute PVT	24 (68.6%)
chronic PVT	11 (31.4%)
Child-Pugh class	
class A, n (%)	8 (22.9%)
class B, n (%)	11 (31.4%)
class C, n (%)	16 (45.7%)
MELD score	
< 15	15 (42.9%)
≥ 15	20 (57.1%)
Esophageal varices	
absences	8 (22.9%)
small	13 (37.1%)
large	14 (40.0%)
Comorbidities	
Essential hypertension	35 (100%)
Type 2 diabetes	24 (68.57%)
Obesity	12 (34.3%)
Psychiatric	6 (17.1%)
Neoplastic antecedents	2 (5.7%)

Evolution of liver function and the degree of liver fibrosis in patients with non-malignant PVT post-SVR during the follow-up

Over the course of the study, an improvement in liver function was observed during antiviral treatment, with improvement of the Child-Pugh score and MELD at the time of SVR (45.7% CP class A, 54.3% CP class B, 100% patients with MELD score < 15). The evolution changes at the 48-week assessment, with a slight increase in the proportion of patients in Child B class (68.6%). At the 3-year assessment/or at the occurrence of the thrombotic event, however, a significant deterioration of liver function was observed compared to the initial situation (62.9% CP class B, 37.1% CP class C; 11.4% MELD <15, 88.6% MELD ≥15) (figs. 1, 2).

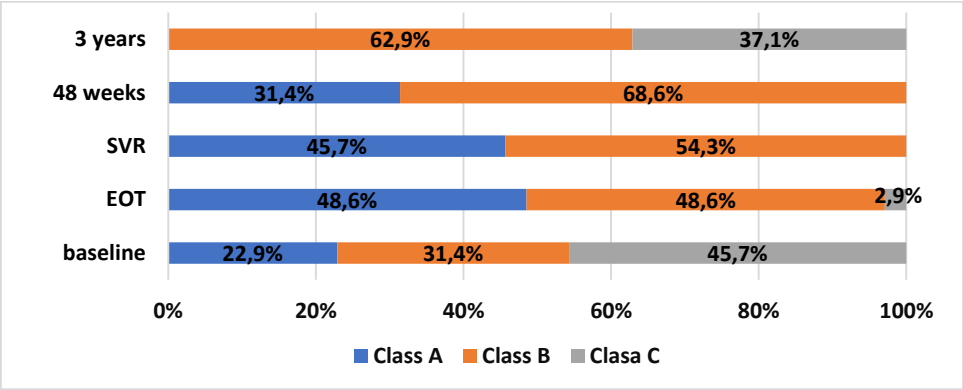

Fig. 1. Evolution of the Child-Pugh score during follow-up in patients with PVT post-SVR

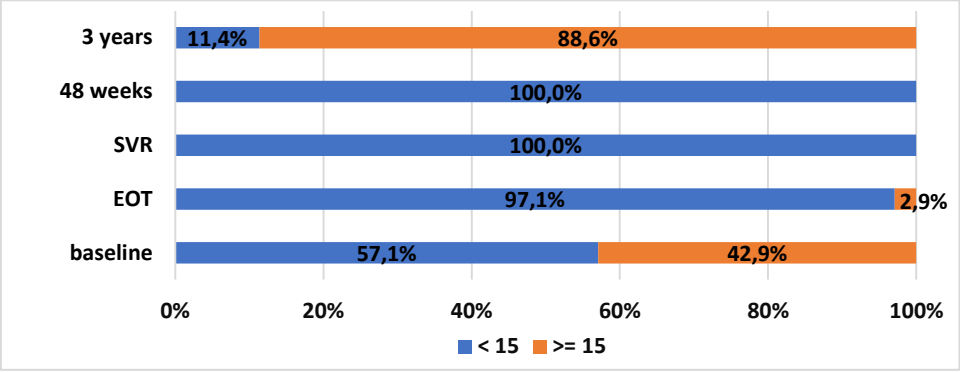

Fig. 2. Evolution of the MELD score during follow-up in patients with PVT post-SVR

Among the patients who achieved SVR with a pre-treatment CPT score ≥ 6 points, 23 (65.71%) showed an improvement in CPT score at the end of treatment (EOT). Among patients whose CPT score decreased ≥ 1 point, the cumulative incidence of non-tumoral PVT was 0%, 2.6%, and 6.5% at 1, 2, and 3 years, respectively, after the end of antiviral therapy, while the rates in those without a decrease in CPT were 8%, 10.8%, and 10.8%. Although the difference did not reach statistical significance (sHR: 0.454 (95% CI: 0.01-2.18; p = 0.331), the incidence of non-tumoral PVT was lower in patients who had an improvement in liver function during treatment, justifying the consideration of liver function pre/post - treatment as risk factors for the development of non-tumoral PVT.

In the case of the variation in the degree of liver fibrosis, a similar process is observed, with a significant decrease in Fibroscan values when evaluating SVR (19.30 kPa from 31.23 kPa to baseline), with the same descending trend at 48 weeks (16.81 kPa) and 3 years (18.21 kPa), but with higher values >12 kPa.

Regardless of whether SVR implies improvement of the liver function, the highest risk of non-tumoral PVT remained in patients with advanced liver disease. Comparative distribution by Child-Pugh classes

of patients with PVT - post SVR: 22.9% patients with Child A, 31.4% patients with Child B, and 45.7% patients with Child C.

Risk factors for non-malignant PVT post-SVR in patients treated with DAA

Among the qualitative parameters evaluated as risk factors for the occurrence of non-malignant PVT in patients with HCV liver cirrhosis treated with antivirals in the studied group, the presence of type II diabetes, ascites, hepatic encephalopathy and the decompensated status of liver disease reached the threshold of statistical significance (tab. II).

TABLE II.

Comparative study of qualitative parameters monitored in patients with PVT post-SVR

		PVT				Total		Pearson X^2	*p*
		Yes		No					
		N	%	N	%	N	%		
Sex	Male	15	42.9%	283	40.7%	298	40.8%	0.06	0.802
	Female	20	57.1%	412	59.3%	432	59.2%		
Age ≥ 65 years	Yes	6	17.1%	226	32.5%	232	31.8%	3.63	0.057
	No	29	82.9%	469	67.5%	498	68.2%		
Obesity	Yes	12	34.3%	181	26.0%	193	26.4%	1.16	0.281
	No	23	65.7%	514	74.0%	537	73.6%		
Comorbidities	Yes	35	100.0%	684	98.4%	719	98.5%	0.56	0.453
	No	0	0.0%	11	1.6%	11	1.5%		
Type 2 diabetes	Yes	35	100.0%	577	83.0%	612	83.8%	7.08	0.008**
	No	0	0.0%	118	17.0%	118	16.2%		
Esophageal varices	Yes	27	77.1%	478	68.8%	505	69.2%	1.09	0.296
	No	8	22.9%	217	31.2%	225	30.8%		
Ascites OR: 8.086 CI 95% (4.01-16.30)	Yes	19	54.3%	89	12.8%	108	14.8%	45.48	0.000**
	No	16	45.7%	606	87.2%	622	85.2%		
Non-selective beta blocker	Yes	0	0.0%	38	7.4%	38	7.3%	0.64	0.542
	No	8	100.0%	473	92.6%	481	92.7%		
Cirrhosis OR: 6.825 CI 95% (3.39-13.70)	Decompensated	19	54.3%	103	14.8%	122	16.7%	37.28	0.000**
	Compensated	16	45.7%	592	85.2%	608	83.3%		
Hepatic encephalopathy OR: 8.643 CI 95% (3.92-19.05)	Yes	11	31.4%	35	5.0%	46	6.3%	39.31	0.000**
	No	24	68.6%	660	95.0%	684	93.7%		

The analysis of quantitative parameters compared between patients with non-malignant PVT and the rest of cirrhotic patients treated with antivirals, revealed statistically significantly increased values for PT, INR, aPTT, ASAT, ALAT, BT, MELD score, and statistically significant values low for platelets, AP and albumin in patients with non-malignant PVT post-SVR (tab. III).

TABLE III.
Comparative study of quantitative parameters monitored in patients with PVT post-SVR

PVT		N	Media	SD	Median	Minimum	Maximum	*p*
Platelets	Yes	35	106428.57	19122.33	110000.00	62000	140000	0.003**
	No	693	136566.09	62960.22	124000.00	16000	475000	
PT	Yes	35	16.76	1.38	16.80	14.60	19.60	0.000**
	No	661	13.48	2.65	12.90	8.30	46.80	
AP	Yes	35	65.17	4.40	65.00	55.0	74.0	0.000**
	No	613	82.94	16.13	85.00	15.0	115.0	
INR	Yes	35	1.88	0.55	2.01	0.77	2.90	0.000**
	No	685	1.35	0.51	1.17	0.82	7.79	
aPTT	Yes	35	44.20	5.30	42.00	39.00	58.00	0.000**
	No	695	32.08	7.23	31.00	22.00	64.00	
ASAT	Yes	35	218.31	73.55	214.00	116.00	359.00	0.000**
	No	695	121.34	79.28	98.00	9.00	625.00	
ALAT	Yes	35	205.48	74.91	204.00	110.00	380.00	0.000**
	No	695	113.80	69.19	93.00	16.00	449.00	
BT	Yes	35	2.34	0.80	2.39	1.18	5.45	0.000**
	No	655	1.23	0.72	1.05	0.16	4.76	
Albumin	Yes	35	2.88	0.70	3.09	0.81	4.22	0.000**
	No	686	3.87	0.60	3.90	1.31	5.29	
MELD	Yes	35	13.71	4.66	12.00	7	21	0.000**
	No	692	10.22	3.45	9.00	6	21	

PT, prothrombin time; AP, prothrombin activity; INR, international normalized ratio; aPTT, activated partial thromboplastin time; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; BT, total bilirubin

DISCUSSION

One of the triumphs of medical therapy in the last two decades, proved by the effectiveness of direct-acting antivirals, is firmly proven by achieving SVR in over 95% of treated patients (12) and which was associated with reducing the progression of chronic hepatitis to cirrhosis, decreasing the risk of decompensation liver and portal hypertension complications, decreasing the need for liver transplantation and decreasing the risk of hepatocellular carcinoma (HCC) (2). Despite effective treatments related to HCV, in addition to the complications and characteristics specific to each progressive stage of liver disease, thromboembolic events could be associated even in patients responding to antiviral treatment.

Non-malignant PVT, an intensely debated topic in recent years, represented the most frequent thromboembolic complica-

tion that might occur in the natural course of liver cirrhosis. The prevalence of PVT varies according to the degree of liver failure and the presence of HCC, being up to 1% in patients with compensated liver cirrhosis, reaching 8% - 25% in liver transplant candidates and 40% in patients with HCC (13). In our study the incidence of non-malignant PVT post-SVR was 4.8%, higher than that reported to date in other studies (4). Stine JG *et al.* reported the experience of seven patients on DAA treatment, followed in a 4-year prospective study, who developed non-malignant PVT (one case with total PVT and six cases with partial PVT) after obtaining SVR (8). During this period 461 subjects were treated with direct antivirals, of which 54.8% (231 patients) were cirrhotic. The incidence of PVT post-SVR was 3% which, although lower than the incidence rates reported in the literature, is not expected given that most patients were not decompensated. At the same time, Russo *et al.* (4) identified 2 cases of non-malignant PVT in a prospective study that included 58 patients treated with antivirals and with SVR, which corresponds to an incidence of 3.44%, lower than that identified in our study. Mandorfer M *et al.* recently reported an incidence of non-malignant PVT of 2.8% in a prospective study of 354 patients with HCV and SVR cirrhosis, much lower than previously reported (5).

The pathogenesis of thrombosis is multifactorial, based on the elements of Virchow's triad: vascular stasis, endothelial injury and hypercoagulability (14, 15, 16). Numerous pieces of evidence stated the fact that the C virus hepatitis could activate hemostasis, and the endothelial damage, the inflammation described at the level of the vascular wall, the changes in the level of the hepatic microcirculation as well as the alteration of the coagulation parameters determined by the HCV infection represent factors that contribute to the occurrence of thrombotic events in advanced liver disease (17).

The majority of patients with non-malignant PVT post-SVR were classified as Child-Pugh B (31.4%) and Child-Pugh C (45.7%), with Child-Pugh A being in the minority (22.9%). Also, most patients with non-malignant PVT post-SVR had a MELD score ≥ 15 at the time of initiation of antiviral treatment. These results obtained in our study were different compared to the data reported in the whole literature. The prospective study by Stine *et al.* (8) included only patients with compensated HCV liver cirrhosis (Child A), which was why the occurrence of PVT post-SVR was an unexpected event, appearing on the background of a perfectly compensated field. In contrast, Russo *et al.* (4) detected non-malignant PVT in 2 patients who were with Child B, a similar situation to the cases reported by Mandorfer B *et al.* (5), where non-malignant PVT post-SVR was found in patients with decompensated liver cirrhosis.

In the studied group, the gender distribution was balanced, with the female gender having a higher weight (57.1%). This distribution of cases is also consistent with the epidemiological data from the specialized literature published for Romania, which reports a higher prevalence of HCV infection in the female sex (18), a fact that justified the larger number of women who addressed for evaluation to initiate antiviral treatment. The mean age of patients with nonmalignant PVT treated with antivirals was 57.86 ± 7.068, similar to the age of patients who experienced thrombotic events reported in other studies (4, 5, 8).

The mean time from SVR to complication was 290.00 ± 116.639 days, with a

minimum of 74 days and a maximum of 450 days. Time to PVT was similar to Stine *et al.* (8) at 272 ± 204 days, with 5 patients diagnosed with PVT < 365 days after obtaining SVR. Mandorfer *et al.* (5) showed that non-tumoral PVT in responders was detected during a mean follow-up of 37.1 months, whereas 8 (4.5%) of patients without SVR were diagnosed with malignant PVT during a mean follow-up of 42.2 months. The shortest period was reported by Russo *et al.* (4), the occurrence of the thrombotic event being at the time of SVR12. A unique case was reported by Garg H *et al.* (19) who described 2 clinical cases with acute nonmalignant PVT discovered during antiviral treatment in 2 patients who received treatment with sofosbuvir and ribavirin. The mechanism underlying these events was interpreted as a simple drug reaction, these antivirals exhibiting idiosyncratic (type B) drug reactions, but they are rare and unpredictable.

Various studies on the efficacy of DAA treatment have shown that achieving SVR in patients with viral hepatitis C cirrhosis was associated with improved MELD and Child-Pugh scores, which reflect improved liver synthesis function (20, 21, 22, 23), a hypothesis that was also confirmed in our study. At the initial assessment, of the 35 patients with PVT post-SVR, almost half (45.75%) were in Child C, one third were in Child B (31.4%), and the remaining 22.9% were part of the Child A. The situation improved along the way, so that at the end of the treatment only one patient (2.9%) remained classified in the Child C, the others being classified in equal percentages in the Child A classes and B, evolution that remained favorable also at SVR12. The results obtained in our study are also confirmed by Gentile *et al.* (24) in an Italian multicenter prospective study of 89 patients with decompensated HCV and responders to antiviral treatment, in whom the rate of change to Child A was 62% 24 weeks after obtaining SVR.

In the present study, the process was also similar to the evolution of the MELD score: at the initial assessment, the proportions of patients with a MELD score below the threshold value of 15 and above it were relatively balanced, with a slight superiority for patients with a MELD score < 15 (57, 1%). At the EOT, the situation improved significantly, only one patient (2.9%) remained with the MELD score > 15, a situation that remained favorable at the time of SVR12. In the SOLAR-1 and SOLAR-2 studies, patients with genotype 1 or 4 decompensated HCV cirrhosis who received DAA showed improvement in both MELD and Child-Pugh (25, 26). Similar results were also reported by Poordad F *et al.* (27) in the ALLY-1 study, where all patients with decompensated cirrhosis and SVR, regardless of genotype, showed improvement in liver function. On the other hand, Marco and Calvaruso showed that in patients with decompensated cirrhosis (ascites, hepatic encephalopathy, bleeding from variceal rupture) treated with antivirals, obtaining SVR does not improve liver dysfunction or mortality (28).

Similarly, in the case of the variation in the degree of liver fibrosis, a similar process was observed, with a significant decrease in Fibroscan values at the SVR assessment, with the same downward trend at 48 weeks and 3 years, but with higher values >12 kPa. These results attest to the fact that there is a risk of thrombotic complications in the portal system even after achieving SVR, the persistence of portal hypertension favoring intravascular blood stasis being considered a key element in the occurrence of portal vein thrombosis (29, 30,

31, 32, 33, 34).

Regardless of whether SVR implies improvement of the liver function, the highest risk of non-tumoral PVT remained for patients with advanced liver disease (Child C). The results of our study are confirmed by Mandorfer *et al.* who observed that an elevated Child-Pugh score was the only independent risk factor for the development of non-malignant PVT post-SVR, with patients with a Child A score having the lowest risk (5).

Among the qualitative parameters evaluated as risk factors for the occurrence of non-malignant PVT in patients with HCV liver cirrhosis treated with antivirals, the presence of type II diabetes, ascites, hepatic encephalopathy and the decompensated status of liver disease reached the threshold of statistical significance. Quantitative parameters correlated as risk factors with non-malignant PVT in the studied group were increased BT and MELD score values, low albumin values, but also the persistence of an increased degree of post-SVR liver fibrosis. Age over 65 years, male gender, obesity and NSBB treatment were not risk factors in the study group. Stine *et al.* mentioned that patients with HCV cirrhosis treated with antivirals have a high risk of developing non-malignant PVT post-SVR, in the context where the 7 patients who developed a thrombotic complication were with Child A (8). This risk is independent of the value prothrombin time, INR or platelet count, the risk of PVT being due rather as a reaction to an overcorrection of an already fragile coagulation balance in the cirrhotic patient.

CONCLUSIONS

Achieving SVR decreases the risk of decompensation among cirrhotic patients, but the risk for thrombotic events still remains. The severity of the liver damage is a risk factor for the occurrence of nonmalignant PVT post-SVR, the majority of patients who developed this complication being Child-Pugh C score. The persistence of liver fibrosis with higher values >12 kPa after obtaining SVR demonstrates the persistence of portal hypertension and implicitly, an increased risk for the occurrence of nonmalignant PVT, which is why these patients must continue to be carefully monitored according to the protocol. Future research should focus on personalizing post-SVR care based on individual risk of complications.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest.

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FACTOR XIII V34L POLYMORPHISM IN A POPULATION OF PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA FROM NORTH-EASTERN ROMANIA

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FACTOR XIII V34L POLYMORPHISM IN A POPULATION OF PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA FROM NORTH-EASTERN ROMANIA (Abstract): Familial hypercholesterolemia (FH) is one of the most frequent inherited genetic disorders of the cholesterol metabolism that has an autosomal dominant or multifactorial inheritance. Factor XIII is also called the factor that stabilizes the fibrin, being an enzyme that makes the bond between the fibrin molecules during the clot formation. The gene for factor XIII is localized on 6p24-25 and can suffer a substitution in 103G>T position (V34L polymorphism), that can decrease the stability of the clot, offering protection for thromboembolic events and a decreased risk for myocardial infarction and coronary artery disease. **Materials and methods:** A cross-sectional study was conducted in the Cardiovascular Rehabilitation Clinic from the Clinical Rehabilitation Hospital from Iasi from 1st December 2020 to 31st of March 2022, included 70 patients diagnosed with certain FH based on the Dutch Lipid Clinical Network score (DLCN) and 20 patients in the control group. **Results:** The homozygous patients had the highest values of the corrected LDL-C and the heterozygous patients had the lowest total bilirubin concentrations. The patients included in the control group that had the homozygous variant had the lowest total cholesterol and non-HDL concentrations. **Conclusions:** This approach enables the implementation of targeted management and treatment approaches to effectively mitigate the risks associated with this condition. The costs of genetic testing decrease over time, the significance of this information is likely to increase even further. **Keywords:** FAMILIAL HYPERCHOLESTEROLEMIA, FACTOR XIII V34L POLYMORPHISM, LIPID PROFILE.

World Health Organization attributes 17.9 million deaths each year to cardiovascular diseases, being thus the leading cause of death worldwide (1). Atherosclerosis is clinically manifested by pectoral angina, stroke, coronary syndrome, peripheral ar-

tery disease, being the central element in the development of cardiovascular pathologies (2).

Familial hypercholesterolemia (FH) is one of the most frequent inherited genetic disorders of the cholesterol metabolism that has an autosomal dominant or multifactorial inheritance (3). Patients might have either a homozygous or a heterozygous genotype, this influencing the severity of the disorder and the age of the cardiovascular disease manifestations (4). Mutations in the LDL-R, APOB, and PCSK9 genes cause the development of the autosomal dominant form. Because of lifelong exposure to very high levels of low-density lipoprotein cholesterol (LDL-C), the patients with FH are often diagnosed after a major cardiovascular event (5, 6).

Primary prevention is extremely important in these patients, due to the fact that more than 90% of the patients with FH from the United States of America have been diagnosed only after a major cardiovascular event (7).

Factor XIII is also called the factor that stabilizes the fibrin, being an enzyme that makes the bond between the fibrin molecules during the clot formation (8). Studies demonstrate that a mutation found in the gene for factor XIII is considered as a protective factor for cardiovascular events. The enzyme is formed from two subunits A and two subunits B (that transport the subunits A in the plasma), factor XIII being activated through the rupture of the N-terminal end of the units A by thrombin and dimer dissociation from the B subunits in the presence of calcium (9). The activated factor XIII makes a bond between the fibrin chains α and γ and between α2-antiplasmin and fibrin, conferring mechanical and proteolytical resistance to the clot. The gene for factor XIII is localized on 6p24-25 and can suffer a substitution in 103G>T position (V34L polymorphism), that can decrease the stability of the clot, offering protection for thrombo-embolic events and a decreased risk for myocardial infarction and coronary artery disease (10, 11). The polymorphism increases the thrombin activation and changes the structure of the fibrine, effect that is correlated with the blood fibrinogen concentration. This mechanism is worth describing it, as in patients with normal fibrinogen concentrations, the polymorphism creates clots in with thinner fibers and less permeability, but it forms thrombi with larger fibers and higher permeability and susceptibility to fibrinolysis in patients with high concentrations of fibrinogen. Because abnormal fiber thickness and altered clot permeability and stability are associated with increased thrombosis risk, these findings suggest that in population studies, both FXIII genotype and plasma fibrinogen concentration should be considered when calculating thrombosis risk (12).

MATERIALS AND METHODS

We performed a cross-sectional study that was conducted in the Cardiovascular Rehabilitation Clinic from the Clinical Rehabilitation Hospital Iasi between 1st December 2020 to 31st of March 2022. We included 70 patients diagnosed with certain FH based on the Dutch Lipid Clinical Network score (DLCN). The inclusion criteria consisted of patients with a DLCN score more than 8 points that signed the informed consent. We excluded all the patients that had secondary causes of hypercholesterolemia, such as patients diagnosed with severe chronic kidney disease or nephrotic syndrome, severe liver disease, hypothyroidism, uncontrolled diabetes mellitus (DM) or patients with a fatty diet. The

group of patients with HF were compared with a control group that included 20 patients with normal lipid profile.

One of the criteria used in the DLCN score is the LDL-C concentration. In our study, we adjusted the value for the patients that had already in the last six months in their medical treatment statins or ezetimibe. Haralambos *et al.* described this correction factor, based on a review that included 71 original articles collected before the establishment of the familial hypercholesterolemia criteria (13).

The anamnesis and physical examination were conducted by a single researcher, that included the patients' medical history, the blood pressure and heart rate, along with the patients' drug history (including lipid lowering therapy). Also, the investigator recorded the blood tests, such as liver function tests, creatinine, calcium, alkaline phosphatase and lipid profile (total cholesterol, LDL, HDL, non-HDL, triglycerides).

DNA was prepared from peripheral blood cells using PureLink® Genomic DNA Kit (Thermo Fisher Scientific). We assessed relevant mutations in FXIII V34L by reverse hybridization using CVD StripAssay kit (ViennaLab Diagnostics, Vienna, Austria).

After the amplification by the polymerase chain reaction (PCR) with biotinylated primers, the PCR products were directly hybridized on the StripAssay® test strips, that contain allele-specific oligonucleotide probes immobilized as parallel lines. Afterwards, the mutant and/or wild type alleles were visualized through an enzymatic color reaction that was visible on the strip.

Positively strained lines were analyzed through the online tool StripAssay® Online Calculator (ViennaLab Diagnostics) and for the gene was analyzed the underlying genotypes.

All the patients signed the informed consent that were included in the study and the ethics committee approval was obtained from both "Grigore T. Popa" University of Medicine and Pharmacy Iasi (certificate of approval dated 15th June 2020) and Clinical Rehabilitation Hospital from Iasi (certificate of approval dated 25th November 2020).

The data analysis was realized using *SPSS 20.0* (Statistical Package for the Social Sciences, Chicago, Illinois). When evaluating the continuous variables, the data were presented as mean ± standard deviation or as a median with interquartile range. When evaluating the categorical variables, the data were presented as the number of cases with percent frequency. We evaluated also the distribution of the variables. When we compared continuous normally distributed variables, we used the Independent Samples t-Test in case of two samples and in case of comparisons of more than two samples we used One-Way ANOVA test. When categorical comparisons were made, we used Chi-square test or Fisher's exact test. In the situations where the distribution of the continuous variables did not satisfy the assumption of normality, the Mann-Whitney U test was performed. When we evaluated the correlations between continuous variables, Spearman correlation coefficient was calculated. A statistical significance was considered at a two-sided p-value less than 0.05.

RESULTS

For our research, we enrolled 70 patients diagnosed clinically with familial hyper-cholesterolemia that were compared with 20 healthy controls. The mean age of the patients diagnosed with FH was significantly higher, but both groups included mostly middle-aged adults. The living environment of both groups was mostly urban.

There were no statistically significant differences regarding the alcohol consumption or the smoking status, but it is worth mentioning that none of the patients from the control group did not smoke more than 10 packs year. Regarding the comorbidities, the patients with FH had significantly more associated diseases compared to the control group. All the above can be observed in first table.

TABLE I.
General characteristics of the studied groups

General characteristics	HF group (n = 70)	Control group (n = 20)	p
Gender n (%)			
Male	29 (41.4%)	7 (35.0%)	0.796
Female	41 (58.6%)	13 (65.0%)	
Living environment. n (%)			
Rural	18 (25.7%)	6 (30.0%)	0.776
Urban	52 (74.3%)	14 (70.0%)	
Age. years (mean ± SD)	54.65 ± 12.81	41.70 ± 13.51	<0.001
BMI. kg/m^2 (mean ± SD)	28.75 ± 4.96	24.95 ± 6.19	0.005
Alcohol consumption	35 (50%)	8 (40%)	0.459
Hypertension	48 (68.6%)	3 (15%)	< 0.001
Myocardial infarction	7 (10%)	3 (15%)	0.686
Chronic ischemic heart disease	12 (17.1%)	0 (0%)	absent
Atherosclerotic cardiovascular disease	36 (51.4%)	3 (15%)	0.004
Basal hyperglycemia	29 (41.4%)	4 (20%)	0.114
Creatinine, mg/dL	0.99 (0.90;1.09)	0.94 (0.88;1.02)	0.246
Ca, mg/dL	9.23 (8.96;9.45)	9.00 (8.75;9.38)	0.020
TGO, UI/L	19.85 (15.92;27.90)	17.35 (13.75;21.55)	0.113
TGP, UI/L	20,20 (16,02;32,95)	17,50 (15.77;27,92)	0,455
Bilirubin, mg/dL	0,53 (0,43;0,70)	0,53 (0,44;0,69)	0,988
Total cholesterol, mg/dL	278,00 (249,50;310,25)	201,00 (173,80;223.75)	0,000
HDL cholesterol, mg/dL	57,82 ± 16,52	46,56 ± 10,24	0,000
Non-HDL cholesterol, mg/dL	221,25 (186,45;253,42)	160,55 (132,30;176,60)	0,000
LDL cholesterol, mg/dL	196,02 ± 47,02	126,17 ± 40,40	0,000
LDL cholesterol corrected, mg/dL	296,29 (224,84;389,75)	130,75 (97,10;155,87)	0,000
Triglycerides, mg/dL	164.70 (104.82;227.57)	100.30 (93.47;144.60)	0.007

The studied polymorphism in the two groups was FXIII V34L. In the group of patients with FH, the cardiovascular and metabolic diseases were observed especially in the ones that had the wild type genotype. When evaluating their biological profile, we observed that they had the highest values of the corrected LDL-C in the homozygous patients, a statistically significant differentiation between them and the patients that had wild type or homozygous genotypes. Moreover, the heterozygous patients had the lowest total bilirubin concentrations, being observed statistical significance between them and the homozygous ones (tab. II).

TABLE II.

Correlations between the polymorphism of Factor XIII V34L and clinical and paraclinical parameters

	Wild Type	Heterozygous	Homozygous	p_1	p_2	p_3
FXIII V34L						
Hypertension	28 (66.7%)	20 (76.9%)	0 (0%)	0.422	absent	absent
Myocardial infarction	6 (14.3%)	0 (0%)	1 (50%)	absent	0.296	absent
Chronic ischemic heart disease	9 (21.4%)	2 (7.7%)	1 (50%)	0.184	0.407	0.062
Basal hyperglycemia	19 (45.2%)	8 (30.8%)	2 (100%)	0.310	0.222	0.049
Atherosclerotic cardiovascular disease	18 (42.9%)	7 (26.9%)	1 (50%)	0.208	1.000	0.497
Alcohol consumption	19 (45.2%)	16 (61.5%)	0 (0%)	0.220	absent	absent
Smoking	15 (35.7%)	10 (38.5%)	0 (0%)	1.000	absent	absent
Heart rate	68.50 (64.00;72.00)	69.00 (63.00;75.00)	63.00 (62.00;64.00)	0.719	0.171	0.296
Calcium	9.22 (8.91;9.45)	9.23 (9.04;9.46)	8.99 (8.98;9.01)	0.631	0.414	0.222
Creatinine	1.00 (0.90;1.08)	0.98 (0.90;1.16)	0.90 (0.90;0.90)	0.830	0.256	0.296
TGO	20.75 (15.70;29.90)	18.90 (16.20;26.20)	16.35 (14.80;17.90)	0.441	0.357	0.339

	Wild Type	Heterozygous	Homozygous	p_1	p_2	p_3
FXIII V34L						
TGP	19.80 (16.10;45.40)	19.90 (15.80;30.10)	27.45 (20.60;34.30)	0.623	0.611	0.381
Total cholesterol	276.00 (243.00;298.00)	285.00 (251.00;317.00)	314.30 (303.60;325.00)	0.233	0.171	0.339
LDL	193.65 (168.40;218.00)	200.05 (175.00;233.90)	217.40 (211.00;223.80)	0.520	0.279	0.529
Corrected LDL	308.54 (225.50;382.60)	249.05 (213.90;366.96)	454.62 (402.84;506.40)	0.256	0.042	0.032
HDL	59.10 (45.40;72.30)	55.30 (48.90;70.80)	44.40 (22.80;66.00)	0.776	0.611	0.476
Non-HDL	208.50 (184.70;243.60)	227.35 (193.80;255.80)	269.90 (259.00;280.80)	0.189	0.089	0.132
Triglycerides	152.10 (103.10;205.00)	185.35 (119.80;245.00)	292.00 (201.50;382.50)	0.246	0.118	0.381
Total bilirubin	0.55 (0.44;0.71)	0.55 (0.44;0.67)	0.34 (0.32;0.37)	0.810	0.104	0.032

P1 – statistical significance between wild type and heterozygous; p2 – statistical significance between wild type and homozygous; p3 – statistical significance between heterozygous and homozygous.

The patients included in the control group that had the homozygous variant had the lowest total cholesterol and non-HDL concentrations. existing statistically significant differences between these parameters and those of the wild type patients. Another aspect that can be observed in Table III is the fact that the patients diagnosed with FH had lower heart rates and higher liver function tests (TGO. TGP) compared to the ones from the control group on each genetic genotype.

TABLE III.

Correlations between the polymorphism of Factor XIII V34L and clinical and paraclinical parameters in the control group

	Wild Type	Heterozygous	Homozygous	p_1	p_2	p_3
FXIII V34L – control						
Hypertension	2 (18.2%)	1 (14.3%)	0 (0%)	1.000	absent	absent
Myocardial infarction	2 (18.2%)	1 (14.3%)	0 (0%)	1.000	absent	absent

	Wild Type	Heterozygous	Homozygous	p_1	p_2	p_3
FXIII V34L – control						
Chronic ischemic heart disease	0 (0%)	0 (0%)	0 (0%)	absent	absent	absent
Basal hyperglycemia	3 (27.3%)	1 (14.3%)	0 (0%)	1.000	absent	absent
Atherosclerotic cardiovascular disease	2 (18.2%)	1 (14.3%)	0 (0%)	1.000	absent	absent
Alcohol consumption	4 (36.4%)	3 (42.9%)	1 (50%)	1.000	1.000	1.000
Smoking status	2 (18.2%)	3 (42.9%)	1 (50%)	0.326	0.423	1.000
Heart rate	72.00 (69.50;73.00)	75.00 (69.50;77.00)	78.00 (78.00;78.00)	0.596	0.103	0.333
Calcium	9.07 (8.81;9.38)	8.87 (8.53;9.10)	9.25 (8.98;9.52)	0.328	0.641	0.333
Creatinine	0.89 (0.86;1.01)	0.96 (0.93;1.05)	1.11 (1.01;1.22)	0.211	0.154	0.222
TGO	18.60 (13.90;24.10)	17.90 (15.90;21.50)	14.45 (12.20;16.70)	0.791	0.410	0.333
TGP	17.20 (14.85;30.30)	17.80 (17.20;23.25)	17.55 (11.00;24.10)	0.536	0.641	0.667
Total cholesterol	213.00 (190.00;242.50)	194.00 (188.50;211.50)	142.50 (139.00;146.00)	0.285	0.026	0.222
LDL/corrected LDL	155.80 (113.50;159.85)	119.90 (117.10;145.50)	88.70 (84.70;92.70)	0.536	0.154	0.222
HDL	46.30 (39.00;58.10)	46.90 (43.85;51.45)	42.20 (40.30;44.10)	0.930	0.923	0.333
Non-HDL	172.80 (148.20;181.25)	140.20 (137.20;166.35)	100.30 (98.70;101.90)	0.328	0.026	0.222
Triglycerides	115.30 (93.65;138.50)	99.20 (96.15;101.80)	124.65 (44.60;204.70)	0.724	1.000	1.000
Total bilirubin	0.51 (0.45;0.62)	0.67 (0.41;0.72)	0.52 (0.48;0.56)	0.860	1.000	0.889

DISCUSSION

In our study, we observed that all homozygous patients with FH were diagnosed with basal hyperglycemia, existing a significant difference between them and the heterozygous ones, results that were not observed in the subjects included in the control group. Phasha *et al.* studied the factor XIII V34L polymorphism in diabetic patients in a South American population. The authors observed that the V34L allele was more frequent in the group of patients without diabetes compared to those diagnosed with this pathology. Thus, they describe that no significant correlations were observed between polymorphism and disease (14). Further studies on this topic will be necessary, which will include a larger number of patients with FH, to establish a definite correlation between the two.

Evaluating the lipid profile of the two groups, we observe that the homozygotes with FH have the highest total cholesterol, LDL, corrected LDL, and non-HDL, while in the control group they had the lowest serum concentrations when compared to the wild type or the heterozygotes. Researchers have shown in a recent study that an increase in factor XIII activity is associated with a higher risk of developing myocardial infarction in young patients, but this activity does not correlate with the V34L variant. They also evaluated several haplotypes of the factor XIII gene and demonstrated that there were no significant differences between the associated polymorphisms and the lipid profile of the patients (15).

The results described by Ambroziak *et al.* are consistent with those of the control group, but not with those of the FH patient group. This fact may be due to the increased serum concentrations of their lipid profile, homozygous patients diagnosed with FH requiring more careful monitoring, but this observation will have to be confirmed later by studies involving a larger group of patients.

The present study is a princeps one, the first in our country, to our knowledge. The results obtained so far are to be completed by integrating a larger number of patients, in a multicenter study.

CONCLUSIONS

Familial hypercholesterolemia is frequently undertreated, resulting in a lack of progress in meeting treatment objectives and an elevated risk of coronary heart disease (CHD). It also serves as an exemplary case for precision medicine, as it seeks to identify through genetic diagnosis a specific subset of individuals who are at high risk of experiencing significant illness and mortality. This approach enables the implementation of targeted management and treatment approaches to effectively mitigate the risks associated with this condition.

National databases and special registries would serve as valuable tools for clinical research and enhancing healthcare planning and patient care. Furthermore, as the cost of genetic testing decreases over time, the significance of this information is likely to increase even further.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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CHRONIC HEPATITIS B INFECTION IN PEDIATRIC POPULATION: A RETROSPECTIVE STUDY FROM A TERTIARY CENTER IN NORTH-EASTERN ROMANIA

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CHRONIC HEPATITIS B INFECTION IN PEDIATRIC POPULATION: A RETROSPECTIVE STUDY FROM A TERTIARY CENTER IN NORTH-EASTERN ROMANIA (Abstract): Chronic viral hepatitis B is the precursor stage of cirrhosis and the 10th leading cause of death worldwide. WHO estimated in 2019 that approximately 296 million people were chronically infected with the hepatitis B virus and over 800,000 deaths. **The aim of study** was to describe characteristics of chronic HBV infection in pediatric population from a tertiary center in North-Eastern Romania. **Materials and Methods**: A retrospective study was conducted on 148 pediatric patients with chronic HBV infection who had visits from 2011 to 2019 to St. Mary's Hospital Iasi (Romania). The HVB infection in study group was analyzed regarding demographic characteristics, distribution of pediatric patients in relation to vaccination status, risk factors, HBV infection stage, and clinical manifestations. The qualitative variables were characterized through frequencies distributions. The quantitative variables were characterized through descriptive statistics (averages, standard deviations). $P < 0.05$ was the threshold for significance. **Results**: Most of the patients (87,83%) had vertical transmission, while 18.91% had horizontal transmission. Most of the pediatric population was vaccinated (87.83%), while 11.48% had incomplete vaccination. The most common risk factor was vertical transmission (81.8%), followed by horizontal transmission, surgical interventions (10.13%), frequent hospitalizations (4.1%), and dental procedures (3.4%). Among patients with HBV infection, most of them presented loss of appetite (16.2%), followed by abdominal pain (12.8%), hepatomegaly (12.2%), and physical asthenia (4.1%). **Conclusions**: Most children from our study were in chronic hepatitis B phase and immune-tolerant phase. Pediatric patients with chronic viral B infection can experience a slow progression of the disease without specific signs and symptoms for a long period, especially when the diagnosis is made at an infant or young child age. **Keywords**: CHILDREN, HEPATITIS B VIRUS, VACCINATION, TRANSMISSION.

Hepatitis B represents a significant health challenge worldwide, characterized by high rates of morbidity and death. Annually, 1.5 million new cases of HBV infection emerge, despite the effectiveness of vaccination and the potential to curb viral replication and spread through medication (1). It is estimated that the global preva-

lence of HBV infection in children up to 5 years of age is 1.3%, significantly lower compared to the 1980-2000 period (4.7%) (2). However, despite increased access to immunization, about 2 million new HBV infections are recorded each year in the 0-5 years age group (3). Given that the majority of HBV infections occur through vertical transmission from mother to child during pregnancy or childbirth, there is an increased risk (over 90%) of chronic HBV infection (over 6 months). HBV infection can progress to hepatic fibrosis, cirrhosis, and hepatocellular carcinoma, although pediatric patients are rarely diagnosed with these conditions (4). However, approximately 3-5% of children with chronic HBV infection develop cirrhosis, while 0.01-0.03% develop hepatocellular carcinoma (5). In the initial stage of HBV infection, typically contracted at birth, the virus shows high replication levels. This is characterized by the presence of HBeAg and extremely high HBV DNA concentrations in the blood, often exceeding 100 million or even 1 billion IU/mL. This stage, known as the HBV infection HBeAg positive (immune-tolerance phase), usually persists for a period ranging from 10 to 30 years. During this time, the likelihood of naturally eliminating HBeAg from the body is quite low. Statistically, after two decades of being infected, the cumulative probability of spontaneously losing HBeAg is about 2% in the first three years, increasing to only 15% after 20 years (6). The second phase of HBV infection, hepatitis B HBeAg positive (often referred to as the immune-clearance or immune-active phase), typically emerges in the third or fourth decade of the disease in individuals who contracted HBV early in life. This phase is marked by the continued presence of serum HBeAg, a gradual reduction in HBV DNA levels, and either increased or variable ALT levels, indicative of HBeAg positive chronic hepatitis. Some patients may undergo multiple exacerbations characterized by temporary loss of HBeAg and periodic elimination of HBV DNA from the blood. In such cases, the recurring episodes of hepatitis increase the likelihood of developing cirrhosis and hepatocellular carcinoma. HBeAg negative hepatitis is identified by the absence of HBeAg in the serum, often accompanied by the presence of anti-HBe antibodies. This condition also features ongoing or variable serum HBV DNA levels that are generally lower than those found in HBeAg positive patients, along with fluctuating or consistently high ALT levels (7). HBeAg negative hepatitis is identified by the absence of HBeAg in the serum, often accompanied by the presence of anti-HBe antibodies. This condition also features ongoing or variable serum HBV DNA levels that are generally lower than those found in HBeAg positive patients, along with fluctuating or consistently high ALT levels (7). HBeAg negative chronic HBV infection, also known as inactive hepatitis phase, is characterized by the presence of antibodies against HBeAg, HBV DNA levels that are undetectable or low (below 2000 IU/mL), and normal ALT levels as defined by standard cutoff values (the upper limit of normal [ULN] is approximately 40 IU/L). Despite having normal serum ALT levels, patients with HBeAg negative chronic infection may still experience histologic inflammation and/or fibrosis (8).

AIM of study was to describe characteristics of chronic HBV infection in pediatric population from a tertiary center in North-Eastern Romania.

MATERIALS AND METHODS

A retrospective study was conducted on 148 pediatric patients with chronic HBV

infection, with at least one medical visit to St. Mary's Hospital Iasi (Romania), from January 1, 2011 to December 31, 2019.

Chronic HBV infection was defined as the presence of hepatitis B surface antigen (HBsAg) for at least 6 months.

The inclusion criteria were:

- age from 0 to 18 years at their initial pediatric visits with the diagnosis of chronic HBV infection;

-the presence of hepatitis B surface antigen (HBsAg) for at least 6 months.

Each patient's demographic characteristics, clinical history, family history of immediate family members, prior HBV vaccination, and laboratory values were extracted from patient charts. Characteristics of HBV vertical transmission were as follows: mother' age and occupation, comorbidities, maternal HBeAg status, type of delivery. Patient charts were reviewed for HBV vaccination and receipt of hepatitis B immunoglobulin (HBIG). The phases of chronic HBV infection were analyzed cross-sectionally according to the following laboratory values: HBeAg status, anti-HBe status, ALT, and HBV DNA levels. The characteristic was based on the American Association for the Study of Liver Diseases (AASLD) hepatitis B guidance 2018 (9). The HVB infection in study group was analyzed regarding demographic characteristics, distribution of pediatric patients in relation to vaccination status, risk factors, HVB infection stage, and clinical manifestations

Statistical analysis. The statistical analyses were performed in SPSS 29.0. The qualitative variables were characterized through frequencies distributions. The quantitative variables were characterized through descriptive statistics (averages, standard deviations). $P < 0.05$ was the threshold for significance.

RESULTS

Table I exposes demographic characteristics of the study population. On age groups, the distribution was as follows: 0-1 yrs. (20.27%), 2-5 yrs. (25.35%), 6-10 yrs. (22.97%), 11-14 yrs. (18.91%), 15-18 yrs. (11.48%). Regarding gender, 52.02% were boys and 47.97% were girls. Regarding the birth type, 80,4% were born naturally and 19.59% were born through C-section with overall median weight at birth 3,200 g. Most of the patients (81.08%) had vertical transmission, while 18.91% had horizontal transmission. Most of the pediatric population was vaccinated (87.83%), while 11,48% had incomplete vaccination. Most of the patients (72.29%) were breastfed, while 27.7% were bottle-fed. Patients from low socioeconomic families (rural provenience) (61.48%) predominated over patients from urban families (38.51%).

Distribution of pediatric patients in relation to vaccination status and birth weight is exposed in figure 1 (93.2% were children with $\geq$ 2,500 g, 4.7% were between 1,500-2,500 g, and 2.0% were < 1,500g) (fig. 1).

According to the study data, 3 children weighed < 2,000 g and 7 children between 1500 and 2,000 g. Among those with LBW (Low Birth Weight), only one was born to a mother with HBV infection, and 2 of them did not have a complete vaccination schedule, meaning they were either not vaccinated by the time of discharge from the maternity hospital or at the age of one month. For those born weighing less than 2,500 g, all were vaccinated. The viral status of the mothers at the time of birth is unknown (tab. II).

The distribution of patients from HBsAg positive mothers. The frequency of administering hepatitis B immunoglobulin to children from HBsAg positive mothers is presented in Fig. 2 (3.4% - received hepatitis B immunoglobulin; 35.1% - did not

receive; 61.5% - unspecified).

The distribution of patients according to risk factors is presented in Table II. It is noted that the most common risk factor is vertical transmission (81.8%), followed by horizontal transmission, surgical interventions (10.13%), frequent hospitalizations (4.1%), and dental procedures (3.4%).

TABLE I.

Demographic characteristics of the pediatric population

Characteristics		Patients (148)
Age (years)	0-1 2-5 6-10 11-14 15-18	30 (20.27%) 39 (25.35%) 34 (22.97%) 28 (18.91%) 17 (11.48%)
Gender	M F	77 (52.02%) 71 (47.97%)
Birth	Natural C-section	119 (80.40%) 29 (19.59%)
Transmission	Vertical Horizontal	121 (81.08%) 27 (18.91%)
Vaccination	Yes No Incomplete	130 (87.83%) 1 (0.67%) 17 (11.48%)
Alimentation in the 1st year of life	Breastfed Bottle-fed	107 (72.29) 41 (27.70%)
Provenience	Urban Rural	57 (38.51%) 91 (61.48%)

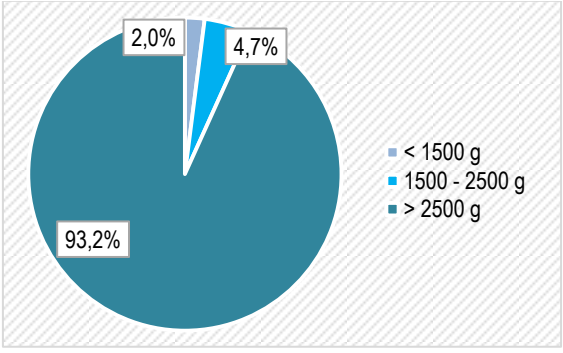

Fig.1. Distribution of pediatric patients in relation to vaccination status and birth weight

TABLE II.

Vaccination status related to birth weight

		Birth weight						Total	
		< 1,500 g		1,500 – 2,500 g		➢ 2,500 g			
		N	%	N	%	N	%	N	%
		Pearson Chi square = 9.333 / p = .053							
Vaccinated	Yes	1	33.3%	6	85.7%	123	89.1%	130	87,8%
	Incomplete	2	66.7%	1	14.3%	14	10.1%	17	11,5%
	No	0	0.0%	0	0.0%	1	0.7%	1	0,7%
Total		3	100,0%	7	100.0%	138	100.0%	148	100.0%

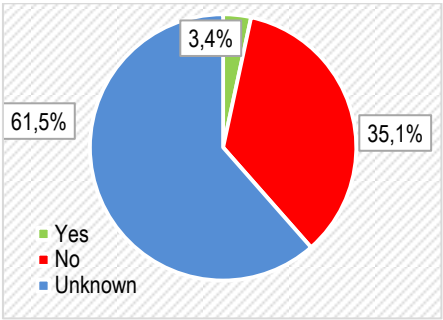

Fig. 2. Ig antihepatitis B administration to children from Ag HBs positive mothers

TABLE III.

Risk factors in HBV infection

	n	%
Tonsillectomy	4	2,7
Tonsillectomy	2	1,4
Dental procedures	5	3,4
Appendectomy	2	1,4
Fractures	2	1,4
Frequent hospitalizations	6	4,1
Frenectomy	1	,7
Other surgical interventions	2	1,4
Laceration on ring finger	1	,7
Polypectomy	1	,7
Intrafamilial transmission	3	2,0
Vertical transmission	121	81,8
Horizontal transmission	27	18,2
Blood transfusions	1	,7
Total	148	100,0

Among the patients, all phases of HBV infection were detected, with the majority (49.3%) being diagnosed in the phase of chronic viral hepatitis B Ag HBe +, followed by HBV infection Ag HBe + in 27.7% of children. Generally, the second phase, that of immunotolerance, predominates in the pediatric age, but in our group, the majority had hepatocytolysis syndrome over x2 VN (normal value) (fig. 3).

Out of a total of 148 children, 19.6% presented with anti-HBe antibodies at the time of diagnosis. Spontaneous seroconversion was predominantly recorded in those with chronic HBeAg- infection (73.9%), and 81.8% of those with chronic HBeAg- hepatitis were positive for anti-HBe antibodies. It is known that seroconversion in the HBe system is more common in the pediatric population than in adults (tab. IV).

The clinical manifestations recorded in the pediatric population with HBV infection are presented in Table V. Among patients with HBV infection, most of them presented loss of appetite (16.2%), followed by abdominal pain (12.8%), hepatomegaly (12.2%), and physical asthenia (4.1%). Other nonspecific signs were rec-

orded such as arthralgia (2%), headache (2%), dysphagia (1.4%), or weight stagnation (1.4%). Other possible clinical manifestations were also sub febrility, sleep disturbances, or scleral and skin sub icterus.

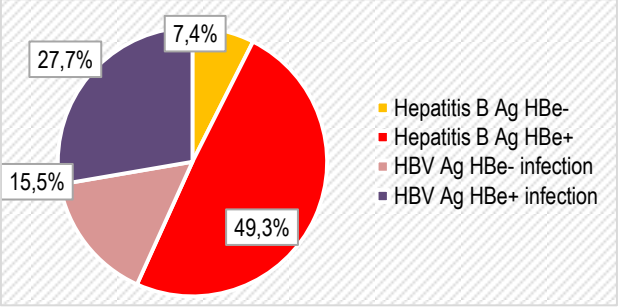

Fig. 3. Distribution of pediatric population related to stage of HVB infection

TABLE IV.

Distribution of pediatric patients related to Ac anti HBe to diagnostic time

		HBV infection stage to la DG								Total	
		VHB Ag HBe+ infection		VHB Ag HBe- infection		VHB Ag HBe+ hepatitis		VHB Ag HBe- hepatitis			
		N	%	N	%	N	%	N	%	N	%
		Pearson Chi-square = 91.130 / p <.001**									
Anti HBe Ab at diagnostic time	negative	39	95.1%	6	26.1%	72	98.6%	2	18.2%	119	80,4%
	positive	2	4.9%	17	73.9%	1	1.4%	9	81.8%	29	19,6%
Total		41	100.0%	23	100.0%	73	100.0%	11	100.0%	148	100.0%

TABLE V.

Clinical manifestation in pediatric population with HBV infection

	n	%
Nausea/vomiting	6	4.1
Loss of appetite	24	16.2
Physical asthenia	6	4.1
Semi-formed stools	6	4.1
Abdominal pain:	19	12.8
- diffuse abdominal pain	11	7.4
- right hypochondrium pain	7	4.7
- epigastric pain	1	.7
Hepatomegaly	18	12.2
Odynophagia	8	5.4
Cough	10	6.8
Rhinorrhea / pharyngeal congestion:	10	6.8
- pharyngeal congestion	3	2.0
- nasal obstruction	2	1.4
- rhinorrhea	5	3.4

	n	%
Nosebleed	6	4.1
Fever	7	4.7
Fatigability	5	3.4
Other:	26	17.6
psychomotor agitation	1	.7
arthralgia	3	2.0
headache	3	2.0
lipothymic states	1	.7
constipation	1	.7
dysphagia	2	1.4
dysphonia	1	.7
overweight	1	.7
jaundice	1	.7
menometrorrhagia	1	.7
menorrhagia	1	.7
metrorrhagia	1	.7
pallor	1	.7
paresthesia of extremities	1	.7
rectal bleeding	2	1.4
weight stagnation	2	1,4
scleral icterus	1	,7
scleral-cutaneous icterus	1	,7
cutaneous icterus, sallow facies	1	,7

DISCUSSION

Although there are many studies that have investigated the risk factors for HBV infection in infants, data from Romania is very scarce and an urgent update is requested. In our country, the major route of HBV infection in children is the vertical transmission which despite universal immune prophylaxis, remains evident and is associated with maternal HBV DNA levels (10). Most children from our study were in chronic hepatitis B phase and immune-tolerant phase, 37.8% were without clinical manifestations, while 36.48% did not have liver enzymes increased.

The immunization schedule is dependent on the birth weight and the infectious status of the mother. All newborns weighing 2,000 grams or more from HBsAg-negative mothers are given the vaccine within the first 24 hours, while those weighing less than 2,000 grams can be vaccinated upon discharge or at the age of one month. If the mothers are HBsAg positive at birth, then the vaccine and HBIG are administered within the first 12 hours, regardless of the newborn's birth weight. If the immunological status of the mother is unknown, the vaccine is administered within the first 12 hours after birth to all newborns (11, 12). The fact that 11.48% patients were incomplete vaccinated and 1 didn't get any vaccines shows that there is a lack in family education regarding the immunization scheme especially since most of them (64.70%) are from urban area. Regarding the HBIG administration, unfortunately is not available in maternities and all the mothers must buy it at a quite high price, even if is indicated to all newborns to HBsAg positive mothers. So, a priority in providing immunoglobulin to children from positive mothers by the government is mandatory. Another aspect is the temporary absence of anti-hepatitis B vaccine in ma-

ternities, and some parents forget or delay the time of vaccination. Until 6 weeks of life is used only the monovalent vaccine. After this age is used the polyvalent vaccine in three doses, first at 6 weeks, the next two at 1 month between them. Also, vaccination is safe and indicated in pregnant women or other high-risk groups (13). Besides that, the evaluation of infants should be done at 9-15 months of life, to be sure that they developed an immune response. Usually those who didn't get antibodies are with low weight birth or other risk factors as chronic diseases, genetic factors, HIV coinfection.

It is already known that the most effective way to prevent vertical transmission of the hepatitis B virus is through the administration of the vaccine and hepatitis B immunoglobulin at birth, reducing the risk from 5-25% to 0.7-1.1% (14). However, the risk is particularly present if the mother was not monitored during pregnancy and did not receive the necessary treatment. In our group, only 5 patients received HBIG in the first hours after birth (5.4%), 52 did not receive it (35.1%), while for the remaining 61.5%, relatives stated they were unaware of this immunization or whether the newborn was immunized (15). The low percentage of HBIG administration indicates a lack of information among pregnant women, a deficient interdisciplinary relationship between the family doctor, obstetrician, neonatologist, and gastroenterologist, and the absence of a national program to ensure the availability of immunoglobulin in maternity wards. We observed a downward trend in those with chronic hepatitis Ag HBe+ in relation to age, with the most diagnoses being in infants and young children, then after the age of 2 years, the number of diagnosed cases gradually decreases until 18 years of age.

We found that most of risk factors, besides vertical transmission, are represented by surgical interventions. It is known that until now, the main mode of hepatitis B virus transmission was through infected blood via inadequately sterilized medical instruments (injections, manicure, cosmetic procedures, tattoos, intravenous drugs, piercing), but today there are disposable needles, blood screening for transfusion, and accessible protective equipment (16). In 3 of the patients, we noted familial transmission, knowing the father's positive history and the mother's negative for HBV infection. This can occur either using personal items (razor blades, toothbrushes, scissors, tweezers, sewing needles, glucose meters) or through close interpersonal contact (scratches, domestic accidents with cuts). Therefore, it is requested education of all family members about periodic HBV testing to identify and promptly treat any new cases, education on hygiene and preventive measures to reduce the risk of transmission and avoiding direct contact with the blood or open wounds of infected individuals without adequate protection measures.

It is known that most children are asymptomatic (17). Indeed, in our study, among those with HBeAg+ infection, 56.1% did not show specific signs and symptoms, the results are roughly the same for those with HBeAg- infection, and among those with chronic HBeAg+ hepatitis, 75.3% presented clinical symptoms and signs such as diffuse abdominal pain, in the right hypochondrium or epigastrium, nausea, vomiting, loss of appetite, and physical asthenia.

CONCLUSIONS

Most children from our study were in chronic hepatitis B HBeAg+ phase and chronic HBeAg+ infection phase (immunotolerant). Pediatric patients with chronic viral B infection can experience a slow

progression of the disease without specific signs and symptoms for a long period, especially when the diagnosis is made at an infant or young child age.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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CHALLENGES IN IMMEDIATE AND LONG-TERM POSTOPERATIVE MANAGEMENT IN PATIENTS WITH CORNEAL TRANSPLANT

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CHALLENGES IN IMMEDIATE AND LONG-TERM POSTOPERATIVE MANAGEMENT IN PATIENTS WITH CORNEAL TRANSPLANT (Abstract): Corneal transplantation is a procedure involving the partial or total replacement of a damaged cornea with a healthy one, serving multiple purposes such as improving visual acuity, for aesthetic reasons, and for tectonic support. The most common procedure used in our clinic is penetrating keratoplasty, which can be burdened with a series of intraoperative and postoperative complications**. The aim:** In this article, we will present our clinic's experience with patients who have undergone and been diagnosed with corneal pathologies through penetrating keratoplasty technique, and the medium and long-term therapeutic difficulties encountered by these patients from 2020 to 2024, at the Ophthalmology Clinic of "Sf. Spiridon" County Clinical Emergency Hospital Iasi. **Materials and methods**: Patients with a history of chemical burns to the cornea, herpetic infection, keratoglobus associated with lagophthalmos, chronic smokers, and those with advanced pseudoexfoliative glaucoma in one eye are included. **Results:** Among the postoperative complications, infections and rejection reactions can occur between 2 weeks and 8 months and are quite challenging to manage. Postoperative infections can be present due to non-conforming sutures, prolonged use of contact lenses, and systemic immunosuppression. Rejection reactions can occur as early as two weeks, with corneal vascularization in the host cornea increasing the risk of graft rejection. Early identification and prompt treatment of inflammation and early infection are facilitating factors in preventing graft rejection. **Conclusions:** Although, in terms of survival, corneal transplantation is the most successful transplant performed in humans, rejection is still the main cause responsible for graft failure. Keratoplasty involves a lengthy healing process; preoperative evaluation and dialogue with the patient are essential to understand the benefits, possible complications, and the need for prolonged treatment. **Keywords:** IMMEDIATE, LONG-TERM, POSTOPERATIVE MANAGEMENT, CORNEAL TRANSPLANT.

INTRODUCTION

Corneal pathologies represent a significant cause of blindness. Visual impairment is due to corneal opacification, keratoconus, Fuchs' endothelial dystrophy, corneal hydrops, marginal degeneration, sphe-

roidal degeneration, pseudophakic bullous keratopathy, other corneal dystrophies, and the failure of a previous graft. Once corneal clarity is lost, transplantation is the current therapeutic procedure with the aim of improving visual acuity (1, 2, 3, 4, 5). Corneal transplantation or keratoplasty is the most common and successful surgery performed worldwide to address a range of corneal pathologies. The first successful corneal allograft was performed by an Irish ophthalmic surgeon, Dr. Samuel Bigger, in 1835 and reported in the Dublin Journal of Medical Science in 1837. He described performing a corneal transplant on a blinded gazelle he encountered while held captive by a nomadic tribe on the outskirts of Cairo (3). However, the world's first human corneal transplant was performed in 1905 by an Austrian surgeon, Eduard Konrad Zirm, marking a significant milestone by performing the world's first full-thickness solid organ transplant (1, 3, 4, 5).

Relative to the number of transplants performed, in the UK, 4,504 corneal transplants were conducted in 2019, while in the USA, there were 85,601 corneas transplanted in the same year. The existence of corneal banks has facilitated such a large number. The USA, Sri Lanka, and Italy are among the few countries with a surplus of tissue donors for export, representing 85%, 9%, and 3% respectively of the total number of exported tissues. Despite the existence of these banks and tissue-exporting countries, at least 55.3% of the world's population does not have access to corneal transplant material (5,8). Corneal transplant procedures are generally classified as penetrating keratoplasty involving the complete removal of the cornea and partial lamellar corneal surgeries, which include anterior lamellar keratoplasty [superficial anterior lamellar keratoplasty (SALK), automated lamellar therapeutic keratoplasty (ALTK), and deep anterior lamellar keratoplasty (DALK)]. Endothelial keratoplasty [Descemet stripping automated endothelial keratoplasty (DSAEK) and Descemet membrane endothelial keratoplasty (DMEK)] broadly fall under this classification (4, 5, 6, 7, 8).

Corneal structure

The cornea is avascular and represents the 1/6 anterior of the outer layer of the eyeball, representing the eye's most important diopter. It is made of six different anatomical layers. The first layer is the epithelium, which consists of squamous cells. The second layer is Bowman's membrane, which has regenerative properties. Most of the cornea, the stroma, contains keratocytes and collagen lamellae that are densely distributed in the anterior part compared to the posterior stroma. Descemet's membrane provides a base for the endothelial cells, which play a key role in maintaining corneal transparency (1, 5, 9). The density of corneal endothelial cells (ECD) decreases progressively throughout life at an average rate of approximately 0.6%. During the first decade of life, the average ECD has been reported to be about 4,640, then it decreases to 2,970 by the age of 30 and continues to decline to 2,400 by the age of 80.

Various performed surgical techniques

Corneal transplantation involves the partial or total replacement of the corneal layers. Currently, the most common type of corneal transplantation is 'penetrating,' which involves grafting a full-thickness corneal button. However, partial thickness grafts or 'lamellar' transplantation are also performed in a significant number of patients. It is indicated for any process lead-

ing to the loss of corneal transparency with preserved neuroretinal integrity and optimal visual potential. Based on the indications, keratoplasties are recommended for therapeutic, tectonic, optical, or cosmetic purposes. Therapeutic keratoplasty is performed to remove the infectious portion of the cornea, mainly in cases of refractory or perforated infectious keratitis. Tectonic keratoplasty provides support and maintains the integrity of the globe. Optical keratoplasty aims to restore vision and has undergone various advancements over time, leading to improved visual quality and postoperative outcomes. Partial removal of the corneal layers, lamellar keratoplasty, has been used to treat anterior corneal scars, keratoconus, and requires a steeper learning curve (3, 4, 5). Rashmi Singh illustrates the types of surgical procedures based on the degree of corneal involvement (4) (fig. 1).

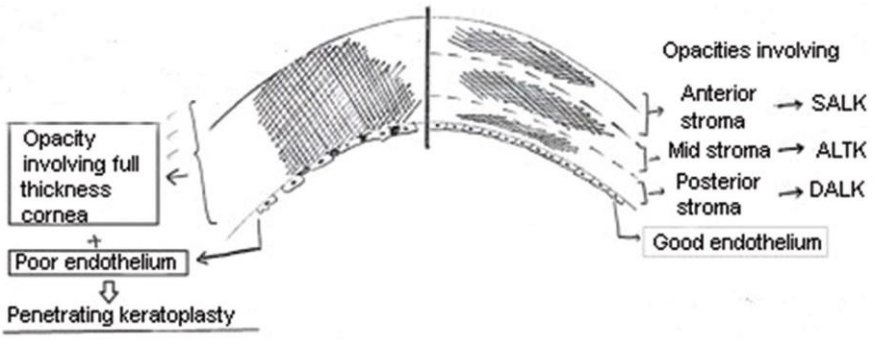

Fig. 1. Diagrammatic representation of the type of keratoplasty depending the on the level of corneal opacities along with endothelial function (Drawn by the authors based on the practical knowledge utilized for decision making in cases of corneal opacity). SALK, superficial anterior lamellar keratoplasty; ALTK, automated lamellar therapeutic keratoplasty; DALK, deep anterior lamellar keratoplasty.

Penetrating Keratoplasty (PK). Zirm successfully performed the first penetrating keratoplasty (PK) in 1905, and until recently, it was the most commonly used corneal transplant technique globally. Due to the rapid implementation of new approaches such as anterior lamellar keratoplasty, the proportion of PK grafts has decreased from 45% to 27% in the last 10 years in the UK. Currently, the most frequent indications are advanced keratoconus not amenable to DALK, tectonic grafts for acute ulcers or infectious keratitis, and the failure of a previous graft. Being the most common procedure for corneal transplantation, PK is generally a successful and efficient treatment for improving visual function. Studies show a graft survival rate ranging from 90% at 5 years to 70% in some studies at 10 years. Failure depends on several factors, primarily the loss of endothelial cells in the donor cornea, with studies reporting a progressive loss over a 10-year post-PK period, leading to late graft failure (1, 2, 4, 5, 9, 10, 11, 12, 13, 14).

Lamellar keratoplasty (LK). During this procedure, the cornea is removed either

superficially (SALK) or deeply (DALK). The clear benefits of this procedure include avoiding complications from open globe surgery (expulsive hemorrhage and endophthalmitis), efficient use of a single cornea, and faster patient recovery (8, 9). Superficial Anterior Lamellar Keratoplasty is indicated for anterior stromal scars after infections, corneal dystrophies, and traumas. It is used for scars occupying 30-40% of the corneal thickness. It is a sutureless surgery that replaces the scar with a graft thickness of 200 μm (4, 5). Deep Anterior Lamellar Keratoplasty (DALK) is performed in cases of deep anterior corneal opacities with good endothelial function, in keratoconus, and certain types of stromal dystrophies. There are two types: pre-Descemet and Descemet's. The pre-Descemet type is done when the cornea is very thin, and there is a risk of perforation. The Descemet's type aims to completely remove the stroma. Many techniques, described using dissection under air or under viscoelastic, have significantly reduced the surgery time and increased the safety of surgical performance (4, 5, 15, 16, 17). A recent meta-analysis comparing the efficiency and safety of DALK vs PK in keratoconus found that the failure rate was similar, but fewer postoperative complications occurred in the DALK group, indicating that, compared to PK, DALK has lower efficiency but higher safety, as demonstrated by other studies (15, 18). Massimo Busin, in a study conducted on patients with keratoconus, used the DALK technique but with the removal of a 6mm central stromal area in 80 eyes without deep stromal scars. In keratoconic eyes without deep stromal scars, combining a larger graft than conventional ones with limited removal of the deep stroma can improve the visual and refractive outcomes of DALK while minimizing the complication rate (19).

Posterior Lamellar Keratoplasty (Endothelial), DMEK, DSAEK. This was described by Melles *et al*. in 1998, involving the total replacement of the posterior area of the affected cornea without affecting the anterior cornea, through a 9 mm limbal incision. Since then, the technique has undergone several improvements. Currently, Descemet's Stripping Automated Endothelial Keratoplasty (DSAEK) represents the current type of the technique, a technically demanding procedure (though less than PLK) that uses a microkeratome to prepare a posterior lamellar donor button, for which the dysfunctional host endothelium is exchanged through a 5 mm scleral tunnel. Once placed and correctly oriented, the graft is pressed anteriorly onto the host's posterior stroma with an air bubble (the pupil is dilated to prevent pupillary block), and the patient maintains a supine position for 24 hours to allow the air bubble to press the graft anteriorly. The advantages of this technique are that visual recovery is faster, it is a closed-globe surgery, avoiding complications like expulsive hemorrhage, but it is challenging to perform. Long-term studies have found that long-term survival rates for DSAEK are comparable or better than for PK. The majority of single-center studies have shown a graft survival rate of 93% or higher at 5 years after DSAEK (1, 5, 6, 20).

The aim of this study is to present our clinic's experience with patients who underwent and were diagnosed with corneal pathologies through the penetrating keratoplasty technique and the medium and long-term therapeutic difficulties encountered in these patients.

MATERIALS AND METHODS

We conducted an observational, retrospective, descriptive study on a series of patient cases who underwent penetrating keratoplasty, focusing on the diagnosis but especially on the difficulties in managing the medium and long-term postoperative care of patients with corneal transplants.

TABLE I.
Surgical and Postoperative Protocol for Corneal Transplant

Disease/Condition	Operative Protocol	Postoperative treatment	Particularities
Keratoconus stage IV Keratoglobus Macular dystrophy Chronic corneal edema Bullous keratopathy Sequela after infectious keratitis	-Decontamination of the periocular skin with 10% Betadine and in the conjunctival sac with 5% Betaine. -Trephination of the donor button with a trephine of the appropriate size after measuring the acceptor patient's cornea with the keratometer. -Marking the acceptor's cornea in 8 or 16 points. -Trephination of the acceptor's cornea. -Excision of the corneal button with left-right scissors. -Injection of miotic and viscoelastic into the anterior chamber (AC). -Fixation of the donor cornea to the recipient bed with 8 or 16 separate sutures, and/or 10.0 Nylon interrupted suture. -Subconjunctival injection with antibiotic + anti-inflammatory and mydriatic.	- Antibiotic and anti-inflammatory eye drops instilled x6/day. - Epithelialization-promoting eye drops and gel instilled x6/day. - Oral corticosteroids 1mg/kg/body in situations at risk of rejection.	- In case of post-herpetic infectious keratitis, add acyclovir 400-800 mg x 5/day. - In patients with keratoglobus: Perform a wide trephination of the cornea (donor diameter 8.25 mm) of the acceptor to avoid the area of large protrusion. -Corticosteroid therapy is essential to prevent graft rejection; sometimes it has been stopped to facilitate epithelialization.

The procedures were evaluated. The inclusion criteria generally followed the recommendations of the national and European transplant guidelines, including: patients with central corneal opacities, keratoconus, corneal dystrophies, with or without intraocular lens implantation, chronic corneal edema, bullous keratopathy, sequelae after infectious keratitis, and keratoglobus. Patients with probable visual potential were included. The surgery was performed by the same surgeon (Dorin Chiseliță) with over 15 years of experience between 2020-2022 and another surgeon (Nicoleta Anton)

in 2024. The surgical technique involved in penetrating optical keratoplasty aimed at functional rehabilitation and improving the quality of patients' life. These patients were registered on the transplant list and monitored, fully evaluated ophthalmologically and for associated general pathologies. All underwent a complete ophthalmological examination, consisting primarily of a detailed medical history related to the ocular pathology. The patients were fully evaluated on equipment and systems, ophthalmologically, being prepared for the surgical procedure under general anesthesia, following the standard follow-up protocol: a comprehensive ophthalmological evaluation involving visual acuity determination, intraocular pressure (IOP) measurement, anterior segment examination and fundus bio microscopy, corneal topography, ocular biometry, ORA, B-mode ocular ultrasound, chromatic sense, ocular motility, tests to detect dry eye syndrome (Schirmer 1, BKT), and OCT (optical coherence tomography) examinations of the optic nerve and macula, as well as interdisciplinary evaluation within the pulmonology and cardiology departments. The operative protocol consisted of penetrating keratoplasty (PK) surgical procedure under general anesthesia and followed the stages that we will detail in first table.

Ethical Approval. During the study, the principles of the Helsinki Declaration were observed. Patients have signed an informed consent, which was attached to the observation sheets.

The postoperative protocol consisted of a series of operative steps and a postoperative protocol according to first table.

Statistical analysis

The data were centralized in a *Micro Soft Excel* database and processed with statistical functions suitable for them, at a significance threshold of 95%.

Simple linear regression allows the study of the relationship between two variables: Y (e.g., number of cases) and X (e.g., time). This linear relationship is graphically illustrated by a regression line according to the formula $Y = a + bX$ (where "b" is the slope of the line, and "a" is the rate at which Y changes at each interval of variable X). The χ^2 test is a non-parametric test that compares 2 or more frequency distributions from the same population, applied when expected events are mutually exclusive. Sometimes, when a frequency in the calculation formula is low, Yates correction is applied to get a higher estimate of the difference. If the p-value is less than the tabular one at the 95% significance threshold, events are not mutually exclusive - in this case, the question arises whether they are dependent. Relative Risk (RR) is the ratio between the risk in exposed patients and the risk in unexposed ones, where the risk in exposed patients is the probability that an exposed individual will develop the disease.

RESULTS

Between 2020 and 2024, in our clinic, the surgeons performed a total of 53 penetrating keratoplasties (16 in 2020, 19 in 2021, 14 in 2022 and 4 in 2024). Apparently, the number of transplant cases over the past 5 years shows a decreasing trend for the upcoming period ($y = 24 - 4x$), but the year 2024 is just beginning and the number of donors is increasing (fig. 2). During the analyzed period, the frequency of female cases was slightly higher (50.9% *vs.* 49.1%), but in 2024, a higher frequency of male cases is noticeable (75% *vs.* 25%; $p = 0.285$).

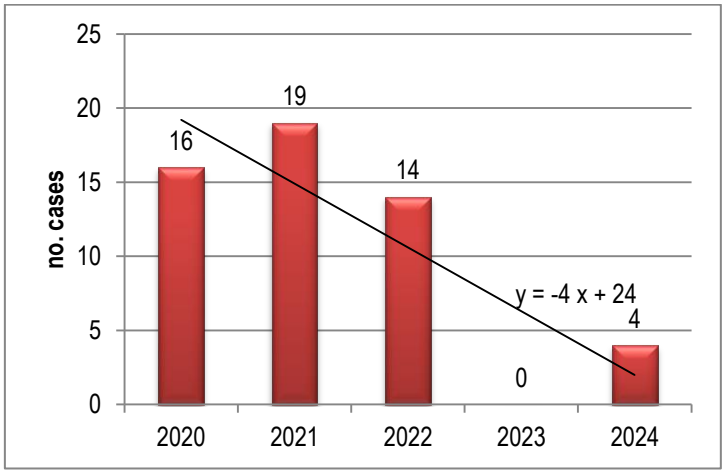

Fig. 2. The number of transplant cases in the period 2020-2024

The number of keratoplasties is due to the small number of donors since Romania did not have a cornea bank during this period. During the analyzed period, the number of complications was not very high, with the most frequent ones being represented by post-traumatic or post-infection corneal leukoma and corneal edema secondary to pseudophakia (fig. 3).

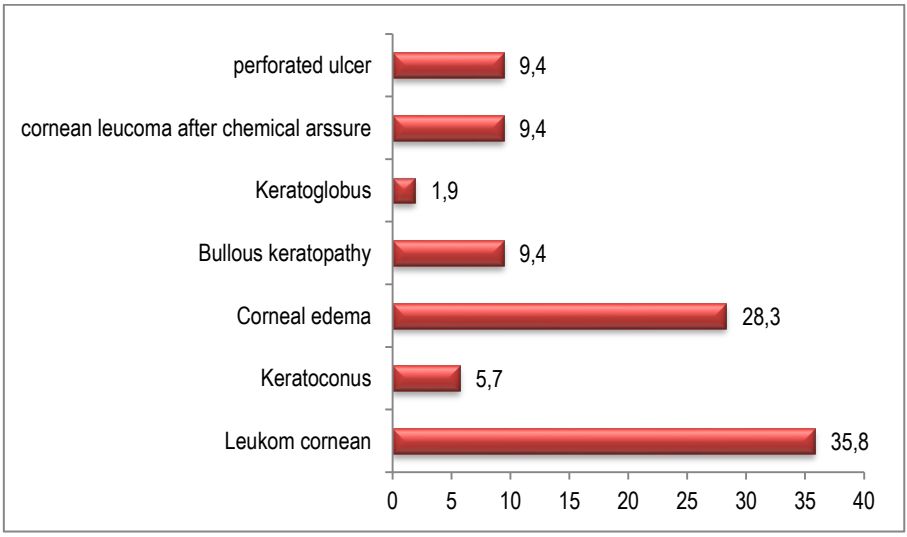

Fig. 3. Pathologies associated with corneal transplantation

From January 2020 to April 2024, a total of 53 keratoplasties were performed, 27 females and 26 males, aged between 16 and 71 years. For this study we selected cases that required special monitoring precautions and needed personalized treatments

and appropriate follow-up. Only 8 out of 53 keratoplasties presented postoperative complications, with one having a severe complication of endophthalmitis leading to enucleation of the eyeball (fig. 4). However, it was found that the risk of complications did not significantly increase in any of the study years (tab. II).

TABLE II.
Distribution of complications over follow-up years

Year	Complications (n=8)		No complications (n=45)		Chi² test	RR	CI 95%
	n	%	n	%			
2020	2	25.0	14	31.1	0.731	0.77	0.17-3.42
2021	4	50.0	15	33.3	0.370	1.79	0.50-6.35
2022	1	12.5	13	28.9	0.920	0.40	0.05-2.95
2024	1	12.5	3	6.7	0.325	1.75	0.28-10.91

The cases selected 2022 had the following pathologies: 2 cases of central corneal leucoma, sequelae of herpetic keratitis, 2 cases of chronic corneal edema in pseudophakia, 1 case of keratoglobus associated with dry eye syndrome and sleep apnea syndrome, corneal dystrophies (3 cases, 1 of Fuchs endothelial dystrophy stage II and 2 of granular), 2 cases after vascularized corneal leucoma secondary to chemical burn, 1 case of corneal leucoma after severe ocular trauma, 2 cases of corneal leucoma, bullous keratopathy, and 2 cases of chronic corneal edema in pseudophakia, associated with pseudo exfoliative and secondary glaucoma. Given these complex cases, surgery is performed under general anesthesia, and special precautions are taken to assess associated general comorbidities.

Case 1 is a 32-year-old patient with a history of ocular chemical burn (var), total vascularized corneal leucoma, trichiasis, and symblepharon for which a KP was performed. Despite appropriate treatment, he experienced early graft failure due to graft opacification secondary to limbal stem cell deficiency and recurrent corneal erosions. The patient did not attend regular follow-up appointments (fig. 4). What is particular about this case is the limbal stem cell deficiency secondary to the burn that contributed to graft failure despite intensive treatment with topical and systemic corticosteroids.

Fig. 4. Graft failure in a patient with corneal transplant, with a history of chemical burn with lime (personal casuistry)

Case 2. A 29-year-old female patient with a history of corneal leucoma post-herpetic keratitis and amblyopia. She underwent penetrating keratoplasty for aesthetic purposes. In addition to standard treatment, she received prophylactic treatment with acyclovir, initially 2 grams per day for a month, followed by a maintenance dose of 400 mg/day, which she continues to take at present. Figure 5 presents the biomicroscopic status before and the day after the transplant.

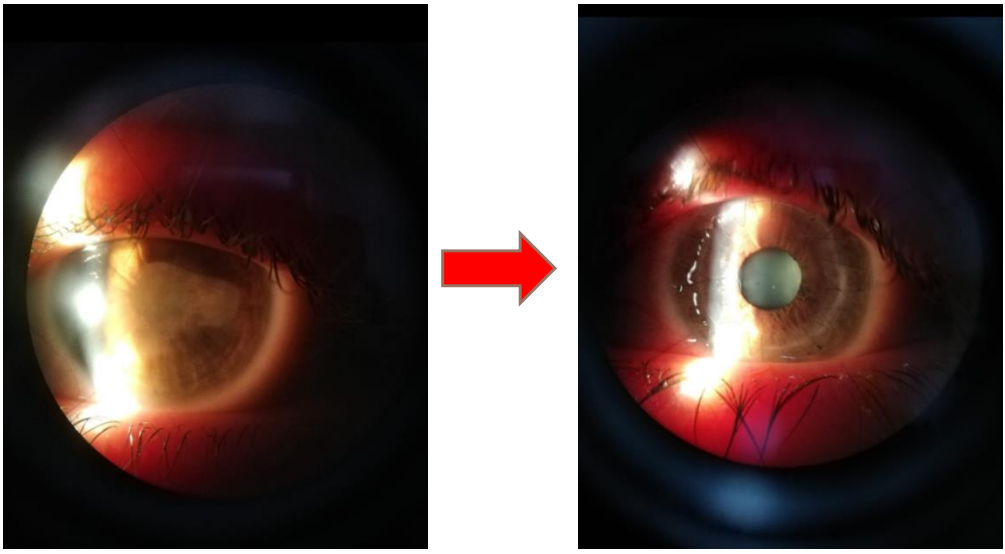

Fig. 5. Images from personal case studies before and after transplant (personal casuistry)

After the first 3 months, the first four interrupted sutures are removed, and all sutures are removed at 1 year. At 1 years and 6 months post-transplant, the evolution is favorable, with vision improving to 0.1 due to keratoplasty, while still keeping up with the dose of acyclovir at 400mg/day (fig. 6).

Case 3 involves a patient known for advanced PEX glaucoma who underwent surgery on the left eye (OS) with visual acuity with hand motion perception (HM), OS pseudophakia due to cataract surgery, and chronic corneal edema in pseudophakia on the right eye (OD). This is a single-eye keratoplasty with favorable postoperative evolution. As a particularity, it required postoperative glaucoma management with continued topical and systemic antiglaucoma medication. The maximum visual acuity achieved is 0.1 without correction, due to severe glaucomatous damage (fig. 7).

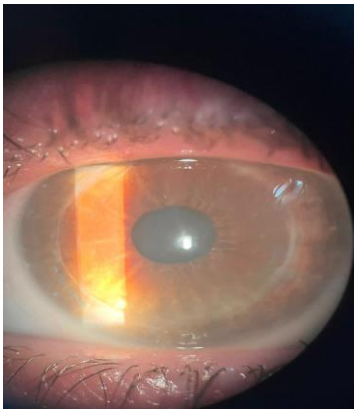

Fig. 6. Clinical status at 1 years and 6 months post-KP (Personal casuistry)

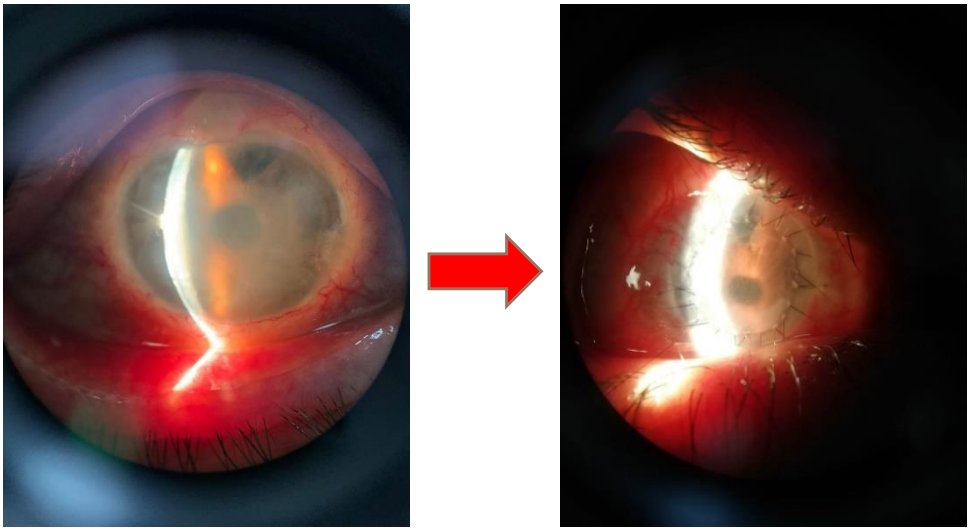

Fig. 7. Clinical status before and after KP (penetrating keratoplasty) (personal casuistry)

Case 4 is that of a 28-year-old female patient with a history of granular corneal dystrophy who underwent keratoplasty in 2020 on the right eye (OD) and in April 2022 on the left eye (OS). An 8 mm corneal button was used, and the evolution was favorable during the first 10 days (fig. 8).

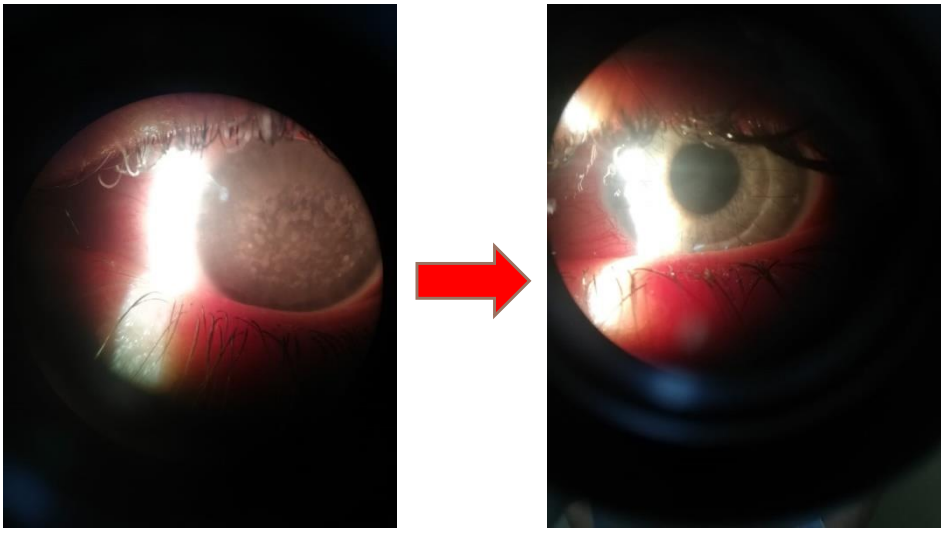

Fig. 8. Clinical status before and after PK (penetrating keratoplasty) (personal casuistry)

A corneal erosion on the graft is made after 30 days, the fluorescein test is positive, and intensive treatment with antibiotics and frequent corneal epithelialization is required. Healing is slow but favorable, with persistent opacity in the lower part of the graft. Her vision remains fairly stable at 22 months post-surgery (fig. 9).

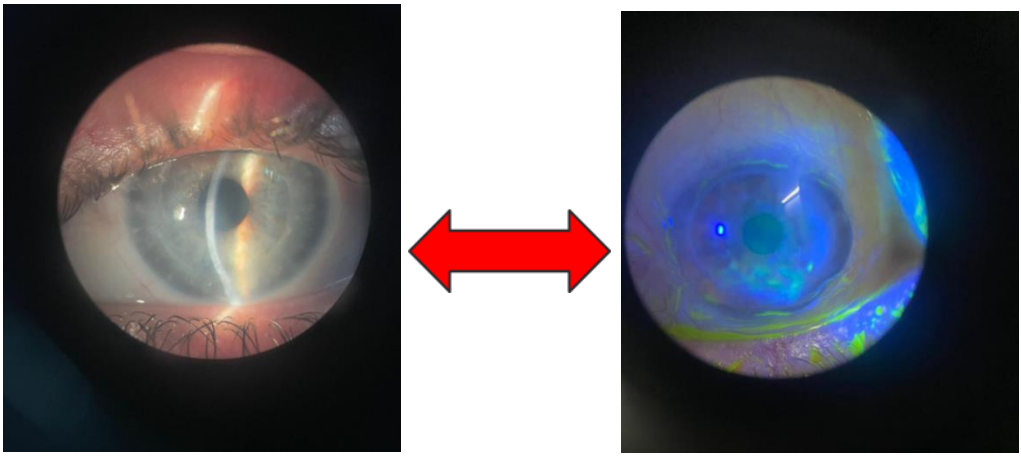

Fig. 9. Clinical status at 22 months post-KP (keratoplasty) (personal casuistry)

Case 5. A 71-year-old male patient from a rural area with Keratoglobus underwent keratoplasty (KP) and was admitted to the Ophthalmology Clinic of "Sf. Spiridon" County Clinical Emergency Hospital in Iași with the following personal pathological history: severe obstructive sleep apnea syndrome, stage II obesity, chronic painless, ischemic heart disease, essential stage II hypertension with increasingly high additional risk, ventricular extrasystolic arrhythmia, aortic cross ectasia, chronic heart failure class II NYHA, hypertriglyceridemia, hepatic steatosis, operated prostate adenoma, bilateral primary gonarthrosis, and bilateral primary coxarthrosis. Ophthalmological examination revealed several pathological aspects: reduced vision in the left eye (OS), severe dry eye syndrome, a diffusely thinned cornea from limbus to limbus in the left eye, its protrusion with a globular appearance, anterior and posterior cortical opacities, and floppy eyelid syndrome (fig. 10).

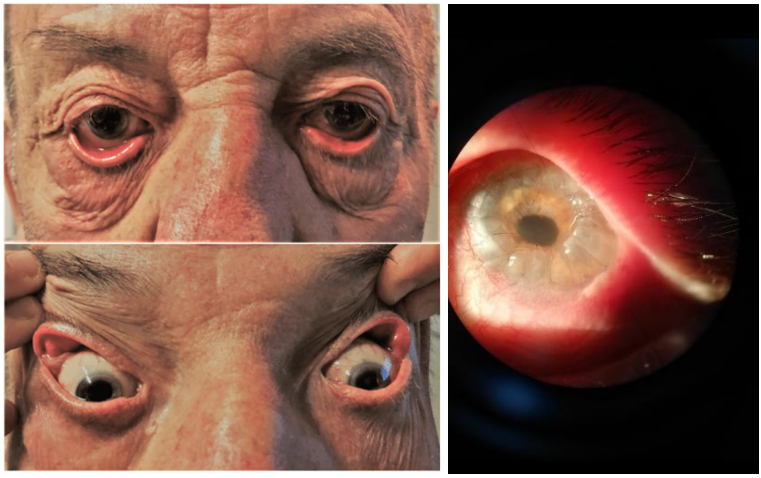

Fig. 10. Clinical status at the beginning, before and after penetrating keratoplasty surgery (personal casuistry)

In addition to the clinical examination and the tomographic examination of the left eye, the diagnosis of Keratoglobus is also significantly influenced by the marked thinning of the cornea across its entire surface (minimum 265 μm) (fig. 11), along with diffuse, concentric bulging, suggestive of the diagnosis. Penetrating keratoplasty was performed along with "open-sky" lens extraction and placement of a multipiece intraocular lens in the capsular bag, with a favorable outcome.

According to the protocols known in corneal transplant surgery, interdisciplinary evaluations are conducted for precise diagnosis, appropriate treatment, and surgical procedure under general anesthesia. The general evaluation also revealed severe obstructive sleep apnea syndrome (Saturation Index = 33.2/hour).

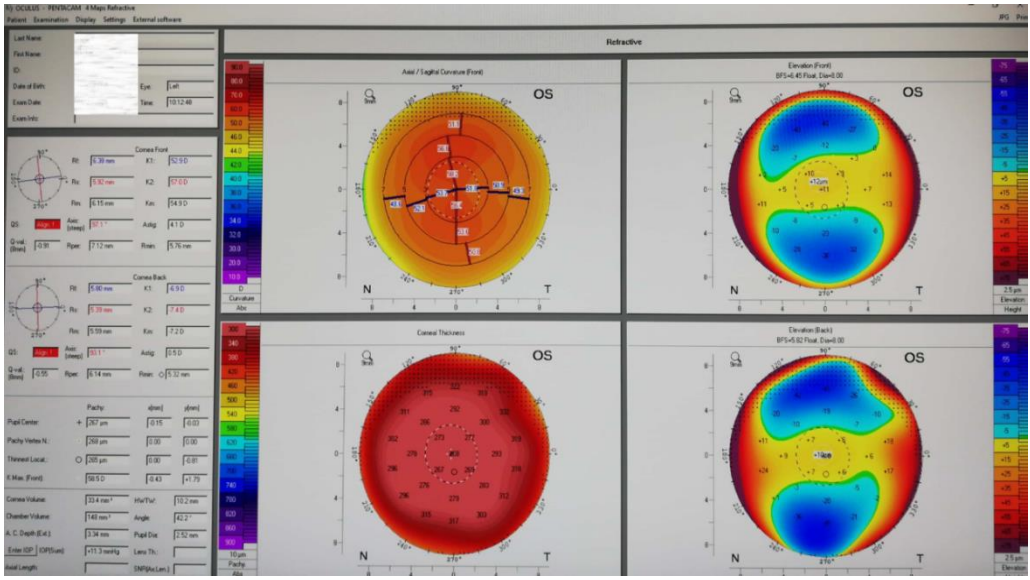

Fig.11. Corneal topography of the left eye (OS). Marked thinning of the cornea across its entire surface (minimum 265 μm) (personal casuistry)

Among the surgical peculiarities, we note the creation of a larger corneal button (8.25 mm) and a slightly decentered trephination due to the specific changes of keratoglobus. The evolution was favorable, and after the removal of the sutures at 1 year, a stable visual acuity was achieved. The cornea fixes diffusely, at 22 months, with a visual acuity of 0.1 without correction. The patient is under chronic treatment with preservative-free artificial tears and epithelializing gels (fig. 12). This patient has associated pathologies requiring both ophthalmological, cardiological and pneumological evaluation. He needs to lose weight and requires positive pressure ventilation 4-14 MB ARR, on a facial mask, for a minimum of 5 hours per night, indefinitely, with respiratory equipment control at 1, 3, 6, 12 months, then annually. Ophthalmologically, it is difficult to quantify the mid and long-term prognosis due to the association of systemic conditions but especially due to the sleep apnea syndrome.

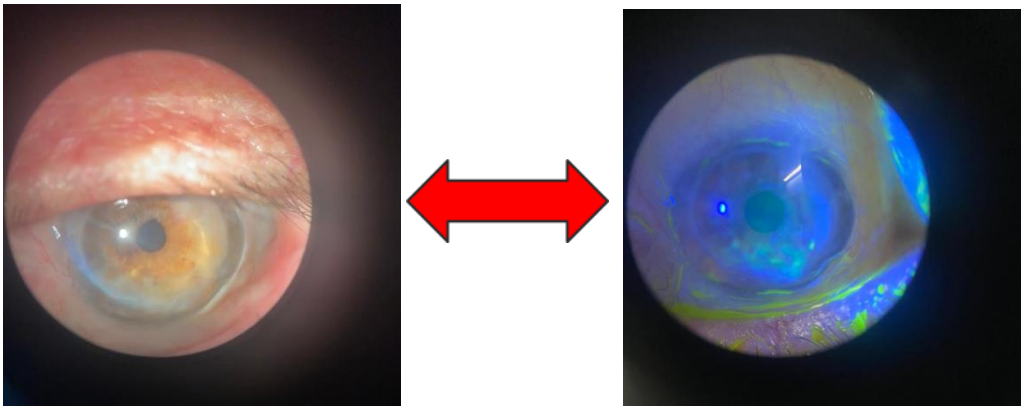

Fig. 12. Clinical status at 22 months post-KP (keratoplasty) (personal casuistry)

DISCUSSION

Corneal transplantation, or keratoplasty, is considered the most successful tissue transplant, largely due to the cornea's "privileged immune" status, which allows a much lower risk of allograft rejection compared to solid organ transplantation. Penetrating keratoplasty (PKP) has become a highly successful procedure due to advancements in eye surgery, corneal surgery, and postoperative treatment. The success of PKP, more than any other ophthalmic surgical procedure, depends on careful and diligent postoperative care. Both the patient and the surgeon must be vigilant about ocular changes so that issues can be promptly addressed and prevent corneal graft failure (19-21). This study highlights the challenges we face in following up with transplant patients and outlines the therapeutic approach in certain situations. When discussing penetrating keratoplasty, the risk of complications is quite high, and the postoperative recovery time is relatively long, sometimes taking years to achieve the best corrected visual acuity. There is a higher risk of allograft rejection compared to other types of keratoplasty, as well as a greater risk of wound dehiscence throughout life due to compromised tectonic resistance stemming from a full-thickness wound. Literature studies show a range of outcomes and complications of penetrating keratoplasty. This procedure is mainly indicated for corneal leukomas, bullous keratopathy, perforated ulcers (2, 21-23) (tab. III).

TABLE III.

Indications for keratoplasty in patients submitted to corneal transplantation

Instructions for KP surgery	Frequency	Studies
Pseudophakic / Aphakic bullous keratopathy	~ 50% of the cases	2, 21, 22, 23
Leucomatous corneal opacity	35-40%	2, 21, 22, 23
Keratoconus and ectasias	1%	2, 21, 22, 23
Impending perforation	8%	2, 21, 22, 23
Melted/Non healing Corneal Ulcer	<1%	2, 21, 22, 23
Perforated Corneal Ulcer		2, 21, 22, 23
Corneal Abscess		2, 21, 22, 23
Failed Corneal Graft	1%	2, 21, 22, 23

Complications of keratoplasty can occur immediately postoperatively or at a distance, and early recognition and treatment are necessary. Therefore, it is extremely important to evaluate on slit-lamp for wound integrity, epithelial defects, corneal edema, IOP (intraocular pressure), iritis, and the possibility of infection on the first postoperative day. Anterior segment OCT examination: CCT (central corneal thickness), ICA (anterior chamber angle depth), and the host-graft junction. As soon as any of these complications manifest or persist, evaluation of the operated eye should be continued daily for a few more days. In table IV, we present possible early and late complications that may occur after penetrating keratoplasty (13, 24-30).

TABLE IV.
Early and late complications after penetrating keratoplasty

Early complications	Late complications
Wound leak	Steroid - induced glaucoma
Primary endothelial failure	Suture - related
Persistent epithelial defect	Infection
Ocular hypertension	Breakage
Urrets-Zavalia syndrome	Irregular astigmatism
Infections	Rejection
Keratitis	•Epithelial
Endophthalmitis	•Stromal
	•Endothelial
	Recurrence of primary disease

Although excellent survival and visual results have been reported for both penetrating and lamellar keratoplasty in the short-term, there are a limited number of published multicenter studies on long-term outcomes. According to literature studies, the graft survival rate is quite high. The graft survival outcome for optical purposes at one and three years is 91.7% and 75.8%, respectively. The Australian Corneal Registry Study (ACGR) reported that the survival of penetrating corneal grafts was 62% after ten years (13, 23, 24). In our patients, out of 14, one presented early graft failure due to limbal stem cell deficiency, secondary to burns which favored graft failure despite intensive topical and systemic corticosteroid treatment. Some cases had a slow favorable evolution. The other cases had a series of complications related to inadequate treatment, frequent graft erosion, corneal ulcer which, after appropriate treatment, had a favorable evolution. One case where we performed a recent transplant had a suture breakage and required reevaluation and treatment. A recent meta-analysis on the incidence of wound dehiscence after keratoplasty showed, from a selection of several articles, that the overall incidence of wound dehiscence was estimated to be 1.9% (95% CI: 0.03, 0.26). Of the included studies, six focused on PK involving 5,611 patients, while three trials examined DALK with a total of 2,101 patients. The analysis revealed that patients undergoing PK were more than twice as likely to experience wound dehiscence compared to those undergoing DALK (25,

26). Other complications described by specialized literature studies include the occurrence of glaucoma. Different studies showed a variable incidence of glaucoma after PK, ranging from 9 to 31% early postoperatively and from 18 to 35% in the late postoperative period. Steroid-induced IOP elevation was the most frequent cause, with an incidence of 38.3%. The most important risk factors in the occurrence of post-PK glaucoma are the lens status (aphakia, pseudophakia) and the disease for which the PK is performed (27, 30, 31). The functional success of a corneal graft depends on many variables, including good surgical technique with adequate donor and host integration, immunological acceptance of foreign tissue, a functional level of astigmatism, and a smooth corneal surface supported by ocular annexes. Corneal infection (keratitis), whether active or healed after keratoplasty, is a major complication that can jeopardize the graft result, leading to failure and poor visual outcome, being a major cause of graft rejection. Microbial keratitis on the graft can occur at variable times after keratoplasty. Studies have reported that most graft infections usually occur within one year of corneal transplantation. Early postoperative infection can occur due to recurrence of the host's disease, infected donor cornea, or intraoperative contamination. Usually, late infections are caused by pathogens acquired from the surrounding environment. The median interval between penetrating keratoplasty and microbial keratitis was 7.6 months. Aggressive corticosteroid treatment in the first six months is a contributing factor to the onset of infections (13, 24). Among late complications, astigmatism is the most frequent cause of suboptimal vision after corneal transplantation despite a clear corneal graft. Based on several studies, 15-31% of patients undergoing PK may develop postoperative astigmatism greater than 5 diopters. Keratometry, photo-keratoscopy, slit-lamp examination, and manifest refraction help in the identification of any tight sutures. Selective suture removal has been shown to hasten visual rehabilitation. Selective suture removal begins at about 6 weeks, and only one or two sutures are removed at one time. The cornea is evaluated every 2 weeks for change in contour, and further sutures are removed (28, 29).

CONCLUSIONS

Although, in terms of survival, corneal transplantation is the most successful transplant performed in humans, rejection is still the main cause responsible for graft failure. Keratoplasty involves a long healing process; therefore, preoperative evaluation and dialogue with the patient are important to understand the benefits, possible complications, and the need for prolonged treatment. Corneal transplantation represents a challenging surgery both in terms of surgical technique and preoperative evaluation and postoperative management.

The alignment of diameters between the host cornea and the donor cornea plays an essential role in centering and ensuring the survival of the transplanted graft.

Suturing techniques are standardized, but the particularities of each case should always be considered, which may require an adapted approach (individual sutures *vs.* continuous suture).

The functional prognosis depends on the patient's condition, the surgery itself, the patient's compliance with treatment and

recommendations, and also on the doctor-patient relationship.

For long-term success after keratoplasty, it is desirable for both the surgeon and the patient to be constantly vigilant to ocular changes so that issues can be promptly addressed. Caring for the corneal transplant is a lifelong commitment. Good interdisciplinary cooperation leads to an increased success rate.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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The author Dorin Chiselita contributed equally to the first and corresponding authors.

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CLINICAL, BIOLOGICAL AND SOCIAL CHARACTERISTICS ASSOCIATED WITH POSITIVE AND NEGATIVE SYMPTOMATOLOGY IN SCHIZOPHRENIA AND BRIEF PSYCHOTIC DISORDER

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CLINICAL, BIOLOGICAL AND SOCIAL CHARACTERISTICS ASSOCIATED WITH POSITIVE AND NEGATIVE SYMPTOMATOLOGY IN SCHIZOPHRENIA AND BRIEF PSYCHOTIC DISORDER (Abstract): Psychotic disorders are prevalent mental health conditions that frequently go underdiagnosed, particularly in their early stages. During the prodromal period, symptoms are frequently underestimated or misattributed. **Materials and methods:** We collected data from the "Socola" Institute of Psychiatry, gathering information from 392 patients hospitalized in 2023 and diagnosed with brief psychotic disorder or schizophrenia. We analyzed classification by age group (20-40, 40-60, and ≥61 years old), rural or urban living environments, level of education, association with depression or anxiety using the Hamilton scales, presence of other psychiatric comorbidities, suicidal ideation or attempts, presence of inflammatory markers (ESR, CRP, and leukocytes), and the treatment plan. **Conclusions:** Our study provided a detailed demographic and clinical profile of patients with brief psychotic disorders and schizophrenia at the "Socola" Institute. The findings highlight the importance of an integrated approach to assessment and management, emphasizing early identification of risk factors and implementation of preventive interventions to promote healthy living and prevent chronic disorders. **Keywords**: PSYCHOSIS, SCHIZOPHRENIA, BRIEF PSYCHOTIC DISORDER, MORTALITY, MEDICATION NON-COMPLIANCE.

Psychotic disorders are common mental disorders, often underdiagnosed, especially in the early stages. The prodromal period is frequently underestimated or misclassified. According to the Global Burden of Diseases, schizophrenia affects 20 million people worldwide, with 1.5 new cases per 10,000 people annually. The World Health Organization (WHO) ranks psychotic disorders among the top 15 causes of disability. Approximately 5% of individuals with at least one psychotic episode commit suicide, while 20% attempt suicide (1).

The costs of treating psychotic disorders exceed those for high blood pressure and diabetes combined. In about 10% of

schizophrenia cases, psychotic decompensations occur within a year, even with monthly monitoring. Early intervention and appropriate treatment in the initial phases are crucial for medication compliance and establishing a therapeutic alliance (2).

The link between the immune system and the brain was established in 1985, with studies showing suppression of immune response in depression. Elevated levels of inflammatory markers are found in patients with major depressive disorder, even without a somatic condition. The coexistence of depression with inflammation and the increased risk of depression with cytokine treatment suggest inflammation's role in depression's pathogenesis (3).

Recent studies indicate a connection between inflammation and psychiatric disorders, particularly schizophrenia. Increased inflammatory markers, such as interleukins (IL-6, IL-1β) and tumor necrosis factor alpha (TNF-α), are found in patients with major depressive disorder and schizophrenia (4-6). Psychological stress can increase pro-inflammatory cytokines, and inflammation may mediate the relationship between diet and mental health, suggesting a high-fiber diet may reduce inflammation and psychotic symptoms (7-9).

Understanding the interactions between the immune system and the brain has significant implications for treating and preventing neuropsychiatric disorders. Recent findings on the link between inflammation and psychosis open new research directions and potential therapeutic approaches (10).

MATERIALS AND METHODS

The study aims to explore the role of inflammation in the pathogenesis of psychotic disorders, particularly schizophrenia. A key objective is to investigate the association between inflammatory markers, such as interleukins (IL-6, IL-1β) and tumor necrosis factor alpha (TNF-α), and the severity of psychotic symptoms.

Another goal is to analyze how inflammation influences treatment response in patients with psychosis. This aspect aims to determine if patients with elevated inflammatory profiles benefit differently from various therapeutic interventions, including antipsychotic treatments and anti-inflammatory strategies.

The study also examines the impact of environmental factors, such as diet and stress, on inflammation and mental health. This includes evaluating how dietary changes, such as adopting a high-fiber diet, might affect inflammation and the severity of psychotic symptoms.

Methodologically, an integrative approach will be used, combining clinical and biological data to understand the interactions between the immune system and the central nervous system in psychotic disorders. This research could lead to new perspectives in treating and managing neuropsychiatric disorders, promoting the development of more effective and personalized therapeutic strategies.

After obtaining the consent of the "Socola" Institute's management, we accessed the electronic databases to identify patients hospitalized in 2023 and diagnosed with brief psychotic disorder or schizophrenia. We selected a total of 392 patients, of which 190 were women and 202 were men.

We classified them by age groups and compared patients from rural areas with those from urban areas and studied their level of education. We also used the Hamilton Depression and Anxiety Scales to determine any association with these disorders and documented the presence of other psychiatric comorbidities. We also assessed

suicidal ideation and suicidal attempts identified in the patient's psychological examination. To complete the analysis, we measured the levels of the inflammatory markers ESH, CRP, and leukocytes, the only relevant laboratory tests available in the "Socola" Institute in 2023, and recorded the types of treatments given to patients.

RESULTS

Analyzing the graph below, we can conclude that there were no major differences between the number of women and men hospitalized in 2023, the percentage being 53% men, compared to 47% women.

Regarding the distribution by age groups, we identified 4 women aged 20-40, 150 women aged 40-60, and 16 women aged over 61 years old (tab. I).

The results presented in second figure indicate a significant rate of psychotic decompensations in women between 40 and 60 years old, which represent 88% of cases. This suggests that women in this age group are particularly vulnerable to decompensated psychotic disorders. Factors that could contribute to this vulnerability include cumulative stress over the years, family and work responsibilities, and possible hormonal changes associated with menopause. Women over 61 years old account for 10% of cases, indicating that although the risk of psychotic decompensation decreases after the age of 60 years old, it does not disappear completely. Age-related health problems, social isolation, and personal loss can contribute to psychological vulnerability in this age group.

On the other hand, women between the ages of 20 and 40 years old have the fewest admissions for psychotic decompensation, accounting for only 2% of cases. This may suggest greater resilience to stress and mental disorders at younger ages or may reflect underdiagnosis or underreporting of these conditions among young women.

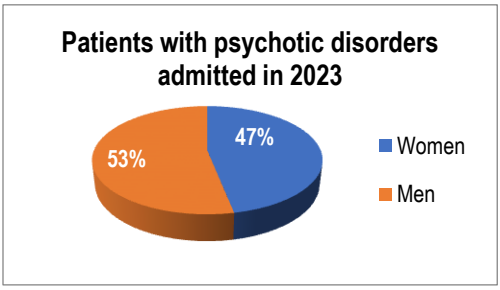


Fig. 1. The ratio of women/men hospitalized in 2023

TABLE I.
Distribution of hospitalized women in 2023 by age group

Age group	Number of women
20-40 y.o.	4
40-60 y.o.	150
≥61 y.o.	16

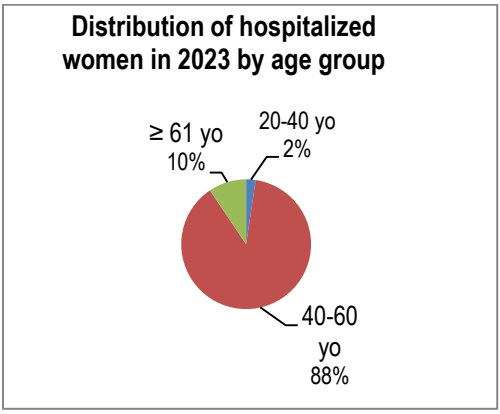


Fig. 2. Distribution of hospitalized women in 2023 by age group

These results emphasize the importance of monitoring and targeted interventions for middle-aged women, who appear to be most affected by psychotic decompensations. It also highlights the need to provide adequate support for older women to help

them manage the psychological challenges associated with aging. At the same time, vigilance must also be maintained for younger age groups to ensure early identification and treatment of mental disorders.

It was found that female patients from the rural area (63%) had a higher hospitalization rate compared to those from the urban area (37%) with a diagnosis of major depressive disorder during 2023 in the Vth Acute Ward of the "Socola" Institute of Psychiatry. This aspect can be explained by the fact that patients from the urban environment have more mental health centers available to which they can go, private offices, outpatient clinics, and perhaps a higher degree of availability and logistical possibilities compared to those from the rural environment for addressing at a doctor in general and psychiatrist in particular (tab. II).

TABLE II.

Distribution according to the environment of origin by age group

Age group	Rural (57)	Urban (33)
20-40 y.o.	4	0
40-60 y.o.	80	70
≥ 61 y.o.	2	14

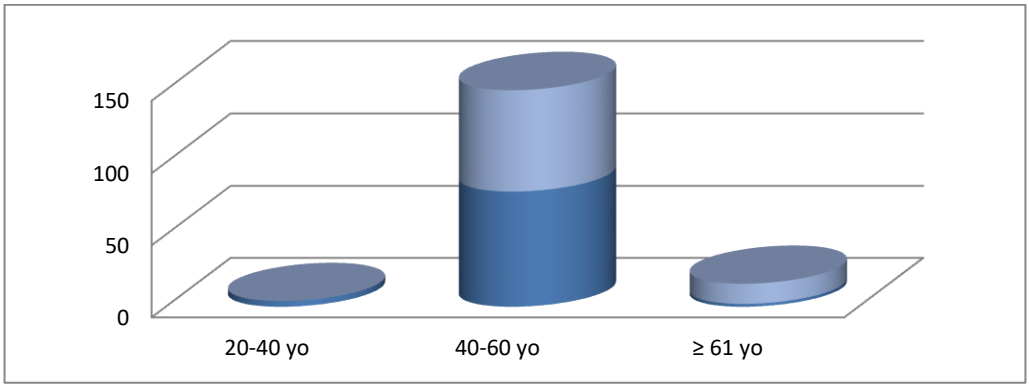


Fig. 3. Distribution by age group and background

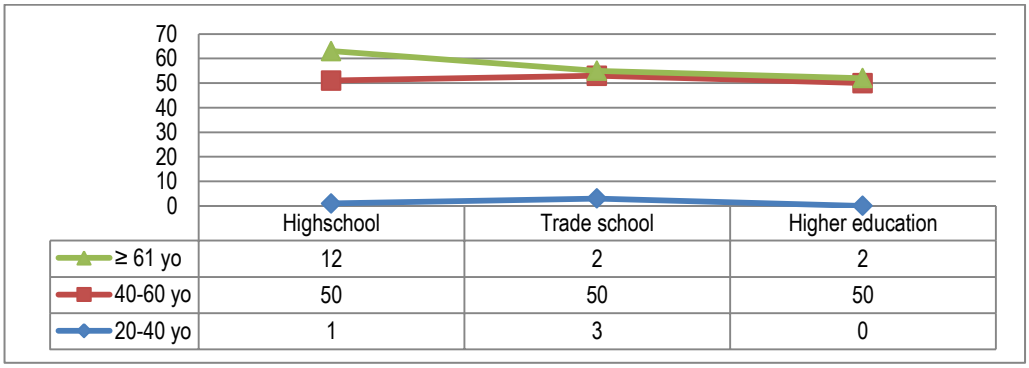
Also, a monthly dispensary for a psychiatric condition leads to a decrease in the frequency of relapses, thus making continuous hospitalization less necessary.

Another aspect to consider is the stigmatization of the psychiatric patient, which unfortunately in Romania is still in continuous development, with all the attempts by the employees of the health system and the mass media to counteract it. Stigmatization and public recognition of a psychiatric condition can often delay presentation to a doctor, with patients first often going through many psychological evaluations and therapies, a fact that would perhaps be characteristic of urban patients, more so than rural ones.

At the same time, patients who have changed their residence from the urban to the rural environment may experience depressive symptoms, the rural environment in Romania being considered in most cases, a disadvantageous environment.

Analyzing the graph and the table above (fig. 4) we can see that most of the patients who requested treatment have high school as their last form of education, especially in the 40-60 age group. In contrast, the lowest addressability was registered among patients with higher education, being only 52

in number. This phenomenon can be explained by the fact that, currently, the predominant form of schooling completed in Romania is high school.



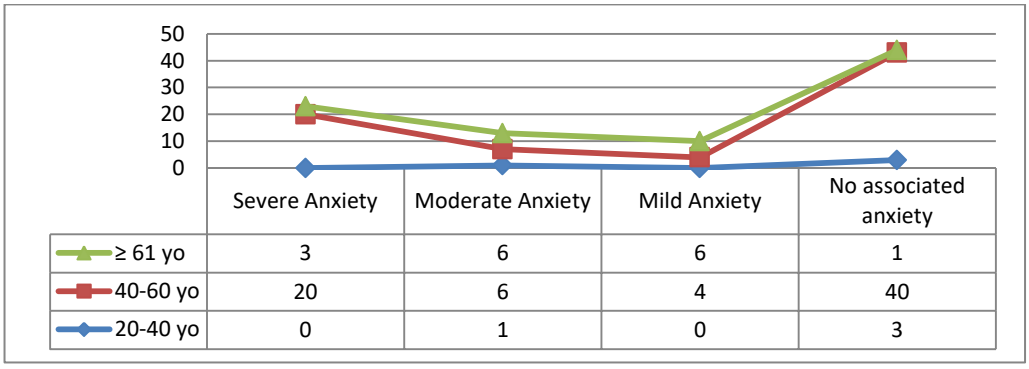
	Highschool	Trade school	Higher education
≥ 61 yo	12	2	2
40-60 yo	50	50	50
20-40 yo	1	3	0

Fig. 4. Distribution according to education level by age group

This finding suggests that education level may influence access to and use of health services. People with higher education, although fewer in number in the presented statistics, could have better access to health information and, implicitly, to prevention and treatment services. They could also benefit from better financial resources and a social support network to contribute to more effective mental health management.

In conclusion, this review highlights the importance of education in accessing and using mental health services and the need for targeted interventions to improve addressability among all educational categories, particularly those with lower levels of education.

The Hamilton Depression Rating Scale is the gold standard for depression scales, unrivaled in general psychiatry. This scale is a measure of depression and includes 17 items, rated on 3-5 degrees, and appears to be a good indicator of the global intensity of the depressive syndrome, sensitive enough to appreciate the change under antidepressant treatment. The scale includes evaluation items of cognitive, behavioral, and especially somatic components (fig. 5).



	Severe Anxiety	Moderate Anxiety	Mild Anxiety	No associated anxiety
≥ 61 yo	3	6	6	1
40-60 yo	20	6	4	40
20-40 yo	0	1	0	3

Fig. 5. Distribution according to severity of depression and association with anxiety, using the Hamilton scale

Most of the time, depression is associated with other comorbidities, either psychiatric or somatic. Thus, for example, in women with depression, we identified 35% with emotionally unstable personality disorder, 25% with dependent personality disorder, 14% with panic disorder, 17% with autolytic ideation, 6% with autolytic attempt and 7% with dementia, in which case I consider that obesity was actually secondary to dementia (fig. 6).

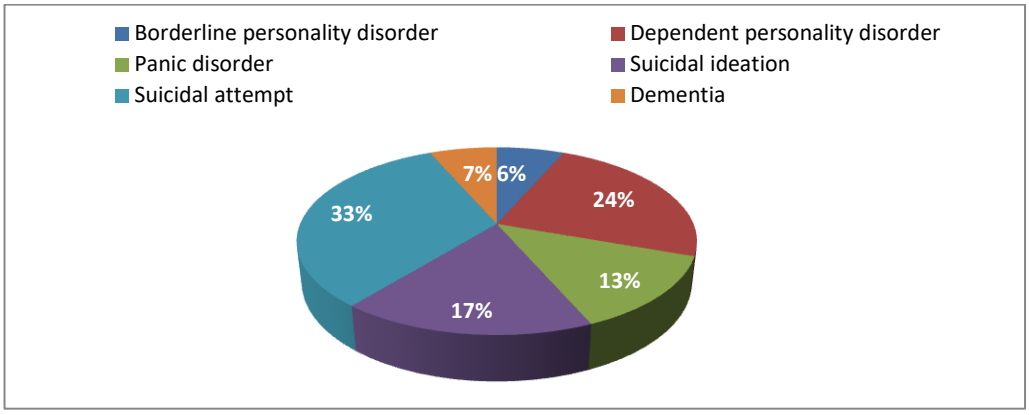


Fig. 6. Associated psychiatric comorbidities

Due to the increase in the suicide rate due to depression, this mental disorder has become one of the most studied psychiatric conditions. Effective management depends on early recognition and diagnosis of depression before it produces suicidal ideation. The clinical diagnosis of depression, beyond the clinical recognition of diagnostic criteria, can be supplemented by the use of specific clinical scales with varying degrees of complexity, from the simplest, such as the MADRS, to the most elaborate, such as the Hamilton scale, which quantifies suicidal ideation. The multidisciplinary approach to depression is reflected in its therapeutic management, from its recognition at the primary ambulatory level to psychiatric evaluation and treatment, with the subsequent recommendation of following a psycho-therapeutic intervention (fig. 7).

The presented results show a clear distribution of patients with autolytic ideation according to age group. In the age group 20-40 years, no patients with autolytic ideation were identified, which suggests that, in this age range, the prevalence of autolytic ideation is very low or nonexistent in the studied sample.

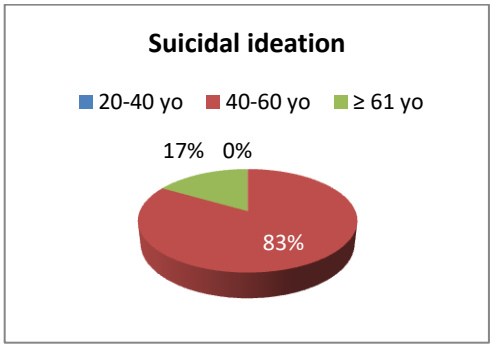


Fig. 7. Association of depression with suicidal ideation

Instead, the 40-60 years old age group is the majority, with a percentage of 78%.

This indicates that women in this age group are most susceptible to autolytic ideation. Possible factors contributing to this vulnerability include work and family stress, menopause, and other hormonal changes, as well as possible health problems and existential crises specific to midlife.

For women over 61, the percentage of autolytic ideation is 22%. Although this percentage is significantly lower than in the 40-60 age group, it still suggests an important concern for mental health among the elderly. Factors such as loneliness, loss of a life partner, health problems, and decreased quality of life may contribute to the increase in autolytic ideation in this age group.

These results emphasize the importance of specific interventions for each age group. For middle-aged women (40-60 years old), psychological support and stress management programs should be developed, as well as mental health monitoring. For older women (over 61 years), it is essential to ensure access to adequate social and medical support, as well as programs to combat loneliness and improve quality of life.

In conclusion, these data highlight the need for personalized prevention and treatment strategies, tailored to the specific needs of different age groups, to reduce the risk of autolytic ideation and improve mental health among women.

The results show that among the cases with associated autolytic attempts, the majority are in the age group of 40-60 years, with 13 patients, while only 3 patients are in the age group of more than 61 years (fig. 8). These data suggest that middle-aged women are more likely to make autolytic attempts compared to older women. The identification of 13 patients with autolytic attempts in this age group suggests a significant vulnerability to psychological and emotional stressors. Possible contributing factors include:

- Stress related to career and family responsibilities;
- Hormonal changes, including menopause, which can affect mood and emotional stability;
- Midlife crises and changes in identity and social roles.

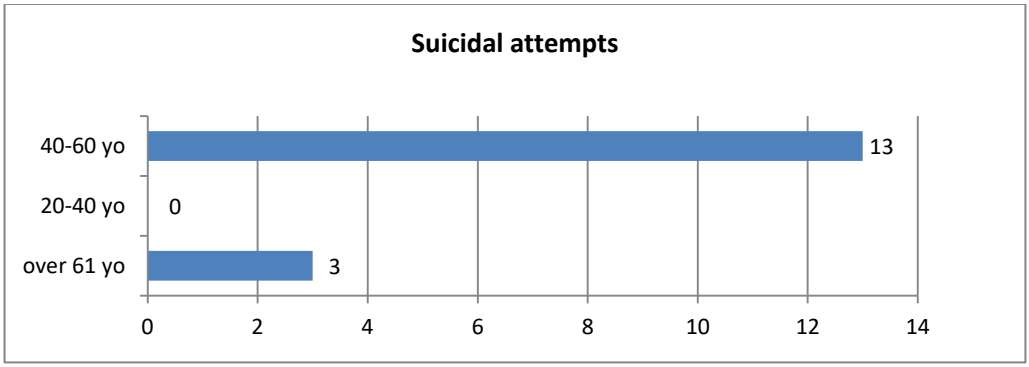


Fig. 8. The association of depression with autolytic attempts

Regarding the identification of 3 patients with autolytic attempts in this age group, although numerically smaller than the 40-60 age group, it is still significant and indicates a mental health problem among the elderly. Possible contributing factors include:

- Social isolation and loneliness, which are common among older people;
- Physical health problems and decreased quality of life;
- Loss of life partner and friends, which can contribute to depression and autolytic ideation.

The results highlight the need for increased attention to the mental health of middle-aged and older women. Tailored interventions that consider risk factors specific to each age group can help reduce autolytic attempts and improve overall well-being.

As particularities in the case of patients who presented suicidal ideation, we encountered in 52% of cases elevated ESR, in 16% of cases CRP at the upper limit, 16% with associated duodenal ulcer, 10% with gastroduodenitis and 6% with GERD (fig. 9).

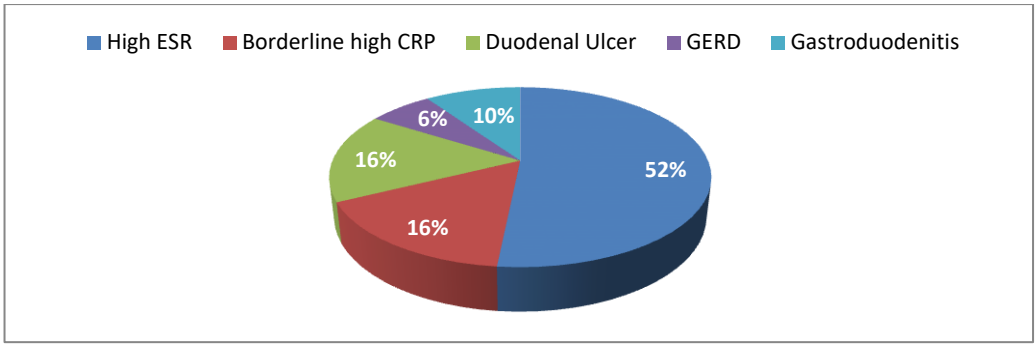


Fig. 9. Peculiarities associated with suicidal ideation

DISCUSSION

Our study identified various inflammatory markers (ESR, CRP, leukocytes) in women with psychotic decompensation hospitalized in 2023. These findings align with recent research on the role of inflammation in psychotic disorders, including schizophrenia. Studies show elevated levels of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in patients with major depressive disorder and schizophrenia (11).

Our results indicate a higher prevalence of psychotic decompensation in women aged 40 to 60 years, supported by studies linking this to hormonal factors, accumulated stress, and family and professional responsibilities (12). In contrast, young women (20–40 years) had fewer admissions, suggesting greater resilience or underreporting of symptoms. Other studies also found an increased risk of severe psychotic symptoms in women aged 40 to 60 years (11, 12).

The majority of hospitalized women were employed or on sick leave, highlighting the role of occupational stress and chronic conditions in psychotic decompensations. Other studies confirm this link, suggesting that psychological support at work can reduce the incidence of these disorders (13). Ehlen *et al.* (2023) emphasized the importance of workplace interventions to reduce stress and improve mental health (14).

Our data show higher addressability among rural women compared to urban women, likely due to reduced access to mental health services and the stigmatization of mental illnesses in rural areas. Studies suggest that telemedicine and public education campaigns can improve access to healthcare in rural areas (15). Mukhamadi-

eva and Shodieva (2021) demonstrated the effectiveness of telemedicine in enhancing access to mental health services in rural areas (16).

Personality disorders and suicidal ideation were frequently identified in patients with depression, consistent with the literature. Studies show that major depression often coexists with other psychiatric disorders, and effective management of these comorbidities and even dietary habits is crucial for suicide prevention (17, 18).

CONCLUSIONS

Analysis of 2023 admissions data for psychotic decompensations showed a slightly higher prevalence in males (53%) compared to females (47%). Among females, women aged 40-60 represented 88% of admissions, indicating increased susceptibility due to factors like work and family stress, menopause, and chronic conditions. In contrast, young women (20-40) had fewer admissions, possibly due to greater resilience or underreporting.

Patients from rural areas had higher admission rates (63%) compared to urban areas (37%) for major depressive disorders. This may be due to limited mental health services and stigma in rural areas. Strategies to improve access, such as telemedicine and public education campaigns, are essential.

Most hospitalized patients had high school education, particularly in the 40-60 age group, while those with higher education were underrepresented. Education level influences access to and utilization of mental health services, with higher education associated with better understanding and resources for managing mental health.

Women aged 40-60 years old were most affected by suicidal ideation (78%), compared to those over 61 (22%). This underscores the need for targeted interventions, including psychological support and monitoring. Autolytic attempts were more common in middle-aged women (13 cases), highlighting their vulnerability to stress.

Biological and clinical features in patients with suicidal ideation included elevated inflammatory markers (ESR), upper limit CRP, and gastrointestinal conditions. These comorbidities suggest a complex interaction between mental and somatic conditions, necessitating a multidisciplinary treatment approach.

Recommendations:

1. Tailored Interventions: Develop psychological support and stress management programs for middle-aged women and improve access for older individuals.

2. Improving Access: Expand mental health services in rural areas and reduce stigma.

3. Education and Prevention: Implement public education campaigns on mental health and promote service utilization across all educational levels.

4. Multidisciplinary Approach: Integrate the assessment and treatment of somatic conditions in managing depression and preventing suicide.

These conclusions highlight the importance of a comprehensive approach tailored to different age groups and socioeconomic categories to improve mental health and reduce psychotic decompensation and suicide risk. The study provides new insights into the determinants of psychotic decompensations and clear directions for developing effective intervention and prevention programs. Continued research is crucial to adapt public health policies to the specific needs of affected populations.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding.

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CLINICAL, BIOLOGICAL CHARACTERISTICS, RISK FACTORS AND OUTCOMES IN HUMAN ENTEROVIRUS CENTRAL NERVOUS SYSTEM INFECTIONS: A PROSPECTIVE STUDY PERFORMED IN A TERTIARY HOSPITAL

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CLINICAL, BIOLOGICAL CHARACTERISTICS, RISK FACTORS AND OUTCOMES IN HUMAN ENTEROVIRUS CENTRAL NERVOUS SYSTEM INFECTIONS: A PROSPECTIVE STUDY PERFORMED IN A TERTIARY HOSPITAL (Abstract): Human enterovirus (HEV) infection is transmitted mainly by the fecal-oral route and has mostly a subclinical, self - limited evolution. This viral central nervous system (CNS)infection could be fatal in various categories of patients. The aim of the current study was to evaluate the clinical-biological characteristics and outcomes of HEV CNS infections in patients admitted to the "Sf. Parascheva" Clinical Hospital of Infectious Diseases Iasi, during 2018-2019. **Materials and methods:** We performed an observational prospective study in patients with confirmed HEV CNS infections (encephalitis / meningitis) by cerebrospinal fluid analysis. Clinical and laboratory data were collected from medical records. **Results:** Sixty - four patients were included, of which 57.81% were diagnosed with HEV encephalitis. All categories of ages were affected, with a median of 67 years in encephalitis and 56 years for patients with meningitis. The most frequent comorbidities were cardiovascular (44.29% cases). The blood neutrophil - lymphocytes ratio (NLR) was statistically significant higher in case of HEV meningitis compared to encephalitis (12.42 ± 4.12 *vs.* 7.97 ± 3.86, p = 0.04). There were significant differences regarding NLR measured in cerebrospinal fluid. The median duration of hospitalization was 5.63 days in HEV encephalitis and 10.92 days in HEV meningitis. There were recorded 23.33% cases of death, all in old, immunosuppressed patients (diabetic patients) with HEV encephalitis. **Conclusions:** HEV CNS infections, are an important cause of mortality in high - risk patients (elderly, immunosuppression). Measurement of NLR could be a useful early diagnostic tool to ensure proper pharmacological management of HEV infections.
Keywords: RISK FACTORS, HUMAN ENTEROVIRUS, CENTRAL NERVOUS SYSTEM, PROSPECTIVE STUDY, TERTIARY HOSPITAL.

INTRODUCTION

Viral central nervous system (CNS) infections (meningitis and encephalitis) are an important cause of morbidity, permanent neurologic sequalae and even mortality among patients. Although they were recorded in all age categories, children and elderly patients, immunocompromised

patients are more strongly affected. The most common etiologies are herpesviruses 1 and 2 (HSV-1 and HSV-2), human herpesvirus 6 (HHV-6), non-polio enterovirus, and arboviruses (West Niles, Zika, dengue, chikungunya), influenza, cytomegalovirus (CMV), Epstein-Barr virus (EBV) (1, 2). Neurological complications, such as encephalitis were reported also in patients with SARS -CoV2 infections (3). Enteroviruses can affect both brain parenchyma and meninges and cause meningoencephalitis.

Diagnosis of enteroviral CNS infections is based on clinical - biological and imagistic data. Medical history, but also history of travel in endemic areas offers useful information to establish a diagnosis.

The International Encephalitis Consortium has proposed the following diagnostic criteria for encephalitis: altered mental status (altered consciousness, personality change) lasting over 24 hours (major criteria) and minor criteria: fever ≥ 38°C, seizures, focal neurological findings, CSF pleocytosis, focal neurological findings on neuroimaging, abnormalities on electroence-phalography without other identified cause (4). However, the criteria proposed have a low sensitivity and specificity of diagnosis, and a new validation score was proposed (5). Clinical features of enteroviral meningitis include triad: fever, neck stiffness and headache. Other neurological symptoms are photophobia and vomiting. Enteroviral CNS infections should be differentiated of other brain infections (brain abscess, other aseptic meningitis caused by *Borellia burgdorferi, Leptospira spp., meningeal tuberculosis, Treponema pallidum, Naegleria fowleri or Cripotccoccus neoformans*), encephalopathy (autoimmune, diabetic ketoacidosis, kidney failure or poisoning) or meningeal invasion in malignancies, autoimmune disorders or drug - induced aseptic meningitis (6, 7). Cerebrospinal fluid (CSF) analysis is the main tool to establish the diagnosis of CNS infection. CSF is clear with pleocytosis (mainly lymphocytes), with high level of proteins and variable levels of glucose depending on etiology. Etiological diagnosis is based on microscopic examination, serological tests (enzyme-linked immunosorbent assay, immunofluorescence, hemagglutination inhibition test) and molecular biology analysis (Polymerase chain reaction, Real-time polymerase chain reaction/qPCR, Reverse transcription polymerase chain reaction/RT-PCR, Nested polymerase chain reaction /nPCR) (1, 2).

The treatment of viral CNS infections is mainly symptomatic and pathogenic. Etiological treatment is available only for infections caused by herpes viruses. However, until a bacterial infection is excluded, antibacterial coverage is used in practical routine (8).

In this context, we conducted a prospective study to assess the clinical-biological characteristics and outcomes of HEV CNS infections in patients admitted to the "Sf. Parascheva Clinical Hospital of Infectious Diseases" Iasi during 2018-2019.

MATERIALS AND METHODS

We performed a prospective observational study which included patients with enteroviral CNS infections. Patients were hospitalized in Clinical Hospital of Infectious Diseases "Sf. Parascheva" Iasi between 2018 - 2019. The study was approved by the Ethics Committee of the hospital, the Ethics Committee of the University of Medicine and Pharmacy "Grigore T. Popa" Iasi. The data was collected in agreement to ensure data protection. The

informed consent was obtained from the patients or their legal representatives before the inclusion in the study.

Inclusion criteria

- written informed consent obtained from the patient or their legal representative
- clinical manifestations: fever, loss of consciousness, photophobia, headache, neck stiffness
- enteroviral CNS infection confirmed by CSF analysis

Exclusion criteria

- other non - enteroviral CNS infections caused by other viruses (including HIV infections)
- bacterial, mycobacterial or fungal CNS infections
- multiple organ dysfunctions
- other non - infectious CNS pathological conditions (cerebral hemorrhage or infarction, malignancies, autoimmune)
- lack of consent of the patients or the legal representative (when applicable)
- patients with incomplete clinical or biological data.

Data collection

Data collected from the medical records included: demographic data (age, gender), medical history, CNS infection history (symptoms at onset, duration), laboratory data (full blood count, CSF analysis, serological tests, PCR), imagistic (cerebral CT), treatment, duration of hospitalization and outcome.

Statistical analysis

Normally distributed data are presented as means ± standard deviations, and nonnormally distributed data are reported as medians and ranges). The chi-squared test was performed for the single-factor analysis of count data and the Kruskal-Wallis was performed for the single-factor analysis of non-normally distributed data. The differences were considered statistically significant at $p < 0.05$. Statistical analysis was performed using XLSTAT 2023.

RESULTS

A total of 64 eligible patients with HEV CNS infections (encephalitis or meningitis) were included in our analysis. Of these, viral meningitis was noted in 61.42% cases, mainly in males (ratio male / female of 3 in meningitis and 1.5 in encephalitis) (fig. 1).

All age groups were affected, the median age being 67 years (23 - 88 years) in HEV encephalitis, respectively 56 years (range 2 - 87 years) in HEV meningitis (tab. I).

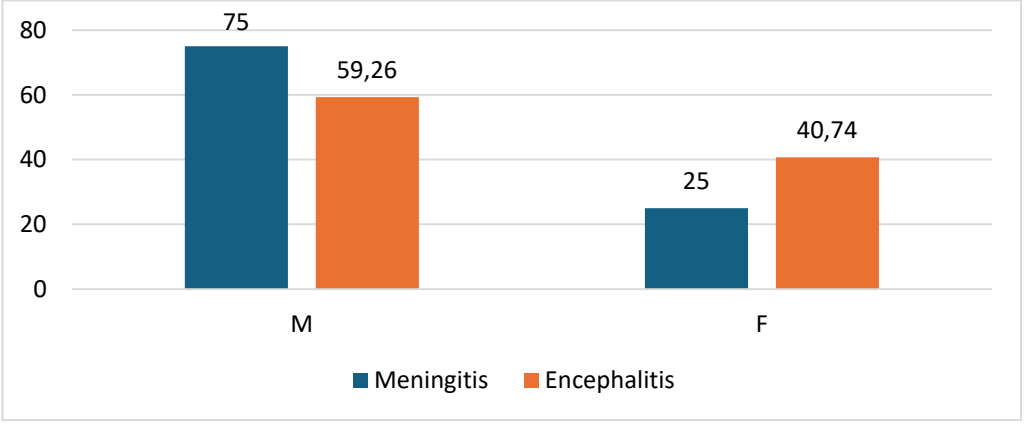

Fig. 1. HEV CNS infections - gender repartition (%)

TABLE I.
Demographic characteristics of patients with HEV CNS infections

	Age (years)		Height (cm)		Weight (kg)	
	Encephalitis (N = 27)	Meningitis (N=37)	Encephalitis (N = 27)	Meningitis (N=37)	Encephalitis (N = 27)	Meningitis (N=37)
Average	67.13	48.87	169.63	157.38	72.81	65
St. dev.	11.13	31.67	7.16	34.205	11.25	27.84
CV (%)	16.58	64.81	4.22	21.73	15.45	42.83
Median	67.13	56	170	172	73	73
Min	59	2	157	84	55	12
Max	75	87	184	183	90	93

Almost all patients described associated diseases (mostly cardiovascular - 44.29% cases and infections - 34.29% cases) (fig. 2).

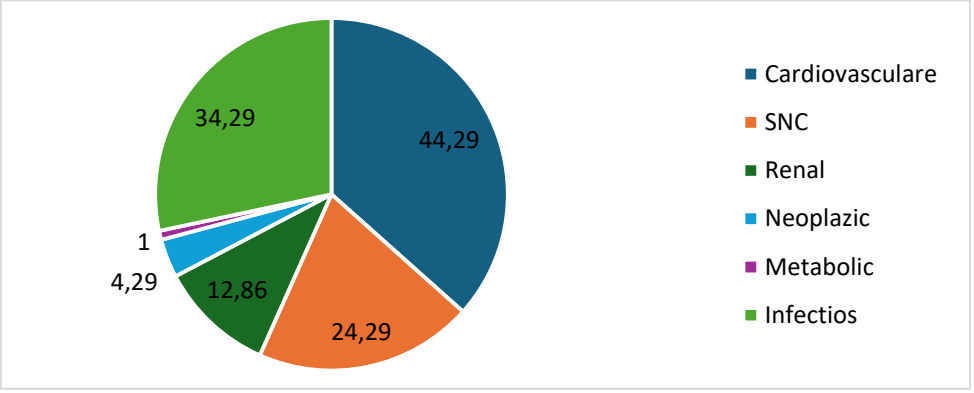

Fig. 2. Comorbidities in patients with HEV CNS infections

Fever was noted in almost all patients with CNS infections (71.43% cases of meningitis *vs.* 56.67% cases of meningitis). Various neurological symptoms were noted in both HEV CNS infections and neck stiffness was identified in both encephalitis and meningitis (fig. 3).

Mean ± standard deviation (CV%) of blood neutrophils to lymphocytes ratio (NLR) was 7.97 ± 3.86 (48.80%) in case of encephalitis, statistically significantly lower than the mean NLR of 12.42 ± 4.12 (33.16%) in case of meningitis (p = 0.04). On other side, NLR in CSF was not statistically different in case of encephalitis and meningitis (0.37 ± 0.28 *vs.* 0.54 ± 0.63, p =0.18) (tab. II).

Although the cellularity in the CSF was not significantly different between HEV encephalitis and meningitis, the biochemical analysis highlighted relevant changes. The level of proteins in CSF was significantly higher in case of meningitis (median of 66 g/l) than in encephalitis (median 1.67 g/l), without significant changes regarding levels of glucose and chloride in CSF.

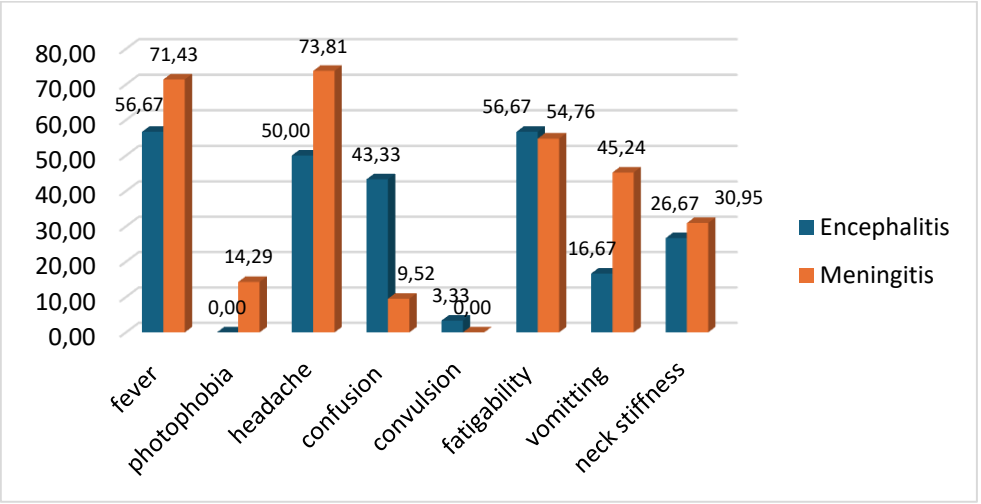

Fig. 3. Symptoms in patients with HEV CNS infections

TABLE II.

NLR ratio

Parameter	NLR			
	Blood		CSF	
	encephalitis	meningitis	encephalitis	meningitis
Mean	7.97	12.42	0.37	0.54
St dev	3.86	4.12	0.28	0.63
CV%	48.40	33.16	74.25	116.00
Median	7.76	3.89	0.39	0.25
Min	0.89	1.23	0.03	0.02
Max	13.58	14.55	0.77	2.03
p	**0.04**		**0.18**	

TABLE III.

CSF analysis - biochemistry results

Parameter	Proteins		Glucose		Chloride	
	encephalitis	meningitis	encephalitis	meningitis	encephalitis	meningitis
Mean	1.43	80.46	7.25	0.72	61.47	10.86
St. dev.	0.58	42.65	0.30	0.39	11.43	15.14
CV %	40.45	53.01	4.15	54.08	18.59	139.37
Median	1.67	66.00	7.33	0.79	59	7.28
Min	0.35	35.00	6.80	0.21	43	6.24
Max	2.10	164.00	7.69	1.4	87	69.6
P	**0.001**		**0.097**		**0.170**	

The mean duration of hospitalization was 15.63 days (range 2 - 34 days) in case of encephalitis and 10.92 days (range 6 - 27) days in case of meningitis. All patients received antibiotic treatment (third generation of cephalosporins, carbapenems, van-

co-mycin). There were recorded 23.33% cases of death, all being noted in old, immunosuppressed patients (diabetic patients) with HEV encephalitis. There were not noted cases of death in HEV meningitis.

DISCUSSION

We evaluated the clinical - biological features, risk factors and outcomes in HEV meningitis and HEV encephalitis. Sixty - four eligible patients were analyzed. The number of cases of meningitis was slightly higher than encephalitis (57.81% *vs.* 42.18%). All categories of ages were affected, with a wild range in case of meningitis (2 - 87 years).

Fever and headache were the main symptoms noted in both encephalitis and meningitis. Even neck stiffness is a specific feature for meningitis, it was noted also in almost 27% cases of HEV encephalitis. On other side, convulsions were noted in only one case of encephalitis. The clinical profile of HEV infections was similar with other published studies (6, 9, 10).

Neutrophils - lymphocytes ratio (NLR) is a biomarker which is very easy to be calculated and offers important prognosis for various disorders. It is calculated as ratio between counts of neutrophils and lymphocytes measured in peripheral blood. Elevated NLR was associated with bacterial or fungal infections, brain abscesses, stroke, myocardial infarction, serious trauma and is a predictor for efficiency of pharmacological treatment in different conditions (such as lymphoma) (11-13). NLR is also a predictive factor in different types of infections, such as sepsis, pneumonia or bacterial meningitis (14). We have identified in our analysis a significantly elevated NLR in case of HEV encephalitis compared with meningitis. NLR value was also elevated in cases of death. However, significance could not be established due to the low number of cases analyzed.

The value of NLR measured in CSF was not statistically different in both enteroviral CNS infections (meningitis and encephalitis). Nugrahanto *et al.* (2024) demonstrated that the value of NLR is significantly increased in case of bacterial meningitis and combined with the value of serum C reactive protein is an important factor for early detection and prognosis in bacterial meningitis. Our study revealed that NLR could be a marker for diagnosis of encephalitis, but this result will be further evaluated in a larger sample sized study (15).

HEV is mostly responsible for subclinical self - limited infections and is identified in almost 1% cases of viral encephalitis. Mortality is elevated in various conditions (agammaglobulinemia, immunosuppressive therapy) or severe systemic diseases (16).

We noted a high rate of use of antibacterial even after a non-bacterial etiology was confirmed. In line with other studies with similar results, the potential of drug toxicity and development of increased antimicrobial resistance is elevated after use of extended spectrum antibiotics in such cases (17).

The small sample size evaluated is the main limitation of the current study. However, this is an interim analysis of patients hospitalized in our clinic between 2018 - 2019. The number of patients was significantly limited by the years of COVID-19 pandemic when the Clinical Hospital of Infectious Diseases was a first - line hospital addressed only for patients with SARS - CoV2 infections. Further evaluations will be performed on eligible patients to obtain more reliable data.

CONCLUSIONS

Human enterovirus central nervous system infections, mainly encephalitis, are an important cause of mortality in patients with risk factors (elderly, immunosuppres-

sion). Measurement of neutrophils - lymphocytes ratio could be a useful early diagnostic tool for differentiation between meningitis and encephalitis to ensure proper pharmacological management of these pathological conditions.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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THE INTERPLAY BETWEEN GASTROINTESTINAL SYMPTOMS AND NUTRITIONAL STATUS IN SYSTEMIC SCLEROSIS PATIENTS

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THE INTERPLAY BETWEEN GASTROINTESTINAL SYMPTOMS AND NUTRITIONAL STATUS IN SYSTEMIC SCLEROSIS PATIENTS (Abstract): Systemic sclerosis (SSc) is a chronic autoimmune disease frequently associated with gastrointestinal (GIT) involvement and malnutrition, which can worsen disease outcomes and quality of life. This study **aimed** to assess digestive involvement and the prevalence of malnutrition in a group of SSc patients and to evaluate the relationship between malnutrition, symptom burden, and disease activity. **Materials and methods:** We included 65 patients with SSc. The UCLA GIT 2.0 questionnaire measured the digestive symptom burden. Malnutrition risk was assessed using the Malnutrition Universal Screening Tool (MUST) and Mini Nutritional Assessment (MNA) scores. We analyzed correlations between malnutrition status, disease activity (EUSTAR-AI index), and digestive symptom burden measured by UCLA GIT 2.0 score. **Results:** Almost 70% of the subjects showed digestive involvement. The prevalence of malnutrition (MNA) was 6.6%, and the risk of malnutrition was 7.7% (MUST). Malnourished individuals experienced significantly higher scores in UCLA (p=0.009), UCLA reflux (p=0.05), UCLA emotional well-being (p=0.001), and UCLA social function (p=0.08), as well as increased disease activity scores (EUSTAR-AI—p=0.027). **Conclusions:** Digestive involvement and malnutrition are frequent among SSc patients, particularly those with higher disease activity scores and GIT involvement. These results highlight the need for rapid gastrointestinal and nutritional management in SSc to improve patient outcomes and quality of life. **Keywords:** SYSTEMIC SCLEROSIS, GASTROINTESTINAL, MALNUTRITION.

INTRODUCTION

Systemic sclerosis (SSc) is a systemic autoimmune disease, with a complex pathogenesis, characterized by skin fibrosis, vasculopathy and internal organs malfunction. The most common symptoms are Raynaud's phenomenon and skin fibrosis (1,2). There are two main subsets of the disease (the limited cutaneous form – lcSSc and the diffuse cutaneous form – dcSSc) that have different developments but also share many characteristics (3). DcSSc is characterized by an early and accelerated organ involvement, a severe prognosis and the presence of anti-Scl 70 antibodies (also known as anti topoisomerase1 antibodies). LcSSc is expressed by a gradual disease development, later

visceral involvement, anti-centromere antibodies positivity, and a better disease prognosis (1,4,5).

After skin, the gastrointestinal tract (GIT) is the second most frequently affected organ. Digestive involvement is observed in 90% of the patients, secondary to progressive fibrosis of the GIT, spanning from the oral cavity to the anus (2,6). This determines the dysmotility and hypomotility of the esophagus, the small intestine, and the gut (7). The GIT involvement causes serious morbidity and affects the survival of these patients (8). The digestive manifestations of SSc may vary depending on the affected area of the GIT (8,9,10). Esophageal symptoms are reported by up to 90% of patients (4). In general, patients experience mild gastroesophageal reflux disease. However, severe dysmotility of the upper and/or lower bowel can also be present in some cases, causing severe manifestations. For example, almost half of the patients experience gastric dysfunction characterized mainly by gastroparesis and gastric antral vascular ectasia (GAVE) (2,11). Dysmotility of the small bowel occurs in up to 80% of the subjects, with the small intestine being considered the second most affected digestive organ (2,12). As far as the colon is concerned, in up to 50% of the patients varying bowel patterns may be observed, alternately constipation and diarrhea or other symptoms such as bloating, distention, malnutrition or bleeding (13,14).

The GIT can be involved in both forms of SSc, although it can be more frequent or more severe in the diffuse phenotype. Other risk factors for severe GIT involvement include male sex, myopathy, and sicca symptoms (15). The severity of digestive symptoms is also associated with emotional distress and depressive disorders (16,17).

It is not entirely understood how the SSc-GIT symptoms develop over time and despite the significant prevalence of GIT disease in SSc, there are few available treatments (18). Severe digestive involvement can lead in time to malnutrition.

In SSc, malnutrition is reported as an independent risk factor for mortality, being associated with cardiac and gastrointestinal involvement, inflammatory status, dysphagia, and chronic intestinal pseudo obstruction (19,20). Malnutrition results from GIT involvement but also intensifies the illness, creating a vicious cycle of deteriorating nutritional status and exacerbating systemic symptoms.

Depending on the assessing methods used for defining the risk of malnutrition, its prevalence in SSc ranges between 18% and 38% (21,20). For example, when using the MUST score, in a Canadian cohort of 586 SSc patients, 10.8% were at medium risk for malnutrition and 17.4% were at high risk. When compared to MUST score for hospitalized patients, the results were similar (21). Higher prevalence of malnutrition were obtained by a French study group that defined malnutrition as one of the following three options: weight loss of > 5% over one month or more than 10% over 6 months, BMI < 21 kg/m^2, or albuminemia < 35 g/L. In their cohort, 38.3% were at high risk according to MUST, 59.2% of the patients were diagnosed with malnutrition, and 25% had severe malnutrition (20).

There are various screening tools for malnutrition and the ESPEN recommends using Nutritional Risk Screening 2002 (NRS – 2002) for patients in the hospital, MUST (The Malnutrition Universal Screening Tool) in the community, and the first part of MNA (Mini Nutritional Assessment) for elders (22).

To assess the digestive involvement in SSc, clinicians can use the University of California at Los Angeles Scleroderma Clin-

ical Trial Consortium GIT 2.0 (UCLA SCTC GIT 2.0) instrument. Although objective studies are the gold standard for diagnosis of GIT involvement, UCLA SCTC GIT 2.0 reflux and distention/bloating scales are sensitive enough and complement the objective methods (23). It is a validated questionnaire, completed by the patient that evaluates seven domains covering a variety of digestive symptoms. It can also highlight the need for further investigations of the GIT, being a useful tool in the screening of SSc patients (24).

It can be very difficult to identify individuals who are at a high risk of experiencing clinically substantial weight loss or malnutrition because GIT symptoms are frequent in SSc patients. In this context, we aimed to explore the range of gastrointestinal symptoms in patients with SSc and evaluate if there are any correlations with the risk of malnutrition.

MATERIALS AND METHODS

We performed an observational study in which we included 65 patients with a SSc diagnosis according to the American College of Rheumatology/European League Against Rheumatism (EULAR) 2013 classification criteria for SSc. The patients were adults admitted to the rheumatology department for revaluation. Patients with mixed connective tissue disease, overlap syndrome, neoplasia, or who refused to participate were excluded.

Information about age, sex, use of immune modulatory treatment, the type of SSc according to LeRoy criteria (25), and other clinically relevant data for our study were taken from the medical records. The modified Rodnan skin score (mRSS) was used for skin involvement assessment. Using venous blood samples, we performed within the hospital laboratory a biochemical evaluation. The hospital laboratory also measured the serum antinuclear antibodies, anti-topoisomerase I, and anticentromere antibody titers. Esophageal reflux presence was considered if patients complained about heartburn or regurgitation. The disease activity was evaluated using the EUSTAR-AI tool (European Scleroderma Trials and Research group Activity Index). Active disease was defined as having an EUSTAR-AI score exceeding 2.5.

We also evaluated the following anthropometric parameters: height (H), weight (W), and body mass index (BMI, calculated as W/H^2). Using the World Health Organization definition for BMI classes our subjects were divided into underweight (BMI under 18.5 kg/m^2), eutrophic (BMI between 18.5 and 24.9 kg/m^2), pre-obesity (BMI between 25.0 and 29.9 kg/m^2) and obesity (BMI greater than 30 kg/m^2).

To evaluate the digestive involvement we used a translated, adapted, and validated version of the UCLA GIT 2.0 questionnaire (26). The questionnaire included 7 categories of symptoms: reflux, distention/bloating, fecal continence, diarrhea, constipation, social function, and emotional well-being. The final score is the average of the scores for each category (except constipation) and may vary between 0 and 2.83. We defined the presence of gastrointestinal involvement if the patients had a total score or at least one of the seven categories > 0. The score was interpreted as previously described by van Leeuwen et al. (27). It was divided into none-mild (a score of 0 or < 0.5), moderate (a score ≥ 0.5 but less than 1.01), and severe (≥ 1.01). For fecal incontinence and distention/bloating items, the symptoms were considered none-mild for a score < 1.01, moderate ≤ 1.61 for distention/bloating and ≤ 2.01 for fecal soiling, and severe if the score was > 1.61 for distention/bloating and > 2.01 for fecal soiling.

The risk of malnutrition was evaluated using the translated version of MNA (Mini Nutritional Assessment) score where malnutrition was defined with a score less than or equal to 7, risk of malnutrition with scores between 8 and 11, and normal nutrition with values greater than 11. We also performed the MUST score and patients were classified as being at low risk (MUST score = 0), at moderate risk (MUST score = 1) or at high risk of malnutrition (MUST score ≥ 2).

The Ethics Research Committee of the Clinical Rehabilitation Hospital from Iasi and the University of Medicine and Pharmacy "Grigore T. Popa" Iasi approved the study and ensured compliance with the principles of the Declaration of Helsinki. Subjects gave written informed consent to undergo the examination.

For the statistical analysis, the tests were performed on SPSS version 26. Categorical variables were presented as proportions and Fisher's test was performed. Continuous variables were expressed as mean and standard deviation. Statistically significant differences between patients with lcSSc and dcSSc were assessed by independent *t* test.

RESULTS

General characteristics. A number of 65 patients with SSc met the inclusion criteria and completed the questionnaires. According to the LeRoy classification criteria, 27 had a limited form of SSc, and 38 had a diffuse subset. The majority were women (86.2%) with a mean age of 57.15 (±10.35) years old. The mean disease duration was 8.48 (±7.09) years. Anticentromere antibodies were present in 38.5% of the patients, while 49.2% of the subjects were positive for anti-SCL70 antibodies.

The demographic and other clinical data are displayed in the first table. The results are expressed as mean ± standard deviation for continuous variables and as number (percentage) for categorical variables. Inflammatory syndrome was considered if either ESR or CRP were elevated. mRSS – modified Rodnan skin score; BMI – body mass index; ILD – interstitial lung disease.

Inflammatory syndrome, described as the presence of ESR (erythrocyte sedimentation rate) and CRP (C reactive protein) above the laboratory normal values, was identified in 52.3% of the patients, more than half of whom had dcSSc.

Interstitial lung disease (ILD) was reported in 63.1% of the patients, mainly in dcSSc subjects (73.7%, *p=0.04)*. Patients with ILD had more often SCL70 antibodies (*p= 0.01)*, higher mRSS skin scores (*p=0.002),* and higher values for muscular enzymes (creatine kinase – *p=0.04* and lactate dehydrogenase – *p=0.014*).

Digestive involvement

Digestive involvement was considered in patients who exhibited gastrointestinal tract symptoms and was present in nearly 70% of those included. Dysphagia was reported in 53.8% of the patients, slightly more frequent in the dcSSc type (55% vs 51%). Early satiety was present in 49.2% of the subjects, of which 63% had lcSSc (*p=0.08)*. Esophageal reflux was spotted in 76.9% of the subjects, with no difference between the two subsets of the SSc. Patients with reflux also had lower interincisive distance (p= 0.05), lower weight, higher CRP levels, and higher EUSTAR-AI scores– with no statistical significance.

Significant differences according to t test for continuous variables and Fisher's exact test for categorical variables comparing lcSSc to dcSSc are highlighted by p values.

The overall UCLA GIT 2.0 score was similar between the two phenotypes of SSc, with the majority of the patients describing none to mild symptoms. Patients with

dcSSc were more likely to have reflux, but the results did not reach statistical significance (0.79 vs 0.69). Furthermore, the reflux symptoms were more serious in dcSSc patients, as 13 vs 5 patients experienced severe UCLA GIT 2.0 scores. UCLA constipation score was higher in lcSSc patients compared to dcSSc patients (0.59 vs 0.36, *p=0.08*) but the difference did not reach statistical significance. Regarding the other domains of the UCLA SCTC 2.0 tool, the scores were similar between the two groups of lcSSc and dcSSc, most of the patients describing none to mild symptoms in all of the categories (tab. II).

Patients with extensive cutaneous involvement, according to mRSS score, had higher UCLA reflux scores (*p=0.07)*.

Anticentromere positivity correlated with higher scores for UCLA (*p=0.048)*, UCLA social function (*p=0.049*), UCLA emotional well-being (*p=0.036),* and UCLA constipation (*p=0.003)*. Furthermore, anticentromere antibodies correlated with moderate digestive involvement according to UCLA severity (*p=0.012)*.

TABLE I.

Characteristics of the study subjects.

General characteristics	Overall (n=65)	lcSSc (n=27)	dcSSc (n=38)	*p*
Age	57.15 ± 10.35	60.44 ± 8.11	54.82 ± 11.20	0.02
Gender, n (%female)	86.2%	100%	76.3%	0.008
Disease duration	8.48 ± 7.09	11.41 ± 7.9	6.39 ± 5.6	0.007
SCL 70 (%)	49.2%	7.7%	78.9%	0.000
Anticentromere antibodies (%)	38.5%	81.5%	8.1%	0.000
mRSS	11.77 ± 6.6	9.67 ± 4.51	13.3 ± 7.5	0.012
ILD	63.1 %	48.1%	73.7%	0.042
Inflammatory Syndrome	52.3%	40.7%	60.5%	0.1
Anemia	67.7%	66.7%	68.4%	1
Dysphagia	53.8%	51.9%	55.3%	0.8
Early satiety	49.2%	63%	39.5%	0.08
Reflux	76.9%	77.8%	76.3%	1
BMI classification				
Underweight (BMI< 18.5)	6.2%	0 %	10.5%	
Healthy weight (BMI = 18.5 – 24.9)	44.6%	51.9 %	39.5 %	
Overweight (BMI = 25 – 29.9)	36.9 %	37 %	36.8 %	
Obesity (BMI >30)	12.3%	11.1 %	13.2 %	
MNA classification				
Normal	43.1 %	41.7 %	58.1 %	
At risk	35.4 %	50 %	35.5 %	
Malnourished	6.2 %	8.3 %	6.5 %	
MUST classification				
Low risk	66.2 %	75 %	80.6 %	
Moderate risk	10.8 %	20.8 %	6.5 %	
High risk	7.7 %	4.2 %	12.9 %	
EUSTAR – AI	1.91 ± 1.25	1.55 ± 1.08	2.17 ± 1.31	0.051
Active disease (≥ 2.5)	27.7 %	14.8 %	37.8 %	0.053
Inactive disease (< 2.5)	70.8 %	85.2 %	62. 2%	

TABLE II.

Results of the UCLA SCTC 2.0 questionnaire

UCLA SCTC 2.0	Overall (n=65)	lcSSc (n=27)	dcSSc (n=38)	*p*
UCLA score	0.43 (0.387)	0.461 (0.41)	0.408 (0.374)	
None-Mild, n (% total)	44 (67.7%)	17 (63%)	27 (71.1%)	
Moderate, n (% total)	15 (23.1 %)	8 (29.6 %)	7 (18.4 %)	
Severe, n (% total)	6 (9.2 %)	2 (7.4 %)	4 (10.5 %)	
UCLA Reflux	0.752 (0.607)	0.69 (0.513)	0.796 (0.669)	
None-Mild, n (%total)	25 (38.5%)	9 (33.3%)	16 (42.1 %)	
Moderate, n(%total)	22 (33.8 %)	13 (48.1 %)	9 (23.7 %)	
Severe, n (% total)	18 (27.7 %)	5 (18.5 %)	13 (34.2 %)	
UCLA Distention	0.788 (0.856)	0.935 (0.916)	0.684 (0.806)	
None-Mild n (%total)	47 (72.3%)	19 (70.4%)	28 (73.7%)	
Moderate, n(%total)	8 (12.3 %)	3 (11.1 %)	5 (13.2%)	
Severe, n (% total)	10 (15.4 %)	5 (18.5%)	5 (13.2%)	
UCLA Fecal soilage	0.123 (0.545)	0.185 (0.622)	0.078 (0.486)	
None-Mild n (%total)	63 (96.9%)	26 (96.3%)	37 (97.4%)	
Moderate, n(%total)	0 (0%)	0 (0%)	0 (0%)	
Severe, n (% total)	2 (3.1%)	1 (3.7%)	1 (2.6%)	
UCLA Diarrhea	0.130 (0.333)	0.074 (0.266)	0.171 (0.372)	0.052
None-Mild n (%total)	54 (83.1%)	25 (92.6%)	29 (76.3 %)	
Moderate, n(%total)	9 (13.8%)	2 (7.4%)	7 (18.4 %)	0.071
Severe, n (% total)	2 (3.1%)	0 (0 %)	2 (5.3 %)	
UCLA Social function	0.254 (0.342)	0. 325 (0.389)	0.205 (0.3)	
None-Mild n (%total)	49 (75.4%)	19 (70.4 %)	30 (78.9%)	
Moderate, n(%total)	13 (20%)	6 (22.2 %)	7 (18.4 %)	
Severe, n (% total)	3 (4.6 %)	2 (7.4%)	1 (2.6%)	
UCLA Emotional well-being	0.53 (0.638)	0.556 (0.631)	0.512 (0.651)	
None-Mild n (%total)	36 (55.4%)	14 (51.9%)	22 (57.9%)	
Moderate, n(%total)	18 (27.7%)	10 (37%)	8 (21.1 %)	
Severe, n (% total)	11 (16.9%)	3 (11.1%)	8 (21.1%)	
UCLA constipation	0.457 (0.535)	0.592 (0.524)	0.361 (0.528)	0.08
None-Mild n (%total)	34 (52.3%)	11 (40.7 %)	23 (60.5 %)	
Moderate, n(%total)	24 (36.9 %)	11 (40.7 %)	13 (34.2 %)	0.054
Severe, n (% total)	7 (10.8 %)	5 (18.5 %)	2 (5.3 %)	

Malnutrition. According to MUST, the risk of malnutrition was high in 7.7% of the patients, moderate in 10.8%, and low in 66.2%. Subjects with a high risk had lower interincisive distance (p=0.03) and higher scores in almost all of the UCLA domains except constipation, but the results did not reach statistical significance.

Based on the MNA, malnourished individuals (6.2% of the subjects) experienced higher values for UCLA (*p= 0.009)*, UCLA reflux (*p=0.05)*, UCLA emotional well-being (*p= 0.001)*, and UCLA social function (*p=0.08)*. Malnourished patients also had higher disease activity scores calculated with the EUSTAR-AI index (*p= 0.027)*. MNA evaluation score also correlated with the presence of dysphagia (*p=0.05)*.

DISCUSSION

In current literature, GIT involvement is present in nearly all SSc patients at various

extents (28). According to the 2012 EUSTAR cohort, gastroesophageal symptoms are the most common, reported in more than 60% of the patients (29). GERD symptoms are more frequently reported (in almost 80% of the cases) if ILD is associated (30). In very early SSc, severe GIT involvement can be present in 15% of the cases, being associated with telangiectasias, higher skin scores, inflammatory myositis, but also with high mortality and low quality of life (31). Severe gastrointestinal involvement can initially lead to unintentional weight loss, which may become significant and result in malnutrition. Male sex, dcSSc, digital ulcers, shorter disease duration, elevated erythrocyte sedimentation rate (ESR)/C-reactive protein, elevated creatine kinase (CK), pulmonary hypertension, aberrant diastolic dysfunction, and interstitial lung disease were among the clinical characteristics linked to significant weight loss (32).

Using the UCLA SCTC GIT 2.0 tool, the severity of the GIT symptoms varies from absent to very mild in 39% of the patients, to mild in 21% of the patients, moderate in 31%, and severe to very severe in 9% of the patients (24). A more recent study that included 834 SSc patients, which used the UCLA SCTC GIT 2.0 questionnaire to evaluate the digestive symptoms, reported none to mild in 73% of the subjects, moderate in 21%, and severe in 7% of the subjects. The same study declared that female sex, smoker status, presence of anticentromere antibodies, and corticosteroid usage were associated with more severe GIT involvement (27).

In the present study, we report that nearly 70% of the patients with SSc experience digestive symptoms, especially gastroesophageal reflux. Alastal *et al*. reported a frequency of 59.24 % for digestive involvement, and GERD was present in 34.8% of the subjects included (33).

The severity of digestive symptoms correlated with the presence of anticentromere antibodies, as these patients reported higher scores for nearly all UCLA domains. These results align with those previously described in the literature (27).

MUST and MNA scores focus slightly differently depending on the questions included: MUST focus more on BMI, recent weight loss, and acute disease, while MNA includes questions on dietary intake, mobility, and psychological stress. According to MNA, 6.2% of the patients in our study group were malnourished. Cano Garcia *et al*. reported lower malnutrition rates using the same index and correlated the results with diffuse skin involvement and sarcopenia (34). According to MUST, 7.7 % of our study group were at high risk of becoming malnourished. We obtained lower rates of malnutrition in our study compared to the existing literature (20,21).

Malnutrition exacerbates GIT symptoms, resulting in a vicious cycle of worsening systemic symptoms and declining nutritional status. Caimmi *et al*. (35) have previously demonstrated that MUST scores correlate with UCLA GIT values. In our case, we obtained higher UCLA scores in malnourished patients, suggesting that digestive involvement impacts their food intake, emotional well-being, and social functioning.

Furthermore, we observed a connection between malnutrition and disease severity measured with the EUSTAR-AI index. A more active disease often involves systemic inflammation, which can increase metabolic demands and reduce nutrient absorption. In our study group, malnutrition seems to be both a consequence of high disease burden and GIT involvement but also a factor that may intensify symptom severity.

The current investigation is constrained by a limited sample size of enrolled pa-

tients. Another limitation is that UCLA GIT is a self-reported questionnaire designed to assess symptoms in the past seven days, potentially introducing bias. Patients with GIT symptoms were not further evaluated with objective measurements to confirm the digestive pathology.

CONCLUSIONS

In conclusion, our data gives information about the high frequency of GIT symptoms in patients with SSc. The assessment of digestive involvement using the UCLA SCTC GIT 2.0 questionnaire provides a systematic approach to characterize the spectrum of GIT manifestations. It facilitates early detection of GIT symptoms and their impact on patient's quality of life, enabling clinicians to further recommend objective methods for the diagnosis of GI disease. Digestive involvement in systemic sclerosis patients often leads to nutritional impairment. Malnutrition can worsen the severity of SSc, creating a cycle where increased disease activity and symptom burden further deteriorate nutritional status.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding.

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SPATIAL-TEMPORAL PATTERNS OF DIABETES PREVALENCE IN ROMANIAN COUNTIES BETWEEN 2012-2021: INSIGHTS FROM THE COMPARATIVE ANALYSIS OF TIME SERIES

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SPATIAL-TEMPORAL PATTERNS OF DIABETES PREVALENCE IN ROMANIAN COUNTIES BETWEEN 2012-2021: INSIGHTS FROM THE COMPARATIVE ANALYSIS OF TIME SERIES (Abstract): Our study investigates the evolution of Diabetes prevalence in Romania across a 10-year period (2012-2021) by applying comparative time series analysis techniques to epidemiological data recorded from all 41 counties of Romania, as well as Bucharest. **Materials and methods**: The main goal was to highlight the similarities and differences between counties, through several methods: the distance-based analysis of the observed values (Euclidean distances) and trends (Manhattan distance), followed by the study of correlations between the standardized Z-scores of the original values and the counties Hierarchical Clustering. **Results:** Our findings revealed significant geographical similarities, confirmed simultaneously by two or more methods among the four involved. These results are exemplified with the particular case of Iasi county, which was found as having a similar behavior with Arges, Dolj and Bihor counties according to the previously stated condition; the in-depth analysis of socio-demographic features explains the detected similarities. **Conclusions:** The study highlights the added value of time series analysis over classical cross-sectional approaches, as it enables the detection of more complex relationships between the raw epidemiological data, expressed through trends, statistical anomalies, or regional clusters. Such results have direct implications in optimizing the public health policy, particularly required in chronic diseases like diabetes**.** **Keywords:** TIME SERIES ANALYSIS; DIABETES MELLITUS; TREND ANALYSIS; EPIDEMIOLOGY.

INTRODUCTION

Time series analysis is of great interest in medical studies, as numerous clinical, epidemiological, and public health phenomena manifest and evolve over time. Time series enable the monitoring of disease incidence and prevalence, highlighting the trends and the possible seasonal components, thus providing a solid foundation for forecasting future cases. The statistical tools used to investigate and quantify the evolution of time series are highly versatile, being primarily focused on their description and modelling in order to detect patterns. This facilitates a better understanding of the phenomenon dynamics over

time and supports the development of more accurate forecasts (1).

The behavior of a time series (2) is quantified through four components: trend (T), representing the general long-term direction; seasonality (S), corresponding to regular short-term fluctuations; cyclicity (C), reflecting irregular long-term variations; and residuals (R), which account for random variations. Time series are described by combining these using two approaches: additive models ($Y = T + S + R$) or multiplicative models ($Y = T \times S \times R$).

The main statistical techniques for analysis aim to characterize the observed behavior by identifying autocorrelations and making forecasts. The autocorrelations assess the dependence degree between the values of the series at different points in time, highlighting therefore the possible patterns. The forecasting extrapolates the series' behavior into the future using well-defined mathematical models: autoregressive models (AR), which estimate future values based on past values; moving average models (MA), which rely on past errors; ARMA or ARIMA models, which combine the previous two and may also include a seasonal component (SARIMA); and Exponential Smoothing models, which account for both trend and seasonality (3, 4, 5). The major challenge lies in identifying the optimal model, which is typically achieved through successive testing of all possible alternatives.

A particular aspect of this analysis is the comparative study of multiple time series (6), aimed at identifying similarities between them and understanding their temporal evolution in order to determine whether phenomena observed in different contexts follow similar or divergent dynamics. The comparative study of time series investigates separately each one of the elements that characterize them. It typically begins with the comparison of absolute values, followed by trend comparison, analysis of shape similarity (through correlations and distance functions), clustering based on their temporal trajectories, and, if applicable, identification of causal or synchronized relationships through cross-correlations (7, 8).

These investigation techniques hold particular importance in the medical field, as they allow to detect common patterns among areas or patients' samples, facilitating further to depict the significant determinants (behavioral, environmental, or the result of certain health policies). Consequently, areas exhibiting similar trends can benefit from coordinated prevention and treatment measures, which optimizes the transfer of best practices and the alignment of public health interventions, improving also their effectiveness and impact in real-time.

A relevant example in this regard is the comparative study of the incidence and prevalence of various pathologies across geographically or administratively distinct areas, aiming to highlight the degree of similarity or divergence between them. Such information becomes the starting point for further analyses regarding potential causes, consequences, and recommended intervention strategies. This is the objective of the present study, in which we propose a reinterpretation, from a new perspective, of data regarding the prevalence of diabetes cases in all counties of Romania over a 10-year period (2012-2021). We approached these data from a global perspective, aiming to compare the regional evolution of this pathology, in order to identify similarities and differences and to provide therefore a reliable basis for well-informed medical decisions and, consequently, for more accurately tar-

geted interventions.

MATERIALS AND METHODS

Database. The database used in our analysis was extracted from a report achieved by the National Institute of Public Health in 2022, regarding the Diabetes Evolution Evidence in Romania, between 2012-2021 (insp.gov.ro). Other newer public data regarding the Diabetes incidence and prevalence in Romania between 2022-2025 were unavailable - that is why we limited our analysis to these data. However, this does not jeopardize the quality of the analysis we intended to carry out, as the territorial similarities we aimed to highlight are not significantly affected by the novelty of the data, depending primarily on the investigated time series accuracy and length.

Methods. The transversal analysis of multiple time series is a sort of statistical comparison between them, conducted in order to identify the existent similarities regarding the direct values, as well as the evolution trends, patterns and periodicity (if any).

The initial approach consists in the visual inspection of the observed values, typically conducted through standard distribution charts. A good practice is to standardize the values, by calculating the corresponding Z-scores - because in this way we eliminate the influence of absolute values, being able to focus better on the trend. The distribution charts usually highlight the general behavior, as well as the trend's direction and the possible points of inflexion.

It follows the analysis of differences between paired values (by noticing how many of them are positive and how many are negative) and of ratios between them (by noticing how many are much higher above 1). A more accurate approach is instead to calculate a distance function between values (9); usually an L-p distance function is recommended, $(x, y) = (\sum |x_i - y_i|^p)^{\frac{1}{p}}$, the most efficient in such cases being the L-2 distance (the Euclidean norm), $D(x, y) = (\sum |x_i - y_i|^2)^{\frac{1}{2}}$ (10). This distance is particularly very popular because it has the properties to be preserved under orthonormal transformations (it is the only one with this property among the class of L-p norms) and to be optimal for estimation in the presence of Gaussian additive noise. Other distance measures recommended in such situations are the Manhattan distance, $D(x, y) = \sum |x_i - y_i|$ and the infinite norm distance, $D(x, y) = \max |x_i - y_i|$ (11). In our study, due to the exposed reasons, we conducted the analysis based on the Euclidean norm.

The final purpose of this analysis is in fact to identify the so-called "significant" similarities between counties; unfortunately, the scientific literature does not provide an appropriate significance test. Therefore, our solution was to calculate the range of Euclidean distances between counties and to report as significant the distances smaller than the 5th percentile, as well as the distances between the 5th and the 10th percentile within this range. Heatmaps and 3D surface charts are the best methods to represent intuitively such results, as well as the tables with similarities.

The next step is to compare the time series evolution, reflected by their trends, patterns or periodicities (12, 13). The easiest way to calculate the trends is through linear regression models; the trend is actually the slope of the regression line between x = the year (between 1 - 2012 and 10 - 2021) and y = the yearly number of new cases for each monitored county. In order to depict the similarities between trends, the available solution is to calculate

again the differences between them, using dedicated measures; we used the Manhattan distance in this regard. The significant similarities between trends were identified also through range analysis - the distances smaller than the 5th percentile and the distances between the 5th and the 10th percentile within the range were reported as significant.

Another available method to depict similarities between trends is based on the Z-scores of the original values (14); since such scores have the property to remove the noise and to preserve the trend, the calculation of correlation coefficients between them is able to provide the significant similarities - they correspond to significant correlation coefficients, bigger than 0.7.

A third method in the same regard is the Z-scores Hierarchical Clustering, based on Ward's method and the Euclidean distance - this is a powerful tool to highlight the time series with similar evolutions (15, 16). Ward's method is an appropriate choice because it minimizes the interval variation of each cluster, in order to create clusters of counties which are similar between them and as different as possible from the others.

We used all these methods on our data, in order to compare their performances in identifying similarities between Romanian counties in which concerns the evolution of Diabetes cases between 2012-2021.

The calculations were made in *SPSS 29.0, MATLAB 2010* and *Microsoft Excel.*

RESULTS

The analysis was carried out on 43 time series (corresponding to the 41 counties of Romania, as well as to the Bucharest area and the whole country).

In the first step of our analysis we proceeded with the direct investigation of the recorded values, represented through distribution charts (fig. 1, 2).

We created the distribution charts for the original values (fig. 1), as well as for the corresponding Z-scores (fig. 2).

Due to the chart's complexity (the increased number of lines) it is rather difficult to analyze them and to compare the cases evolution in different counties; anyway, the Z-scores chart emphasizes clearly that we are dealing in all cases with ascendant trends.

In the next step of our analysis we calculated the Euclidean distances between all possible paired values, in order to identify the similarities between counties, according to the proposed criteria of significance, based on percentiles. The chart of Euclidean distances between counties in which concerns the yearly number of Diabetes cases is presented in third figure.

The range of variation is 431700.93, the minimal Euclidean distance being equal with 1740.46 and the maximal one being equal with 433441.39. As we already stated, we divided the total range of variation in 11 intervals, defined by the percentiles 0.05, 0.1, 0.2 … 0.9 and we grouped the pairs of counties falling within each interval of variation (tab. I).

The next step of the study was the investigation of the recorded time series trends, by analysing first the differences between slopes, calculated using the Manhattan distances. The chart of Manhattan distances between trends is presented in fourth figure.

The range of variation is 7985.60, the minimal Manhattan distance being equal with 0.66 and the maximal one being equal with 7985.60. We divided again the total range of variation in 11 intervals, defined by the percentiles 0.05, 0.1, 0.2 … 0.9 and we grouped the pairs of counties falling within each interval of variation (tab. II).

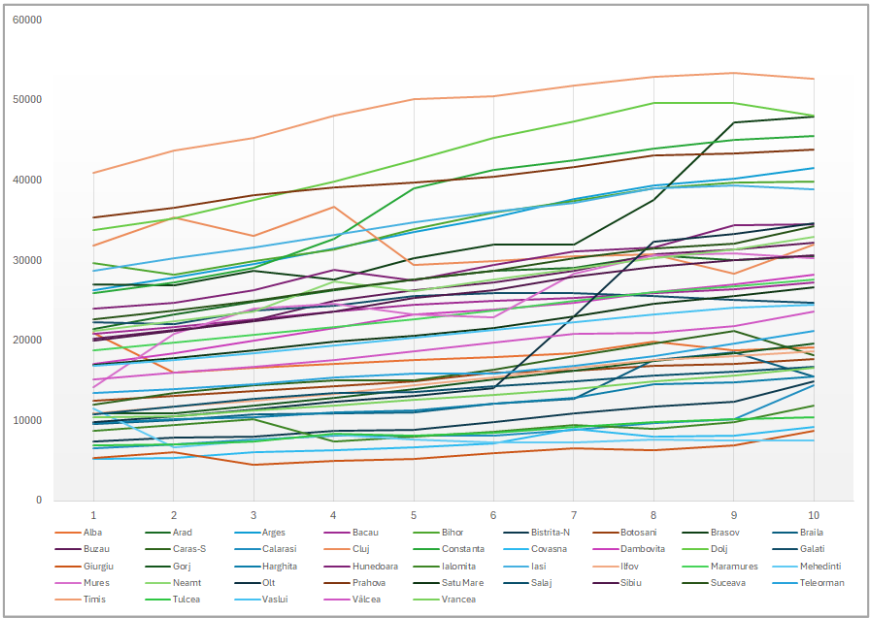

Fig. 1. The recorded number of diabetes cases in Romanian counties between 2012-2021 (the original values distribution chart)

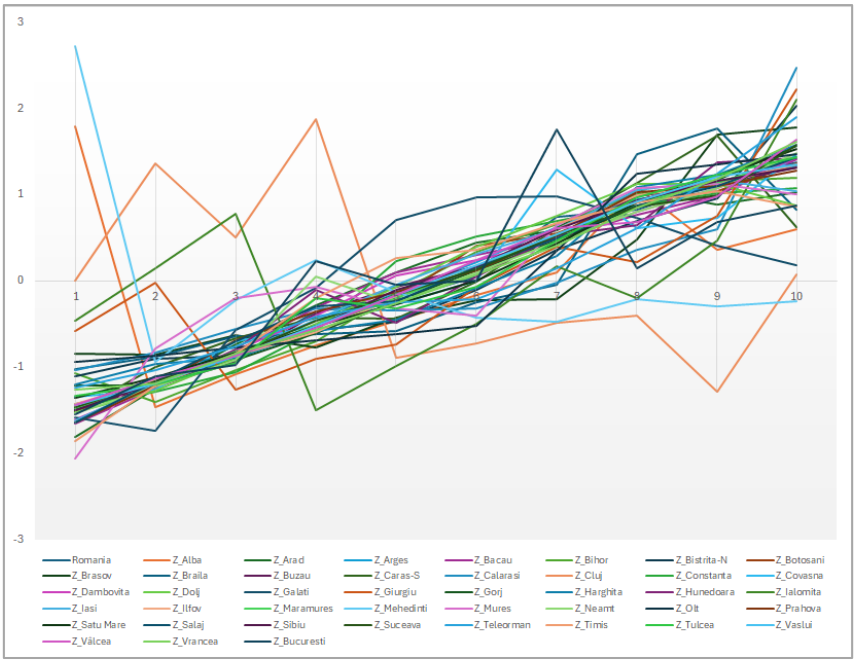

Fig. 2. The recorded number of diabetes cases in Romanian counties between 2012-2021 (the Z-scores distribution chart)

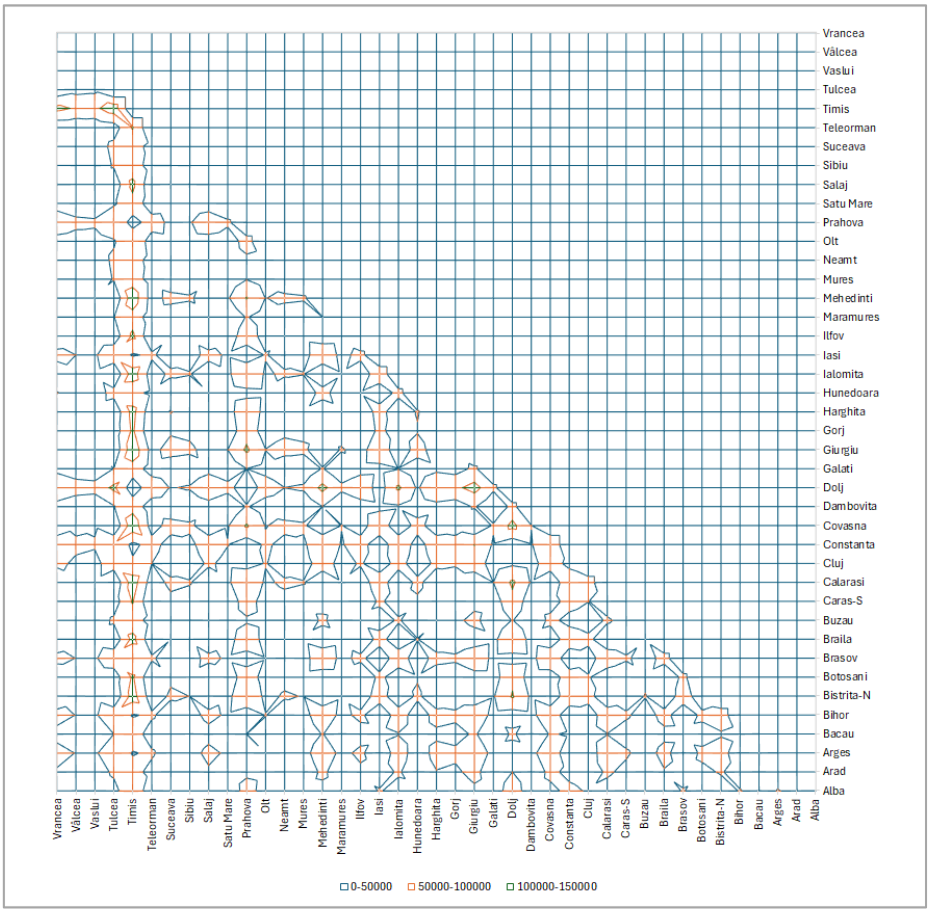

Fig. 3. Euclidean distances between counties (the yearly number of Diabetes cases)

TABLE I.

The intervals of variation between counties (distances) - defined by percentiles

Interval of variation		Pairs of Counties*
Percentile 0.05	1740.46 - 5917.15	BH - AG; BZ - AR; CS - BT; CL - BN; GL - BC; GR - CV; GJ - BT; HR - BR; IL - CL; IS - AG / BH; IF - BT / CS / GJ; MM - BC / DB; NT - AR / BZ; SM - DB / MM; SJ - BT / GJ / HR / IF; SB - AR / BZ / NT; SV - AR / BZ / HD / NT; TR - BT / CS / IF; TL - CL / CV / IL; VS - SM; VL - VS; VN - BR / GJ / HR / SJ.
Percentile 0.05 - 0.1	5917.16 - 9612.90	BR - BT; CV - CL; DB - BC; GL - DB; GJ - BR / CS; HR - BN / BT / GJ; HD - AR / BZ; IL - BN / CV; IF - BR / HR; MM - GL; MH - CL / CV / GR / IL; MS - BZ; NT - HD; SJ - BR / CS; SB - BC / DB / GL / MM / MS; SV - SB; TL - AB / GJ / SJ; TL - BN / GR / MH; VS - DB / MM; VL - AB / SM / TR; VN - BT / IF.
Percentile 0.1 - 0.2	9612.91 - 16543.46	BC - AR; BT - AB; BV - AG / BH; BR - BN; BZ - BC; CS - BR; CL - BR; CT - AG / BH; CV - BN; DB - AR / BZ; GL - AR / BZ; GR - BN / CL; GJ - AB / BN; HR - CS / CL / CV; IL - BR / GR / HR; IS - BV / CT; IF - AB / BN; MM - AR / BZ; MH - BN; MS - AR / BC / DB / GL / HD / MM; NT - BC / DB / GL / MM / MS; PH - DJ; SM - AB / BC / BZ / GL / MS; SJ - AB / BN / IL; SB - HD / SM; SV - BC / DB / GL / MM / MS; TR - BR / HR / SM; TL - BR / HR; VS - AB / BC / CS / GL / MS / SB / TR; VL - BT / CS / DB / GJ / IF / MM / SJ; VN - BN / CS / CL / IL / TR / TL.

Interval of variation		Pairs of Counties*
Percentile 0.2 - 0.3	16543.47 - 23625.29	AG - AR ; BC - AB; BT - BN; BR - AB; CS - BN; CL - BT; CJ - AR / AG / BH; CT - BV; CV - BR; DB - AB / CS; DJ - CT; GL - AB; GR - BR; GJ - CL; HR - AB / GR; HD - AG / BC / BH / BV / CJ / DB / GL; IL - BT / GJ; IS - CJ / HD; IF - CL / IL; MM - AB / CS / HD; MH - BR; NT - AG / BH / CJ; PH - AG / BH / CT / IS; SM - AR / BT / CS / GJ / IF / NT / OT; SJ - CL / CV / MH; SV - AG / BH / BV / CJ / IS / SM; TL - BN / DB / IL / MM; TM - DJ; TL - BT / GJ / IF / SJ; VS - AR / BT / BZ / GJ / IF / NT / SJ; VL - BC / BR / GL / HR / MS / SB; VN - AB / CV / GR / MH / VL.
Percentile 0.3 - 0.4	23625.30 - 29433.53	BH - AR ; BN - AB; BT - BC; BV - AR; BZ - AB / AG / BH / BV; CS - BC; CL - CS; CJ - BC / BV / BZ; CV - BT; DB - BT; DJ - AG / BH; GL - CS / CJ; GR - BT; GJ - CV / DB / GR; HD - CT; IL - AB / CS; IS - AR / BZ / DJ; IF - CV / DB / GR; MM - BT / GJ / IF; MH - BT / CS / GJ / IF; MS - AB / CS / CJ; NT - BV / IS; OT - AB / BT / CS / DB / GJ / IF / MM / MS; PH - BV; SM - BR / HD; SJ - DB / GR / SM; SB - AB / AG / BH / CJ / IS / OT; TR - BC / CL / GL / MH / MS / OT; TM - PH; TL - CS / TR; VS - BR / HR / HD / OT / SV; VL - AR / BN / BZ / NT / OT / SV; VN - SM / VS.
Percentile 0.4 - 0.5	29433.54 - 36532.13	AR - AB; BC - AG; BH - BC; BV - BC; CS - AR / BZ; CL - AB; CT - AR / BZ / CJ; CV - AB / CS; DB - AG / BH / BR / CJ; DJ - BV; GL - AG / BH / BT; GR - CS; GJ - BC / GL; HR - DB; HD - AB; IS - BC / GL; IF - BC / GL; MM - AG / BH / BR / CJ / HR; MH - AB; MS - AG / BH / BT / BV / GJ / IS / IF; NT - AB / CS / CT; OT - AR / BC / BR / BZ / GL / HR / NT; PH - CJ / HD; SM - BN / CJ / HR; SJ - BC / GL / MM / MS / OT; SB - BT / BV / CS / GJ / IF; SV - AB / CT / OT; TL - AR / BZ / CV / GR / NT / SB; TL - AB; VS - BN; VL - CL / HD / IL / TL; VN - BC / DB / GL / MM / OT.
Percentile 0.5 - 0.6	36532.14 - 45127.89	BN - BC; BT - AR; BR - BC; BZ - BT / BR; CJ - AB; CT - BC; DB - BN / BV; DJ - CJ; GL - BV / BR / CT; GR - AB; GJ - AR / BZ; HR - BC / GL; HD - BT / CS / DJ; IL - DB; IS - DB; IF - AR / BZ; MM - BN / BV / IL / IS; MS - BR / CT / HR; NT - BT / GJ / IF; OT - BN / CL / HD / IL; PH - AR / BZ / NT; SM - AG / BH / BV / CL / IL / IS / MH; SJ - AR / BZ / NT; SB - BR / CT / HR / SJ; SV - BT / CS / GJ / IF / PH; TL - HD / SV; TM - CT / IS; TL - OT / SM; VS - AG / BH / BV / CL / CJ / CV / IL / IS / MH / TL; VL - CJ / CV / GR / MH; VN - AR / BZ / MS / NT / SB.
Percentile 0.6 - 0.7	45127.90 - 54981.64	AG - AB; BH - AB; BN - AR; BV - AB; BR - AR; BZ - BN; CL - BC; CJ - BT ; CS; CV - BC; DB - CL / CT / CV; DJ - AR / BZ; GL - BN / CL; GR - DB; HR - AR / BZ; HD - BR / GJ / HR; IL - BC / GL; IS - AB; IF - HD; MM - CL / CT / CV / GR; MH - BC / DB / GL / MM; MS - BN / CL / IL; NT - BN / BR / DJ / HR; OT - AG / BH / BV / CJ / CV / GR / IS / MH; PH - BC / DB / GL / MM / MS; SM - CT / CV / GR; SJ - HD; SB - BN / CL / DJ / IL / PH; SV - BR / DJ / HR / SJ; TL - CJ; TM - AG / BH / BV; TL - BC / DB / GL / MM / MS / SB; VS - CT / GR; VL - AG / BH / BV / IS; VN - HD / SV.
Percentile 0.7 - 0.8	54981.65 - 68035.67	BT - AG / BH; BV - BT; BR - BV; CS - AG / BH / BV; CL - AR / BZ; CJ - BR; CT - AB / CS; CV - AR / BZ; DJ - BC / DB; GL - CV / DJ; GR - AR / BC / BZ / GL; GJ - AG / BH / BV / CJ; HR - CJ; HD - BN / CL; IL - AR / BZ / HD; IS - BT / CS / GJ; IF - AG / BH / BV / CJ / IS; MM - DJ; MH - AR / BZ; MS - CV / DJ / GR / MH; NT - CL / CV / GR / IL / MH; OT - CT; SM - PH; SJ - AG / BH / BV / CJ / IS; SB - CV / GR / MH; SV - BN / CL / CV / IL / MH; TL - AG / BH / BV / CT / IS; TM - CJ / HD / SV; TL - AR / BZ / HD / NT / SV; VS - PH; VL - CT / PH; VN - AG / BV / CJ.
Percentile 0.8 - 0.9	68035.68 - 90569.12	BN - AG / BH; BV - BN; BR - AG / BH; CL - AG / BH / BV; CJ - BN / CL; CT - BN / BT / BR; CV - AG / BH / BV / CJ; DJ - AB / BT / CS; GR - AG / BV / CJ; GJ - CT / DJ; HR - AG / BH / BV / CT; HD - CV / GR; IL - AG / BH / BV / CJ; IS - BN / BR / CL / CV / HR / IL; IF - CT / DJ; MH - AG / BH / BV / CJ / HD / IS; OT - DJ; PH - AB / BT / BR / CS / GJ / HR / IF / OT; SM - DJ; SJ - CT / PH; SV - GR; TL - DJ / PH; TM - AR / BC / BZ / DB / GL / MM / MS / NT / SM / SB; TL - AG / BH / BV / CJ / IS; VS - DJ / TM; VL - DJ; VN - BH / CT / IS / PH.
Percentile 0.9 - 1	90569.13 - 433441.39	CT - CL; CV - CT; DJ - BN / BR / CL / CV; GR - BH / CT / DJ; HR - DJ; IL - CT / DJ; IS - GR; MH - CT / DJ; PH - BN / CL / CV / GR / IL / MH; SJ - DJ; TM - AB / BN / BT / BR / CS / CL / CV / GR / GJ / HR / IL / IF / MH / OT / SJ / TR; TL - CT / DJ / PH / TM ; VL - TM; VN - DJ / TM; Bucuresti - all counties.

* the 2-letters abbreviation codes for Romanian counties were used (https://enciclopediaromaniei.ro/wiki/)

It follows therefore that the smallest distances between counties, which belong to the 0.05 percentile and can be characterized as significant, are recorded between BH - AG / IS; BZ - AR / NT / SB / SV; CS - BT / IF / TR; CL - BN / IL / TL; GL - BC; GR - CV; GJ - BT / IF / SJ / VN; HR - BR / SJ / VN; IS - AG; IF - BT / SJ / TR; MM - BC / DB / SM; NT - AR / SB / SV; SM - DB / VS; SJ - BT / VN; SB - AR; SV - AR / HD; TR - BT; TL - CV / IL; VL - VS; VN - BR. It can be noticed, for example, that Iasi county is the most similar with Bihor and Arges counties in which concerns the number of Diabetes cases recorded between 2012-2021.

The smallest distances between trends, which belong also to the 0.05 percentile and can be characterized as significant, are recorded between AB - IL; AR - GJ / MM; BC - CL / HR / SJ / VN; BH - BZ; BN - SJ / VN; BT - SJ; BR - CS / IF / PH / VS / VL; BZ - TM; CS - IF / VS / VL; CL - HR / SJ; CV - TL; DM - HD / **IS** / NT / SB; GL - GR; GJ - MM; HR - SJ / VN; HD - **IS**; **IS** - NT / SB / SV; IF - PH / VS / VL; NT - SB / SV; PH - VL; SB / SV; VS - VL. The analysis reveals that Iasi county has the most similar trends with Dambovita, Hunedoara, Neamt, Sibiu and Suceava counties.

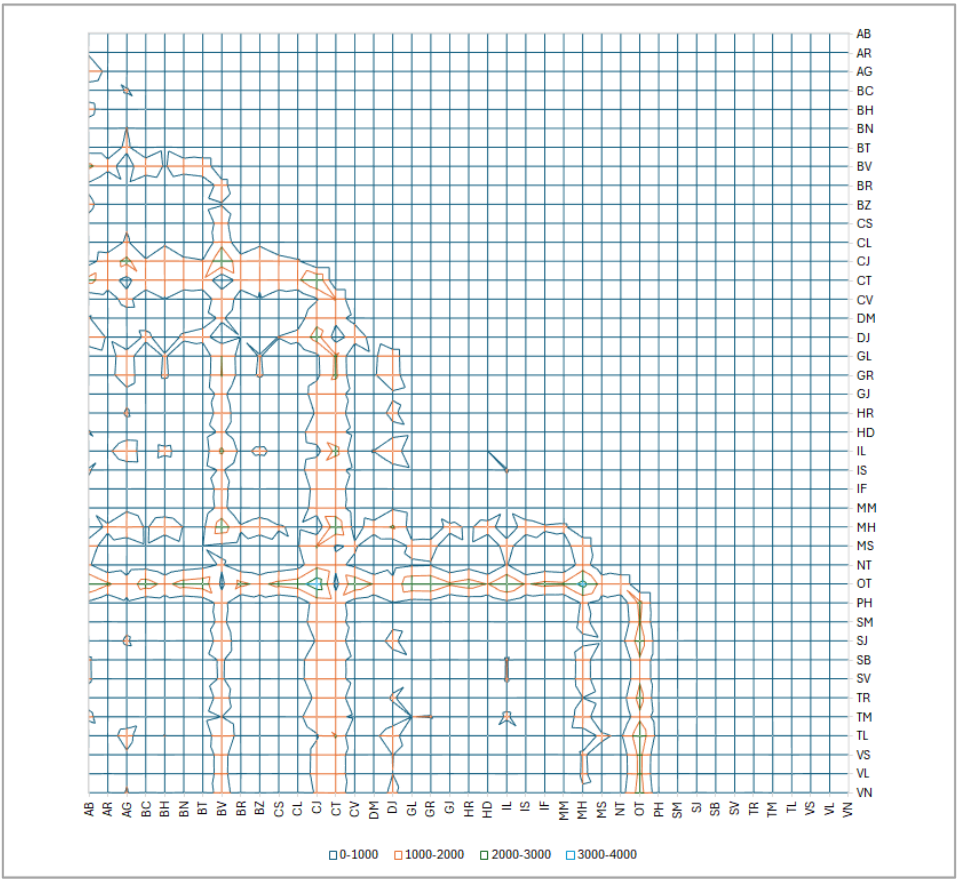

Fig. 4. Manhattan distances between trends (the yearly number of Diabetes cases)

TABLE II.

The intervals of variation between trends (distances) - defined by percentiles

Interval of variation		Pairs of Counties*
Percentile 0.05	0.66 - 50.44	AB - IL; AR - GJ / MM; BC - CL / HR / SJ / VN; BH - BZ; BN - SJ / VN; BT - SJ; BR - CS / IF / PH / VS / VL; BZ - TM; CS - IF / VS / VL; CL - HR / SJ; CV - TL; DM - HD / IS / NT / SB; GL - GR; GJ - MM; HR - SJ / VN; HD - IS; IS - NT / SB / SV; IF - PH / VS / VL; NT - SB / SV; PH - VL; SB / SV; VS - VL.
Percentile 0.05 - 0.1	50.45 -18.77	AR - BR / PH / SM / VL; BC - BN / BT; BH - TM; BN - CL / HR; BT - CL / HR; BV - CT; BR - GJ / MM; CS - PH / TR; CL - VN; CV - GL; DM - SV; GL - TL; GR - IL / TL; GJ - PH / SM; HD - NT / SM / SB / SV; IS - TM; IF - MM / TR; MM - PH / SM / VS / VL; NT - TM; PH - VS; SJ - VN; SB - TM; SV - TM; TR - VS / VL / VN.
Percentile 0.1 - 0.2	118.78 -29.45	AB - GL / GR; AR - CS / DM / HD / IS / IF / TR / VS; AG - DJ / MS; BC - CS / IF / TR / VS; BH - DM / IS / MS / NT / SB / SV; BN - BT / BR / CS / IF / PH / SJ / VS / VL; BT - CV / TR / TL / VN; BR - SM / TR / VN; BZ - DM / HD / IS / MS / NT / SB / SV; CS - GJ / HR / MM / SM / VN; CL - CV / TR / TL; CV - GR / SJ; DM - GJ / MM / SM / TM; GL - IL; GJ - HD / IS / IF / NT / SB / VS / VL; HR - TR / VS; HD - MM / TM; IL - TL; IS - SM; IF - SM / VN; MM - TR; MS - TM; NT - SM; PH - SM / TR; SM - SB / SV / VS / VL; SJ - TR / TL; VS - VN; VL - VN.
Percentile 0.2 - 0.3	229.46 - 30.96	AB - CV / TL; AR - BN / NT / SB / SV / TM / VN; BC - BR / CV / GL / PH / TL / VL; BH - HD; BN - CV / GJ / MM / SM; BT - CS / GL / GR / IF / VS / VL; BR - CL / DM / HR / HD / IS / NT / SJ / SB; BZ - SM; CS - CL / HD / SJ; CL - GL / IF / PH / VS / VL; CJ - MH; CV - HR / IL / VN; DM - IF / PH / VS / VL; GL - HR / SJ; GR - SJ; GJ - SV / TR / TM / VN; HR - IF / PH / TL / VL; HD - IF / PH / VS / VL; IS - MM / MS / PH; IF - SJ; MM - NT / SB / SV / VN; MS - NT / SB / SV; NT - PH; PH - SJ / SB / SV / VN; SM - TR / TM; SJ - VS / VL; TL - VN.
Percentile 0.3 - 0.4	330.97 - 58.91	AB - BT / MH; AR - BC / BH / BT / BZ / CL / HR / SJ; AG - BH / BZ / TM; BC - GR / GJ / MM / SM; BH - GJ / MM / SM; BN - DM / GL / GR / HD / TL; BT - BR / GJ / IL / MM / PH; BV - DJ; BR - SV / TM; BZ - GJ / MM / PH; CS - CV / DM / IS / NT / SB / SV; CL - GR / GJ / IL / MM / SM; CV - TR / VS; DM - MS / TR; DJ - MS; GL - TR / VN; GR - HR / VN; GJ - HR / SJ; HR - MM / SM; HD - MS / TR; IL - MH / SJ; IS - IF / TR / VS / VL; IF - NT / SB / SV / TM; MM - SJ / TM; NT - TR / VS / VL; PH - TM; SM - SJ / VN; SB - TR / VS / VL; SV - VS / VL; TR - TL; TM - VL.
Percentile 0.4 - 0.5	458.92 - 85.64	AB - BC / CL / HR / SJ / VN; AR - CV / MS; AG - BV / DM / HD / IS / NT / SB / SV; BC - DM / HD / IL / IS / NT / SB; BH - BR / CS / DJ / IF / PH / VS / VL; BN - IL / IS / NT / SB / SV; BT - SM; BR - BZ / CV / GL / TL; BZ - CS / DJ / IF / VS / VL; CS - GL / GR / TM / TL; CL - DM / HD; CT - DJ; CV - IF / MM / PH / VL; DM - HR / SJ / VN; DJ - TM; GL - IF / MH / VS / VL; GR - MH / TR / VS; GJ - MS; HR - HD / IL / IS / NT / SB; HD - SJ / VN; IL - VN; IS - VN; IF - TL; MM - MS; MS - SM; NT - VN; PH - TL; SB - VN; SV - TR / VN; TR - TM; TM - VS; TL - VS / VL.
Percentile 0.5 - 0.6	585.65 - 765.27	AB - BN / CS / CJ / IF / TR / VS / VL; AR - AG / GL / GR / TL; AG - CT / GJ / SM; BC - BH / BZ / SV / TM; BH - BN / TR / VN; BN - BZ / TM; BT - DM / HD / IS / NT / SB / SV / TM; BR - GR / IL / MS; BZ - CL / HR / SJ / TR / VN; CS - IL / MS; CL - IS / NT / SB / SV / TM; CJ - IL; CT - OT; CV - GJ / HD / MH / SM; DM - DJ; DJ - HD / IS / NT / SB / SV; GL - GJ / MM / PH / SM; GR - GJ / IF / MM / PH / VL; GJ - TL; HR - SV / TM; IL - IF / PH / TR / VS / VL; IS - SJ; IF - MS; MH - TL; MS - PH / VS / VL; NT - SJ; SM - TL; SJ - SB / SV / TM; TM - VN.
Percentile 0.6 - 0.7	765.28 - 1005.47	AB - AR / BR / GJ / MM / PH / SM; AR - DJ / IL; AG - BR / CS / IF / MM / PH / TR / VS / VL; BC - MH / MS; BH - BT / BV / CL / CV / HR / SJ; BN - MH / MS; BT - BZ / MH / MS; BV - BZ / MS / OT; BR - DJ; BZ - CV / TL; CL - MH / MS; CJ - CV / GL / GR / TL; CT - MS; CV - DM / IS / NT / SB / SV / TM; DM - GL / GR / TL; DJ - GJ / IF / MM / PH / SM / VL; GL - HD / IS / NT / SB / SV / TM; GR - HD / IS / NT / SM / SB / SV; GJ - IL; HR - MH / MS; HD - TL; IL - MM / SM; IS - TL; MH - SJ / TR / VN; MS - SJ / TR / VN; NT - TL; SB - TL; SV - TL; TM - TL.

Interval of variation		Pairs of Counties*
Percentile 0.7 - 0.8	1005.48 -355.93	AB - BH / BZ / DM / HD / IS / NT / SB / SV / TM; AR - BV / MH; AG - BC / BN / BT / CL / CV / HR / SJ / VN; BC - CJ / DJ; BH - CT / GL / GR / IL / TL; BN - CJ / DJ; BT - CJ / DJ; BV - DM / GJ / HD / IS / MM / NT / SM / SB / SV / TM; BR - MH; BZ - CT / GL / GR / IL; CS - CJ / DJ / MH; CL - CJ / DJ; CJ - HR / SJ / TR / VN; CT - DM / HD / IS / NT / SB / SV / TM; CV - MS; DM - IL; DJ - HR / OT / SJ / TR / VS / VN; GL - MS; GR - MS / TM; GJ - MH; HD - IL; IL - IS / NT / SB / SV / TM; IF - MH; MM - MH; MH - PH / SM / VS / VL; MS - TL.
Percentile 0.8 - 0.9	1355.94 -969.47	AB - AG / DJ / MS; AR - CJ / CT; AG - GL / GR / IL / OT / TL; BC - BV / CT; BH - CJ / MH / OT; BN - BV / CT; BT - BV / CT; BV - BR / CS / CL / CV / HR / IF / PH / SJ / TR / TL / VS / VL / VN; BR - CJ / CT; BZ - CJ / MH / OT; CS - CT; CL - CT; CJ - DM / GJ / HD / IS / IF / MM / NT / PH / SM / SB / SV / TM / VS / VL; CT - GJ / HR / IF / MM / PH / SM / SJ / TR / VS / VL / VN; CV - DJ; DM - MH / OT; DJ - GL / GR / IL; HD - MH / OT; IL - MS; IS - MH / OT; MH - MS / NT / SB / SV / TM; MS - OT; NT -OT; OT - SB / SV / TM.
Percentile 0.9 - 1	1969.48 -985.60	AB - BV / CT / OT; AR - OT; AG - CJ / MH; BC - OT; BN - OT; BT - OT; BV - CJ / GL / GR / IL / MH; BR - OT; CS - OT; CL - OT; CJ - CT / DJ / MS / OT; CT - CV / GL / GR / IL / MH / TL; CV - OT; DJ - MH; GL - OT; GR - OT; GJ - OT; HR - OT; IL - OT; IF - OT; MM - OT; MH - OT; OT - PH / SM / SJ / TR / TL / VS / VL / VN; Bucuresti - all counties.

* the 2-letters abbreviation codes for Romanian counties were used (https://enciclopediaromaniei.ro/wiki/)

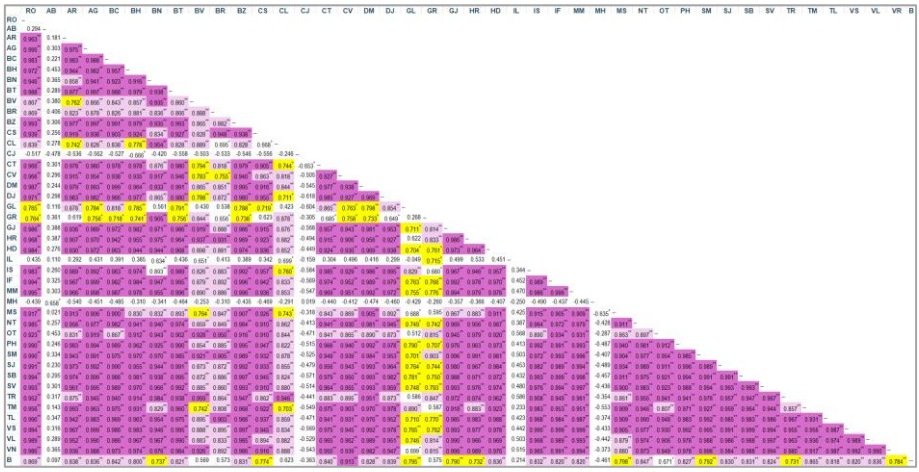

Fig. 5. The correlation matrix between counties
(the Z-scores of the yearly number of Diabetes cases)

We calculated also the correlation coefficients between the Z-scores of the original values, as another method to identify the significant similarities between trends, according to the theory.

The correlations matrix (figure 5) shows strong and significant correlations between almost all counties, including Bucuresti and the whole country. Anyway, there are a few counties not correlated at all (Alba, Cluj, Ialomita and Mehedinti), as well as a few others weaker correlated, even significant - namely Brasov, Braila, Calarasi, Galati and Giurgiu. Iasi county is the strongest correlated with Arges, Buzau and Dolj and the weakest correlated with Bistrita-Nasaud,

Brasov, Braila, Galati and Bucuresti. These results confirm partially the previous ones, but the correlations analysis seems to be less sensitive, because it identifies strong and significant correlations between almost all investigated counties.

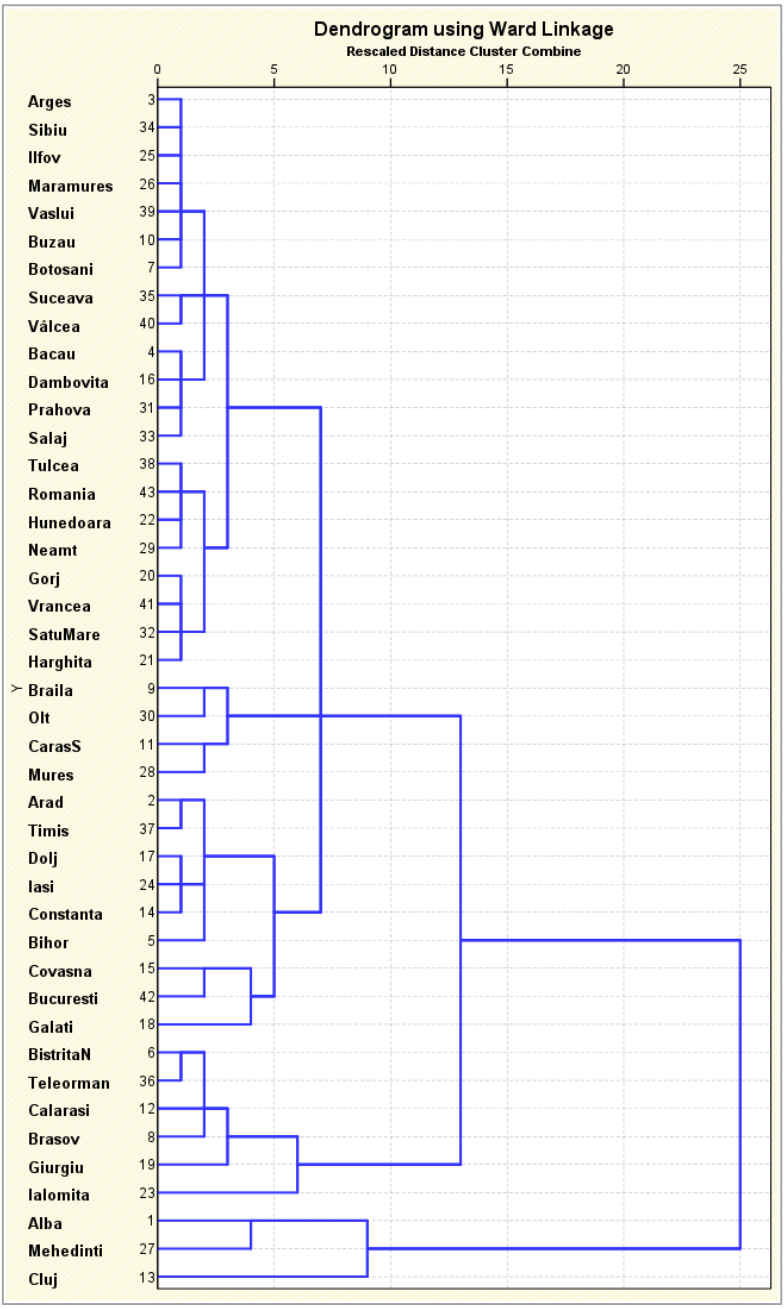

Fig. 6. The dendrogram of the counties according to the hierarchical clustering

Finally, we applied the Hierarchical Clustering of the 41 counties and Bucharest, also in order to identify the similar evolutions. The dendrogram (fig. 6) highlights 5 main clusters, as it follows:

- Cluster 1 - Arges, Sibiu, Ilfov, Maramures, Vaslui, Buzau, Botosani, Suceava, Valcea, Bacau, Dambovita, Prahova, Salaj, Tulcea, Romania, Hunedoara, Neamt, Gorj, Vrancea, Satu Mare, Harghita: these counties are very similar in behavior between them, as well as with the whole country;
- Cluster 2 - Braila, Olt, Caras-Severin and Mures;
- Cluster 3 - Arad, Timis, Dolj, Iasi, Constanta, Bihor, Covasna, Bucuresti and Galati;
- Cluster 4 - Bistrita-Nasaud, Teleorman, Calarasi, Brasov, Giurgiu, Ialomita;
- Cluster 5 - Alba, Mehedinti, Cluj.

DISCUSSION

Each of the presented methods provides slightly different similarity patterns between the counties of Romania regarding the evolution of diabetes cases over the 10-year monitoring period. This is not necessarily surprising, as the working methodologies differ, and the statistical parameters used as reference points also vary. However, the usefulness of employing these tools in parallel lies in identifying potential points of convergence in the results, which indicate significant similarities in the evolution of the time series under investigation.

In the specific case of Iași County, which was used for exemplification, three points of convergence can be observed: Argeș County, which exhibited similarities both in the calculation of Euclidean distances between annual case numbers and in the correlation of trends; Bihor County, identified through both Euclidean distance calculations and hierarchical clustering; and Dolj County, highlighted through the trend's correlation and hierarchical clustering. It can therefore be concluded that the time series of diabetes cases recorded in Iași between 2012 and 2021 shows the highest degree of similarity with the corresponding time series recorded in the aforementioned counties-namely, Argeș, Bihor, and Dolj.

TABLE III.

Socio-demographic indicators relevant for Diabetes prevalence

	Iasi	**Arges**	**Dolj**	**Bihor**
Total population (2025)	988621	606726	663167	608277
% population > 65 y.o. (2024)	17.03%	20.98%	20.87%	18.63%
Average age (2023)	39.0	43.9	43.1	41.4
Population density (/ km^2)	140	83	81	73
Urban (%) / Rural (%)	~50/50	~55/45	~55/45	~60/40
GDP per capita (2022)	12,027 EUR (average)	13,660 EUR (average)	11,898 EUR (average)	12,139 EUR (average)
Average net monthly income (2024)	5,177 RON	4,824 RON	4,750 RON	4,506 RON
County Performance Index (2024)	8	7	5	9
Diabetes: prevalence (%, 2024)	~6.5%	~6.5%	~6.5%	~14.8%
Diabetes: mortality rate (/100000 inhabs 2023)	19.8	59.4	2.3	1.1

(http://cumstam.ro / http://statistici.insse.ro)

It is interesting to discuss about the reasons of these similarities, because, apparently, there are no obvious resemblances between these counties. Iasi belongs to the Moldavia region, while Arges and Dolj belong to Oltenia and Bihor belongs to Transylvania. A more in-depth analysis, however, highlights relevant similarities (tab. III).

Firstly, the topography of the four counties is relatively similar - characterized by hilly and plain areas. Moreover, all four counties have a population of over 600,000 inhabitants, with an urban population share between 50% and 60% and extensive rural areas. Consequently, the population's lifestyle is relatively urbanized, though not highly sophisticated, which leads to common nutritional risks (mainly a hypercaloric diet, characterized by high consumption of processed foods, sweets, and sugary drinks, sedentary behavior, and average access to health education).

All four counties are located in regions with medium levels of economic development - neither extremely poor nor highly affluent - which favors unhealthy dietary habits, primarily represented by a hypercaloric diet and consumption of processed foods, that are tasty but relatively inexpensive. Another relevant aspect is that the population's age structure is relatively aged, which increases the prevalence of chronic diseases, including diabetes.

Each county has at least one medical university center or a county-level reference hospital, ensuring a relatively uniform process of diagnosis, convergence in reporting and monitoring cases, as well as standardization of treatment and prevention protocols. All four counties have equally benefited from national prevention and screening programs implemented through family medicine, resulting in comparable levels of health education. These shared features are significant arguments to support the detected similarities in the prevalence of diagnosed cases during the monitored period.

An interesting aspect - one that requires further analysis - is the evolution observed in 2024: while in Iași, Argeș, and Dolj the prevalence of diabetes remained close to the national average of 6.5%, in Bihor a significant worsening was recorded, reaching 14.8% - more than double the national average. This can be a relevant subject for future researches.

CONCLUSIONS

Therefore, the comparative analysis of time series provides essential insights into the temporal and spatial evolution of diseases' prevalence, offering an approach that integrates the recorded absolute values with trends, disease dynamics, and potential anomalies - such elements cannot be identified through classic cross-sectional studies. Tools such as heatmaps, clustering, and cross-correlation allowed the optimal visualization of inter-county relationships and the identification of geographic areas exhibiting similar epidemiological behavior.

Such information has direct applicability in calibrating better the screening and prevention campaigns, and improve significantly the resources allocation, allowing to tailor optimally the public health policies to the specific context of each county. This spatial-temporal integrative approach is crucial to put in practice the concept of

evidence-based medicine, especially in the case of chronic diseases with slow progression, multifactorial causes, and major impact on the healthcare system such as Diabetes Mellitus.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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PROGNOSIS OF POSTOPERATIVE LUMBAR DISCITIS FOLLOWING LUMBAR DISC HERNIATION SURGERY: AN ANALYSIS OF RISK FACTORS

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PROGNOSIS OF POSTOPERATIVE LUMBAR DISCITIS FOLLOWING LUMBAR DISC HERNIATION SURGERY: AN ANALYSIS OF RISK FACTORS (Abstract): Postoperative lumbar osteodiscitis is a rare but serious complication following spinal disc surgery, with a prevalence of 3-5% of cases. It is commonly caused by septic or aseptic factors, and early diagnosis is essential for effective management. The study **aims** to evaluate the risk factors associated with the development of osteodiscitis following lumbar disc herniation surgery, particularly focusing on lifestyle-related factors and comorbidities. **Materials and methods:** A retrospective, cross-sectional study was conducted between 2018 and 2024, analyzing clinical observation records from two hospitals in Bucharest. The study included 85 patients who developed postoperative lumbar osteodiscitis. Patient data, including risk factors such as smoking, alcohol consumption, poor hygiene, and comorbidities, were collected and analyzed. The association between these factors and the duration of symptom onset, as well as the impact on postoperative pain scores (VAS), was examined. **Results:** The study found that lifestyle-related factors, particularly smoking, were prevalent in 27 out of 85 patients. The mean duration until the onset of symptoms was not significantly influenced by these factors. Patients with comorbidities were typically older, with a significant difference in mean age between those with and without comorbidities. A reduction in VAS scores was observed post-treatment for all patients, although no statistically significant differences were found in pre- and post-treatment VAS scores related to lifestyle factors. **Conclusions:** Lifestyle factors such as smoking, alcohol use, and poor hygiene do not significantly affect the time to symptom onset in osteodiscitis, but they are associated with a higher risk of complications. The management of postoperative osteodiscitis remains predominantly conservative with antibiotics, although surgical intervention may be necessary in cases of resistance or neurological complications. Proper follow-up is crucial for ensuring complete eradication of infections. **Keywords**: SPONDYLODISCITIS, SPINAL INFECTION, OSTEOMYELITIS, ANTIBIOTIC THERAPY, PAIN MANAGEMENT, SURGICAL INTERVENTION, CHRONIC INFLAMMATION.

INTRODUCTION

Infections of the lumbar spine may occur following surgical intervention on a degenerative spinal disc and can be either septic or aseptic in nature. Lumbar disc surgery, particularly discectomy proce-

dures, may lead to postoperative infections involving the intervertebral disc (discitis), the vertebral bodies (osteomyelitis), or both, referred to as osteodiscitis. These spinal pathologies seldom present in isolation and are typically encompassed under the term spondylodiscitis. Postoperative lumbar infections may further extend into the epidural space, facet joints, or paravertebral tissues.

Postoperative lumbar osteodiscitis is an uncommon yet serious complication, accounting for approximately 3-5% of cases. It must be carefully differentiated from primary vertebral endplate infections with secondary disc involvement. Therefore, the term spondylodiscitis or vertebral osteomyelitis is often more appropriate to describe this condition (1). The risk of postoperative spinal infection increases due to various preoperative, intraoperative, and postoperative factors, with incidence rates ranging from 0.7% to as high as 20% in some reports (2). Diagnosis of postoperative osteodiscitis relies on a multidisciplinary approach, integrating clinical symptoms, laboratory investigations, bacteriological cultures obtained via disc aspiration, and radiological findings (3-6). This condition represents a serious complication that can severely impair quality of life, resulting in neurological deficits, spinal deformities, or segmental instability (4-6).

A recent study in Denmark reported an increase in spondylodiscitis incidence from 2.2 to 5.8 per 100,000 individuals per year between 1995 and 2008, while in Germany, the estimated incidence ranged from 30 to 250,000 cases annually, based on data from the Federal Statistical Office in 2015 (7). In Western countries, the incidence remains under 1%, whereas in India, it ranges from 4% to 10% (8).

Postoperative osteodiscitis is a rare but severe complication following intervertebral disc surgery. This clinical entity was first described by Frank Turnbull in 1953 (9-11). It predominantly affects the lumbar spine, with single-level involvement observed in approximately 65% of cases, contiguous multilevel disease in 20%, and noncontiguous multilevel involvement in 10%. The condition typically manifests around 6 to 7 weeks post-discectomy and is often associated with incomplete surgical procedures or recurrent disc herniations (2-9). The infection may be septic-commonly due to contamination by skin flora such as Staphylococcus aureus—or aseptic, resulting from intraoperative trauma and vascular compromise of the disc. The latter may be associated with spinal tuberculosis, which spreads along the spinal ligaments and involves the anterior aspects of adjacent vertebral bodies, potentially leading to vertebral angulation and progressive kyphosis (12).

MATERIALS AND METHODS

This study is a descriptive cross-sectional analysis based on secondary data extracted from general clinical observation charts, collected between 2018 and 2024, at the Bucharest University Emergency Hospital and the Dr. Victor Babeș Clinical Hospital for Infectious and Tropical Diseases, Bucharest. A range of variables were recorded from these observation charts and compiled into a structured database for analysis.

The working hypothesis of this research is that specific patient-associated factors may influence the clinical course of osteodiscitis and contribute to the occurrence of its complications. Postoperative osteodiscitis often presents with nonspecific

clinical symptoms, and the majority of patients tend to receive a delayed diagnosis. The cornerstone of effective treatment for this infection lies in an accurate and timely diagnosis, which should always be based on a comprehensive correlation between the patient's clinical presentation, laboratory test results, and imaging findings.

The primary objective of this study is to evaluate the association between various factors and the development of osteodiscitis-related complications, based on a sample of 85 patients identified across two medical institutions in Bucharest during the specified period.

To ensure ethical compliance, informed consent for data protection and study inclusion was obtained and signed by each patient or, where applicable, their legal guardians. The study also involved cooperation with infectious disease departments outside the University Hospital, enabling follow-up of operated patients, and ensured access to preoperative and postoperative neuroimaging investigations.

The target population consisted of patients diagnosed with osteodiscitis and hospitalized after varying intervals from surgical intervention for disc herniation located at different levels within the thoracic, lumbar, and/or sacral spinal segments.

Statistical evaluation was performed based on patient subgrouping by age, sex, residence, education level, behavioral risk factors, and presence of comorbidities. Data were analyzed using the independent samples t-test to compare VAS scores and symptom onset timing between subgroups, and Welch's t-test was applied for comparing mean ages in groups with unequal variances. The Chi-square (X^2) test was used to assess the association between education level and area of residence. Normality was tested using the Shapiro-Wilk test, and variance equality with Levene's test. Statistical significance was considered at $p < 0.05$. Results were presented in structured tables and graphs to illustrate subgroup distributions and comparative outcomes.

The final study population comprised 85 consecutively hospitalized patients, with 64 cases recorded at the Bucharest University Emergency Hospital and 21 cases at the Dr. Victor Babeș Clinical Hospital**.**

RESULTS

The study included a total of 85 hospitalized patients diagnosed with postoperative osteodiscitis. Of these, 64 patients were admitted to the University Emergency Hospital Bucharest, while 21 patients were treated at the Dr. Victor Babeș Clinical Hospital for Infectious Diseases and Tropical Medicine.

With respect to sex distribution, among the 64 patients from the University Emergency Hospital Bucharest, 30 were female and 34 were male. At the Dr. Victor Babeș Clinical Hospital, 13 were female and 8 were male (fig. 1).

This distribution reflects a predominance of male patients in both medical institutions, with the overall male-to-female ratio slightly favoring men (42 males *vs*. 43 females).

The age distribution of the 85 patients included in this study was stratified into standard 5-year age intervals, highlighting the demographic structure of the cohort. As shown in the figure, the largest concentration of cases was observed in the 60-64, 65-69, and 70-74 age groups, with a predominance of female patients in these intervals.

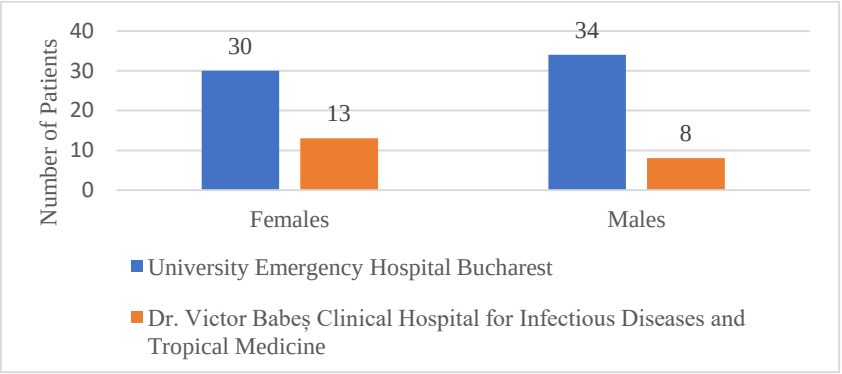

Fig. 1. Distribution of patients with postoperative osteodiscitis by sex and hospital of admission.

In contrast, male patients were more frequently represented in the older age groups (75-79 and 65-69), with a notable presence also in the 55-64 range. Fewer cases were recorded in the younger age brackets, with only isolated instances of patients under 40 years of age, indicating that postoperative osteodiscitis predominantly affects an older adult population, particularly those aged 55 and above (fig. 2).

This pattern supports the hypothesis that age-related comorbidities, immune senescence, and degenerative spinal pathology may contribute to increased vulnerability to postoperative infectious complications.

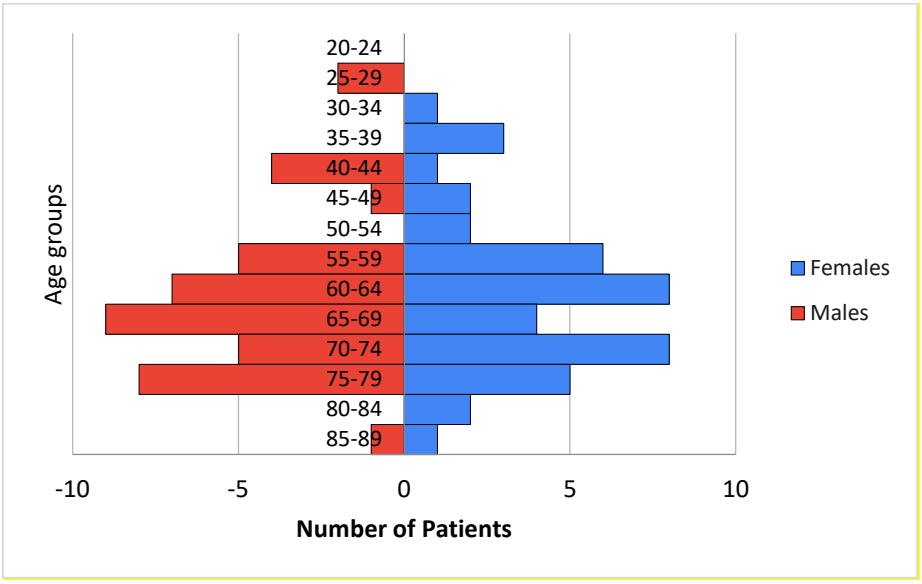

Fig. 2. Distribution of postoperative osteodiscitis patients by age group and sex. (Female patients represented in blue; male patients in red. Age groups are shown in 5-year intervals.)

The population analyzed in this study was also categorized based on their residential environment-rural or urban-and sex. Out of the total 85 patients (fig. 3):

- Among female patients, 24 resided in rural areas, while 19 were from urban areas.
- Among male patients, 23 had a rural residence, and 19 lived in urban areas.

This data indicates a slightly higher prevalence of osteodiscitis among patients from rural settings in both sexes. The marginal difference between rural and urban populations may reflect unequal access to timely surgical interventions, postoperative monitoring, or early diagnostic imaging in rural regions.

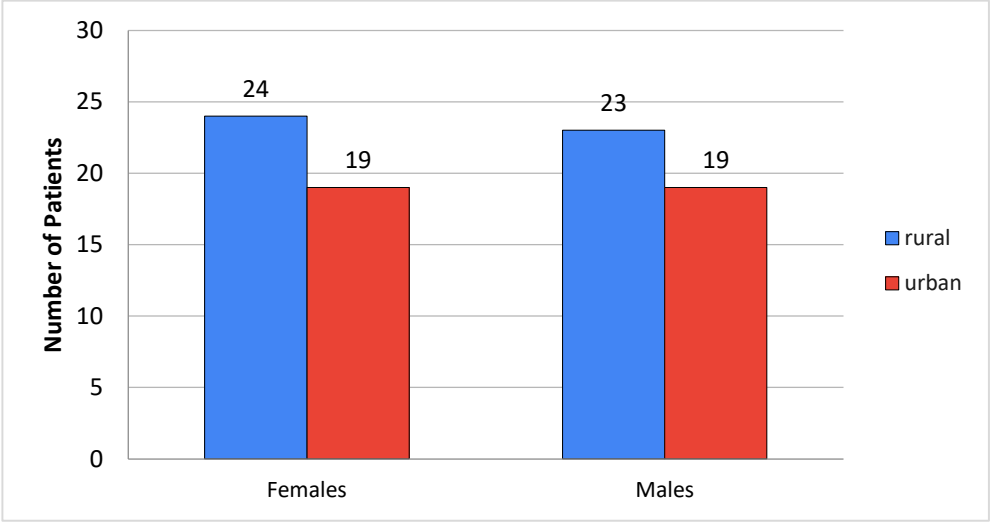

Fig. 3. Distribution of patients diagnosed with postoperative osteodiscitis by sex and area of residence. (Rural patients represented in blue; urban patients in red.)

In the assessment of predisposing conditions, several behavioral risk factors were identified among the patient cohort, with smoking, alcohol consumption, and poor hygiene emerging as the most frequently reported. A total of 13 patients were active smokers, making this the most prevalent behavioral factor in the study. Additionally, 7 patients reported chronic alcohol use, while another 7 exhibited poor personal hygiene, which may have contributed to increased microbial colonization or delayed postoperative healing (fig. 4)

These findings emphasize the importance of comprehensive preoperative screening that includes modifiable behavioral risk factors. Patient education and counseling prior to surgery could play a key role in lowering the incidence of postoperative complications such as osteodiscitis.

Average time until the onset of osteodiscitis symptoms varied depending on lifestyle-related factors. Symptoms appeared latest in smokers, at around 15.7 weeks, while non-smokers experienced symptoms earlier, at approximately 9.5 weeks. Similar trends were observed for patients with poor hygiene and those consuming alcohol, who developed symptoms slightly later compared to those without

these factors.

These differences were not statistically significant, indicating that lifestyle-related factors did not have a clear impact on the timing of symptom onset. No significant differences were observed between male and female patients either ($p \geq 0.05$) (Table 1).

Older patients were more likely to present with comorbidities (tab. II and fig. 5).

The number of patients with comorbid conditions was notably higher in the older age groups compared to those without comorbidities.

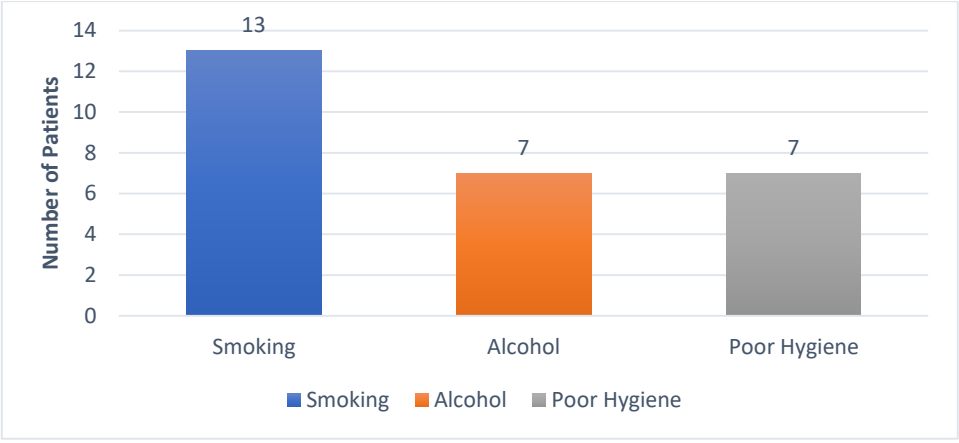

Fig. 4. Distribution of behavioral risk factors (smoking, alcohol consumption, and poor hygiene) among patients diagnosed with postoperative osteodiscitis.

TABLE I.

Average time (weeks) to symptom onset according to lifestyle-related factors in patients with postoperative osteodiscitis.

Lifestyle-related factor	The presence or absence of lifestyle- related factors	Mean duration (weeks) until symptom onset	Number of patients
Smoking	Smoking	15.69 (SD±12.399)	13
	Non-Smoking	9.53 (SD±7.083)	72
Alcohol	Alcohol Consumption	14.57 (SD±8.829)	7
	No Alcohol	10.10 (SD±8.241)	78
Poor Hygiene	Poor Hygiene	16.14 (SD±15.742)	7
	Normal Hygiene	9.96 (SD±7.291)	78
Total		**10.47 (SD±8.328)**	**85**

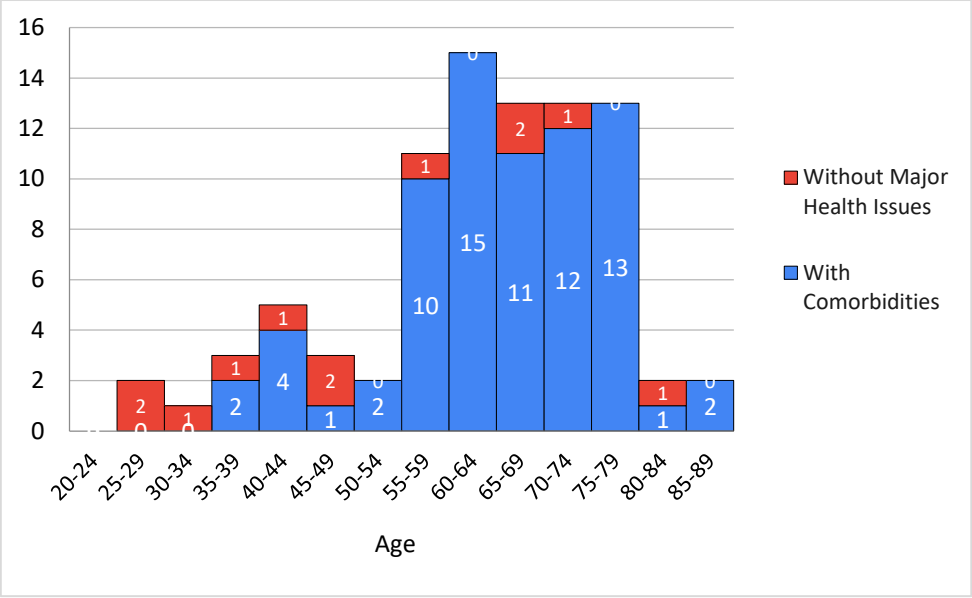

Fig. 5. Distribution of patients with and without comorbidities across age groups. (Blue bars indicate patients with comorbidities; red bars indicate those without major health issues.)

Patients with comorbidities had a higher mean age (65.15 years), compared to those without comorbidities (54.19 years), suggesting a possible age-related vulnerability to multiple health conditions in the postoperative context (tab. II).

TABLE II.

Presence of comorbidities and mean age of patients with postoperative osteodiscitis

Comorbidities	Number of Patients	Median Age
Yes	69	65.15 (SD±10.403)
No	16	54.19 (SD±18.985)
Total	**85**	**62.64 (SD±13.548)**

Patients from urban areas were more likely to have higher education, with a greater frequency of individuals holding university degrees compared to those with only secondary education. In contrast, patients from rural areas predominantly had medium-level education, with significantly fewer reporting higher education qualifications.

The association between education level and area of residence was found to be statistically significant (Chi-square test, $p < 0.05$) (fig. 6).

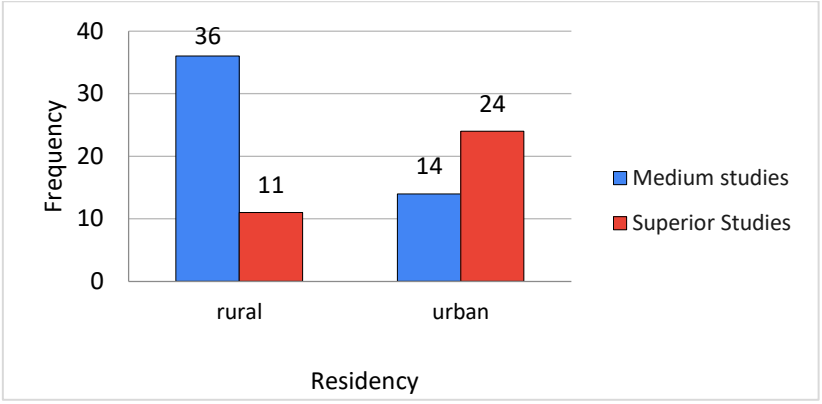

Fig. 6. Distribution of education level by area of residence among patients with postoperative osteodiscitis.

Pre-treatment VAS scores tended to be higher among patients who reported alcohol consumption, while lower average scores were observed in non-smokers and those without alcohol use. Despite these numerical differences, the variations in both pre-treatment and post-treatment VAS scores across all risk factor categories-including smoking, alcohol use, and poor hygiene-were not statistically significant (independent samples t-test, $p \geq 0.05$).

This suggests that the presence of these lifestyle-related risk factors did not significantly influence pain intensity as measured by the VAS scale, either before or after treatment in the studied population (tab. III).

TABLE III.

Distribution of patients according to risk factors and their pre-treatment and post-treatment VAS scores.

Gender	Risk Factors	Presence of Risk Factors	Number of Patients	Pre-treatment VAS score	Vas Score Post-Treatment
Females	Smoking	Yes	7	7.43 (SD±1.512)	3.43 (SD±2.370)
		No	36	7.69 (SD±1.327)	4.50 (SD±2.063)
	Alcohol	Yes	2	9.50 (SD±0.707)	7.50 (SD±0.7070
		No	41	7.56 (SD±1.305)	4.17 (SD±2.048)
	Poor Hygiene	Yes	4	7.25 (SD±0.5)	3.00 (SD±1.155)
		No	39	7.69 (SD±1.398)	4.46 (SD±2.162)
Males	Smoking	Yes	6	8.33 (SD±1.506)	5.17 (SD±1.329)
		No	36	7.81 (SD±1.091)	4.36 (SD±1.710)
	Alcohol	Yes	5	8.20 (SD±1.095)	5.00 (SD±1.414)
		No	37	7.84 (SD±1.167)	4.41 (SD±1.707)
	Poor Hygiene	Yes	3	8.67 (SD±1.528)	5.33 (SD±1.155)
		No	39	7.82 (SD±1.121)	4.41 (SD±1.697)

Gender	Risk Factors	Presence of Risk Factors	Number of Patients	Pre-treatment VAS score	Vas Score Post-Treatment
Total	Smoking	Yes	13	7.85 (SD±1.519)	4.23 (SD±2.088)
		No	72	7.75 (SD±1.207)	4.43 (SD±1.883)
	Alcohol	Yes	7	8.57 (SD±1.134)	5.71 (SD±1.704)
		No	78	7.69 (SD±1.241)	4.28 (SD±1.886)
	Poor Hygiene	Yes	7	7.86 (SD±1.215)	4 (SD±1.633)
		No	78	7.76 (SD±1.261)	4.44 (SD±1.931)
Total			**85**	**7.76 (SD±1250)**	**4.40 (SD±1.904)**

Postoperative VAS scores decreased in all subgroups, regardless of gender or the presence of lifestyle-related risk factors. On average, the mean VAS score dropped from 9.83 preoperatively to 7.71 postoperatively in patients without poor hygiene, and from 9.86 to 7.57 in smokers.

Although alcohol users had the highest preoperative and postoperative VAS scores (10.00 and 9.00, respectively), this group had a very small sample size (n=3), limiting the interpretability. Patients without alcohol consumption had a more notable pain reduction, from 9.80 to 7.60. Across all groups, the pain reduction averaged around 2 points on the VAS scale (tab. IV).

TABLE IV.

Distribution of patients by risk factors and their preoperative and postoperative VAS scores

Gender	Risk Factors	Presence of Risk Factors	Number of Patients	Pre-op VAS Score	Post-op VAS Score
Females	Smoking	Yes	4	10.00 (SD±0.00)	7.50 (SD±0.577)
		No	15	9.73 (SD±0.458)	7.80 (SD±1.014)
	Alcohol	Yes	1	N/A	N/A
		No	18	9.78 (SD±0.428)	7.67 (SD±0.907)
	Poor Hygiene	Yes	3	9.67 (SD±0.577)	7.67 (SD±0.577)
		No	16	9.81 (SD±0.403)	7.75 (SD±1.00)
Males	Smoking	Yes	3	9.67 (SD±0.577)	7.67 (SD±1.528)
		No	11	9.91 (SD±0.302)	7.73 (SD±0.7860
	Alcohol	Yes	2	10.00 (SD±0.00)	9.00 (SD±0.00)
		No	12	9.83 (SD±0.389)	7.50 (SD±0.798)
	Poor Hygiene	Yes	0	N/A	N/A
		No	14	9.86 (SD±0.363)	7.71 (SD±0.914)
Total	Smoking	Yes	7	9.86 (SD± 0.378)	7.57 (SD±0.9760)
		No	26	9.81 (SD±0.402)	7.77 (SD±0.908)
	Alcohol	Yes	3	10 (SD±0.00)	9 (SD±0.00)
		No	30	9.80 (SD±0.407)	7.60 (SD±0.855)
	Poor Hygiene	Yes	3	9.67 (SD±0.577)	7.67 (SD±0.577)
		No	30	9.83 (SD±0.379)	7.71 (SD±0.914)

DISCUSSION

In current spine surgery literature, the sudden onset of severe pain following a pain-free interval after lumbar discectomy often raises suspicion for recurrent disc herniation. However, this distinction is typically made through MRI evaluation only after a 6-month postoperative period. In the present study, all patients showed clinical improvement within the first eight weeks, regardless of treatment strategy. Follow-up was conducted systematically using blood tests or plain radiographs at 1, 3, 6, and 12 months post-discharge, and then annually. Fusion status was assessed between 6 and 12 months via radiography and computed tomography. Importantly, no neurological deficits were observed during either conservative or surgical treatment phases.

Patients who underwent surgical intervention reported a higher rate of satisfaction at the 12-month follow-up compared to those managed conservatively. This group also benefited from significantly shorter hospital stays, reduced periods of bed rest, a quicker tapering of antibiotic regimens, and earlier relief of clinical symptoms. However, surgery was associated with higher overall costs-including the need for blood transfusions-and a higher complication rate, as supported by existing studies (3, 7).

The treatment of postoperative osteodiscitis relies primarily on targeted antibiotic therapy initiated after biopsy and guided by pathogen identification, cultures, and sensitivity profiles. Strict bed rest, nutritional support, analgesics, orthotic bracing, and physical therapy are also essential components of the conservative management strategy. Immobilization of the affected spinal segment, commonly using a rigid brace, is typically recommended for approximately two weeks or until acute pain subsides. Numerous studies have demonstrated that long-term conservative treatment can achieve success rates ranging between 70% and 83% (3, 4, 9, 13-17).

Some authors advocate for early surgical intervention, particularly via debridement followed by antibiotics, arguing it may yield better outcomes than antibiotics alone. In cases where treatment is initiated within two weeks of symptom onset, six weeks of antibiotic therapy is often considered sufficient for resolution (3, 4, 5, 7, 18).

These findings collectively underscore the importance of early diagnosis, individualized therapeutic decision-making, and multidisciplinary management to optimize outcomes in patients with postoperative osteodiscitis.

CONCLUSIONS

Lifestyle-related factors such as alcohol consumption, smoking, and poor hygiene were identified in 27 out of the 85 patients included in the study, with smoking being the most frequently observed. No statistically significant differences were found in the time to symptom onset between subgroups based on the presence or absence of these risk factors. Although higher pre-treatment VAS scores were noted among alcohol users and lower scores among non-users and non-smokers, these differences were not statistically significant either. However, the presence of at least one lifestyle-related risk factor (smoking, alcohol use, and/or poor hygiene) was significantly associated with

the development of complications.

The diagnosis of postoperative lumbar osteodiscitis should rely on a combination of clinical history, physical examination, biochemical markers, and appropriate neuro-radiological imaging, particularly MRI. Most cases can be successfully managed with conservative antibiotic therapy, guided by the identification and sensitivity profile of the responsible pathogen. Surgical intervention is reserved for patients who fail to respond to conservative treatment, those with antibiotic-resistant infections, and those presenting with neurological complications such as acute paraplegia, intractable lumbar pain, sepsis, abscess formation, spinal instability, or severe kyphosis.

Long-term follow-up extending up to one year postoperatively is essential to ensure complete eradication of the infection and to monitor for potential late-onset complications (2, 19-23).

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interests, and they received no specific funding regarding this scientific research.

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THE IMPORTANCE OF CONTINUOUS GLUCOSE MONITORING AND DIETARY DECISIONS IN GESTATIONAL DIABETES MELLITUS

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THE IMPORTANCE OF CONTINUOUS GLUCOSE MONITORING (CGM) AND DIETARY DECISIONS IN GESTATIONAL DIABETES MELLITUS (Abstract): Gestational diabetes mellitus (GDM) poses significant risks to both the mother and fetus if not managed appropriately. Continuous glucose monitoring (CGM) provides comprehensive glycemic profiles that support individualized dietary interventions. **Materials and methods:** We discuss here about 44 pregnant women diagnosed with GDM, illustrating how CGM data can be used to optimize glycemic control through specific nutritional strategies. Continuous interstitial glucose measurements were obtained using the Dexcom ONE+ CGM sensor, with monitoring initiated at 27-28 weeks and continued through 39 weeks of gestation. **Results:** Fasting blood glucose averaged 112 mg/dL. One-hour postprandial glucose differed by meal type (ANOVA, $p<0.001$), with high-carbohydrate meals producing the highest values, followed by fruits, protein/low-carb meals and snacks (Tukey *post-hoc*). Two-hour postprandial glucose showed a similar pattern (ANOVA, $p<.001$). Differences between CGM readings and true glucose levels were larger in participants who experienced false low-glucose alarms, and this difference was statistically significant (t-test, $p<0.001$). **Conclusions:** Our data highlights the value of integrating CGM technology with personalized nutritional plans in the effective management of GDM. **Keywords:** GESTATIONAL DIABETES MELLITUS, CONTINUOUS GLUCOSE MONITORING, DIET.

INTRODUCTION

Gestational diabetes mellitus (GDM) is a type of hyperglycemia diagnosed or developed during pregnancy, often divided into classes of diet-controlled and pharmacological treatment. Identification of GDM is crucial because it increases the risk of developing type 2 diabetes in the long term and reduces adverse pregnancy outcomes. However, there is no consensus on the diagnostic criteria for GDM or the optimal timing of pregnancy screening. Approaches to the management of GDM have evolved over several decades because of the high rates of perinatal mortality among women with diabetes during pregnancy (1, 2).

According to a recent assessment by the International Diabetes Federation, GDM has been diagnosed in 1 in 6 live births worldwide. Approximately 7% of pregnancies in the United States are complicated by some type of diabetes, with GDM accounting for 86% of these cases. In Europe, the estimated prevalence of GDM is 10.9% (3). The evolution of diagnostic criteria has reflected ongoing scientific research and changing standards in maternal-fetal medicine. The release of placental hormones causes marked insulin resistance, with human placental lactogen being the main hormone associated with increased insulin resistance. In patients with GDM, conditions or factors cause maternal pancreatic β-cell dysfunction or delayed β-cell response, leading to decreased insulin secretion and maternal hyperglycemia. Maternal obesity in early pregnancy, due to higher levels of free fatty acids, also promotes abnormally increased insulin resistance. Genetic etiology has also been discovered in some studies on DGM, with several studies discovering genetic polymorphisms involved in functions such as insulin secretion, glucose metabolism, and insulin resistance in patients with type 2 diabetes (1, 3, 4, 5).

Frequent glucose monitoring is crucial for achieving optimal glycemic control, especially during pregnancy. The American Diabetes Association recommends at least daily fasting and postprandial testing, with a minimum of four tests per day (6). However, adherence to traditional self-monitoring of blood glucose (SMBG) is often suboptimal due to barriers such as pain, inconvenience, disruption of daily activities, and social stigma. Pregnant women with gestational diabetes (GDM) also face difficulties completing the prescribed testing regimen, leading to increased preeclampsia and higher HbA1c levels at delivery (7, 8, 9, 10).

CGM, or continuous glucose monitoring, is now considered ideal for managing blood glucose levels in people with type 1 diabetes (T1D) and T2D treated with intensive insulin therapy, basal insulin therapy, and patients with a history of severe hypoglycemia. CGM measures interstitial glucose levels and transmits readings to the user's smartphone app or wearable receiver every 1 to 5 minutes. It displays trend arrows that indicate the direction and rate of change in glucose levels and can predict glucose changes over the next 20 to 60 minutes. It has been shown to lower HbA1c, reduce severe hypoglycemic events, increase time in target glucose range, and reduce time in hypoglycemic ranges (6, 11).

This study aims to investigate the management of gestational diabetes mellitus (GDM) in by utilizing continuous glucose monitoring (CGM) technology, specifically the Dexcom system, with nutritional surveillance.

MATERIALS AND METHODS

This study focused on the evaluation of glucose variability and the influence of diet in 44 pregnant women with gestational diabetes mellitus (GDM). By analyzing their glucose trends along with food intake, this study aimed to understand how real-time glucose data can guide dietary choices, improve glycemic control, and support the overall management of GDM during pregnancy.

The participants used a Dexcom ONE+ CGM sensor (Dexcom, Inc., San Diego, CA) to continuously record interstitial glucose values from March 23rd to June

10th, 2025. The Dexcom ONE+ is a compact, discreet, and reliable continuous glucose monitoring system for type 1, type 2, and gestational diabetes (12). The sensor, worn on the back of the upper arm, continuously measured interstitial glucose levels every five minutes for up to 10 days. The data were transmitted in real time to the Dexcom ONE mobile application, allowing participants to view glucose trends, set personalized alerts, and record daily events such as meals, snacks, and exercise.

During the follow-up period, data on blood glucose levels and food intake were collected and subsequently analyzed to assess glucose response to specific meals, time in range (defined as 70-140 mg/dL, according to recommended targets for gestational diabetes), glycemic variability, and patterns of hyperglycemia or hypoglycemia.

All participants were diagnosed with GDM and were monitored from 27-28 weeks of gestation until 39 weeks. The mean body mass index (BMI) of the cohort was 29.5, classifying them on average as overweight according to World Health Organization (WHO) criteria (13). The diagnosis was established based on standard oral glucose tolerance test (OGTT) criteria. OGTT results demonstrated elevated fasting plasma glucose (mean = 116 mg/dL, reference target <95 mg/dL) and 1-hour post-load glucose (mean = 165 mg/dL, reference target <140 mg/ dL), while 2-hour post-load glucose remained within normal limits (mean = 99 mg/dL, reference target <115 mg/dL). These findings met the diagnostic threshold for GDM.

RESULTS

Fasting blood glucose (FBG) values ranged from 83 to 97 mg/dL, with a mean of 112 mg/dL(SD=5), reflecting generally well controlled basal glycemia prior to meals.

A One-way ANOVA analysis was conducted, and it revealed a significant effect of meal type on 1-hour postprandial glucose (PPG_1h), $F(3, 40) = 488.231$, $p<0.001$. Post-hoc comparisons using the Tukey HSD test indicated that high-carbohydrate meals resulted in significantly higher glucose levels (M = 150.92 mg/dL, SD = 2.42) than protein/low-carb meals (M = 115.25 mg/dL, SD = 3.79), snacks (M = 110.5 mg/dL, SD = 2.17), and fruits (M = 130.4 mg/dL, SD = 2.22) (fig. 1).

In addition, a similar pattern was observed for 2-hour postprandial glucose (PPG_2h). One-way ANOVA showed a significant effect of meal type, $F(3, 40) = 223.764$, $p<0.001$, with Tukey post-hoc comparisons revealing that high-carbohydrate meals (M = 111.92 mg/dL, SD = 1.85) produced higher glucose levels than fruits (M = 103.6 mg/dL, SD = 2.22), protein/low-carb meals (M = 95.33 mg/dL, SD = 2.57), and snacks (M = 89.90 mg/dL, SD = 1.82) (fig. 2).

CGM measurements revealed discrepancies compared to glucometer readings. Participants without false hypoglycemic alarms had a mean CGM difference of 23.19 mg/dL (SD = 0.89), whereas participants experiencing false alarms had a mean difference of 28.71 mg/dL (SD = 0.72). An independent-samples t-test was conducted to compare the difference between CGM and glucometer readings in participants. Levene's test indicated that the assumption of equal variances was met, $F = 0.693$, $p = 0.410$. The t-test revealed a significant difference between the groups, $t(44) = -20.31$, $p<0.001$, with a mean difference of -5.53 mg/dL (95% CI [-6.08, -4.98]) (fig. 3).

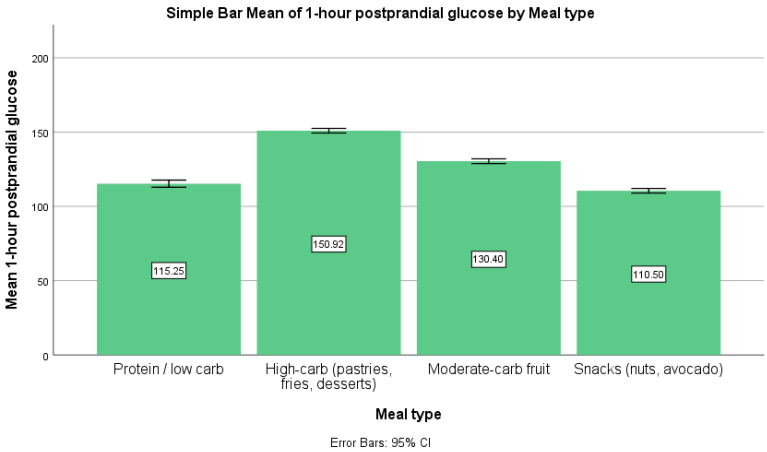

Fig. 1. Mean 1-hour postprandial glucose levels (mg/dL) by meal type in 44 pregnant women with gestational diabetes mellitus. Error bars represent ± standard deviation. One-way ANOVA revealed a significant effect of meal type on 1-hour glucose, $F(3, 40) = 488.231$, $p<0.001$. Tukey HSD post-hoc tests showed that high-carbohydrate meals had higher glucose than protein/low-carb meals (-35.67 mg/dL, $p<0.001$), fruits (-20.52 mg/dL, $p<0.001$), and snacks (-40.42 mg/dL, $p<0.001$). Fruits were higher than protein/low-carb meals (15.15 mg/dL, $p<0.001$) and snacks (19.90 mg/dL, $p<0.001$). Snacks were higher than protein/low-carb meals (4.75 mg/dL, $p = 0.002$).

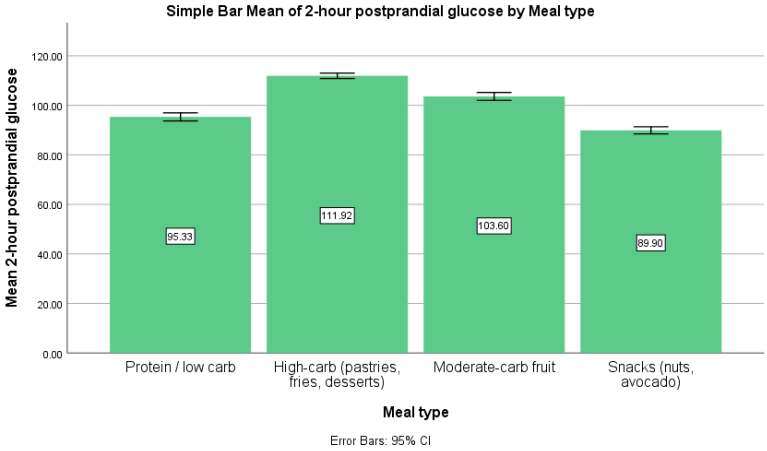

Fig. 2. Mean 2-hour postprandial glucose levels (mg/dL) by meal type in 44 pregnant women with gestational diabetes mellitus. Error bars represent ± standard deviation. One-way ANOVA showed a significant effect of meal type on 2-hour glucose, $F(3, 40) = 223.764$, $p<0.001$. Tukey HSD post-hoc tests indicated that high-carbohydrate meals resulted in higher glucose than protein/low-carb meals (16.67 mg/dL, $p<0.001$), fruits (8.40 mg/dL, $p<0.001$), and snacks (22.00 mg/dL, $p<0.001$). Fruits resulted in higher glucose compared to protein/low-carb meals (8.27 mg/dL, $p<0.001$) and snacks (13.60 mg/dL, $p<0.001$). Snacks were higher than protein/low-carb meals (5.33 mg/dL, $p<0.001$).

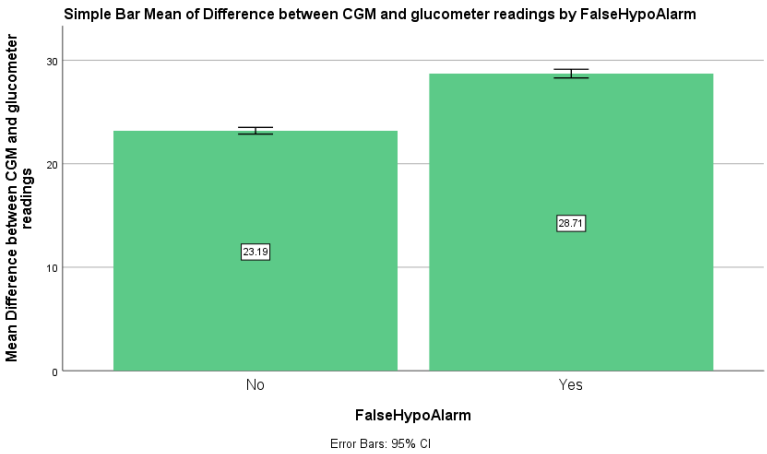

Fig. 3. Mean difference between CGM readings and glucometer measurements (mg/dL) in participants with and without false hypoglycemic alarms. Error bars represent ± standard deviation. Independent-samples t-test showed a significant difference, t (44) = -20.31, p<0.001, with participants experiencing false alarms having higher CGM discrepancies (28.71 ± 0.72 mg/dL) than those without false alarms (23.19 ± 0.89 mg/dL).

DISCUSSION

Our data illustrates how continuous glucose monitoring (CGM), combined with personalized dietary interventions, can effectively guide clinical decisions to improve glycemic control in GDM. Participants' glycemic profiles revealed significant differences in postprandial glucose depending on meal type, with high-carbohydrate meals produced significantly higher 1-hour and 2-hour postprandial glucose levels compared with protein / low-carbohydrate meals, snacks, and fruits, which showed lower postprandial values (ANOVA and Tukey *post-hoc*, p<0.001).

Postprandial hyperglycemia after ingestion of refined carbohydrates and high-fat meals reflects previous research findings emphasizing the impact of meal composition on glucose fluctuations in GDM (14, 15). Similarly, CGM measurements showed slightly larger discrepancies in participants experiencing false low-glucose alarms, confirming the t-test result (p<0.001).

In contrast, meals rich in fiber, protein, and unsaturated fat - such as vegetables, nuts, avocados, and dairy - were associated with improved glycemic control, consistent with randomized controlled trials demonstrating the benefits of low-GI, high-fiber diets in the management of GDM (16).

Body weight remained relatively stable across participants, consistent with dietary and activity patterns. These dietary patterns not only attenuate postprandial glucose peaks but also contribute to satiety and improved insulin sensitivity (17). This data supports the clinical focus on dietary counseling tailored to individual glycemic responses, made more feasible by CGM technology.

Challenges associated with CGM accuracy, particularly false hypoglycemia alarms and calibration issues, have been documented in other studies (9, 18). These findings emphasize the need for healthcare providers to integrate CGM data with capillary glucose measurements and clinical judgment.

Despite these limitations, CGM offers unparalleled granularity in glucose monitoring, allowing for real-time feedback and more precise dietary adjustments (19, 20).

Taken together, these aspects reinforce current guidelines that advocate for personalized strategies for managing GDM, combining technology (CGM) and diet optimization (6). This also opens new discussions about the use of AI in nutritional epidemiology (21, 22). Future research should focus on larger cohorts to validate the utility of CGM in predicting adverse outcomes and refining dietary recommendations based on individual glycemic responses.

CONCLUSIONS

Our data highlights the critical role of continuous glucose monitoring (CGM) combined with personalized dietary management in optimizing glycemic control in gestational diabetes mellitus (GDM). CGM use allowed identification of meal types that produced higher postprandial glucose levels, while protein/low-carbohydrate meals and snacks were associated with more stable glycemia. CGM provided more detailed glucose monitoring than standard glucometer readings, highlighting its value for guiding real-time clinical decisions. Future studies should investigate CGM-guided dietary strategies in larger cohorts to validate these findings and refine recommendations for GDM management.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

PATIENT CONSENT STATEMENT

The participants provided informed consent for the use of their anonymized medical data for educational and research purposes. No medical actions were implemented based on the data obtained, and all care decisions were made by her obstetric care team.

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THE CITY EFFECT: HOW URBAN LIVING SHAPES PSYCHOTIC RELAPSE RISK

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THE CITY EFFECT: HOW URBAN LIVING SHAPES PSYCHOTIC RELAPSE RISK (Abstract): Urbanization is a rapidly intensifying global trend and a significant determinant of population mental health. Growing evidence demonstrates that individuals exposed to urban environments experience a markedly higher risk of developing psychotic disorders, particularly schizophrenia, with effects that show clear dose-response patterns based on timing and duration of exposure. This study **aimed** to examine relapse burden in a hospital-based cohort of patients with psychotic disorders, with particular focus on urban residence and environmental correlates. **Materials and methods:** We conducted a six-month observational cross-sectional study including 60 hospitalized patients with schizophrenia-spectrum or acute psychotic disorders. Relapse vulnerability was operationalized using prior psychiatric hospitalizations. A subgroup of 31 patients underwent depressive symptom assessment and biomarker analysis, alongside 19 healthy controls. Sociodemographic, lifestyle, and environmental exposures were recorded. Descriptive statistics and correlation analyses were performed. We complemented the observational findings by a targeted literature synthesis. **Results:** Half of our cohort had 3 or more hospitalizations, indicating substantial relapse burden. Within the acute psychotic subgroup, 65% were of urban origin. Smoking (41.7%) and occupational chemical exposure (16.7%) were notable environmental factors. The predominance of urban residence among relapse-prone patients aligns with epidemiological evidence supporting an exposure-gradient relationship between urbanicity and psychosis. **Conclusions:** Urbanicity appears to function as a cumulative environmental correlate of psychotic relapse. Integrating epidemiological, psychosocial, and neurobiological perspectives may inform targeted prevention strategies and urban mental health policies aimed at reducing relapse risk. **Keywords**: URBANICITY, PSYCHOSIS, SCHIZOPHRENIA, STRESS.

INTRODUCTION

Urbanization represents a major demographic shift of the century with projections estimating that two-thirds of the population will be urban by 2050 (1). This change has paramount implications for public mental health (2, 3). Emerging evidence demonstrates a consistent link between urbanicity and increased risk of psychotic disorders, particularly schizophrenia (2-4).

Studies dating back to 1939 have con-

sistently reported an increased prevalence of psychosis in densely populated socially disorganized urban areas (4). These findings have been replicated by modern large-scale cohort studies across Europe and North America (5). Additionally, a dose-response association between urban living and psychosis has been established, since prolonged urban exposure in early life leads to higher psychosis likelihood later in life (6).

This association is multifactorial, involving interactions between social, environmental, and biological processes (7). Numerous psychosocial adversities are concentrated within urban areas (i.e. social fragmentation, deprivation, minority stress, and exposure to violence) (7).

Important neurobiological changes occur in the context of urbanicity (8). A central hypothesis is that chronic exposure to urban stressors potentiates sensitization of stress-response circuits, particularly HPA axis and mesostriatal dopaminergic pathways (3, 9). Urban upbringing has been associated with hypercortisolemia and heightened HPA axis reactivity in both healthy individuals and those at high risk for psychosis (4). Chronic activation of the HPA axis may lead to glucocorticoid dysregulation, hippocampal vulnerability, and exaggerated responses to daily stressors (1, 10). Individuals raised in urban areas show stronger amygdala and perigenual anterior cingulate cortex activation during social stress tasks, suggesting heightened threat sensitivity (8). These stress-induced neural alterations mirror patterns seen in individuals with prodromal or established psychosis (6). Furthermore, urbanicity is strongly associated with increased striatal dopamine synthesis capacity, a core biological abnormality in schizophrenia (4, 8, 9). Positron-emission tomography (PET) imaging studies show that individuals exposed to urban environments demonstrate greater mesostriatal dopaminergic responsivity to stress (8, 9). This dopaminergic sensitization appears to mediate the link between social defeat and psychosis (3, 8, 9). Migrant status, minority stress, and chronic social exclusion also correlate with increased dopaminergic firing (4).

Despite decades of research, the mechanisms that connect urbanicity to psychotic outcomes remain only partially elucidated, while the burden of psychosis in cities continues to grow (1). A better understanding of these pathways is crucial for targeted public health interventions, reshaping urban environments, and improving outcomes for high-risk populations (4). Concurrently, we must acknowledge the role of mental health services in preventing psychotic decompensations (4). Counteracting the cumulative effects of urban stress requires accessible and culturally sensitive mental health services (4). Coordinated specialty care (e.g. assertive community treatment, family therapy, and integrated psychosocial support) reduces relapse and improves long-term functioning in psychotic disorders (1, 11). Early intervention services for individuals at high clinical risk can delay or prevent the evolution of prodromal symptoms to full psychosis, particularly in urban settings (6, 11). Mobile crisis teams and home-visiting programs are essential for patients who struggle with adherence or experience social isolation in dense city environments (12). Considering that every child is a developing adult, preventive strategies should target high-risk developmental periods, when urban stressors exert the strongest neurobiological effects (4,

13). In this regard, public health strategies should integrate mental health indicators into urban development frameworks to ensure that infrastructure decisions account for their psychological impact (7, 11). Consequently, these strategies may facilitate the improvement of patients' quality of life in the context of their own cultural and value systems (1, 14, 15).

The aim of our study was to characterize relapse vulnerability in a hospital-based cohort of patient with psychotic disorders by examining socio-demographic, lifestyle, and environmental factors, with a particular focus on urbanicity as a cumulative exposure correlate. We used prior psychiatric hospitalization history as a proxy for relapse burden, we described relapse-pattern distributions and evaluated candidate modifiers such as smoking and occupational chemical exposure. These observational findings were complemented by a targeted synthesis of current evidence on the mechanisms linking urban environments to psychosis onset and relapse, integrating epidemiological, psychosocial, neurobiological, and policy-level perspectives.

MATERIALS AND METHODS

We conducted a hospital-based observational cross-sectional study, complemented by a synthesis of current literature. Our study was performed at Socola Institute of Psychiatry over a recruitment period of approximately six months, allowing continuous monitoring of psychiatric admissions.

The primary observational cohort consisted of 60 hospitalized patients diagnosed with schizophrenia-spectrum disorders or acute psychotic disorder who fulfilled predefined inclusion and exclusion criteria. Within the larger cohort, a clinically characterized subgroup of 31 patients diagnosed with acute psychotic disorder was further analyzed for biological and symptom-related correlation. A control group of 19 individuals without psychiatric or inflammatory pathology was included for comparative purposes in the biomarker analyses.

Inclusion criteria consisted of: patients over the age of 18, diagnosis or acute psychotic disorder, voluntary hospitalization and written informed consent, Hamilton Depression Rating Scale (HAMD) score $\geq$ 26, absence of active inflammatory somatic disease, no recent surgery or vaccination in the previous three months, minimum basic education level ($\geq$ 8 completed classes). Exclusion criteria were represented by: major endocrine dysfunction, substance abuse capable of altering hormonal or inflammatory markers, severe chronic somatic illness, inability to provide informed consent.

All participants underwent structured clinical evaluation. Depressive symptom severity was measured using HAMD. In the acute psychotic subgroup, inflammatory and hormonal biomarkers were assessed using standardized laboratory methods, with blood sampling performed under controlled conditions.

Data were entered into a secured database and analyzed using SPSS software. Descriptive statistics were used to characterize socio-demographic and clinical variables. Categorical variable was expressed as absolute numbers and percentages. Correlation analysis (Pearson coefficient) was applied for continuous biological and clinical variables. Given the observational cross-sectional design, analyses were exploratory and descriptive in nature, without causal inference.

To complement the observational findings, a targeted narrative review was conducted using PubMed, Scopus, and Google Scholar. The search strategy included combinations of the following terms: urbanicity, urban environment, psychotic disorder, psychotic relapse, and psychotic decompensation. Only English-language, peer-reviewed publications were included. The aim of this addition was to integrate epidemiological, psychosocial, and neurobiological evidence regarding urban exposure and psychosis relapse into the interpretation framework of the observational findings.

RESULTS

The observational cohort comprised 60 hospitalized patients diagnosed with schizophrenia-spectrum disorders or acute psychotic disorder. Within this group, 31 patients with acute psychotic disorder underwent biomarker and depressive symptom assessment, alongside a control group of 19 individuals without psychiatric or inflammatory pathology.

Relapse vulnerability was operationalized using prior psychiatric hospitalization history. The distribution was as follows: 20 patients (33.3%) had 1-2 prior hospitalizations, 18 patients (30%) had 3-5 prior hospitalizations, and 12 patients (20%) experienced over 5 hospitalizations. Collectively, 50% of the cohort had at least 3 prior admissions, indicating a substantial subgroup with recurrent or chronic psychotic decompensation. This tendency toward higher admission frequency suggests a non-random concentration of relapse burden within the sample and supports the interpretation of the cohort as relapse-prone rather than first-episode predominant.

In the acute psychotic subgroup, Pearson correlation analysis between TNF-α and HAMD score revealed a negligible linear association ($r = 0.06310$) and a statistically non-significant result ($p > 0.005$). Therefore, at least in this subgroup, inflammatory burden, does not independently account for depressive symptom intensity in acute psychosis. This finding supports a multifactorial vulnerability model rather than a single biomarker-driven mechanism.

Within the acute psychotic subgroup (n=31), 65% (n=20) resided in urban areas, compared to 35% (n=11) from rural environments. Large-scale epidemiological studies have consistently showed that the incidence of psychosis is higher in densely populated cities compared to rural regions with effects sizes ranging from 1.5- to 3-fold according to the specific urban context (4, 6). This association is independent of socioeconomic factors, family psychiatric history, and substance use (1, 3). However, psychotic risk is dependent on the length of exposure, being minimal for individuals born and raised in rural areas, increasing for those from suburban zones, and maximal for patients living in inner cities (1, 4, 6). Moreover, early life can be highlighted as a critical period during which urban-related stressors may shape stress reactivity systems and neurodevelopment (1, 7). In this respect, longer exposure during childhood and early adolescence heightens psychotic vulnerability considerably (1, 3). Although inferential testing between urbanicity and hospitalization frequency was limited by available aggregated data, the predominance of urban origin within a clinically unstable subgroup is consistent with an exposure-gradient hypothesis. The descriptive over-representation of urban residents among relapse-prone patients aligns with the aforementioned epidemiological models.

Cross-cultural studies strengthen the association between urbanicity and psychosis with both Western European and North American cohorts reporting a higher incidence for schizophrenia and non-affective psychoses for residents of urban centers (1, 3, 4). Moreover, similar patterns have been observed in low- and middle-income countries, where urbanization is rapid and associated with social inequality, making it apparent that the link between psychotic disorders and urban living transcends economic, social, cultural, and healthcare contexts (7, 16). Additionally, migration leads to a two- to four-fold increase in psychosis rates in migrant residents, particularly of the second generation, of large cities compared to natives (1, 4). This is especially frequent when the migrants belong to socially marginalized ethnic or cultural groups (1, 4, 7).

Numerous social and environmental risk factors are concentrated within urban areas (3). However, they do not act independently, but rather cumulatively to increase psychotic risk (3, 7). One of the main factors we were able to identify is social fragmentation, characterized by high residential mobility, single-person households, and weak neighborhood cohesion (4, 17). This phenomenon is widely associated with higher admission rates for first-episode psychosis and psychotic relapses (4). In addition to social fragmentation, social isolation raises the risk of psychotic relapse through decreased social buffering, increased sensitivity to stressors, and increased loneliness (7, 18). At the same time, perceived social exclusion may intensify threat vigilance and internalized stigma, destabilizing individuals that are predisposed to psychotic relapse (19, 20). Along the same lines, social deprivation (i.e. unemployment, poor educational attainment, housing instability, and material poverty) amplifies psychosocial stress by increasing social comparison, reducing perceived control, and inducing chronic hypervigilance (1, 3). All of the latter are known factors that might destabilize vulnerable individuals and increase the risk of psychotic relapses (3). This vulnerability appears to be more important in young-to-middle adulthood. In the acute psychotic disorder subgroup 71% of the patients (n=22) were between 20-40 years, representing the peak psychosis relapse years. This result reduces confounding from geriatric comorbidity, since only one patient (3%) was over 61 years.

Deprivation may also influence psychical neighborhood quality, including chronic noise exposure, crowding, substandard housing, and lack of safe areas, which contribute to cumulative psychosocial stress (1, 21). Consequently, deprivation is a contributive factor to victimization and high crime rates (1, 3, 22). The former acts as an acute stressor that may precipitate relapse in individuals with pre-existing vulnerability (1, 22). In this regard, exposure to community violence has been shown to disrupt sleep, alter cortisol rhythms, and impair emotional regulation, all contributing to psychosis (22, 23). Not least, high urban density raises exposure to noise, crowding, and overstimulation, which dysregulate stress response and increase psychosis vulnerability (1, 6). Sensory overload, whether by constant auditory stimuli, frequent interpersonal proximity, or visual complexity, can contribute to cognitive fatigue and perceptual instability, particularly in high-risk individuals (3, 24, 25). Chronic noise exposure has been identified as not only a precipitant for psychotic

relapses, but also for triggering other known risk factors for psychosis such as sleep disturbance and circadian dysregulation (26, 27). Additionally, we identified occupational exposure to toxic chemical environments to be another risk factor that is more prevalent in urban areas. In our cohort, 16.7% (n=10) of patients reported occupational exposure to toxic chemical environments. While numerically smaller than the smoking subgroup, this exposure category represents a discrete environmental risk subset. Given its lower prevalence, statistical power for subgroup comparison is limited. However, the presence of nearly 1 in 6 patients underscores the role of environmental factors in relapse.

We also identified substance use and medication non-adherence as key precipitants of psychotic relapse. In our cohort, 41.7% of patients (n=25) were smokers, while 58.3% denied smoking. This prevalence exceeds general population smoking rates in most European samples, indicating behavioral enrichment within the psychosis cohort. Substance use is more prevalent in urban areas (1, 28). These substances may partially mediate the association between urban living and psychotic relapse (1, 28). Although cross-tabulation between smoking and hospitalization categories was not available for inferential testing, the substantial smoking burden within a relapse-prone sample suggests a potential interaction between nicotine use and illness chronicity. From a statistical standpoint, the near 42% smoking rate represents a clinically meaningful exposure cluster warranting further stratified analysis in future studies. Additionally, patients living in high-stress environments are more likely to experience daily disruptions, reduced social support, and lower treatment continuity, all of which contribute to poor adherence (6, 29). Homelessness, unstable housing, and frequent address changes common in deprived urban zones can reduce access to outpatient psychiatric care and timely follow-up as well (4, 30).

Our analysis of the scientific literature revealed several protective factors against psychotic relapse in vulnerable patients, including physical environmental resources, social buffers, and individual-level attributes. Firstly, access to green spaces has been consistently associated with lower stress levels, which results in reduced psychiatric symptom burden and improved overall mental health (4, 31). Exposure to natural environments improves cognitive restoration by enhancing attention and working memory, while reducing perceived stress (1, 31). Studies of youth show that green-space deprivation correlates with increased psychotic symptoms, illustrating the protective effect of these spaces (3, 31). Secondly, strong community cohesion has been shown to protect against the adverse mental health consequences of urban living (4, 32). Higher social capital reduces loneliness, mitigates social defeat, and increases coping capacity in vulnerable individuals (5, 32). Cultural identity and co-ethnic density also play mitigating roles with minority individuals experiencing fewer psychotic relapses when living in communities with higher ethnic representation (5, 33). Moreover, positive family environments appear to decrease stress-related cortisol responses and help prevent symptom exacerbation (3, 34). Another factor that seems to moderate the relationship between urban stress and psychotic relapses is psychological resilience (6). Cognitive-behavioral interventions have been shown to lower psychotic experiences in

high-stress environments by enhancing coping skills and reducing catastrophic thinking (3, 35, 36). Higher cognitive reserve may also moderate the impact of sensory overload and social stress via top-down neural regulation (24, 37). Social skills training, structured psychoeducation, physical activity, or mindfulness are also tied to enhanced ability to seek support during early signs of decompensation by improving stress reactivity and emotional regulation (1, 4, 36). Finally, early intervention acts as a buffer against psychotic decompensations due to urban stress, particularly for individuals with controlled psychotic symptoms (6, 36). Urban areas have a better availability of specialized psychiatric services, which can facilitate rapid treatment response (3). Coordinated specialty care (i.e. psychotherapy, family interventions, and case management) significantly reduce relapse rates in high-risk individuals (1, 36). However, disparities in service accessibility within cities mean that protective effects rely heavily on socioeconomic status and local system capacity (4).

CONCLUSIONS

Our study aimed to clarify the connection between urbanicity and the risk of psychosis and psychotic relapse in a hospital-based cohort. Urbanization exerts a significant and consistently replicated influence on the risk of psychosis, affecting both the onset and course of schizophrenia-spectrum disorders across diverse populations. Epidemiological studies demonstrate strong associations between urbanicity and increased psychotic risk, which is further supported by evidence showing that migration, minority stress, socioeconomic deprivation, and social fragmentation impact vulnerable group within cities. Moreover, mechanistic studies reveal that urban stress affects neurobiological pathways central to psychosis. These neural changes align with the patterns observed in high-risk and first-episode populations, suggesting that urbanicity functions as a meaningful environmental risk factor rather than a simple social correlate. Urban stressors additionally contribute to symptomatic destabilization and relapse among individuals with established psychosis.

At the same time, we identified several protective factors that can buffer the adverse effects of urbanicity. Policy interventions that enhance environmental quality, reduce inequality, and strengthen social infrastructure have shown promise in mitigating mental health disparities. Incorporating mental health priorities into city planning is increasingly recognized as crucial for improving urban population's quality of life.

Ultimately, future research is necessary and must integrate epidemiology with urban neuroscience, high-resolution environmental modelling, and gene-environment interaction framework to fully characterize the processes through which cities shape psychotic risk. Addressing the risk of psychotic relapse in urban areas requires coordinated interdisciplinary action across psychiatry, public health, and urban planning to transform cities into supportive, health-promoting spaces.

CONFLICT OF INTERESTS AND FUNDING

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IMPACT OF CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR MODULATORS ON RESPIRATORY, NUTRITIONAL, AND INTESTINAL PARAMETERS IN CHILDREN WITH CYSTIC FIBROSIS: A SINGLE CENTER OBSERVATIONAL STUDY

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IMPACT OF CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR MODULATORS ON RESPIRATORY, NUTRITIONAL, AND INTESTINAL PARAMETERS IN CHILDREN WITH CYSTIC FIBROSIS: A SINGLE CENTER OBSERVATIONAL STUDY (Abstract): Highly effective cystic fibrosis transmembrane conductance regulator (CFTR) modulators have substantially changed the course of pediatric cystic fibrosis (CF) by improving CFTR function, respiratory outcomes, and nutritional status. The extent to which modulator therapy reverses severe exocrine pancreatic insufficiency and chronic low-grade intestinal inflammation in children remains incompletely defined. **Aim:** To characterize the multidimensional impact of CFTR modulator therapy on CFTR function, respiratory morbidity, nutritional status, exocrine pancreatic function, and intestinal inflammation in a real-world pediatric CF cohort. **Materials and methods:** In this prospective, single-center observational study (April 2024-January 2026), we included children aged 1-17 years with confirmed CF. The main treatment cohort comprised 32 genetically eligible children who initiated and maintained CFTR modulator therapy (ETI) for at least 12 months. Sweat chloride, anthropometric z scores, respiratory microbiology, liver function, and vitamin D status were assessed at predefined timepoints. In a digestive sub cohort of 31 treated children, fecal elastase, fecal calprotectin, and fecal neutrophil gelatinase-associated lipocalin (NGAL) were measured according to treatment duration and compared with values in 22 age matched healthy controls without digestive disease. Respiratory morbidity was evaluated from the frequency of infectious pulmonary exacerbations, airway microbiology, and lung function; spirometry was performed in children aged ≥6 years and ventilatory impairment was categorized as normal, mild, moderate, or severe. **Results:** After 12 months of CFTR modulator therapy, mean sweat chloride decreased from 114.1 to 78.3 mmol/L, indicating a marked improvement in CFTR function at cohort level. Weight for age and body mass index (BMI) z scores improved significantly, whereas height for age z scores remained essentially unchanged, consistent with a predominantly weight-driven short-term nutritional recovery. The mean annual rate of infectious pulmonary exacerbations declined from 4.72 at baseline to 0.41 at 6 months, reflecting a rapid and sustained reduction in respiratory morbidity. Diagnosis before 6 months of age was associated with a higher risk of severe exocrine pancreatic insufficiency and vitamin D deficiency, delineating an early onset, severe digestive phenotype. **Conclusions:** In children

with CF, CFTR modulator therapy confers major clinical and biological benefits in terms of CFTR function, respiratory morbidity, and weight-related nutritional recovery. However, established severe exocrine pancreatic insufficiency and persistent low-grade intestinal inflammation appear only partially reversible, suggesting that the digestive phenotype of CF is less amenable to correction once structural damage has occurred. Incorporating fecal elastase and fecal inflammatory biomarkers into routine follow-up, alongside anthropometry, respiratory outcomes, and hepatic monitoring, may enhance multidimensional disease surveillance and more precisely guide long-term nutritional and hepatodigestive management in the era of highly effective CFTR modulators. **Keywords:** CYSTIC FIBROSIS, CFTR MODULATORS, FECAL CALPROTECTIN, ELEXACAFTOR, TEZACAFTOR, IVACAFTOR, SWEAT CHLORIDE, NGAL, PANCREATIC INSUFFICIENCY.

INTRODUCTION

Cystic fibrosis (CF) remains one of the most common autosomal recessive multisystem genetic disorders in childhood. It is caused by mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) gene, which impair epithelial chloride and bicarbonate transport, leading to dehydrated secretions and a complex respiratory, digestive, nutritional, and hepatopancreatic phenotype (1, 2). Over the last decade, pediatric CF care has entered a new therapeutic era with the development of CFTR modulator therapies, particularly highly effective combinations such as elexacaftor / tezacaftor / ivacaftor (ETI), which target the basic molecular defect rather than acting solely at the symptomatic level (1, 2). Current standards of care acknowledge that these therapies have profoundly changed the prognosis of CF and have required a redefinition of how clinical response, long-term monitoring, and residual disease phenotypes are evaluated in the modulator era (1).

The respiratory benefits of CFTR modulators are well established. Pivotal trials and their extensions have shown that ETI leads to marked improvement in CFTR function, reflected by a significant reduction in sweat chloride concentration, together with improved lung function, fewer pulmonary exacerbations, and better overall clinical status (2). These effects were initially demonstrated in adolescents and adults with eligible genotypes. They were subsequently confirmed in children aged 6-11 years and, more recently, in those aged 2-5 years, supporting the concept that early intervention may alter disease trajectory before irreversible structural damage is fully established (3-5). In particular, phase 3 pediatric studies of ETI have shown favorable efficacy and safety profiles, reinforcing the central role of modulator therapy in contemporary pediatric CF management (3-5).

Beyond pulmonary improvement, increasing attention has been directed toward the impact of modulators on nutritional status. Malnutrition and growth failure remain major determinants of prognosis in pediatric CF and are driven by maldigestion, chronic inflammation, recurrent respiratory infection, and increased energy expenditure (6). In the CFTR modulator era, several studies and recent reviews have consistently reported improvements in body weight and body mass index, suggesting that partial restoration of CFTR activity may reduce the metabolic burden of disease and improve nutrient utilization (3, 5, 6).

However, the magnitude of these benefits is hetero-geneous, and nutritional recovery is not uniform across all anthropometric parameters, with linear growth often responding more slowly than weight gain (6). These observations support the need for an integrated longitudinal evaluation of nutritional response under modulator therapy, particularly in children, for whom growth and developmental windows are of major clinical relevance (6).

In contrast to the robust respiratory and weight-related response, the extrapulmonary digestive benefits of CFTR modulators remain incompletely defined. The exocrine pancreas is one of the earliest and most severely affected organs in CF, and exocrine pancreatic insufficiency often develops in the first months of life (7, 8). Although emerging evidence suggests that modulator therapy may increase fecal elastase levels and allow partial recovery of pancreatic function in a small subgroup of patients, especially those treated early and with less advanced residual damage, most children with severe exocrine pancreatic insufficiency do not achieve full pancreatic sufficiency (7,8). A recent review emphasized that, although the paradigm of exocrine pancreatic disease is beginning to shift in the era of highly effective modulator therapy, meaningful functional recovery remains limited to a minority of patients, and objective monitoring with fecal elastase continues to be recommended in clinical practice (8).

Another insufficiently clarified domain is persistent intestinal inflammation in CF. A growing body of evidence supports the existence of a CF-associated enteropathy characterized by dysbiosis, altered intestinal permeability, maldigestion, and low-grade mucosal inflammation (9-11). Earlier studies demonstrated that fecal inflammatory markers, particularly fecal calprotectin, may be elevated in children with CF, and this intestinal inflammation has been associated with more severe clinical phenotypes and impaired growth (9, 10). More recent systematic reviews, however, indicate that the interpretation of fecal calprotectin in CF remains complex, as it may reflect both intestinal inflammation and a broader systemic inflammatory burden (11). In parallel, newer biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL) have attracted interest for the assessment of epithelial and neutrophil-driven inflammation, although experience in pediatric CF remains limited (12, 13). Emerging data suggest that modulator therapy may only partially improve intestinal inflammation, while complete normalization is not consistently observed, particularly in patients with longstanding or advanced digestive disease (12, 13).

In this context, real-world studies in pediatric cohorts remain essential. Clinical trials of CFTR modulators often include highly selected populations, relatively limited follow-up periods, and underrepresentation of severe or complex digestive phenotypes (2-5). In everyday clinical practice, individual response to modulator therapy is influenced by age, genotype, pre-existing pulmonary and digestive structural damage, baseline nutritional phenotype, and duration of treatment exposure (1, 6, 8). Therefore, a multidimensional evaluation of children treated with CFTR modulators-including parameters of CFTR function, respiratory morbidity, growth, exocrine pancreatic function, and fecal inflammatory biomarkers-is needed to better understand which components of disease are reversible and which persist as residual phenotypes in

the era of highly effective modulators (1, 7, 8, 12, 13).

Against this background, the present study aimed to assess the impact of CFTR modulator therapy on respiratory, nutritional, and intestinal parameters in a monocentric pediatric CF cohort under real-world conditions, integrating a longitudinal before-and-after treatment analysis with a cross-sectional comparison against healthy controls.

MATERIALS AND METHODS

We conducted a prospective, single-center observational study at a regional pediatric CF referral center between April 2024 and January 2026. The study combined a longitudinal component, assessing repeated measurements before and after CFTR modulator initiation, with a cross-sectional comparison of digestive biomarkers between children with CF and healthy controls.

Inclusion criteria: children aged 1-17 years with confirmed CF were consecutively enrolled during routine clinical follow-up. The diagnosis of CF was established based on a compatible clinical phenotype, an abnormal or intermediate sweat chloride test confirmed according to current standards, and the identification of two pathogenic or likely pathogenic CFTR variants. **Exclusion criteria** included non-CF gastrointestinal disease, known inflammatory bowel disease, malignancy, other major comorbidities unrelated to CF, and incomplete follow-up during the study period.

The main treatment cohort consisted of 32 genetically eligible children with CF who initiated and maintained CFTR modulator therapy and had available longitudinal follow-up data. A digestive sub-cohort of 31 treated children was included in the analysis of fecal biomarkers. For comparison of inflammatory and pancreatic fecal markers, 22 apparently healthy age-matched children without a history of inflammatory bowel disease, chronic malabsorption, or chronic gastrointestinal infection were enrolled as controls.

Eligible patients received CFTR modulator therapy according to national recommendations, based on age and genotype. Treatment regimens included corrector/potentiator combinations, with most children receiving elexacaftor/ tezacaftor/ ivacaftor (ETI). Dosing was adjusted for age and body weight. Participants were evaluated at baseline, before treatment initiation, and at regular follow-up visits, for at least 6-12 months for systemic outcomes, with a longer observation period available for selected digestive and hepatic parameters.

At each visit, the following variables were recorded: demographic and genetic characteristics; anthropometric data (weight, height, BMI, and corresponding age and sex adjusted z scores); respiratory outcomes (number of pulmonary exacerbations, hospitalizations, and antibiotic courses); respiratory microbiology; selected biochemical parameters (including liver enzymes and vitamin status); and sweat chloride concentration. Sweat chloride was measured using standardized procedures at baseline and after at least 12 months of CFTR modulator therapy. Infectious pulmonary exacerbations were recorded longitudinally at baseline and during follow-up and were defined in accordance with European CF working group recommendations and criteria used in recent real-world ETI studies.

Lung function was assessed by spirometry in children aged ≥6 years, in line with international and national guidelines that

recommend spirometry as the standard for objective evaluation of ventilatory function from this age, when cooperation is generally reproducible. The main parameter analyzed was forced expiratory volume in 1 second (FEV_1), expressed as a percentage of the predicted value for age, sex, and height. FEV_1 was recorded at baseline and at scheduled follow-up visits, and the severity of ventilatory impairment was categorized as normal, mild, moderate, or severe, according to current recommendations.

Fecal samples were collected from spontaneously passed stool during periods of clinical stability, in the absence of acute gastroenteritis and without recent exposure to antibiotics likely to substantially alter the intestinal microbiota. Samples were stored refrigerated, transported promptly to the laboratory, and frozen until analysis.

The following fecal biomarkers were measured: fecal elastase (µg/g) as a marker of exocrine pancreatic function; fecal calprotectin (µg/g) as a marker of intestinal inflammation; and fecal lipocalin 2/NGAL (ng/g) as an additional biomarker of mucosal inflammation and barrier dysfunction. Measurements were performed using commercially available ELISA kits validated for pediatric use, according to the manufacturers' instructions; all samples were analyzed in duplicate, and results were expressed per gram of stool.

Study Objectives. The primary objectives of the study were: (1) to assess changes in sweat chloride following initiation of CFTR modulator therapy; (2) to evaluate changes in nutritional status, as reflected by weight for age, height for age, and BMI z scores; and (3) to characterize changes over time in the frequency of infectious pulmonary exacerbations and, where available, FEV_1, given its role as a major prognostic marker in CF.

Secondary objectives were: (1) to describe fecal elastase, calprotectin, and NGAL levels in children treated with CFTR modulators; (2) to compare fecal biomarkers between children with CF and healthy controls; and (3) to explore associations between digestive biomarkers, exocrine pancreatic insufficiency, vitamin D status, respiratory profile (including FEV_1, when available), and hepatic markers.

Statistical Analysis. Statistical analyses were performed using Python (Pandas and SciPy libraries). Continuous variables were summarized as mean ± standard deviation or median and interquartile range (IQR), according to their distribution. Categorical variables were described as frequencies and percentages. Normality was assessed using the Kolmogorov-Smirnov, Anderson-Darling, D'Agostino-Pearson, and Shapiro-Wilk tests.

Paired comparisons for longitudinal outcomes were performed using paired t tests or corresponding non-parametric tests, as appropriate. Comparisons between the CF and control groups were carried out using independent samples t tests or Mann-Whitney tests, depending on data distribution. Repeated measures ANOVA was used to evaluate changes in pulmonary exacerbation frequency over time. Associations between categorical variables were analyzed using Fisher's exact test, with relative risks and 95% confidence intervals reported where relevant. A two-sided p value <0.05 was considered statistically significant.

Ethical Considerations. The study was approved by the institutional ethics committee and conducted in accordance with the Declaration of Helsinki, national regulations

governing biomedical research in minors, respectively, by the Ethics Committee of the Sf. Maria Emergency Children Hospital, Iasi (approved number 31600/26.10.2023) and by the Research Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy Iasi (approved number 434 /24.04.2024). Written informed consent was obtained from the legal guardians of all participants, and assent was additionally obtained from older children and adolescents, where appropriate. All data were anonymized before analysis.

RESULTS

Baseline Characteristics. The study included 32 children with CF treated with CFTR modulators and 22 healthy controls, who were used for digestive biomarker comparisons. The mean age at CF diagnosis in the treated cohort was 29.63 months (95% CI: 15.63-43.62), and the median age at evaluation was approximately 12 years. Most treated children carried severe CFTR variants functionally classified as class I/II, including 18 patients (56.3%) homozygous for F508del. More than half of the treated patients were diagnosed before 6 months of age, mainly in the context of abnormal newborn screening or meconium ileus. All children receiving modulators had exocrine pancreatic insufficiency, and approximately 90% had severe pancreatic insufficiency (fecal elastase <100 µg/g). At modulator initiation, 61% of patients met criteria for malnutrition. Baseline demographic and clinical characteristics of the cohorts are summarized in first table.

TABLE I.

Baseline demographic, genetic, and clinical characteristics of the study population

Variable	CF modulator group (n = 32)	Healthy controls (n = 22)
Age at evaluation, years	~12 (median)	~12 (median)
Age at diagnosis, months	29.63 (95% CI: 15.63-43.62)	
Female sex, n (%)	18 (56.3%)	**11 (50.0%)**
508del homozygous, n (%)	18 (56,3%)	-
Diagnosis before 6 months of age, n (%)	~54%	-
Exocrine pancreatic insufficiency, n (%)	32 (100%)	0
Severe EPI (fecal elastase <100 µg/g), n (%)	~90%	0
Malnutrition at treatment initiation, n (%)	61%	0

The median age at evaluation in the CF modulator group was approximately 12 years, comparable to the age range of the control group, and the sex distribution was similar (56.3% *vs.* 50% female), making a major confounding effect of these variables on subsequent comparisons unlikely. The mean age at diagnosis in the treated group was 29.63 months, although more than half of the patients were identified before 6 months of age, reflecting early diagnosis, often in the context of abnormal newborn

screening or suggestive digestive manifestations.

From a genetic standpoint, most children carried class I/II CFTR variants, and F508del homozygotes accounted for 56.3% of the cohort, indicating a mutation profile dominated by minimal function genotypes. All patients receiving modulators had exocrine pancreatic insufficiency, and approximately 90% had severe EPI (fecal elastase <100 µg/g), in contrast to the complete absence of pancreatic involvement in the control group. At treatment initiation, 61% of children with CF were malnourished, whereas none of the healthy controls met malnutrition criteria. Taken together, these data show that although the groups are comparable in terms of age and sex, the CF modulator cohort is characterized by a severe genotype, early disease onset, and a profoundly abnormal digestive phenotype with severe EPI and nutritional deficit, justifying careful assessment of the response to modulator therapy in this high-risk population.

CFTR Function and Nutritional Response to Modulator Therapy.

Changes in CFTR function, anthropometric parameters, lung function, and respiratory morbidity following initiation of CFTR modulators are summarized in second table. After 12 months of treatment, a robust pharmacodynamic response was observed, reflected by a marked reduction in sweat chloride concentration. Mean sweat chloride decreased from 114.1 mmol/L (95% CI: 105.4-122.8) at baseline to 78.3 mmol/L (95% CI: 69.8-86.8) at 12 months, corresponding to a mean change of −35.78 mmol/L (95% CI: −46.00 to −25.57; $t(31) = 7.144$; $p < 0.001$). This magnitude of response is consistent with values reported in clinical trials and real-world studies of ETI and indicates substantial correction of CFTR dysfunction at the cohort level.

Nutritional recovery during the first year of treatment was predominantly driven by weight gain. Weight for age z scores improved from −1.03 to −0.17, and BMI for age z scores from −1.01 to 0.13, with both changes being highly statistically significant ($p < 0.0001$). In contrast, height for age z scores remained virtually unchanged (−0.65 at baseline *vs.* −0.65 at 12 months; $\Delta = +0.057$ SD; $p = 0.717$), suggesting that early nutritional benefit is expressed mainly through weight restoration, whereas linear growth likely requires longer observation intervals to show detectable improvement.

In accordance with current recommendations for pediatric lung function testing, standardized spirometry was performed only in children aged ≥6 years, in whom cooperation is generally reproducible. In this subgroup with spirometry available at 12 months, mean FEV_1 increased from 62.4% to 77.4% of the predicted value, corresponding to a mean gain of 14.9 percentage points (95% CI: 8.5-21.3; $p = 0.0001$). This improvement in $ppFEV_1$ falls within the range of increases reported in clinical and observational ETI studies in children and adolescents (approximately 9-14 percentage points) and confirms that, in our pediatric cohort, modulator therapy is associated with a substantial functional gain in lung function.

In parallel, the mean number of infectious pulmonary exacerbations declined from 4.72 episodes per year at baseline to 0.41 episodes during the first 6 months of treatment, a reduction that was highly statistically significant ($p<0.0001$). The

concomitant decrease in sweat chloride, improvement in weight-related z scores, increase in ppFEV$_1$, and abrupt fall in exacerbation frequency support a major and rapid impact of CFTR modulators on CFTR function, nutritional status, and respiratory morbidity. Overall, under real-world conditions, CFTR modulator therapy in children with CF is associated with a rapid and clinically meaningful improvement in CFTR function, a substantial correction of weight deficit, and a relevant enhancement of lung function, whereas the trajectory of statural growth appears, at least in the short term, to be less clearly influenced.

TABLE II.
Changes in CFTR function, anthropometry, and respiratory outcomes following CFTR modulator therapy.

Outcome	Baseline	Follow-up	Effect estimate	p value
Sweat chloride (mmol/L)	114.1	78.3 at 12 months	−35.78 mmol/L (95% CI: −46.00 to −25.57)	<0.001
Weight-for-age z-score	−1.03	−0.17 at 12 months	+0.86 SD	<0.0001
BMI-for-age z-score	−1.01	0.13 at 12 months	+1.06 SD equivalent	<0.0001
Height-for-age z-score	−0.65 SD	−0.65 SD	+0.057 SD	0.717
Infectious pulmonary exacerbations	4.72/yr.	0.41 at 6 months	Marked reduction over time	<0.0001
pp FEV$_1$ % predicted	62.4 %	77.4 at 12 months	+14.9 percentage points (95% CI: +8.5 to +21.3)	p= 0.0001

*ppFEV$_1$ calculated in the subgroup of 20 children aged ≥6 years with complete FEV$_0$-FEV$_{12}$ data

Respiratory Parameters and Microbiological Profile. Changes in respiratory morbidity after initiation of CFTR modulator therapy are detailed in Table 2 and in this subsection. Infectious pulmonary exacerbations showed an abrupt reduction during the first 6 months of treatment, with the mean number of episodes decreasing from 4.72 ± 1.20 episodes per year at baseline (95% CI: 4.29-5.15) to 0.41 ± 0.76 episodes in the first 6 months (95% CI: 0.13-0.68; $p < 0.0001$). Repeated measures ANOVA (timepoints: baseline, 1, 3, and 6 months) demonstrated a highly significant overall effect of time on exacerbation frequency (Greenhouse - Geisser corrected $F(1.692, 52.44) = 140.7$; $p < 0.0001$), indicating a strong and sustained therapeutic impact of modulators on respiratory morbidity.

Trend analysis suggested a predominantly linear downward pattern, with a rapid decline in exacerbations immediately after treatment initiation and subsequent stabilization at very low values. This profile is consistent with real-world ETI data in adolescents and adults and confirms the major early impact of modulator therapy on exacerbation burden in the pediatric population. Together with the increase in ppFEV$_1$ and improvement in nutritional parameters, these findings support an im-

portant overall respiratory benefit in the cohort analyzed.

Concerning respiratory microbiology, chronic colonization with *Pseudomonas aeruginosa* and/or *Staphylococcus aureus* showed clinically plausible associations with disease severity and bronchiectasis burden; however, these relationships did not reach statistical significance in the present cohort, likely due to the relatively small sample size and limited number of events. Nevertheless, the observed trends are consistent with our previous data demonstrating a correlation between *S. aureus* and *P. aeruginosa* colonization and bronchiectasis development in the extended center cohort, suggesting that the microbiological profile remains an important determinant of respiratory severity even in the modulator era.

Exocrine Pancreatic Function and Intestinal Inflammation. The profile of digestive biomarkers in children with CF treated with CFTR modulators and in healthy controls is presented in table III. Compared with healthy controls, children with CF on modulator therapy had significantly higher levels of fecal inflammatory biomarkers. Median fecal calprotectin was 92.4 µg/g (IQR: 30.7-196) in the CF group versus 18.8 µg/g (IQR: 5-40) in controls ($p = 0.0028$), with approximately half of the CF children having values above 100 µg/g. Median fecal NGAL was also higher in children with CF than in controls (1.685 µg/g *vs.* 0.915 µg/g; $p = 0.0008$), whereas values in the control group were often very low or undetectable.

Fecal elastase remained profoundly reduced in the CF cohort, with a median value of 1.2 µg/g (IQR: 0.5-20), while all controls had values ≥200 µg/g ($p < 0.0001$). This confirms the persistence of severe exocrine pancreatic insufficiency despite exposure to highly effective modulator therapy, in contrast to isolated reports of partial pancreatic function recovery in a small subset of patients.

Longitudinally, neither fecal calprotectin nor fecal NGAL showed a clear trend towards normalization under treatment. Median calprotectin values remained broadly similar across exposure categories (approximately 108 µg/g at 0-12 months, 69 µg/g at 12-24 months, and 91 µg/g at >24 months; $p = 0.73$), and fecal NGAL exhibited a similar pattern ($p = 0.72$). Although several individual patients demonstrated decreases in calprotectin over time, none achieved complete normalization below 50 µg/g, suggesting the persistence of concomitant low-grade intestinal inflammation in most children, in line with published data on CF-associated enteropathy.

Exocrine pancreatic function also remained essentially unchanged over the course of longitudinal follow-up. Fecal elastase values remained below 100 µg/g in almost all patients, and no child reached the threshold for pancreatic sufficiency (>200 µg/g); only one patient shifted from severe to moderate exocrine pancreatic insufficiency. Fecal calprotectin and NGAL correlated positively with each other and inversely with fecal elastase, supporting the presence of a persistent digestive phenotype characterized by low-grade intestinal inflammation and severe exocrine pancreatic insufficiency. Figures 1-3 graphically illustrate these between-group differences and the inter-individual variability of fecal biomarkers, liver function, and intestinal inflammation in CF patients treated with modulators.

TABLE III.

Digestive biomarker profile in children with CF treated with CFTR modulators and in healthy controls.

Biomarker	CF group	Healthy controls	p value
Fecal calprotectin (µg/g)	92.4 (IQR: 30.7-196)	18.8 (IQR: 5-40)	0.0028
Fecal NGAL (µg/g)	1.685	0.915	0.0008
Fecal elastase (µg/g)	1.2 (IQR: 0.5-20)	≥200 in all controls	<0.0001

Vitamin D and Hepatic Profile

The evolution of hepatic parameters during CFTR modulator therapy is presented in table IV. Children diagnosed before 6 months of age had a significantly higher risk of severe exocrine pancreatic insufficiency and vitamin D deficiency, suggesting that an early and severe digestive phenotype is closely linked to minimal function CFTR genotypes and early pancreatic involvement.

Under CFTR modulator therapy, the hepatic profile remained globally stable, with only modest and transient changes in liver enzymes. Median ALT increased slightly at 6 months, followed by a decline to values below baseline after more than 24 months (29.3 (23.1-41.4) U/L at baseline *vs.* 24.9 (21.5-30.5) U/L at >24 months; p = 0.009), with a similar pattern observed for AST (28.2 (21.8-38.3) U/L at baseline *vs.* 22.8 (19.4-28.5) U/L at >24 months; p = 0.006). GGT, total bilirubin, and INR remained stable and within normal limits throughout follow-up, in line with studies reporting minimal or no hepatic changes under ETI.

No significant correlations were identified between peak ALT values and fecal calprotectin or NGAL levels, suggesting that mild variations in transaminases were largely independent of intestinal inflammatory burden. However, all cases of mild hepatic abnormalities occurred in children with severe exocrine pancreatic insufficiency and severe CFTR genotypes, whereas no child with residual pancreatic function exhibited hepatic changes, supporting a close relationship between a severe digestive phenotype and hepatic vulnerability.

TABLE IV.

Evolution of hepatic parameters during CFTR modulator therapy.

Parameter	Baseline median (IQR)	6-month median (IQR)	>24-month median (IQR)	p-value (baseline *vs.* >24 months)
ALT (U/L)	29.3 (23.1-41.4)	35.6 (23.8-45.0)	24.9 (21.5-30.5)	0.009
AST (U/L)	28.2 (21.8-38.3)	34.6 (20.4-42.3)	22.8 (19.4-28.5)	0.006
GGT (U/L)	17.4 (15.2-22.5)	18.0 (15.0-25.0)	18.2 (15.2-21.5)	0.270
Total bilirubin (mg/dL)	0.51 (0.36-0.63)	0.50 (0.40-0.70)	0.54 (0.39-0.64)	0.920
INR	1.02 (0.98-1.03)	1.01 (0.99-1.04)	1.01 (0.98-1.04)	0.150

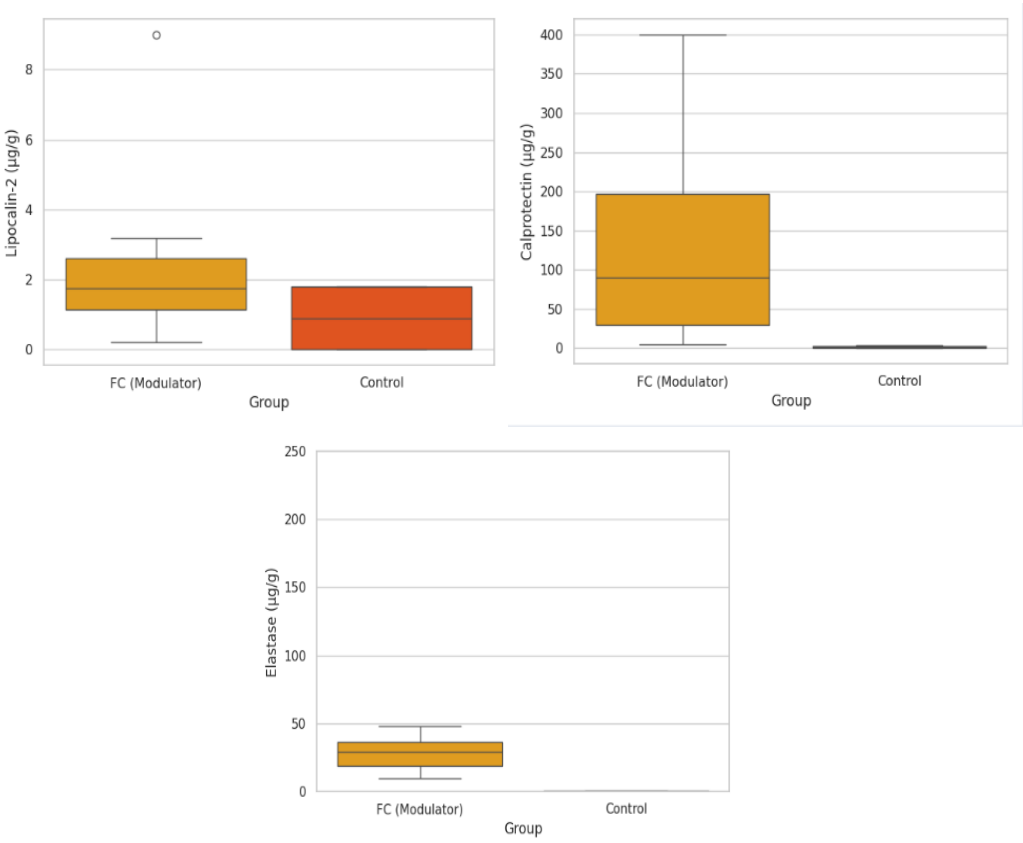

Fig. 1. Fecal digestive biomarkers in children with CF receiving CFTR modulator therapy compared with healthy controls.

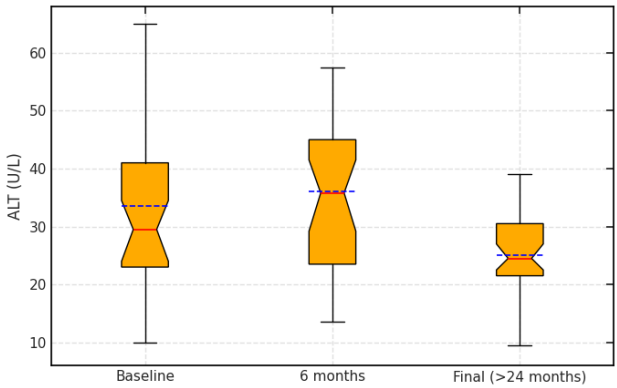

Fig. 2. ALT evolution at baseline, 6 months, and >24 months of CFTR modulator therapy, illustrating a transient increase at 6 months followed by a decline below baseline values ($p < 0.01$), suggestive of a modest improvement in hepatic profile.

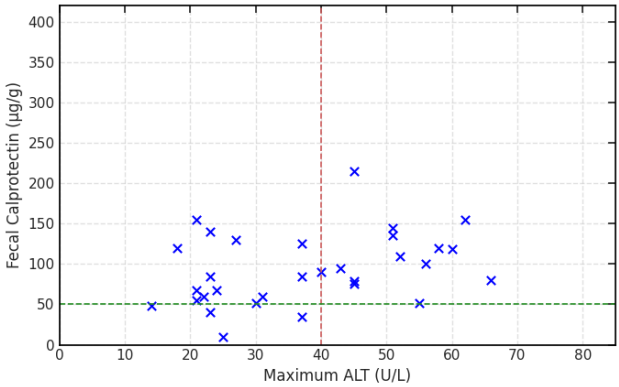

Fig. 3. Correlation between liver function and intestinal inflammation in CF patients treated with modulators.

DISCUSSION

This pediatric real-world study shows that CFTR modulator therapy elicits a strong, yet organ-specific, therapeutic response in children with cystic fibrosis. The most pronounced benefits were observed in CFTR function, respiratory morbidity, and weight-related nutritional recovery, whereas severe exocrine pancreatic dysfunction and low-grade intestinal inflammation were only minimally reversible in most patients, which is consistent with other studies in the literature. (7, 8, 12, 14).

The marked reduction in sweat chloride confirms a robust pharmacodynamic effect of CFTR modulators under routine clinical conditions. Although the magnitude of sweat chloride decline in this cohort was somewhat lower than that reported in tightly selected pediatric ETI trials, the change remained clinically meaningful and likely reflects real-world heterogeneity in genotype, baseline disease severity, preexisting structural damage, and treatment adherence (2-5, 14, 15). Similar variability has been reported in observational studies, in which a substantial proportion of ETI-treated patients retain sweat chloride values above 60 mmol/L despite overall improvement, underscoring that CFTR correction is heterogeneous outside trial settings (14-16). Collectively, these findings support the role of sweat chloride as a useful, but not fully deterministic, biomarker of treatment response in the modulator era.

Respiratory improvement in our cohort was substantial. The abrupt decline in infectious pulmonary exacerbations during the first six months of therapy supports the concept that highly effective modulators interrupt the cycle of mucus obstruction, infection, and inflammation that drives CF lung disease progression (2-5, 14). This pattern is consistent with observational cohorts and registry data showing marked reductions in pulmonary morbidity after the introduction of ETI, including fewer exacerbations, hospitalizations, and antibiotic courses (3-5, 14, 15). The gain in ppFEV$_1$ in the subgroup with spirometry data was also in line with real-world pediatric series, which report meaningful improvements in lung function sustained over 12 months and beyond (3-5, 14-16). Nevertheless, persistent chronic airway colonization in some children indicates that established infection

and bronchiectatic structural lung disease are not fully reversible through CFTR correction alone, and that airway microbiology remains an important determinant of long-term respiratory outcomes (14-16).

From a nutritional standpoint, treatment was associated with substantial improvements in weight and BMI for age z scores, whereas height for age remained essentially unchanged during the first year of follow-up. This dissociation suggests that improved energy balance and reduced inflammatory burden may drive early weight catch-up, while linear growth likely requires longer follow-up and may remain constrained by prior disease burden, long-standing exocrine pancreatic insufficiency, and cumulative nutritional deficits. A similar early divergence between weight and height trajectories has been reported in longitudinal registry analyses, including recent Danish data showing that height z scores remained largely unchanged two years after ETI initiation despite significant gains in weight and BMI (17). These observations support the notion that statural deficits represent a more fixed component of the CF phenotype once established and may not fully normalize even in the context of highly effective CFTR modulation.

The digestive findings are among the most clinically relevant aspects of this study. Severe exocrine pancreatic insufficiency persisted in nearly all children despite modulator exposure, and there was no meaningful recovery of fecal elastase in the vast majority of patients. This observation supports the concept that, once acinar destruction and fibrosis are established, restoration of epithelial ion transport alone is insufficient to reestablish exocrine pancreatic function. (18) Reports of pancreatic recovery under CFTR modulators have been largely confined to infants and very young children treated early, before advanced structural damage is present. For example, extension data from ivacaftor trials in children aged 2-5 years showed sustained improvements in fecal elastase, suggesting that early modulation may delay or mitigate pancreatic deterioration in selected genotypes (18-20). In older patients with established structural disease-such as our cohort with a median age of 12 years-exposure to elexacaftor/tezacaftor/ivacaftor appears insufficient to restore exocrine function, in line with PROMISE GI findings showing no clinically significant change in fecal elastase after six months of ETI in patients aged ≥12 years (18-21).

Similarly, fecal calprotectin and NGAL remained elevated compared with healthy controls and did not normalize over time, despite an excellent respiratory and weight response. In line with previous work, fecal calprotectin levels in children with cystic fibrosis are frequently above normal and often reflect overall disease burden rather than overt gastrointestinal symptoms (22). The inverse relationship between fecal inflammatory markers and fecal elastase in our cohort suggests that severe exocrine pancreatic insufficiency and intestinal inflammation may represent interconnected components of a common digestive phenotype rather than independent abnormalities (18, 20). Prospective multicenter data show that CFTR modulators reduce intestinal inflammation, with a significant fall in fecal calprotectin-particularly under elexacaftor / tezacaftor/ ivacaftor-yet a substantial proportion of patients retain abnormal values after 6-12 months of treatment, indicating only partial resolution of CF-associated enteropathy (12).

The pattern of liver enzyme dynamics

observed in this cohort - a modest early increase followed by longer-term normalization - is consistent with emerging evidence on the hepatic effects of elexacaftor/tezacaftor/ivacaftor. In pediatric cohorts evaluated with shear wave or transient elastography, initiation of elexacaftor/tezacaftor/ivacaftor has been associated with mild, mostly transient elevations in transaminases and a subsequent reduction in liver stiffness among children with cystic fibrosis and hepatobiliary involvement (23). In our study, GGT, total bilirubin, and INR remained stable and within normal limits, and late ALT and AST values fell below baseline, supporting a favorable hepatic safety profile overall that is in line with systematic reviews of CFTR modulator therapies and their impact on liver biochemistry and non-invasive liver disease markers (24). Persistence of vitamin D deficiency in children with severe exocrine pancreatic insufficiency further underscores that CFTR modulators do not eliminate the need for structured digestive and hepatobiliary management. Pancreatic enzyme replacement, supplementation with fat-soluble vitamins, and close nutritional and hepatic monitoring should therefore not be relaxed solely based on respiratory or anthropometric improvement (24).

This study has several strengths. It prospectively evaluates a real-world pediatric cohort, integrates systemic and digestive outcomes, incorporates healthy controls for fecal biomarker comparison, and addresses a clinically relevant but insufficiently explored question: whether highly effective CFTR modulators fully reverse the digestive phenotype of CF. However, several limitations must be acknowledged. The study was single-center and included a modest sample size, limiting statistical power for subgroup analyses, and the number of untreated CF comparators was too small to support robust parallel inferences. Follow up duration varied across outcomes, and some analyses were restricted to subgroups with available longitudinal digestive data. In addition, the observational design precludes causal inference, and the study did not include microbiome sequencing or standardized imaging of structural gastrointestinal disease.

Despite these limitations, the present findings have important clinical implications. In pediatric CF, treatment success should not be defined solely by improved respiratory outcomes and weight gain. The persistent burden of digestive disease remains clinically relevant and warrants dedicated longitudinal evaluation. A multidimensional follow-up model incorporating fecal elastase, fecal inflammatory biomarkers, vitamin status, and hepatic surveillance, alongside respiratory outcomes, may better capture overall disease activity in the era of highly effective CFTR modulators and support more precise long-term nutritional and hepato-digestive management strategies.

Future research directions. The introduction of highly effective CFTR modulators has fundamentally altered the natural history of cystic fibrosis, transforming the prognosis of pediatric patients from one defined by progressive functional decline into an on-treatment prognosis that is conditioned by the degree of individual response and the magnitude of residual risk. Building on the observations from this study, several priority avenues for future research emerge.

The persistence of exocrine pancreatic insufficiency and intestinal inflammation,

reflected by elevated fecal calprotectin and NGAL, indicates that CF-associated enteropathy is not fully reversible in patients with established structural lesions (12, 16-18)/ Future studies should incorporate longitudinal fecal microbiome sequencing to elucidate the mechanisms underlying persistent dysbiosis and its relationship with intestinal inflammation in the modulator era. In parallel, prospective cohorts of infants diagnosed through newborn screening are needed to determine whether ultra-early initiation of CFTR modulators can prevent the development of this residual digestive phenotype and avert irreversible pancreatic acinar destruction (12, 18, 19-22).

Although the rapid recovery of weight and BMI for age z scores represents a major therapeutic success, this accelerated trajectory requires a re-evaluation of traditional nutritional strategies. The shift from a historical risk of undernutrition to an emerging phenotype characterized by a risk of overweight and obesity warrants detailed investigation, including the potential long-term cardiometabolic consequences. Future research should therefore longitudinally assess body composition (lean mass versus fat mass), lipid profile, and the risk of cystic fibrosis-related diabetes (CFRD), to tailor enzyme replacement, caloric intake, and dietary counselling to the new treatment-induced metabolic trajectories (17).

The mild and transient fluctuations in liver enzymes (ALT/AST) observed under elexacaftor/tezacaftor/ivacaftor in this cohort underscore the importance of rigorous pharmacovigilance. Future studies should correlate serum biomarkers (e.g., APRI) with noninvasive structural assessments such as transient elastography (Fibro Scan) to differentiate drug-related hepatotoxicity from progression of CF-associated liver disease and to define evidence-based surveillance protocols. In the same long-term safety domain, further work is needed to standardize baseline and follow-up ophthalmologic assessment in children treated with CFTR modulators, in light of pediatric reports of cataract development following early ivacaftor or ETI exposure in utero and during breastfeeding (23-26).

As respiratory morbidity, particularly pulmonary exacerbations, declines dramatically under modulator therapy, classical endpoints progressively lose their ability to discriminate long-term prognosis (23, 24, 27). Future research should validate dynamic prognostic models that integrate sensitive functional markers of small airway disease, such as the Lung Clearance Index, together with high-resolution imaging scores (e.g. chest CT bronchiectasis and air-trapping indices) (24, 26). Such a multidimensional approach may unmask silent structural lung disease and the persistence of niche chronic infections, thereby guiding individualized treatment decisions under real-world conditions, from the newborn period until adulthood (24, 26-29).

CONCLUSIONS

In this real-world pediatric cohort, CFTR modulator therapy was associated with marked clinical benefits, including substantial reductions in sweat chloride, infectious pulmonary exacerbations, and improvements in ppFEV$_1$ and weight/BMI-for-age z scores, indicating a rapid shift from progressive functional decline toward improved respiratory stability and nutritional recovery. However, severe exocrine pancreatic insufficiency and low-grade intestinal inflammation persisted in almost all children, with fecal elastase remaining profoundly reduced and fecal calprotectin

and NGAL only minimally improved, suggesting that the digestive phenotype of cystic fibrosis is only partially reversible once structural damage has occurred. The hepatic profile with elexacaftor/tezacaftor/ivacaftor was generally favorable, with only mild, transient ALT/AST elevations and stable GGT, bilirubin, and INR. Yet, enzyme fluctuations in children with severe exocrine pancreatic insufficiency and high-risk genotypes underscore the need for ongoing hepatobiliary surveillance. Overall, pediatric cystic fibrosis care in the modulator era should be judged not solely by gains in lung function or weight, but by sustained, multidimensional control of respiratory, pancreatic, intestinal, hepatic, and nutritional health across the life course, supported by early initiation of CFTR modulators and lifelong, multidisciplinary follow-up.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest.

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REDUCTION OF SUBJECTIVE STRESS PERCEPTION IN FEMALE PATIENTS WITH AUTOIMMUNE HYPOTHYROIDISM AFTER RESTORING EUTHYROID STATUS WITH LEVOTHYROXINE TREATMENT

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REDUCTION OF SUBJECTIVE STRESS PERCEPTION IN FEMALE PATIENTS WITH AUTOIMMUNE HYPOTHYROIDISM AFTER RESTORING EUTHYTOID STATUS WITH LEVOTHYROXINE TREATMENT (Abstract): Autoimmune Thyroid Diseases (AITDs), comprising Graves' Disease and Hashimoto's Thyroiditis have a multifactorial origin and stress, as an environmental trigger, has been incriminated as the most frequent risk factor for the development of mood disorders. Also, hypothyroidism in Hashimoto's Thyroiditis is associated with different neuro-psychiatric conditions such as concentration, memory and attention deficits, depressive mood and anxiety disorder, particularly in the female sex. **Aim:** to evaluate the subjective perception of stress in women with newly diagnosed autoimmune hypothyroidism and the influence on stress perception of restoring euthyroidism with levothyroxine (LT_4) treatment. **Materials and methods:** we conducted a prospective study over a one-year period (October 2014 - September 2015) that included 17 female patients newly diagnosed with autoimmune hypothyroidism, with no history of psychiatric disorders or treatments and no other major ailments. Perceived stress was measured using four standardized questionnaires before and after instituting LT_4 therapy. **Results:** All patients included in our study, with a mean age of 43.8 ± 10.8, reported a stress level in the medium range. After LT_4 replacement treatment was initiated and euthyroidism was restored, subjective stress levels measured with all four questionnaires were lower compared to baseline. Furthermore, when we divided patients into subgroups, based on their reproductive status, psychometric scores of patients in the reproductively active subgroup before and after LT_4 treatment, were significantly lower after restauration of euthyroidism, but no significant reductions were observed in the menopausal subgroup. **Conclusions:** further research is needed, using more objective stress measurement methods, for a more reliable determination of the impact of LT_4 treatment and restauration of the euthyroid state in patients with autoimmune thyroid dysfunction. **Keywords**: AUTOIMMUNE THYROID DISEASE, STRESS PERCEPTION, PSYCHOMETRIC.

The spectrum of autoimmune thyroid disease (AITD) comprises two main disorders, Graves' disease (GD) and Hashimoto's Thyroiditis (HT). Although GD and HT represent two very different clinical phenotypes, they are both characterized by a dis-

ruption in the immune tolerance and an aberrant attack of the host through formation of antibodies that trigger thyroid antigens: thyroperoxidase (TPO), thyroglobulin (Tg) and thyroid stimulating hormone-receptor (TSH-R). This is histologically translated through the infiltration of the thyroid gland by B and T lymphocytes (1).

Even though the exact mechanisms behind AITD are not yet fully understood, its origin is multifactorial, environmental triggers acting on a specific genetic background (2, 3). AITDs are the leading cause of thyroid dysfunction, particularly in the modern countries, after the implementation of salt iodination programs (4). Taken together, AITDs have a prevalence that exceeds 5% in the general population and an even higher prevalence in women (5). AITDs are commonly associated with other autoimmune disorders, forming various phenotypes of the polyglandular autoimmune syndrome, which is also more prevalent in the female sex, over 85% of patients being women (6, 7, 8).

In recent years, the key role of stress (particularly psycho-social stress, psychological trauma and daily hassles) as an environmental trigger in the physiopathology of AITD has been the central subject for several studies (9, 10). There is accumulating evidence in the literature of a connection between stress and emotion-based pathologies and AITD, possibly through immunologic mechanisms and the activation of the two major axes involved in the pathophysiology of stress, the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic adreno-medullary system (SAM) (11, 12). Numerous studies have already shown that patients with GD reported significantly more stressful and negative life events than controls in the months anterior to the onset of symptoms (13) and an increase of GD incidence during or after wartime (14). In contrast to the considerable information published on the association between stress and the onset of GD, there have been fewer studies about the relationship between stressful events and HT and the results seem to be contradictory (9, 15).

Stress has also been incriminated as the most frequent risk factor for the development of mood disorders such as major depressive disorder (16) and hypothyroidism in HT is associated with different neuropsychiatric conditions such as concentration, memory and attention deficits, depressive mood and anxiety disorder (16). Also, evidence shows that female sex has a higher susceptibility to stress and ailments related to an emotional imbalance, as well as the fact that AITDs are 5 to 10 times more common in women (5). Accordingly, the aim of this study was to evaluate the subjective perception of stress in women with newly diagnosed autoimmune hypothyroidism and the influence on stress perception of restauration of the euthyroid status after levothyroxine (LT_4) treatment.

MATERIALS AND METHODS

This prospective study was conducted over a one-year period between October 2014 and September 2015 in the Endocrinology Clinic of the „Saint Spiridon" County Hospital in Iași, Romania. The experimental protocol, in conformity with the international ethical standards and was formally approved by the Local Ethics Committee (Protocol number 14,750 in July 17th2014). All subjects were recruited among patients visiting the Endocrinology Clinic diagnosed *de novo* with autoimmune hypothyroidism and provided written consent before the start of the study.

Patients were selected to participate in the study based on the following inclusion

criteria: female, 18 to 65 years old, non-obese (body mass index, BMI=18.5-29.9 kg/m^2), no history of psychiatric diagnoses and treatments, no other significant illnesses and newly diagnosed with autoimmune hypothyroidism based on positive serum thyroid antibodies (TPO and/or Tg, with a cut-off value of 40 IU/ml for both) and high thyroid stimulating hormone (TSH), as measured by a chemiluminescent method ("Sf. Spiridon" Clinical Emergency Hospital Iasi, Romania), with normal values ranging from 0.4-4.0 μIU/ml. Males, patients who were already diagnosed with thyroid dysfunction or thyroid cancer or had a partial or total thyroidectomy, patients with documented depression-anxiety disorders or who were taking antidepressive or anxiolytic medications, as well as noncooperative patients were excluded. For this study, we also excluded patients with AITD who were hyperthyroid or euthyroid.

Stress perception was evaluated in our patients by four standardized questionnaires. These questionnaires were self-administered before the clinical examination, but the investigators checked the degree of completion and interviewed patients on the missing information, without forcing answers from them. Depressive and/or anxiety symptoms was assessed using the Hamilton depression (HAM-D) and Hamilton Anxiety (HAM-A) scales. The Perceived Stress Scale (PSS) was used to measure the individual subjective stress perception scores in the study population by testing, on a scale from 1 to 5, how unpredictable patients view their lives and the extent to which they feel they can control the small challenges they face daily. The 51-items Daily Hassles (DH) scale was administered to evaluate patients' perception of stress regarding daily demands at home, at work, in their social or personal lives.

All patients were prescribed levothyroxine (LT_4) in the appropriate dosage and were called for reevaluation in 6-8 weeks. When the thyroid function normalized under LT_4 treatment, patients received again the four psychometric questionnaires.

The descriptive data are presented as means and standard deviation (SD). Statistical analysis was performed using Sigma Plot 11 (Systat Software Inc., San Jose, California) and *SPSS v.20* for Windows (SPSS Inc., Chicago, Illinois). Comparisons of numerical variables before and after LT_4 treatment were made using a paired Student's t-test. Statistical significance was set at $p < 0.05$.

RESULTS

After applying inclusion and exclusion criteria, 17 newly diagnosed patients with autoimmune hypothyroidism have agreed to participate in our survey. The characteristics of the study population are presented in table I.

TABLE I.

General and biochemical characteristics of the study population (n=17)

Age (years)	43.8 ± 10.8
BMI (kg/m^2)	26.6 ± 5.1
Smoker (n/%)	3 (0.51%)
Sleep pattern (hours/night)	7.2 ± 1.2
Physical activity (min/day)	58.8 ± 65.3
TSH (μIU/ml)	10.9 ± 11.2
Thyroid volume	10.9 ± 5.3

Data are expressed as the mean ± SD.

The study group interviews conducted at enrollment, when patients were in the hypothyroid state, showed a stress level in the medium range as measured with both PSS and DH questionnaires and the Hamilton scales for depression and anxiety showed mild depression, but no anxiety disorder. After LT_4 replacement treatment was initiated and euthyroidism was restored, subjective stress levels measured with the PSS questionnaire were significantly lower (p=0.046) compared to baseline (fig. 1). The scores for DH, HAM-D and HAM-A questionnaires were also reduced in the euthyroid state, on LT_4 therapy, compared to baseline, however, not statistically significant (p=0.79, p=0.190, p=0.397, respectively).

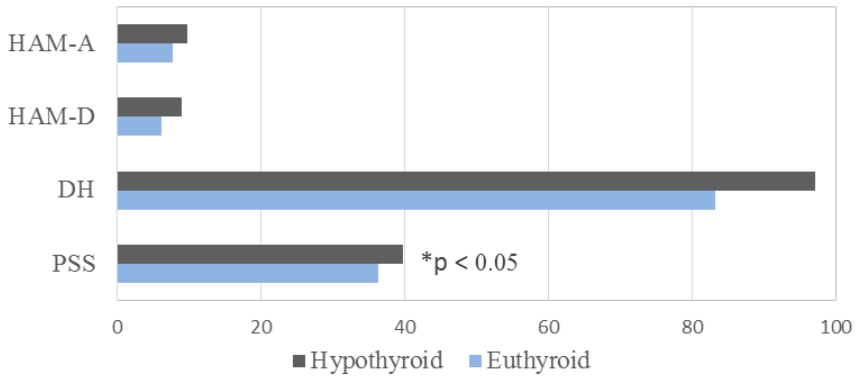

Fig. 1. Psychometric scores of the study group before and after LT_4 treatment

We divided the study group into two smaller, more homogenous subgroups: reproductively active women (n=10), with a mean age of 37.6 ± 8 and menopausal women (n=7), with a mean age of 52.7 ± 7.7. When we compared psychometric scores of patients in the reproductively active group before and after LT_4 treatment, we found a significant reduction in the scores of all four scales after restauration of euthyroidism with LT_4 therapy (tab. II).

In the menopausal subgroup, however, patients had only minimal reductions in stress levels as measured by the PSS and DH questionnaires (p=0.82 and p=0.99, respectively), while they reported an increase in Hamilton depression and anxiety scale scores (tab. III). These results were not statistically significant (p=0.38 and p=0.12, respectively).

TABLE II

Psychometric scores in women of reproductive age before and after LT_4 treatment

	Before LT_4 treatment	After LT_4 treatment	*p*
PSS	37.9 ± 8.2	32.9 ± 5.0	0.031
DH	101.4 ± 32.5	78.3 ± 8.3	0.035
HAM-D	9.0 ± 7.2	3.3 ± 2.3	0.039
HAM-A	10.6 ± 8.3	5.0 ± 1.9	0.056

Data are expressed as the mean ± SD.

TABLE III

Psychometric scores in menopausal women before and after LT_4 treatment

	Before LT_4 treatment	After LT_4 treatment	*p*
PSS	42.3 ± 6.1	41.9 ± 5.1	0.82
DH	91.1 ± 28.6	91.3 ± 19.8	0.99
HAM-D	9.0 ± 5.9	11.0 ± 6.1	0.38
HAM-A	8.4 ± 6.9	12.1 ± 1.9	0.12

Data are expressed as the mean ± SD.

When we compared psychometric scores of the two subgroups, while there were no significant differences between women in the reproductively active and the menopausal subgroups in any of the four questionnaires administered at enrollment, before the LT_4 therapy was instituted (fig. 2), after restoring the euthyroid status, during LT_4 treatment, women of reproductive age had significantly lower scores in PSS, HAM-D and HAM-A scales (p=0.034, p=0.042 and p=0.049, respectively) and a lower stress levels, however statistically non-significant (p=0.227) as measured with the DH questionnaire (fig. 3).

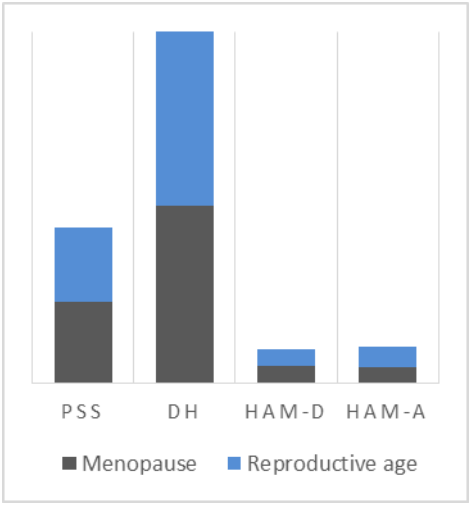

Fig. 2. Psychometric scores of subgroups before LT_4 treatment (hypothyroid state).

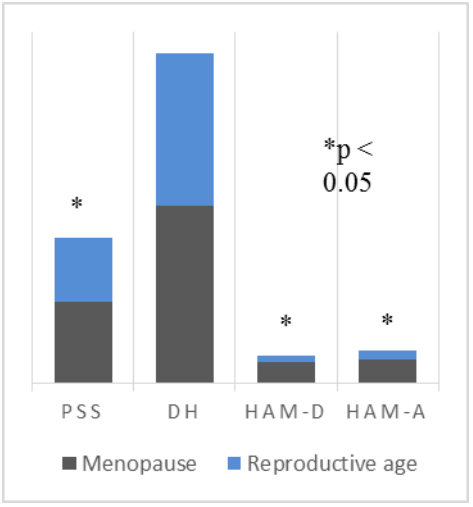

Fig. 3. Psychometric scores of subgroups during LT_4 treatment (euthyroid state).

DISCUSSION

In this report, we evaluated the impact of LT_4 treatment and restoration of the euthyroid status on the subjective perception of stress in a naïve population of women with autoimmune hypothyroidism and no history of psychiatric illnesses or other significant ailments, using four standardized questionnaires.

Stress is a key factor incriminated in the onset of various chronic conditions, as part of modern lifestyle (11). In consequence, we were not surprised that the female population included in this study reported stress perception scores in the medium range and mild depressive disorder.

As we mentioned in a previous systematic review article (10), there is a very small number of studies that evaluated the impact of stress on the onset and propagation of HT, particularly in female patients, although it is already well known that AITDs, as well as other autoimmune disorders, are caused by emotional imbalances and stress and that the prevalence of these conditions is higher in this population group.

Our main finding was that stress was perceived differently by reproductively active subjects compared to the menopausal patients in our study. It is noteworthy the fact that while in the menopausal subgroup there are no significant differences between scores of PSS and DH questionnaires before and after the administration of LT_4 treatment, in the subgroup of women of reproductive age we observed that after the restoration of euthyroidism with LT_4 therapy, the mean scores regarding subjective stress perception and general wellbeing were significantly improved. Furthermore, this research showed that in reproductively active female patients the correction of autoimmune hypothyroidism also reduces depressive and anxiety symptoms that patients reported in the hypothyroid state. In contrast, in the menopausal subgroup, depressive mood and anxiety scores tended to worsen after patients achieved euthyroidism.

We could argue that menopausal women have a more advanced age compared to reproductively active patients and they tend to have a higher risk of developing other conditions such as cardiovascular problems, which could lead patients to perceive differently daily events compared to young, healthy women (17).

Our study has some limitations. First, the small sample size may not be representative for the general population, although we tried to include only patients with no significant pathologies and no history of psychiatric disease or treatments. Another major limitation, as for every interview-based report, is recall-bias that stands from data reported from patients' memories, that is also subjective to the patients' mood in the moment of the interview.

CONCLUSIONS

The results of our study showed that female patients with autoimmune hypothyroidism have a medium stress level and there is a tendency of a reduction in the subjective stress perception after restoration of euthyroidism with LT_4 treatment in patients of reproductive age, but not in menopausal women.

We consider of essential importance the use of objective stress measurement methods, for a more reliable determination of the impact of LT_4 treatment and restauration of the euthyroid state in patients with autoimmune thyroid dysfunction.

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IMAGE RECONSTRUCTION IN CARDIAC SPECT IMAGE PROCESSING

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IMAGE RECONSTRUCTION IN CARDIAC SPECT IMAGE PROCESSING (Abstract): **Aim:** The purpose of this study was to assess the effects of two reconstruction techniques of myocardial perfusion images (MPI) in cardiac patients considered at low risk for myocardial perfusion abnormalities. **Materials and methods**: We evaluated 36 patients (10 males, 26 females; median age 61 years) with rest MPI studies by comparing their myocardial SPECT images reconstructed using two different algorithms. All patients underwent myocardial SPECT imaging with 99m Tc Tetrofosmin at rest, using a Symbia Evo Excel SPECT or a Siemens e.cam dual head system. SPECT images were reconstructed using filtered back projection method, and an iterative reconstruction method using the 3D Flash algorithm. For the quantitative evaluation of the reconstructed images by two methods, the contrast, contrast to noise ratio and signal to noise ratio were calculated. Using a standard template of coronary artery distribution and the polar map displays, the summed rest scores (SRS) were calculated according to the standard coronary artery distribution. **Results:** 3D Flash reconstruction algorithm significantly increases image contrast by 31% ($p < 0.005$). There were significant differences between FBP and 3D FLASH reconstruction in reducing the perfusion artifacts in right coronary artery (RCA) territories. Summed Rest Score (SRS) showed a significant decrease by 64 % ($p<0.01$) between the long-established FBP reconstruction and 3D Flash algorithm. **Conclusions**: 3D Flash-based reconstruction showed a significant improvement in image contrast and a decrease in many artifacts at the level of the inferior wall. **Keywords:** SPECT, MYOCARDIAL PERFUSION IMAGING, FBP RECONSTRUCTION, FLASH 3D.

Single-photon emission computed tomography (SPECT) myocardial perfusion imaging (MPI) is widely used for direct assessment of myocardial perfusion (1, 2). Image reconstruction of the raw data is an essential step in the processing of cardiac SPECT images (3). The most commonly employed method that has been used for many years was filtered back-projection (FBP) algorithm. It is computationally fast, easy to implement and widely applied in clinical imaging. However, FBP reconstructed images are degraded by many factors, including distance-dependent blur, attenuation, scatter and noise which limits spatial resolution, degrade the image contrast and distort the shapes of the reconstructed objects (4).

Increased availability of computer resources and improvements in mathematical computation has led to the introduction of iterative reconstruction (IR) methods as alternatives for FBP in the heart. It is now widely accepted that IR algorithms can provide some advantages over the conventional FBP, by modeling the emission and detection process and compensate for scattering. Iterative reconstruction methods can produce images that are of higher spatial resolution, lower noise, higher contrast and improved detection of myocardial perfusion defects (5-7). Moreover, depth-dependent resolution recovery (RR) with SPECT has been developed, and its positive impact on the resolution has been reported (8).

The proposed Siemens Flash 3D is a SPECT tomographic reconstruction algorithm based on Ordered Subsets Expectation Maximization (OSEM) iterative method that incorporates a measured 3-D beam model for collimation in the iteration process (9, 10). Using proprietary algorithms, a new SPECT tomographic reconstruction algorithm was developed for use on Siemens gamma cameras. With Flash 3D all slices are reconstructed simultaneously and not successively in the interactive calculation. The full information content of the reconstruction volume is used by Flash 3D in the reconstruction, providing increased accuracy over earlier approaches that relied on less sophisticated resolution recovery methodologies of the projection data.

The goal of the current investigation was to evaluate the introduced Flash 3D reconstruction algorithm in MPI, and determine whether this image reconstruction protocol optimized for resolution recovery produced adequate imaging outcomes as compared with conventional FBP.

MATERIAL AND METHODS

Subjects. The study involved 36 patients - 10 (28%) males, 26 (72%) females, median age 61 years, range 41-80 years, with known or suspected diastolic heart failure and low risk for myocardial ischemia. All subjects underwent rest imaging according to *American Society of Nuclear Cardiology* (ASNC) imaging guidelines. The study was conducted according to the principles of the Declaration of Helsinki and was approved by the local ethics committee.

Hemodynamic data, including heart rate, systolic blood pressure, and diastolic blood pressure, were recorded at the beginning at the study.

SPECT Imaging Protocol. All patients underwent resting myocardial perfusion SPECT. Images were acquired at 45 minutes after intravenous administration of 15-20 mCi (555-740 MBq) of 99 mTc-Tetrofosmin. All studies were completed with a 90°-angled dual-head Symbia Evo Excel or Siemens e.cam gamma camera equipped with low-energy, high-resolution collimators. Patients were placed in the supine position with arms up, and automatic body contouring was used to obtain the optimum detector-to-patient distance. Emission data were acquired with a 20% energy window centered at 140 keV. Further acquisition parameters were 3° per step, 180° each head, and 25 s per projection.

Data analyses. All data were reconstructed with both FBP (Butterworth filter, order 5, cut-off 0.35) and 3D Flash (10 iterations, eight subsets, Gaussian filter, FWHM= 9.60) using the same workstation (fig.1).

Reconstructed image quality was characterized using three quantitative criteria: contrast (C), the contrast to noise ratio

(CNR) and signal to noise ratio (SNR). The image contrast was calculated between two regions of interest (ROIs) drawn inside the LV wall and cavity. The noise was estimated as the standard deviation of the mean number of counts of the myocardial ROI. CNR and SNR were determined as the ratio between contrast over the noise, respectively as the ratio between the mean number of counts over the noise (fig. 2).

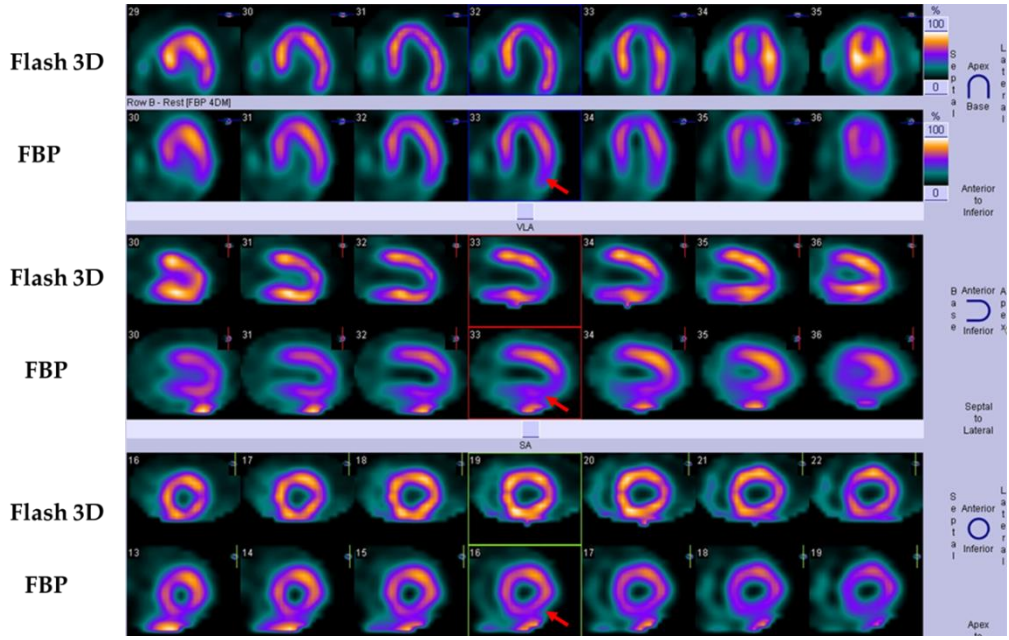

Fig. 1. Comparison of images reconstructed with FBP and 3D Flash. Note the presence of an inferior and lateral wall perfusion defect on FBP reconstructed images indicating inhomogeneous myocardial perfusion at the level of the inferior and lateral wall (arrow). Images reconstructed with Flash 3D shows no defect, suggesting that this was an artifact. Also, a better contrast between the left ventricular walls and cavity and better visualization of the right ventricle.

$$C = \frac{A_{Myo} - A_{Cavity}}{A_{Myo} + A_{Cavity}} \times 100$$

$$CNR = \frac{C}{\sigma Myo}$$

$$SNR = \frac{A_{Myo}}{\sigma_{Myo}}$$

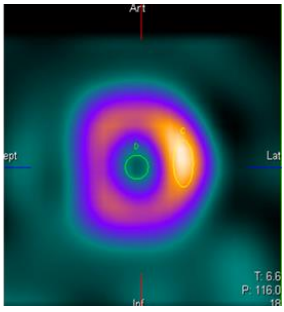

Fig. 2. Calculation of the image contrast (C), contrast to noise ratio (CNR) and signal to noise ratio (SNR) and the corresponding ROI´s.

Polar map displays of extent and severity were automatically generated for both methods and compared with a normal database using Quantitative Perfusion SPECT (QPS, Cedars-Sinai Medical Center, Los Angeles, CA, USA) software suite. The summed rest score (SRS) was used to measure cardiac perfusion inhomogeneity. For each coronary artery distribution, a perfusion score was automatically assigned, by comparing the polar maps of the patients with a database for normal persons under the same physiological conditions, gender, and isotope used (99mTc). A reconstructed image was interpreted without artifacts if the radiopharmaceutical distribution was homogeneous throughout the polar map display and the quantified perfusion score was 0 as compared with the database (fig. 3). A score of one, which represents an equivocal or mild reduction in perfusion, was considered an artifact introduced by the reconstruction algorithm.

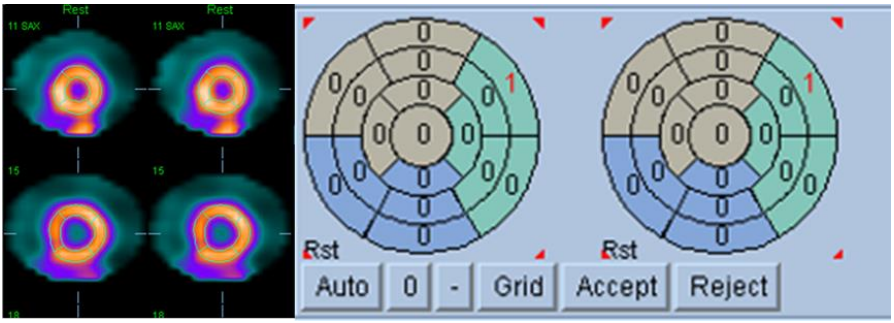

Fig. 3. Summed Rest Score (SRS) depicting mild reduction of tracer uptake in the territory of LCx artery.

Statistical analyses. Student's t-test for paired samples was employed to compare image quality parameters and the estimates of left ventricle perfusion abnormalities (summed rest scores) between the two reconstruction methods. Numeric results are expressed as mean ± SD. All statistical tests were performed with MedCalc for Windows (version 11.3.8.0 MedCalc Software, Mariakerke, Belgium). In all analyses, a p-value of less than 0.05 was considered statistically significant.

RESULTS

Image Quality Evaluation. 3D Flash reconstruction algorithm significantly increases image contrast by 31% ($p<0.005$). 3D Flash also demonstrated that, at identical dose levels, the contrast to noise ratio and signal to noise ratio are slightly decreased (tab. I).

TABLE I

Image quality evaluation parameters during stress and at rest.

Parameter	FBP	3D Flash
Contrast (%)	11	42*
Contrast to Noise Ratio	0.71 ± 1.49	0.63 ± 0.36
Signal to Noise Ratio (SNR)	9.34 ± 2.93	8.56 ± 3.23

*p < 0.005 vs. FBP; FBP - filtered back projection.

Perfusion abnormalities induced by reconstruction method. A total of 108 coronary artery territories were evaluated for perfusion abnormalities with both SPECT

reconstruction methods. The coronary artery territories to which the abnormal perfusion was found were as follows: on images reconstructed by FBP defects were found in 7 left anterior descending artery (LAD) territories in 16 left circumflex artery (LCx) territories and in 16 right coronary artery (RCA) territories, while on images reconstructed using 3D Flash in 2 LAD, 10 LCx and in 9 RCA territories. There were significant differences between FBP and 3D FLASH reconstruction in reducing the perfusion artifacts in RCA territories (16 vs. 9, $p= 0.008$) and no statistically significant differences in LAD territories (7 vs. 2, $p>0.05$) or LCx territories (16 vs. 10, $p>0.05$). Summed Rest Score (SRS) showed a significant decrease by 64 % ($p<0.01$) between the long-established FBP reconstruction and 3D Flash algorithm.

DISCUSSION

The present study was performed to assess the feasibility of the 3D Flash reconstruction algorithm which has been reported to improve noise properties by applying compensation techniques for photon attenuation, Compton scatter and depth-dependent resolution as compared to conventional reconstruction method using FBP (10, 11, 12). Our results indicate that there is an increase in image contrast between the myocardial wall and the left ventricular cavity by 31% for 3D Flash reconstructed images. Differences in perfusion scores were encountered in individual coronary artery territories with Flash 3D against FBP, except the RCA vascular territory where the mean difference in perfusion scores was significantly increased. Concerning individual vascular territories, the disagreements between the two types of reconstruction were mostly in the RCA and LCx arteries. This is probably due to the effect of sub diaphragmatic activity and diaphragmatic attenuation especially the case of the inferior wall. This finding may suggest that, although the compensation techniques for the photon attenuation and scatter might be effective with Flash 3D, it does not decrease the difficulties associated with the inferior wall attenuation artifacts (13). Nevertheless, the use of a standard template of coronary arteries distribution in MPI perfusion analysis may have also influenced. For instance, if a perfusion defect lies in the RCA territory, slightly increased perfusion defect size in Flash 3D reconstruction will incorporate part of the LCx territory as well (14). Summarizing, Flash 3D algorithm provides superior results as compared to conventional FBP by increasing the image contrast between the myocardial wall and the left ventricular cavity. With FBP reconstruction the CNR and SNR values were higher, with a trend toward decreased values in Flash 3D reconstructed images that are consistent with a slight increase in image noise. This could be related to the increased number of iterations and subsets used in our study (10 iterations, eight subsets) which may have an additive effect on noise as it has been suggested by other researchers (15). Anyhow, Flash 3D reconstructed images are improved quantitatively by increasing the absolute values of image contrasts, while CNR and SNR values remain approximately equal to those measured in the FBP reconstructed images.

CONCLUSIONS

Our study proved that Flash 3D algorithm was superior to conventional FBP reconstruction by increasing image contrast and diminishing inferior wall artifacts. Both FBP and iterative methods are useful, but the latter are now preferred since they offer more accurate modeling of physical processes.

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PREVALENCE OF MATERNAL HYPOTHYROIDISM COMPLICATED WITH PREECLAMPSIA: A RETROSPECTIVE ANALYSIS OF 60 CASES

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PREVALENCE OF MATERNAL HYPOTHYROIDISM COMPLICATED WITH PREECLAMPSIA: A RETROSPECTIVE ANALYSIS OF 60 CASES (Abstract): **Aim:** We sought to determine whether there is an increased prevalence of hypothyroidism in pregnancies complicated with preeclampsia. **Material and methods:** We performed a retrospective analysis of 60 pregnancies complicated with preeclampsia and managed in our departments. FT3, FT4 and TSH levels were recorded, as well as the mode of delivery, and were compared to a control group of healthy normotensive women of similar demographic data. **Results:** Median gestational age was the same among study and control group. Cesarean section rate was higher in the study group, whereas median birth weight was higher in control group, but both differences did not reach statistical significance. More low birth weight infants were delivered by hypothyroid mothers and this was marginally significant. In preeclamptic women mean TSH value is significantly increased when is compared to mean TSH at healthy normotensive women of similar demographic data. Free triiodothyronine (FT3) and free thyroxine (FT4) levels were similar among preeclamptic and normotensive pregnant women. **Conclusions:** Subclinical hypothyroidism is more common in preeclamptic than in normotensive pregnant women. **Keywords**: MATERNAL HYPOTHYROIDISM, PREECLAMPSIA, PREVALENCE, PREGNANCY.

It is well documented that during pregnancy occur substantial, but reversible physiologic changes in thyroid function and some authors implicate for that the elevated levels of circulating estrogens (1, 2). While preeclampsia occurs in about 8% of all pregnancies, it is observed in 16.7% of pregnancies complicated by subclinical hypothyroidism and in 43.7% of pregnancies with overt hypothyroidism (3). There are a few reports that have studied thyroid hormone status in preeclamptic women showing that hypothyroidism is more common than in normotensive women, and indicating that increased incidence of hypothyroidism might correlates with the severity of preeclampsia (4, 5).

The physiologic mechanism that would explain the higher incidence of hypothyroidism in preeclamptic women is not yet delineated, but low concentrations of thyroid hormones are attributed to decreased

plasma protein concentrations and elevated levels of endothelin, a vasoconstrictor produced by vascular endothelium (6). In the latter case, vascular endothelial damage, which raises levels of endothelin, is implicated in the pathogenesis of subclinical hypothyroidism in preeclampsia. To shed light on this aspect, we evaluated thyroid hormone levels in pregnant women with preeclampsia in the third trimester and correlated it with obstetric outcome.

MATERIAL AND METHODS

In the present study, we evaluated thyroid hormonal status of 60 pregnant women who were admitted in the 3rd Department of Obstetrics and Gynecology of the University of Athens and Rea Hospital of Athens during the last six years with a diagnosis of preeclampsia at the third trimester of their pregnancy.

Free triiodothyronine (FT3), free thyroxine (FT4) and thyroid stimulating hormone (TSH) were evaluated at the time of diagnosis of preeclampsia. TSH was measured by immunoradiometric assay and the normal nonpregnant level in our hospital is 0.1-5.5 mIU/L. Also, we recorded the mode of delivery, gestational age at delivery and birth weight.

Control group consisted of equal number of healthy normotensive pregnant women, with similar demographic data (age, race, residence, weeks of gestation). In both groups - study and control - women with other pathological conditions were excluded. Women with other conditions associated with adverse obstetric and neonatal outcomes, such as diabetes, multiple gestation, other endocrine diseases, infections, rheumatologic diseases, were also excluded.

Preeclampsia was diagnosed on those cases where blood pressure was at least 140/90 mmHg in two consecutive measurements, after the 20th week of gestation. In all the preeclamptic cases proteinuria was diagnosed with urine test at the moment of admission.

Results are expressed as median values with their range interval where necessary. Comparisons between two groups by the Wilcoxon signed rank test and Mann-Whitney test where appropriate. Statistical analysis of demographic data and hormone levels between groups were performed with the *t-Student* test and X^2 test (for delivery route).

RESULTS

In the present study 60 pregnant women were investigated concerning their thyroid hormonal status and the possible relationship between hypothyroidism and preeclampsia.

Mean gestational age at the time of admission and diagnosis was 35.2 ±2.6 weeks in the study group and 36.1±2.5 weeks in the control group. Median age of the preeclamptic women was 29.2±5.6 years and 28.8±4.2 years at the control group. There was no statistically significant difference between age and week of gestation between the two groups.

On the contrary, there were statistically significant differences regarding gestational age at delivery and birth weight among control and study group. Preeclamptic women delivered earlier (37.1±1.2 weeks) than the control group (38.6±1.1 weeks) ($p<0.001$).

Furthermore, mean birth weight in the control group was 3.2±0.3 kg, whereas in study group was 2.85±0.35 kg ($p<0.05$). The mode of delivery (vaginal *vs* cesarean section) occurred to be without significant statistical difference: 50 women (83.3%) of the study group delivered with cesarean section, compared to 46 (76.6%) of the study group (tab. I).

TABLE I.

Clinical and demographic characteristics

Characteristics	Control group	Study group	p-value
Maternal age (years)	28.8±4.2	29.2±5.6	NS
Gestational age at admission (weeks)	36.1±2.5	35.2±2.6	NS
Gestational age at delivery (weeks)	38.6±1.1	37.1±1.2	<0.001
Delivery mode			
Vaginal	14	10	NS
Cesarean	46	50	NS
Birth weight (kg)	3.2±0.3	2.85±0.35	<0.05

Concerning the thyroid hormone status, mean FT3 and mean FT4 levels were not significantly different (NS). TSH mean levels were significantly higher in the preeclamptic group ($p<0.001$) (tab. II).

TABLE II.

FT3, FT4, TSH levels in the study and in the control group (*p<0.001: Mann-Whitney test)

Thyroid hormone status	Normal range	Study group		Control group	
		Mean	SD	Mean	SD
FT3 (pg/mL)	1.7-5.2	3.45	2.2	2.8	1.50
FT4 (ng/mL)	0.6-2.4	0.7	0.42	0.7	0.42
TSH*(mIU/L)	0.5-5.1	4.4	3.6	2.4	2.15

In the control group only 5 women (8.34%) had TSH levels higher than 5.1 mIU/L, whereas the study group had 28 cases (46.67%) (tab. III).

TABLE III.

Distribution of normal and abnormal TSH levels

	TSH <5.1 (mIU/L)	TSH >5.1 (mIU/L)
Control group	55 (91.66%)	5 (8.34%)
Study group	32 (53.33%)	28 (46.67%)

DISCUSSION

There are a few reports addressing the question whether hypothyroidism and preeclampsia are associated, or if hypothyroidism when appearing in preeclampsia is a marker of adverse obstetric outcome.

Davis et al (7) showed that among 16 women with overt hypothyroidism, 7 were preeclamptic and 5 had a history of chronic hypertension. Morbidity and mortality in this population was substantially higher and was associated mostly to *abruptio placentae* and preeclampsia.

It is known that hypothyroidism can lead to vascular smooth muscle contraction in systemic and renal vessels, resulting in

increased peripheral vascular resistance, diastolic hypertension, and decreased tissue perfusion. Interestingly there are a few case reports suggesting that hypothyroidism can also cause proteinuria, indicating that maybe hypothyroidism is the cause for preeclampsia and not the other way around. Ogata et al (8) reported a case of a 65-year-old woman with proteinuria, edema, weight gain, and renal dysfunction due to discontinuation of T4 therapy, with renal biopsy revealing focal segmental proliferative glomerulonephritis. Restoration of thyroid hormone levels lead to recovery of renal function. Lastly, Alfadda & Tamilia (9) describe a case of overt hypothyroidism in a 20-week pregnant woman that was associated with a preeclampsia-like syndrome and fetal death.

In the present study, we found that mean TSH is significantly increased in preeclamptic patients when compared to control group. We didn't registered differences among the study and control group regarding FT3 and FT4. These results are in accordance to other reports where subclinical hypothyroidism is more common in preeclamptic than normotensive pregnant women (2, 4, 5).

We should point out that Leung et al (10) report that women with overt or subclinical hypothyroidism before pregnancy had a higher incidence of gestational hypertension, implying that incidence of low birth weight infants in women with overt hypothyroidism is due to increased incidence of preterm delivery, which is related to gestational hypertension. In this study, maternal hypothyroidism did not correlate with adverse obstetric and neonatal outcomes.

Mild changes in thyroid hormonal status may occur due to various stress factors, irrespectively to thyroid diseases, as well as to decreased plasma albumin concentrations (11). Also, there are suggestions that the decrease of thyroid hormones is caused by the loss of protein and protein-bound hormones in the urine (12).

Lao et al (4) found that preeclamptic women with lower thyroid hormones and increased TSH levels were more likely to deliver small for gestational age (SGA) infants than euthyroid women. These results were confirmed by Kaya et al (5) that showed that serum total T4 and total T3 concentrations in women with SGA infants were significantly lower than in women with AGA infants. These two investigators though did not agree on the possible correlation between birth weight and TSH levels. Whereas Lao et al (4) also reported a negative correlation Kaya et al (5) found that total T4 and T3 levels in preeclamptic and eclamptic women correlated positively with birth weights. Basbug et al (14) in agreement to Kaya et al (5) results revealed significant correlations between birth weights, total T4 and T3 levels in preeclamptic and eclamptic women.

Lao et al (4) showed that total T4, total T3, and free T3 correlated significantly with plasma albumin and urate concentration, indicating that thyroid hormonal status could reflect severity of preeclampsia, since decreased plasma albumin concentrations, correlate with low birth weights in preeclampsia. This conclusion is also supported by Basbug et al (13), that showed that maternal endothelin levels correlated with all thyroid function tests.

There are indications that low birth weight infants might have a mechanism to prevent decreases in free T4 concentrations associated with total T4 concentrations (14) and this hypothesis explains why a signifi-

cant correlation between birth weight, total T4, and total T3 exists, but not between birth weight, free T4, and free T3 among infants of preeclamptic and eclamptic subjects.

CONCLUSIONS

In preeclamptic women mean TSH value is significantly increased compared to mean TSH at healthy normotensive pregnant women of similar demographic data.

Free triiodothyronine (FT3) and free thyroxine (FT4) levels were similar among preeclamptic and normotensive pregnant women.

Maternal hypothyroidism did not correlate with adverse obstetric and neonatal outcomes.

Our study results support the statement that subclinical hypothyroidism is more common in preeclamptic than in normotensive pregnant women.

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CLINICAL, LABORATORY AND THERAPEUTIC PROFILE OF LICHEN PLANUS

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CLINICAL, LABORATORY AND THERAPEUTIC PROFILE OF LICHEN PLANUS (Abstract): **Aim:** Retrospective study of the clinical manifestations and clinical forms of lichen planus, investigation methods and therapeutic means using data from patients admitted to the Dermatology Clinic of the "Sf. Spiridon" County Clinical Emergency Hospital Iasi, in the interval January 1st-December 30th, 2015; the obtained results were compared with the data in the literature. **Material and methods:** Forty-five patients were included in the study. The data were interpreted by using various statistical notions: arithmetic mean, percentage distribution, standard deviation, coefficient of variation, "t-Student" test. Histopathological examination was performed in most cases to enhance diagnosis certainty. **Results and discussion:** The analysis of the study group (29 women and 16 men) produced many results in agreement with literature data. In 2015 the prevalence of lichen planus in the patients admitted to the Clinic of Dermatology, regardless of clinical form was 4.01% with a female preponderance (female/male ratio 2: 1). The mean age of the study patients was 55.31 years and 79.98% of total cases were in the fourth and six decades of life. The main infectious risk factor for the development and progression of lichen planus is hepatitis C virus (13.3%). The most common form of lichen planus (88.8%) was the cutaneous form. **Conclusions:** Lichen planus is a chronic autoimmune disease with undulating course and different clinical subtypes that can affect multiple body regions**. Keywords**: LICHEN PLANUS, PAPULE, AUTOIMMUNE DISEASES, LICHENOID REACTIONS.

Lichen planus is a mucocutaneous inflammatory dermatosis of uncertain etiology, with chronic or subacute course, often self-limited (1). The classic description of lichen planus eruption is given by the 5 Ps: purple, planar, polygonal, pruritic, papules (2).

The estimated prevalence of lichen planus in the general population is in the range of 0.2% to 4%. It occurs more fre-

quently in the fifth or sixth decades of life, predominantly in women. Lichen planus in children is uncommon, but cases with familial aggregation have been reported (3).

The cause of lichen planus is still unknown. The etiologic hypotheses include genetic, infectious, psychological and autoimmune factors (1).

Lichenoid eruptions (LDE-probably a type IV hypersensitivity reaction) are reactive mucocutaneous lesions that are nearly identical clinically and histologically with those in idiopathic lichen planus. Drugs more frequently associated with LDE are the synthetic antimalarials, gold salts, D-penicillamine, methyldopa, B-blockers, angiotensin-converting enzyme inhibitors, spironolactone, nonsteroidal anti-inflammatory drugs, thiazide, phenothiazine derivatives, allopurinol, tetracycline, sulphonylurea (1).

Notable is the association of lichen planus with such autoimmune diseases as systemic lupus erythematosus, myasthenia gravis, dermatomyositis, ulcerative colitis, chronic active hepatitis, pemphigus vulgaris, dermatitis herpetiformis, bullous pemphigoid, alopecia areata, vitiligo, Sjogren's disease, systemic sclerosis. It supports the immunological hypothesis in the pathogenesis of lichen planus (1).

MATERIAL AND METHODS

This open and retrospective study was conducted on a sample of 45 patients admitted with a diagnosis of lichen planus to the Dermatology Clinic of the "Sf. Spiridon" Clinical Emergency Hospital Iasi between January 1st and December 30th, 2015.

Data on clinical manifestations, clinical forms, investigation methods and therapeutic approaches were collected. The obtained data allowed us to classify the patients by gender, age, area of residence, etiology, affected areas, clinical form of lichen planus, association with certain diseases, type of administered treatment and its efficacy, and to compare the obtained results with the literature data. The clinical and histopathological examinations proved to be essential in making the diagnosis. In patients who experienced multiple hospitalizations we focused on the criteria for evaluating treatment efficacy and occurrence of side effects.

For data interpretation, we used various statistic notions: arithmetic mean, percentage distribution, standard deviation (to highlight dispersion of data around the calculated value), coefficient of variation (which is an index of dispersion used to compare statistical series), student's t test (indicating the presence or absence of a significant difference between the two means and between two statistical series that could be correlated with the intervention of objective factors).

RESULTS AND DISCUSSION

In 2015 the prevalence of lichen planus in the patients admitted to the Clinic of Dermatology, regardless of clinical form was 4.01%. Per the literature data, the estimated prevalence of lichen planus in the general population is in the range of 0.2% to 4% (3), with geographic area variations.

Among the study patients the urban/rural ratio was 1:1.5. This difference could be related to prolonged occupational sun exposure (farming), multiple studies suggesting a relationship between exposure to ultraviolet (UV) light and disease severity, while improvement in lesions is obtained using UV protection (4).

There was a female preponderance in our study (female/male ratio 2: 1) (fig. 1), result in accordance with the literature data (5).

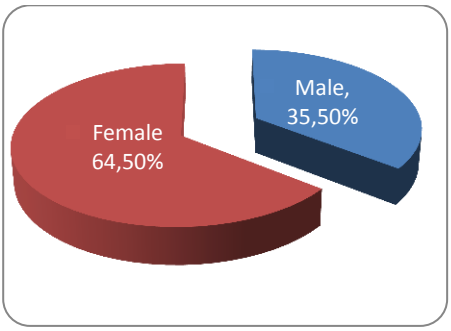

Fig. 1. Sex distribution of the lichen planus patients

The mean age of the study patients was 55.31 years (range 9 to 87 years). The data are consistent with the literature data, per which lichen planus may occur at any age, with a peak incidence in the third to the sixth decades of life (5). In this study, 79.98% of total cases were in the fourth and six decades of life (fig. 2). A case of childhood lichen planus was recorded in a 9-year-old girl, accounting for 2.22% of all cases, but without familial aggregation of lichen planus (6).

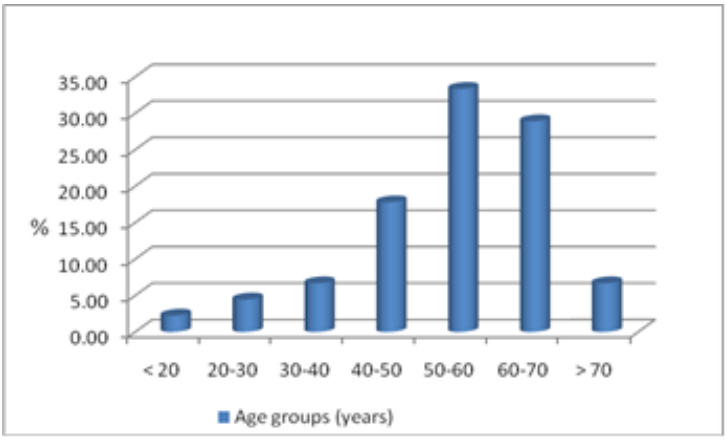

Fig. 2. Percentage distribution by age-group of lichen planus patients

As for the clinical manifestations of lichen planus, the study protocol aimed to analyze the following: disease history, general and local physical examination, association with other diseases. The onset was insidious in most cases, with a progressive development of symptoms and lesions.

Of the medications administered prior to the occurrence of the disease the most common were the drugs for cardiovascular diseases (66.66%) and non-steroidal anti-inflammatory drugs (15.6%), drug classes known as having an important role in the onset or further progression of lichen planus.

Another factor responsible for the development and progression of lichen planus is the infectious one, hepatitis C virus being the most studied agent. Laboratory investigations revealed that 6 (13.3%) of the study patients had chronic hepatitis C, a high percentage considering that the seroprevalence of hepatitis C virus in the general population is about 5% (7). These patients presented mucocutaneous involvement. Other viral infections incriminated are those with herpes family of viruses: herpes simplex, Epstein-Barr, cytomegalovirus and herpesvirus 6. *Helicobacter pylori* infection (8) was detected in 3 patients.

Local physical examination is useful for the classification of lichen planus cases based on clinical form, lesion morphology and anatomical region.

Of the 45 patients, 40 (88.8%) had cutaneous LP, 5 presented only mucosal involvement (11.1%), and 15 patients both cutaneous and mucosal involvement, accounting for 33.3% of all cases (per the literature data, 30-70% have lichen planus with mucosal involvement (5)).

In the 40 cases of lichen planus with cutaneous involvement, the most common morphological subtype was represented by the classic form (18 cases, 45%) (fig. 3).

Fig. 3. Flat, polygonal, erythematous-violet, small, shiny, isolated, itchy papules on the flexor surface of the hand (classic lichen planus)

The second most common subtype was the atrophic form. Long-term use of potent and super potent topical corticosteroids may lead to the development of this form of lichen planus. In the present study, only 2 of the 7 patients with atrophic lichen planus were known to have lichen planus and were on treatment, the remaining 5 being at their first hospital admission.

Other common subtypes were the hypertrophic subtype, 5 patients (fig. 4), and follicular subtype, 5 patients (fig. 5), 4 of them women presenting alopecia plaques on the scalp and one male patient with lesions on the chest.

Fig. 4. Multiple papular, itchy, violaceous lesions, arranged in a reticulate fashion (hypertrophic lichen planus)

Fig. 5. Flat, follicular, polygonal, erythematous-violet, bright, isolated papules on the scalp (follicular lichen planus)

Of the 45 patients included in this study 20 (44.4%) presented mucosal involvement. Oral lesions were the most common, and most patients were females (tab. I).

Of the 13 patients with oral involvement 7 presented the reticular form, which is the most common form. Five patients had the erosive form, the symptoms consisting of pain, burning sensation, sometimes dysphagia. One of these 7 patients,

an 87-year-old male, presented atrophic-erosive lichen planus lesions (fig. 6).

TABLE I
Distribution of mucosal lesions by anatomical areas

Affected mucosal region	Number of patients		Total	%
	Males	Females		
Oral cavity	4	9	13	60
Genital mucosa	3	7	10	40
Total	7	16	23*	100

*the total corresponds to 23 patients as some of them presented both oral and genital involvement

Fig.6. Exulceration, slightly depressed, irregular contour, painful, covered by a pseudo membrane

Seven patients (24.13%) presented vulvovaginal involvement, of which 5 (17.24%) with the erosive form, sometimes in association with atrophic lesions. Three (10.34%) of these presented complications, the most common being represented by the agglutination and resorption of the labia minora, accompanied in 2 cases (6.89%) by the narrowing the vaginal ostium. Included in this group was a case (3.44%) of vulvo-vaginal-gingival syndrome, the patient associating erosive vulvovaginal and oral lichen planus at the level of jugal mucosa.

Genital involvement was found in 3 males (18.75%), 2 with the erosive form and one with the classic papular form.

In this study, the diseases most frequently associated with lichen planus were hypertension, diabetes mellitus and chronic venous insufficiency.

A number of autoimmune diseases have been found to be associated with lichen planus: alopecia areata (2 cases, 4.44%), autoimmune thyroiditis/Graves' disease (6 cases, 13.3%), systemic lupus erythematosus (3 cases, 6.66%), vitiligo (3 cases, 6.66%), vasculitis (1 case, 2.22%), plaque morphea (1 case, 2.22%), scleroderma (1 case, 2.22%), pemphigus vulgaris (2 cases, 4.44%), psoriasis (2 cases, 4.44%), making a total of 21 patients, representing 46.6% of the study group. Also, the association with hypersensitivity reactions is mentioned in numerous studies (9): arthritis (3 cases, 6.66%), allergies to various compounds (8 cases, 17.7%), asthma (1 case, 2.22%), gastritis (2 cases, 4.44%), atopic dermatitis (1 case, 2.22%), making a total of 15 patients (33.33%). The results are consistent with the data in the literature and support the autoimmune theory (1).

The triad of oral lichen planus, diabetes mellitus and hypertension, association known as Grünspan's syndrome was found in two patients (4.44% share).

The results of laboratory investigations are nonspecific, excluding direct and indirect immunofluorescence, immunohistochemistry and histopathology.

Histopathological examination was performed in most cases to enhance diagnostic certainty, in agreement with the data in the literature (10). The most common changes are the orthokeratotic hyperkeratosis, hypergranulosis, acanthosis, tapered rete ridges, band-like lymphoplasmocytic inflammatory infiltrate at the dermo-epidermal junction the presence of Civatte bodies (apoptotic keratinocytes). These are exemplified in the following iconography,

with images of biopsies collected from our patients (fig. 7, 8, 9, 10).

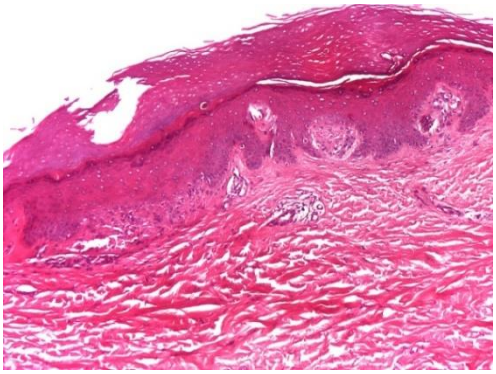

Fig. 7. Hyperkeratosis, hypergranulosis, acanthosis – Classic lichen planis. HE 4x

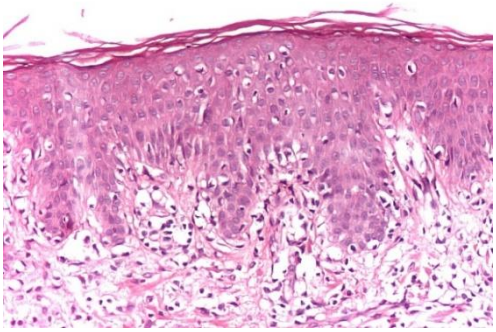

Fig. 8. Spongiosis with exocytosis, Civatte bodies – Lichenoid eruption. HE 10x

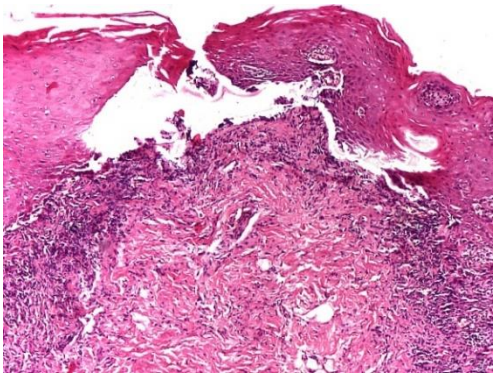

Fig. 9. Submucosal bullae – Oral lichen planus. HE 4x

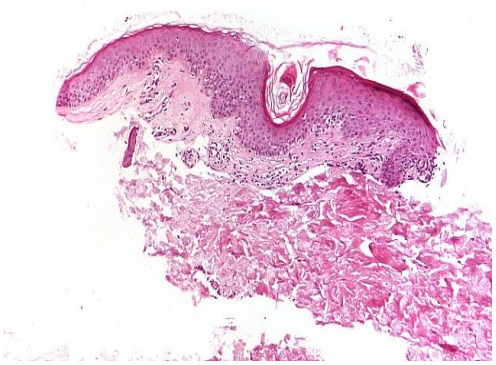

Fig. 10. Discrete perifollicular inflammatory infiltrate – Follicular lichen planus. HE 4x

Regarding the treatment of lichen planus, the following ones were mainly administered: topical (88.88%), systemic (17.7%) and intralesional corticosteroid therapy (2.2%), therapy with immunosuppressive agents (2.2%), PUVA therapy (2.2%), antihistamines (93.3%), vitamin C (88.8%) and calcium gluconate (82.2%) (as adjuvants to the main therapy). The main treatment option was topical corticosteroids and in 6 cases (13.3%) was also associated systemic therapy. Systemic corticosteroid therapy was used in 8 cases (17.7%) of lichen planus with severe multiple of both the skin and mucous membranes. Thus, 4 of these cases (8.8%) had rash extended and oral lesions insufficiently responsive to topical treatment, and 3 cases (6.6%) with rash, erosive oral and genital, only one case of lichen planus eruptive generalized unresponsive to topical therapy. Individualized treatment regimens were tailored for each patient, their efficacy, occurrence of possible side-effects and the interaction with the medication used to treat the associated diseases associated being closely monitored.

The course and prognosis are generally favorable, most patients (73.3%) showing

complete or partial remission of the lesions on treatment. In the patients (26.6%) with persistent lesions the treatment was reconsidered. Clinical forms with negative evolution or absence of remission were the mucosal lichen planus cases, both the oral and genital mucosa. 6 patients (13.3%) had various complications. Thus, three of them (6.6%) suffered diffuse or localized cicatricial alopecia, one man (2.2%) with erosive lichen planus genital complicates with phimosis, and two females (4.4%) develop agglutination of labia minora. Given the risk of complications, sometimes debilitating in lichen planopilaris and mucosal lichen planus, and of malignant transformation of the erosive mucosal forms, patients were informed about these risks and periodic clinical, biological and therapy reassessment visits were recommended.

CONCLUSIONS

The profile of the patients enrolled in this study is consistent with the data in the literature. Thus, our findings emphasize frequently with the association between liver damage and lichen planus, especially HCV infection (6 patients, 13.3%) and cirrhosis (3 cases, 6.6%). In our study, dyslipidemia was found in 48.88% of cases with lichen planus, the most frequent association was made with the cutaneous form (35.5%) followed by the oral (22.2%) and genital form (8.88%). In 2016, Lai YC et al (12) published similar results regarding the association between lichen planus and dyslipidemia. Although, palm-plantar lichen is very rare (1-2% according the literature), in our study it was present in 10% of cases, being characterized by multiple, bilateral lesions associated with severe pruritus. Forward studies will explain this statistics results.

Lichen planus may have a psychosocial impact affecting the quality of life of patients, our study revealing that 8.8% of cases were diagnosed with depression. Giving attention to pathogenesis, clinical picture and an early diagnosis are essential for selecting the most appropriate treatment. Our data referring to the therapeutic options have been aligned with European standards of treatment. Given the undulating nature of the disease, periodic monitoring of disease course and therapeutic efficacy are required.

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NEWS

CDH17 IS A MORE SENSITIVE MARKER FOR GASTRIC ADENOCARCINOMA THAN CK20 AND CDX2

Metastatic cancers can be difficult to diagnose in a clinical setting with most being carcinomas. In the past, a panel consisting of cytokeratin (CK) 7, CK20, and CDX2 antibodies has been used to assess gastrointestinal (GI) tumors of unknown primary. Expression of CK7 is observed in a wide variety of carcinomas, thus, it is an important marker in diagnosis. CK20 expression is specific to certain types of cancer and is typically used in combination with CK7 to distinguish different types of tumors. CDX2 is a homeobox gene that encodes an intestine-specific transcription factor and is expressed in the nuclei of epithelial cells throughout the GI tract. Immunohistochemical expression of CDX2 protein in primary and metastatic colorectal carcinomas has been previously documented. CK7 along with CK20 or CDX2 markers constitute an important panel for the diagnosis of GI tumors versus tumors of unknown origin. However, in difficult cases, expression patterns may vary and the presence or absence of these markers may not lead to a definitive diagnosis. Cadherin 17 (CDH17), also called liver-intestine cadherin, is a cell-adhesion protein that is transcriptionally regulated by CDX2, and is involved in tumor invasion and metastasis. CDH17 is a novel diagnostic marker for GI tract carcinoma with greater sensitivity than CDX2 in colon cancers. Recently, a mouse monoclonal CDH17 (clone IH3) antibody has been developed to aid pathologists in interpreting GI adenocarcinomas, especially in cases of unknown primary origin. There have been conflicting results with CDH17 antibodies, even when using the same designated clone as presented in this study. This study will present an immunohistochemical evaluation in a side by-side comparison with antibodies CDH17, CK20, and CDX2 in various neoplastic tissues, with emphasis on GI adenocarcinomas. CDH17 may also be helpful in cases that are negative for CK7, CDX2, and CK20. The study results confirm that CDH17 expression analysis may be of significant value in the diagnosis of GI tumors and those of unknown origin (Altree-Tacha D, Tyrrell J, Haas T. CDH17 Is a More Sensitive Marker for Gastric Adenocarcinoma Than CK20 and CDX2. *Arch Pathol Lab Med* 2017; 141: 144-150).

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ATHEROSCLEROSIS IN THE YOUNG ADULT: FEWER HYPOTHESES, MORE FACTS

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ATHEROSCLEROSIS IN THE YOUNG ADULT: FEWER HYPOTHESES, MORE FACTS (Abstract): Several studies have reported early atherosclerotic changes in arterial wall of young adults, although they usually become symptomatic after age 40 years. Initially, some sporadic and limited data were available to document the atherosclerotic lesions in young adults; early subclinical assessment were later possible using accurate ultrasound and other imaging techniques. Our aim was to a review of eloquent studies proving different type of atherosclerotic lesion in the young adult. Long-term follow-up and further analysis of this population demonstrated important relationship with traditional and some non-traditional, risk factors. The data comprised in the reviewed studies are arguments in favor of a more refined atherosclerotic risk assessment and appropriate preventive strategy in young adult.
Keywords: ATHEROSCLEROSIS, YOUNG ADULT, CARDIOVASCULAR RISK, NON-INVASIVE CARDIOVASCULAR TESTING.

Atherosclerosis is an active systemic pathobiological process, usually clinically expressed and complicated in adulthood and elderly (1). However, previous autopsy studies (2), or, more recently clinical investigations based on the latest subclinical methods (vascular and intravascular ultrasound, coronarography, computed tomography and magnetic resonance) showed subclinical or complicated atherosclerotic lesions in young adults, or even in teenagers or children (1-3). In Bogalusa Heart Study (2), an interesting approach was developed, combining autopsy information about the extent of vascular damage, with patient's history providing data about the presence of cardiovascular risk factors, collected prior to death. Significantly and persistently elevated blood pressure levels, adipose mass and dyslipidemia in young adults are markers of risk for progressive coronary atherosclerosis. Abdominal and visceral obesity, most commonly encountered and associated with metabolic syndrome, were strongly correlated, especially in males, with coronary atherosclerosis and, less well with aortic involvement (4, 5).

Most cardiovascular risk factors assessed in clinical studies are correlated with the initiation and progression of the atherosclerotic disease. Atherosclerotic transformation of the arterial wall is the faster the more factors such as dyslipidemia, inflammation and thrombosis are precociously activated. Smoking, sedentary lifestyle and obesity are factors increasingly

encountered in teenagers and their overtime persistence will lead to early complications of atherosclerosis. Myocardial infarction is a frequent complication of coronary atherosclerosis in young adults, frequently represented by an isolated single lesion, still complicated by ulceration and thrombosis due to the persistence of a conglomeration of cardiovascular risk factors (2, 6).

Atherosclerosis in young adults was initially studied by sporadic autopsy information and later clinical studies. One of the main limitations of the systematic interpretation of the data in the clinical studies remains the arbitrary delimitation of the age intervals defining young adult. Some of the published articles have used mixed cohorts of patients, aged under or up to 18 years, or occasionally set an upper age limit for the young adult of 35 years and even 45 years. The first anatomical observation and invasive studies reported mainly the complications of atherosclerosis such as stroke or myocardial infarction, while the modern approach is non-invasive and allows imaging assessment of subclinical atherosclerosis in several categories of young adults (1, 2, 4-6). This review outlines the most significant data published on adults aged 18 to 45 years, with emphasis on subclinical atherosclerosis beside the presence and extent of complications.

EPIDEMIOLOGICAL APPROACH

The epidemiological data were collected from morphological and clinical surveillance studies. Some of the earliest findings were obtained from autopsy specimens collected from young soldiers killed in war battles (7, 8). At a mean age of 22 years, 70% of them already had coronary atherosclerotic lesions (8). Post-mortem coronary angiography showed minor changes in 45% and raised lesions in 5% of 105 soldiers killed in Vietnam war (7, 8). Bogalusa Heart Study provided significant data, describing the postmortem arterial morphological findings in subjects enrolled before their death and followed-up for a period for the description of the specific cardiovascular risk factors. The study enrolled 204 patients aged 2 to 39 years. Early atherosclerotic lesions, represented by fatty streaks, were reported in 50% of subjects aged 2 to 15 years and in 85% of subjects aged 21 to 39 years (8). Advanced atherosclerotic lesions, represented by raised fibrous - plaque, were mentioned in 8% of subjects aged 2 to 15 years and in 69% of subjects aged 26 to 39 years (2, 6, 8). PDAY Study (Pathobiological Determinants of Atherosclerosis in Youth) reported autopsy findings from 2876 subjects aged 15 to 34 years. Advanced atherosclerotic lesions were described in the abdominal aorta in 20% of patients aged 15 to 34 years and 40% of subjects aged 30 to 34 years. Right coronary artery showed pathological changes in 10% of subjects aged 15 to 19 years and in 30% of those 30 to 34 years (8, 9).

Autopsy studies have inherent limitations (artifacts caused during the processing of morphological samples or inconsistencies in the absence of *in vivo* hemodynamic conditions). Therefore, this kind of studies were followed by clinical evidence obtained with invasive or non-invasive methods. Tuzcu EM et al. (1) reported results obtained by coronary intravascular ultrasound performed within about 1 month after transplantation in 262 heart transplant recipients, the organ donors being young asymptomatic subjects (146 men and 116 women, mean age 33.4±13.2 years). Atherosclerotic lesions were present

in 136 patients (51.9%). Atherosclerosis prevalence is increasing significantly with age (17% in subjects aged under 20 years and 85% in subjects older than 50 years), without gender differences (52% in men and 51,7% in women) (1). In other studies, subclinical atherosclerosis was noninvasively assessed and it proved to be more frequent in young subjects with metabolic syndrome (5, 10), obesity and other cardiovascular risk factors (10-12). CARDIA Study demonstrated an increased incidence of coronary affectation in young adults with abdominal obesity (12).

ASSESSMENT OF SUBCLINICAL ATHEROSCLEROSIS

Over 20 years of research were needed to establish new methods and cardiovascular biomarkers that could be properly representative for the presence of subclinical atherosclerosis. The new methods have high applicability and maximum benefit if they prove fidelity in detection of atherosclerotic lesions, reproducibility and cost-efficiency, are non-invasive and standardized. Some of these methods have been developed more and are used to screen large populations and guide medical intervention to reduce the cardiovascular risk (13).

After 1990, accurate measurements of carotid intima - media thickness (cIMT) were possible using high - resolution B-mode ultrasonography. This imaging technique is non-invasive and accessible in large communities of all ages, gender and origin. Most of the studies demonstrated that cIMT could be correlated with aortic and coronary atherosclerosis, reflecting, at the same time, the adaptation of the arterial system to increased intravascular stress (13, 14). Accurate measurements require a standardized technique, approaching the common carotid, internal carotid and carotid bifurcation at several arterial sites. Currently, ultrasound apparatus should be equipped with a transducer greater than 8 MHz. A cIMT greater than 1 mm is considered abnormal. In term of prediction, it is important that single or average values measured for cIMT have the same power in correlation with future coronary events (13-15). Carotidian atherosclerosis is an independent predictor of stroke and myocardial infarction (16, 17).

Ankle-brachial index (ABI) has started as a simple and inexpensive test for diagnosis of peripheral arterial disease (PAD). In this regard, the most used was the protocol for the determination of ABI with the Doppler method. Usually, per the American Heart Association (AHA) protocol for the determination of the ABI with Doppler method, the calculation is made for each leg with the higher of the 2 ankle pressures (determined in tibialis posterior and dorsalis pedis arteries) in relation to the highest brachial systolic blood pressure (SBP) (18).

The sensitivity is ~ 90% and specificity ~ 98% in the presence of stenosis $\geq$ 50% in leg arteries. An ABI < 0.9 is considered abnormal. In terms of risk, the studies have reported a high incidence of cardiovascular morbidity and mortality in patients with pathological ABI values (19-21). The relevance of risk prediction is still debatable in persons $\geq$ 50 years old, where pathological values for ABI were more frequently found. Therefore, the importance of ABI as a predictor of atherosclerosis is more valuable in persons < 50 years old (17). Some authors have proposed a shift in the calculation of ABI, for the PAD diagnosis, and, particularly, when it is used as a general indicator of atherosclerosis and cardiovascular risk. For example, using the lower ankle

pressure instead of the higher ankle pressure between tibialis posterior and dorsalis pedis for each leg, ABI was associated with higher sensitivity, but with lower specificity in the diagnosis of PAD (22). In 831 patients who underwent coronarography, ABI was calculated using both formulas and the comparison showed that with the modified version more patients at high cardiovascular risk were identified (23).

The detection of coronary calcium by electron beam-computed tomography (EBCT) and helical computed tomography (CT) led to the identification of asymptomatic but high-risk coronary artery disease patient (17). Coronary artery calcification (CAC) score was correlated with pathological findings of atherosclerosis in coronary arteries (17, 24). Current guidelines discuss the indications for coronary CT, namely asymptomatic patients at high cardiovascular risk, stable patients with suspected coronary disease or with acute chest pain, in the preoperative evaluation of patients before noncoronary cardiac surgery and in the follow-up of cardiac transplant patients (25). CAC is an independent risk factor for the future cardiac ischemic events, stratification depending on its values. In a cohort with 4,4052 asymptomatic subjects, for a CAC reported as 0, 10-year risk of all-cause mortality was < 1% (26, 27). A CAC score ≥ 400 (Agatston units) indicate the subjects at high risk for future coronary events that should be referred for coronary angiography. However, absence of coronary calcium will not exclude the existence of noncalcified plaque (28).

Magnetic resonance is more valuable, but, at the same time, a more expensive imaging method of atherosclerosis. The use of this technique could bring new information about the remodeling of the arterial wall and vulnerable plaque in most of the arteries (coronary, carotid and peripheral arteries) and could serve as a reference point in monitoring therapy (29).

At the same time, some noninvasive, reproducible and safe tests have been developed for the assessment of the functional properties of the endothelium (brachial artery flow-mediated dilatation, pulse wave and contour analysis, pulse amplitude tonometry). The analysis of pulse wave velocity (PWV) is made by a device that calculates pulse wave propagation velocity between 2 arteries (carotid and femoral or radial) (30). Carotid-femoral pulse wave velocity > 12 m/s indicates an increased risk for future cardiovascular events.

Of the serum markers, C-reactive protein (CRP) or interleukin-6 are important for the expression of systemic inflammation and were correlated with cardiovascular events (31). Particularly CRP was introduced in different risk stratification models.

SOME PATHOGENIC HYPOTHESES

Usually, individual characteristics known as cardiovascular risk factors will influence the progression of atherosclerosis in time. Less evidence was collected about the pathogenesis of accelerated atherosclerosis in children, adolescents or young adults under the age of 45 years. Accelerated atherosclerosis was described in case of homozygous familial hypercholesterolemia (32). The disease is characterized by a genetic heterogeneity and a phenotypic variability with impaired functionality of the low-density lipoprotein (LDL) receptor, increased levels of LDL-cholesterol and lipoprotein (a), associated with low levels of high density lipoprotein cholesterol (HDL-cholesterol) (in some patients an accelerated turnover of HDL apoA-I and a decreased

HDL-cholesterol efflux could be present) (33). The current guidelines recommend Doppler echocardiographic evaluation of the heart and aorta annually and coronary CT every 5 years for the screening of subclinical atherosclerosis in children and young adults with familial hypercholesterolemia (33).

Epidemiological and clinical studies (34) also demonstrated a cluster of cardiovascular risk factors (hypertension, obesity, hyperglycemia, smoking, family history) in relationship with unhealthy lifestyle in these patients. Some of them meet the criteria for metabolic syndrome, namely insulin resistance, associated with increased risk of developing diabetes and cardiovascular diseases (5,35). Confounding factors could be present occasionally, in patients with thrombotic complications of some hereditary or acquired systemic diseases in the absence of significant atherosclerotic lesions. Early atherosclerosis in young women was particularly related to autoimmune processes or to high levels of lipoprotein (a) (36).

ELOQUENT STUDIES ON ATHEROSCLEROSIS IN YOUNG ADULTS

As mentioned above, the early development of atherosclerotic lesions in children and young adults was first documented in autopsy studies (2, 7). Although the atherosclerotic changes were demonstrated after death, in the Bogalusa Heart Study the approach was because the subjects were also followed-up before death for the description of cardiovascular risk factors and, partially, for the assessment of subclinical atherosclerosis by carotid ultrasound (6, 10, 37).

Vascular ultrasound and other validated methods for detecting subclinical atherosclerosis have permitted in vivo assessment of the atherosclerotic lesions. More clinical studies were published after 2,000 year and the most eloquent are presented below (tab. I).

TABLE I

The main clinical studies on atherosclerosis in young adult

Reference	Population (n)	Age range(y)/ Mean age (y)	Gender Male/Female (n)	Method for the assessment of atherosclerosis	Atherosclerosis outcome
1. Tuzcu et al. 2001 (1)	262	NR/ (33.4±13.2)	146/ 116	Intravascular ultrasound	Prevalence of atherosclerosis 52% in men and 51.7% in women, 17% in individuals < 20 years, 37% in age 20-29 years and 60% in age 30-39 years
2. Lee et al. (2007) (12) CARDIA Study	2,951, as part of the CARDIA Study	18-30 at CARDIA year 0/NR	NR	Computed tomography for CAC	Abdominal obesity was associated with early atherosclerosis
3. Mattsson et al. (2008) (5)	2,163	NR/ (32±5)	999/ 1,164	Ultrasound measurement of cIMT, and FMD	MS was associated with increased cIMT in both sexes

Reference	Population (n)	Age range(y)/ Mean age (y)	Gender Male/Female (n)	Method for the assessment of atherosclerosis	Atherosclerosis outcome
4. Knoflach et al. (2009) (36)	205	18-22/ 20.6±1	0/ 205	Ultrasound measurement of cIMT	Autoimmune processes, high lipoprotein (a), environmental exposure to tobacco smoke accelerated early atherogenesis in young women
5. Skilton et al. 2014 (38), Cardiovascular Risk in Young Finns Study	696	24-45/ 31.9±5	320/ 376	Ultrasound measurement of cIMT, and FMD	cIMT was increased in people with larger versus normal birth weight
6. Spring et al. 2014 (39), CARDIA Study	3,538, as part of the CARDIA Study	18-30 at CARDIA year 0/NR	1,535/ 2,003	Ultrasound measurement of cIMT, computed tomography for CAC	Healthy lifestyle changes during young adulthood are associated with decreased risk for subclinical atherosclerosis in middle age
7. Bhuiyan et al. 2015 (40), Bogalusa Heart Study	461, as part of the Bogalusa Heart Study	24-43/ 35.6	0/461	Ultrasound measurement of cIMT	cIMT was same in early menarcheal age (< 11 yrs.) vs normal menarcheal age (≥11yrs.) in white women but higher in black women
8. Eikendal et al. 2015 (41), USE-IMT initiative	3,067	NR/ 32-42	1,828/ 1,239	Ultrasound measurement of cIMT, recording of the new cardiovascular disease events	55 first-time myocardial infarctions or stroke occurred during a follow-up of 16,3 years and the events were related to cIMT
9. Reis et al. 2015 (42), CARDIA Study	4,061, as part of the CARDIA Study	18-30 at CARDIA year 0/NR	2,043/ 2,018	Recording of the new cardiovascular disease events	During a 24.8 year, median period of observation, were 125 incidents cardiovascular disease, 62 coronary heart disease and 33 heart failure events
10. Gidding et al. 2016 (43), CARDIA Study	3,008, as part of the CARDIA Study	18-30 at CARDIA year 0/NR	NR	Computed tomography for CAC and AAC	During the follow-up period, the CAC increased from 3 to 5 and AAC remain 2 between the age 35±3.6 to 40.2±3.6

Reference	Population (n)	Age range(y)/ Mean age (y)	Gender Male/Female (n)	Method for the assessment of atherosclerosis	Atherosclerosis outcome
11. Hartiala et al. 2016 (44), Cardiovascular Risk in Young Finns Study	589, as part of the Cardiovascular Risk in Young Finns Study	39-45/ NR	NR	Computed tomography for CAC	Mean levels of LDL-C, total cholesterol, Apo-B, and SBP levels across the 27-year period were significantly higher among those with CAC vs. those without
12. Eikendal et al. 2016 (45) The Atherosclerosis Risk in Young Adults (ARYA) Study	736	NR/ 28.4±0,9	344/ 392	Ultrasound for carotid intima-media echogenicity quantified as gray-scale median and for cIMT	Adolescent BMI related to cIMT and echogenicity in young adults; SBP only related to cIMT

AAC - abdominal aortic calcium, Apo-B - apolipoprotein B, BMI - body mass index,
CAC - coronary artery calcification, cIMT - carotid artery intima-media thickness,
FMD - brachial artery flow-mediated dilatation, LDL-C - low density lipoprotein cholesterol,
MS - metabolic syndrome, NR - not reported, SBP - systolic blood pressure

CONCLUSIONS

Our review has demonstrated an increased incidence of subclinical atherosclerosis in young adults. Based on the main selected studies, early atherosclerosis was related to a cluster of traditional risk factors (hypertension, obesity, hyperglycemia, smoking, dyslipidemia) and some non-traditional risk factors (birth weight), or more particular for the young women (lipoprotein (a), autoimmune processes). Further improvement in the methods of subclinical atherosclerosis assessment will consistently contribute to a better prediction of future cardiovascular morbidity and of outcome following the therapeutic strategies.

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IS LIVER BIOPSY NECESSARY IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE?

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IS LIVER BIOPSY NECESSARY IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE? (Abstract): Nonalcoholic fatty liver disease is the most common cause of liver disease in the Western world. Liver biopsy is considered the gold standard in the diagnosis and progression of the disease and its usefulness cannot be neglected in terms of research. But in current clinical practice, liver biopsy tends to be replaced by less expensive and noninvasive methods allowing the identification of cardiovascular and metabolic risks. Given the fact that a small percentage of individuals with nonalcoholic fatty liver disease will progress to cirrhosis, attention will be focused more on cardiovascular risk as nonalcoholic fatty liver is now regarded as a distinct component of the metabolic syndrome. The aim of the clinician is to identify the early stages of fatty liver, using in this purpose simple and easily accessible methods. Many techniques have been proposed for the diagnosis of nonalcoholic fatty liver, from simple clinical factors (anthropometric indices measuring, blood pressure), laboratory biomarkers, imaging methods and scores, which should allow early treatment. This review describes different methods for identifying nonalcoholic fatty liver and various stages of this disease. **Keywords:** NAFLD, LIVER BIOPSY, NON-INVASIVE DIAGNOSIS

Nonalcoholic fatty liver disease (NAFLD) is a major problem of the 21st century and is the primary cause for liver disease in the western Europe. Even if it is generally benign, it can be associated with major hepatocellular impairment, inflammation and necroptosis, nonalcoholic steatohepatitis (NASH) being present in 20-30% of the subjects. Few of them will develop hepatic cirrhosis. Moreover, the issue of fatty hepatocytes goes further, because it increases the incidence of cardiovascular disorders and doubles the risk of diabetes mellitus, irrespective of the severity of hepatic impairment (1). Also, the cardiovascular risk is doubled by metabolic syndrome, and approximately one third of the subjects with NAFLD have metabolic syndrome (2).

Liver biopsy can be seen as a useful method for scientific research and liver disease staging. However, for the NAFLD patient, non-invasive and cheap methods are needed, which will allow the early diagnosis of the liver disease and the under-

lying cardiovascular risk.

NAFLD DIAGNOSIS

Currently, the available non-invasive methods for NAFLD diagnosis are represented by a set of symptoms and signs, and a combination of clinical and laboratory tests. Although some of these markers are usually useful for diagnosing an NAFLD patient, they do not have a high specificity and sensitivity capable of distinguishing between NAFLD and NASH and determining the degree of fibrosis.

Liver biopsy is currently the gold standard for NAFLD diagnosis because it allows the exclusion of different causes of liver injury and it can estimate the severity of adipose infiltration in the liver, inflammation of the lobes, hepatocyte ballooning and fibrosis. Even though it is the gold standard, liver biopsy has some limits; it is an invasive procedure, it is associated with certain risks and its analysis can be influenced by pathologist's subjective judgment (3).

An important issue that requires mentioning is the quality of non-invasive methods compared to liver biopsy for diagnosing NASH. Currently, the available non-invasive methods for NASH diagnosis are represented by signs and symptoms, biochemical tests and combinations of physical examination and laboratory tests. Once the NAFLD diagnosis is made, its severity should be assessed, which is useful to predict the disease course. When determining the NAFLD stage, 2 factors are related to severity: degree of fibrosis and of inflammation (4).

Physical examination is non-specific for assessing the severity of NAFLD. The conditions usually associated with the severity of this disease include obesity, old age, diabetes mellitus and high blood pressure.

Typical biomarkers – only one biomarker cannot diagnose NAFLD or differentiate among steatohepatitis, NASH or liver cirrhosis. Even though a slightly elevated serum aminotransferase level is the first change noted in NAFLD patients, the transaminases are within normal ranges in most of these subjects. Moreover, all the histologic changes seen in NAFLD subjects may be associated with normal transaminases. Serum gamma-glutamyl transpeptidase (GGT) is usually elevated in NAFLD subjects and is reportedly associated with increased mortality. Despite this, the diagnosis of NAFLD cannot be made solely based on GGT levels (5).

Inflammation – a proinflammatory status typical to metabolic syndrome was shown to be related with the course of steatohepatitis and liver damage in NAFLD. Inflammatory markers may also have a diagnostic potential. A study (6) described a relationship between cytokine level and NASH. Moreover, the level of TNF-alpha correlates with the degree of inflammation and fibrosis. It has been found that adiponectin level is notably lower in the early stage of NASH as opposed to simple steatosis. Another study (7) reported a relationship between leptin, hormone produced in the adipose tissue, and the histologic appearance of hepatic parenchyma. Hyaluronic acid (HA) and CC-chemokine ligand 2 (CCL-2), which are also markers of inflammation, were also proved to be elevated in NASH subjects.

Fibrosis – Markers of fibrosis are represented by HA and type IV 7s collagen. In a study of 112 NAFLD subjects it has been noted that these 2 biomarkers could exclude late stages of fibrosis. In NAFLD patients, assessing HA levels has proved to be helpful in predicting advanced stages of fibrosis (8).

Hepatocyte apoptosis – has a major role

in liver damage in NAFLD subjects. Lately, a particular product of liver cell apoptosis, caspase–cleaved fragments of Cytokeratin 18 (CK18) have proved to be significantly elevated in NASH patients, compared to steatosis patients or healthy subjects. Moreover, it has been shown that the serum CK18 level, measured by ELISA test, was significantly higher in NASH patients, compared to controls and steatohepatitis patients. CK18 proved to be a predictor of NASH. NASH patients who underwent metabolic surgery, procedure which was shown to have a favorable effect on BMI, had lower CK18levels (9).

NON-INVASIVE METHODS

Regarding biomarkers, different scores have been proposed in order to recognize NAFLD.

SteatoTest - is a mix between FibroTest (which is a marker of fibrosis in patients with hepatitis B or C and alcoholic liver disease) and ActiTest (surrogate marker of hepatocyte necrosis in hepatitis B and C) and body mass index (BMI), triglycerides, cholesterol and blood sugar levels, adjusted for gender and age (10).

Fatty liver index (FLI) (11) is a mixed method based on four parameters: waist circumference, BMI, triglycerides and GGT, which allows the prediction of fat deposition in the liver with an 84% certainty. The score was initially used to diagnose subjects at high risk of steatosis, who needed ultrasonography and lifestyle changes; then it was certified as marker of prognosis, used to predict the intima media thickness, coronary diseases, insulin resistance, but also the nine-year incident diabetes and atherosclerosis (12).

Lipid accumulation product (LAP) is calculated using the waist circumference and the serum triglyceride levels; it was reported as a predictor for the risk of diabetes, cardiovascular diseases and mortality of all cause in patients with high risk of cardiovascular disease. The natural LAP logarithm, adjusted for gender, has a sufficient accuracy in recognizing subjects with liver steatosis who need ultrasonography (13).

HAIR Score – is useful for predicting NASH in patients suffering from severe obesity (BMI>35kg/m^2). This score is based on 3 factors; their presence being scored 1 point each: 1. Hypertension: 1 point; 2. ALT> 40UI/L: 1 point; 3. Insulin resistance index >5.0: 1 point. The total score is obtained by summing the scores for the 3 factors. A HAIR score of 3 practically confirms NASH (14).

BAAT Score – the presence of only one of the factors below rules out septal fibrosis and liver cirrhosis: 1. BMI>=28kg/m^2: 1 point; 2. Age >=50: 1 point; 3. ALT (alanine aminotransferase) >= 2x (normal value): 1 point; 4. Triglycerides>=1.7mmol/L (150mg/dl): 1 point. A final score of 1 or 0 has a negative prognostic value of 100% for fibrosis (sensitivity 100% and specificity 46%) (15).

NICE Model - is based on the presence of the metabolic syndrome (using International Diabetes Federation criteria), ALT levels and caspase-cleaved CK18 fragment levels (assessed through biopsy). A score of over or equal to 5 allows for the accurate diagnosis of NAFLD in patients with morbid obesity (16).

NASH Score System for Morbidly Obese - consists of 6 parameters, namely obstructive sleep apnea, type 2 diabetes, hypertension, ALT>=27U/L, AST >=27U/L and Caucasian race. Two points are assigned to Caucasian race and one point is assigned to the rest of the five components. The probability of NASH is determined based on the summed points (17). Another study (18) reported that approximately 90% of the

patients with early-stage NASH could be predicted by assessing the serum adiponectin level, serum type IV collagen 7S level and insulin resistance. Resistin and adiponectin are synthesized especially in fat tissue and play a major role in insulin resistance. Low levels of adiponectin are present in NAFLD patients.

IMAGING METHODS

Imaging methods cannot make the difference between NASH and steatosis, but the diagnosis they provide orient therapy designed to reduce the cardiovascular risk.

Ultrasonography - is an available, non-invasive and cheap method used to detect hepatic steatosis; its specificity is 66-95% and sensitivity 60-94%; the assessment of liver steatosis is subjective, on a three-point scale (severe, moderate and intermediate) (19).

Computed tomography (CT) - permits a better analysis, superior to ultrasound, by measuring the mean liver attenuation in Hounsfield units (HU), but there is variability in interpreting hepatic attenuation on CT scans (19).

Magnetic resonance imaging (MRI) – for assessing liver steatosis two methods can be used: magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (H-MRS). MRI has a 100% accuracy in detecting liver steatosis. Even though it is a highly accurate method for detecting and dynamically quantifying steatohepatitis, it is expensive and not readily available (20).

Elastography (Fibroscan) – Multiple meta-analyses suggest that liver stiffness measurement using Fibroscan is a useful method for the evaluation of early stages of cirrhosis and severe hepatic fibrosis.

Acoustic radiation force impulse (ARFI) – is a method by which the elastic properties of a surface are evaluated, giving real-time images in mode B. Unlike elastography, this method is not restricted to subjects with high BMI (21).

Magnetic resonance elastography – method used to study the elastic properties of an area of interest in the liver tissue (22).

CONCLUSIONS

Due to the high prevalence of NAFLD, we believe that cheap and non-invasive methods are needed in order to diagnose this hepatic impairment. Liver biopsy, regarded as the “golden standard”, has few benefits for patients (especially those with early-stage NAFLD who are more at risk of cardiovascular disease than hepatic complications); it is useful, however, in medical research, even if the ethical aspect remains a matter of debate.

We conclude that non-invasive methods (risk scores, biomarkers and imaging methods) can replace liver biopsy in early stages of NAFLD. Further studies are required to validate new non-invasive diagnosis methods.

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INFLUENCE OF SLEEP ON OBESITY IN CHILDREN

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INFLUENCE OF SLEEP ON OBESITY IN CHILDREN (Abstract) Childhood obesity is a global epidemic with long term implications. The main cause of obesity is an increase in calorie intake and a decrease in physical activity, but also there is clear evidence suggesting a link between the duration and quality of sleep and obesity risk. Good sleep habits are involved in increased ability to concentrate at school, improvement of general state, immune system development, increased quality of life. On the other hand, there are several mechanisms by which chronic sleep deprivation induces weight gain: disturbance of hormones that control hunger center, increased time for meals, reduced physical activity, metabolic changes. Recently, nighttime sleep duration has declined, in contrast with the increasing prevalence of obesity. Childhood sleep habits have a long term effect on weight, with repercussions even into adulthood. This is the reason why there is increasing interest to include sleep quality on the list for childhood obesity prevention. Sleep represents an important and independent risk factor of obesity in children and adolescents and it should be taken into consideration in the management of obesity. **Keywords**: OBESITY, SLEEP, CHILDREN

Childhood obesity is a global epidemic with long-term implications: cardiovascular diseases (high blood pressure, dyslipidemia), endocrine dysfunctions (diabetes type 2, insulin resistance), pulmonary complications (obstructive sleep apnea, asthma, effort intolerance) (1). Recent statistical studies have shown that the prevalence of obesity increased dramatically, particularly in boys, and especially in rural areas (2). Considering all these aspects, special attention should be paid to risks factors and prevention of childhood obesity.

It is well known that the main cause of obesity is an increased calorie intake in combination with decreased physical activity (3). But it is widely accepted that other significant factors are also involved in this process. Thus, literature data show convincing evidence that shorter sleep duration and poorer sleep quality is an important and independent risk factor of obesity in children (4).

BENEFITS OF SLEEP

In order to understand the consequences of sleep deficit, we must first know the benefits of a quality sleep. Good sleep habits are involved in increased ability to concentrate at school, improvement of general state, immune system development, increased quality of life. Along with diet

and exercise, sleep plays an important role in growth, development and maturation of body functions, especially during childhood, when more sleep is needed than in adulthood. Adequate sleep also plays a key role in the regulation of homeostasis and hormonal systems involved in somatic and intellectual development, brain maturation, information processing and memory consolidation (5, 6, 7).

Insufficient sleep has been linked to emotional, social, cognitive and behavioral disturbances, such as poor concentration, depression, unsatisfactory academic results, suicidal tendency. All these negative effects improve with increased sleep duration both in childhood and adolescence (8).

HOW SLEEP AFFECTS BODY WEIGHT

Besides all these benefits, sleep plays a role in body weight. To better understand this mechanism a series of studies and researches have been conducted. Their conclusion was that lack of sleep increases the appetite because it influences the hormones regulating the hunger center. Among these hormones are leptin and ghrelin. Leptin is considered a marker of energy stores. Low leptin levels signal the nervous system of a depletion of energy reserves, which leads to increased appetite. On the other hand, ghrelin, the hormone released by gastric cells, is a marker of energy deficit. So, higher ghrelin levels stimulate the appetite and storage of ingested calories (9). There are studies which show that sleep deprivation increases ghrelin level and decreases the leptin level with a corresponding increase in hunger and appetite, especially for foods rich in fats and carbohydrates (10).

Another element through which sleep influences body weight is the availability of more time for meals. People who sleep less at night will consume more food than people with normal sleep duration, because they have more time available to think about food. Sleep also influences physical activity. People who do not get enough sleep are tired and therefore will reduce the exercise duration. Sleep-deprived people tend to spend more time watching television and less time in playing sports (11, 12). Laboratory experiments show that sleep deprivation lowers body temperature, which leads to lower energy consumption and finally to accumulation of extra weight (11, 13).

The reduction of sleep duration produces metabolic changes in the body. There are several hormones that fluctuate during the light/dark cycle, being affected by sleep: growth hormone, melatonin, cortisol (14). Sleep deprivation is associated with a decline in glucose tolerance and insulin sensitivity, which effectively prevents the body to metabolize sugar from food (15). It was also found that short sleep duration is associated with a pre-sleep growth hormone secretion in addition to the normal hormone secretion (6, 16). These increased growth hormone levels may induce a transient insulin resistance in muscle tissue, resulting in a significant increase of insulin resistance in other tissues (17). Also, elevated evening cortisol concentrations are likely to result in reduced insulin sensitivity on the following morning (6, 17). Other complications associated with insulin resistance and obesity in childhood and adolescence include metabolic syndrome, fatty liver and polycystic ovary disease (18).

In addition, sleep deprivation could decrease energy expenditure by reducing the thyroid stimulating hormone (TSH), and

this fact has as consequence a reduced rate of the basal metabolism or a disturbance of neural circuits which control body weight (14).

IMPACT OF SLEEP ON CHILDHOOD OBESITY

Recently, night time sleep duration has declined, in contrast with the increasing prevalence of obesity. It has been shown that when children aged 10-17 years were offered optimal sleep conditions for several days in a raw, they used to sleep an average of 9 hours/day. This demonstrates that the body needs at least 9 hours of rest per day. In contrast with these physiological needs, the statistics show that teenagers get 7 hours of sleep or less (5, 6, 7). Also, over the past century sleep duration decreased by 0.75 minutes per year, with a more significant decrease on school days, for older children, and especially for boys (19).

Studies aimed at illustrating the effect of sleep deprivation on body weight in children have been conducted. Thus a British study in over 8000 children measured weight, height and body mass index. Other risk factors probably involved in obesity, such as maternal education, intrauterine and perinatal factors, food habits, family and demographic characteristics were also considered. The results showed that sleep is an independent risk factor, children getting less than 10.5 hours/night of sleep at age 3 having a 45% higher risk of obesity at age 7 compared to those getting at least 12 hours/night (4). Similarly, a study in 915 US children aged 6 months, 1 year and 2 years aimed at predicting the effects of sleep duration on body mass index (BMI), subscapular and triceps skinfold thicknesses, and overweight. The conclusion was that an average daily sleep duration of less than 12 hours during the first year of life increases by 2 times the risk of obesity at age 3 (20).

Childhood sleep habits have a long term effect on weight, with consequences well into adulthood. This was demonstrated by a study on over 1000 children from New Zealand, followed from birth until age 32 using information on the hours of sleep at age 5, 7, 9 and 11 years. It has been shown that each 1 hour reduction in sleep duration during infancy is associated with a 50% higher risk of obesity at the age 32 (21). Furthermore, late bedtimes and late wake up times are associated with an unfavorable weight status profile, both in children and adults, regardless of gender (22).

GOOD SLEEP HABITS

Regarding the optimal duration of sleep to be recommended to parents for their children, the body needs change as the child grows from infancy up to childhood and adolescence. The sleeping duration recommendations of the American Sleep Foundation are: 0 – 3 months: 14 – 17 hours/day; 4 – 11 months: 12 – 15 hours/day; 1 – 2 years: 11 – 14 hours/day; 3 – 5 years: 10 – 13 hours/day; 6 – 13 years: 9 – 11 hours/day; 14 – 17 years: 8 – 10 hours/day; 18 – 25 years: 7 – 9 hours/day (23).

To effectively advise parents of overweight children, it is important to investigate aspects related to duration and quality of sleep, in order to assess whether they get enough sleep, difficulty awakening in the morning, drowsiness or excessive daytime fatigue associated with concentration difficulties at school or with homework, difficulties in putting the child to sleep at night, systematic nigh awakenings. All these elements indicate the existence of sleep

problems. In this case, it is important to correct children′s sleep issues, which can prevent both obesity and related health consequences.

Some recommendations for a better quality sleep are:

- Follow a regular sleep schedule (go to bed and wake up the same time every day);
- Relaxing activities before bedtime;
- Avoid television, computer use or video games at least 3 hours before bedtime
- Avoid before bedtime heavy food as well as foods and beverages with stimulating effect such as chocolate, caffeine containing sodas (24, 25).

CONCLUSIONS

In conclusion, sleep is an important and independent risk factor of obesity in children and adolescents. Even though obesity prevention is based on nutrition and physical exercise, sleep habits should be also taken into consideration for a more efficient management of obesity. A healthy sleep implies enough hours of restorative sleep, without interruptions (to be able to pass through all stages of sleep), adapted to the sleep needs of each period of childhood, and suitable to the natural biological rhythm of the child. Once learned the discipline of a quality sleep, the child has a better compliance regarding food and physical exercise discipline. Considering all the chronic medical complications that obesity can cause, we can strongly affirm that a healthy sleep is one of the keys to maintaining a normal weight, contributing to a healthier life both now and in the future.

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NADPH OXIDASE: STRUCTURE AND ACTIVATION MECHANISMS (REVIEW). NOTE I

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NADPH OXIDASE: STRUCTURE AND ACTIVATION MECHANISMS (Review). NOTE I (Abstract) NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase), with its generically termed NOX isoforms, is the major source of ROS (reactive oxigen species) in biological systems. ROS are small oxygen-derived molecules with an important role in various biological processes (physiological or pathological). If under physiological conditions some processes are beneficial and necessary for life, under pathophysiological conditions they are noxious, harmful. NADPH oxidases are present in phagocytes and in a wide variety of nonphagocytic cells. The enzyme generates superoxide by transferring electrons from NADPH inside the cell across the membrane and coupling them to molecular oxygen to produce superoxide anion, a reactive free-radical. Structurally, NADPH oxidase is a multicomponent enzyme which includes two integral membrane proteins, glycoprotein $gp91^{phox}$ and adaptor protein $p22^{phox}$, which together form the heterodimeric flavocytochrome b558 that constitutes the core of the enzyme. During the resting state, the multidomain regulatory subunits $p40^{phox}$, $p47^{phox}$, $p67^{phox}$ are located in the cytosol organized as a complex. The activation of phagocytic NADPH oxidase occurs through a complex series of protein interactions. **Keywords:** REACTIVE OXYGEN SPECIES, NADPH OXIDASES, NOX, STRUCTURE, LOCALIZATION, FUNCTIONS.

Oxidative stress (SO) is essentially the result of an imbalance between the endogenous production of free radicals derived from oxygen (ROS) or nitrogen (RNS), and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants (1, 2, 3). This molecular imbalance in ROS metabolism has been proven both at the cellular level and throughout the body. ROS have an opposing, context-depending dual role.

Under normal circumstances, the production and removal of ROS, that occurs in distinct extracellular or (sub)cellular spaces, are strictly controlled by effective endogenous defense mechanisms that block their excessive production. Some reactive oxygen species are necessary for life (especially superoxide anion radical O^{2-}, hydrogen peroxide H2O2) (4) having a pivotal role in mediating important functions in normal cellular physiology by their contri-

bution to regulating different physiological processes such as cellular defense, signaling mechanisms, steroid synthesis, activation of G protein-coupled receptors, regulation of transcription factors, gene expression (5).

Overproduction of ROS results in oxidative stress with various pathological consequences such as atherosclerosis, hypertension, vascular remodeling after angioplasty, myocardial infarction, asthma, stroke, etc. (6, 7, 8, 9).

Sources of ROS. Of the many sources of ROS we mention the redox enzymes NADPH oxidase, mitochondrial respiratory chain, xanthine oxidase, lipoxygenase, and cyclooxygenase, which are continuously interacting with each other. In biological systems, ROS have the superoxide radical anion O^{2-} as one of the most important representative. A typical enzyme belonging to this redox group that generates ROS is NADPH oxidase, with important biological functions (10). NADPH oxidases are present in the higher life forms (animals, plants), but also in fungi, but not in prokaryotes and most unicellular eukaryotes. The enzyme is expressed in professional phagocytic cells, multiple types of blood cells (such as neutrophils, monocytes, eosinophils, macrophages, mast cells) (11) involved in antibacterial, antifungal, antimicrobial defense by reactions with free radicals; the enzyme is also involved in innate immunity (12). Phagocytic NADPH oxidase becomes active upon stimulation and then generates superoxide anions. An alternative scenario in that phagocytic NADPH oxidase directs the ions inside and outside the vacuole formed by endocytosis producing changes in pH and ion flow (13). NADPH oxidase and NADPH oxidase-like enzyme also described in non-phagocytic cells have been identified in almost every type of tissue, but functionally distinct from phagocytic oxidases (14). Non-phagocytic NADPH oxidases are constitutively active enzymes that produce superoxide radical anion intracellularly in a slow and sustained manner and act as a signaling molecule influencing the transcription factors, molecules involved in inflammation, cell growth etc (15).

The cellular compartments in which NADPH oxidases are expressed include: the nucleus, endoplasmic reticulum, endosomes, phagosomes, mitochondria, and extracellular space (16).

Structure of NADPH oxidase. In mammals, NADPH oxidase is a plasma membrane-bound multicomponent complex (heteroprotein) that faces the extracellular space, consisting of 7 isoforms (homologues). ROS is produced by NADPH oxidase through NOX protein, thus differentiating the enzyme from other oxidases. NADPH oxidases are collectively referred to as the NOX family: NOX 1, NOX 2 (identical to membrane glycoprotein $gp91^{phox}$, also known as a subunit β, suffix phox refers to **ph**agocyte **ox**idase) (17), NOX 3, NOX 4, NOX5 and associated dual oxidases – Duox 1 and Duox 2 (18, 19, 20). The catalytic subunits of NOX 1, NOX 2, NOX3 and NOX 4 form a macromolecular complex with $p22^{phox}$ (known as subunit α) as a common enzyme activator subunit. NOX 5 Duox1 and Duox2 are $p22^{phox}$-independent isoforms. Duox 1 and 2 have additional peroxidase domains termed this way due to homology to peroxidases which catalyze dismutation of superoxide anion to hydrogen peroxide – H2O2 (13). Therefore, each of these isoforms has a core catalytic

subunit, the so-called NADPH-oxidase (NOX), two dual oxidase subunits (Duox) and 5 regulatory subunits.

Isoenzymes of NADPH-oxidase (NOX) are distinguished by the following features: subcellular localization, tissue and subcellular patterns of expression, structure (the nature of the catalytic subunits determines the adaptation of regulatory subunits), mechanism of enzyme activation, ROS-generating catalytic mechanism and functions (1, 10,21).

Physiological functions of NADPH oxidases: the enzymes catalyze the transfer of electrons in cytosol across the membrane from NADPH, which oxidizes, to molecular oxygen, which is reduced to produce the superoxide radical anion (O^{2-}), generator of other ROS species. The NADPH oxidases was originally identified as a component of innate host defence. In phagocytes, this complex enzyme is activated to produce superoxid anion and other secondarily derived ROS, wich promote killing of invading micro-organisms. Another non phagocytic NADPH oxidases system is present in almost every body organ being physiologically involved in signal transduction, cell proliferation and differentiation, posttranslational processing of proteins (4, 18).

Mechanism of action of NADPH oxidase Although all NOX enzymes have the same function in ROS generation, the activation and control mechanisms, the nature of the ROS produced, subunit requirements, and intracellular distribution varies between isoforms (22). Some NOX are membrane proteins with catalytic functions, other have cytosolic localization and regulatory functions (23). All NOX family members share a common core structure made up of 6 transmembrane domains. The catalytic core of the classic phagocytic enzyme – NADPH-oxidase (NOX 2) as of the other isoforms consists of two membrane-integrated proteins (subunits): $gp91^{phox}$, glycosylated protein (24), (considered the patriarch of NOX family) and $p22^{phox}$ (non-glycosylated adapter protein) which together form a large subunit, the flavocytochrome b558 complex (heterodimeric flavohemoprotein b558). $Gp91^{phox}$ subunit consists of 6 transmembrane domains and the cytoplasmic C-terminal region which contains two binding sites for FAD (flavin adenine dinucleotide) and for the electron donor NADPH. All NADPH oxidases except NOX 5, Duox 1 and Duox 2 have similar topological structure of the catalytic core $gp91^{phox}$. The N-terminal domain of NOX 5 binds calcium, Duox1 and Duox 2, in addition to the NOX 5 structure present at the N-terminal transmembrane α-helix a domain homologous to peroxidase (25).

The regulatory subunits play important roles in: maturation and expression of NOX and Duox subunits in biological membranes, and in enzyme activation and spatial organization of the various components of the enzyme complex (26). Some isoforms of NADPH oxidase contain GTP-ase (guanine triphosphate hydrolase), Rac 1 and Rac 2, which modulate their activity (27).

The activation of phagocyte NADPH oxidase occurs through a series of complex protein interactions in response to numerous agonists (27). In unstimulated cells, components $p40^{phox}$, $p47^{phox}$ and $p67^{phox}$ are grouped in the cytosol into a complex (25).When the resting cell is exposed to a variety heterogeneous stimuli (chemical, physical, environmental, biological factors)

(24), the peripheral cytosolic component becomes highly phosphorylated able to form a complex which migrates to the membrane where it associates with flavocytochrome b558 and together with other components – $p40^{phox}$, $p47^{phox}$ and $p67^{phox}$ – forming the active oxidase which transfers electrons from the substrate (NADPH) to molecular oxygen with formation of superoxide radical anion (27, 28), precursor of other toxic ROS (17). The activation process is strictly controlled, involving in addition to phosphorylation multiple conformational changes. This enzymatic complex is involved in many key biological functions such as cell growth, proliferation, differentiation, migration, defense, signaling and regulation of gene expression. Excessive ROS production attributed to the increased NOX activity, causing cellular oxidative stress, may contribute to the occurrence of a wide variety of diseases: vascular disease, inflammation, endothelial dysfunction, cell proliferation, fibrosis, angiogenesis, cardiovascular remodeling, cardiac aging, renal remodeling in hypertension, atherosclerosis, diabetes, heart failure, myocardial ischemia-reperfusion injury.

CONCLUSIONS

NADPH-oxidases is a proteic complex that, being stimulated, is capable to produce O^{2-}, by transfering an electron from NADPH to O2. It is a multicomponent family enzyme, with a complex structure made of membrane proteine complex, with cathalitic properties; in the same time, the enzyme has citosolic structures with regulatory properties. The tisulary distribution of NADPH-oxidases and their activating mechanisms are of large variety.

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LEVELS OF MUTANS STREPTOCOCCI AMONG SCHOOLCHILDREN IN IASI, ROMANIA

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LEVELS OF MUTANS STREPTOCOCCI AMONG SCHOOLCHILDREN IN IASI, ROMANIA (Abstract): **Aim:** To evaluate the dental and salivary density of *mutans streptococci* among Iasi schoolchildren. **Material and methods**: This study cross-sectional included 533 schoolchildren aged 7-12 years. The density of *mutans streptococci* on dental surface (mesial and occlusal surfaces of teeth 1.6 and 3.6) and in saliva was assessed using Dentocult® SM Strip mutans kits (Orion Diagnostica Oy) according to manufacturer's instructions. Subjects were given one of the following scores: 0 = $<10^4$ CFU/ml; 1 = $<10^5$ CFU/ml; 2 = 10^5–10^6 CFU/ml; 3 = > 10^6 CFU/ml. **Results**: A *mutans streptococci* density score 3 was predominant both in saliva and on the mesial and occlusal surfaces of the first permanent molars. Scores 2 and 3 were predominant in younger children, while in 12-year-old children the four scores were in similar percentages**. Conclusion**: High levels of *mutans streptococci* prevail among Iasi schoolchildren, highlighting the need to implement complex caries prevention programs. **Keywords**: SCHOOLCHILDREN, MUTANS STREPTOCOCCI, DENTAL CARIES.

Oral diseases are the result of a complex interaction between etiologic factors and a number of risk factors, modulated by protective factors. Pointing out the risk factors enables the identification of those elements that act to damage oral health, which further makes possible the implementation of measures aimed at eliminating the action of these factors.

Detailed and accurate knowledge of the distribution of oral diseases and their determinants in the population is a compulsory stage for developing strategies to improve preventive programs and planning dental services.

The aim of the present study was to evaluate the dental and salivary density of *mutans streptococci* (*MS*) among schoolchildren in Iasi town.

MATERIAL AND METHODS

The study was cross-sectional, clinical and microbiological and was conducted in 2014. The study sample was selected according to the following criteria: children aged 7 to 12 years, attending schools in various districts of Iasi town; schools that have a dental office. The sample was selected using the probabilistic method and included 533 children (50.47% males and 49.53% females).

Examinations were performed in the

dental offices in schools.

The density of *MS* on dental surface (mesial and occlusal surfaces of teeth 1.6 and 3.6) and in saliva was assessed using Dentocult® SM Strip mutans kits (Orion Diagnostica Oy) according to manufacturer's instructions. For the assessment of dental density, special tips were used to take bacterial plaque samples; these were subsequently spread on the four sites of the square-tipped strips. For salivary density evaluation, the patient was invited to chew a paraffin pellet for 1 minute, in order to facilitate the transfer of *MS* from tooth surfaces into the saliva; the patient was instructed to eliminate any excess saliva, then the rough surface of the round-tipped strip was pressed against the saliva remaining on patient's tongue. The two strips (with dental and salivary samples) were then placed, with the smooth surfaces clipped and attached to the cap, in the selective culture broth; the vials were labeled with patient's data, then incubated at 35-37°C for 48 h; after incubation, the strips were removed from the culture broth and dried, then the density of the grown colonies was compared to the chart provided by the producer, according to the following scores: 0 = $<10^4$ CFU (colony-forming unit)/ml; 1 = $<10^5$ CFU/ml; 2 = $10^5 – 10^6$ CFU/ml; 3 = > 10^6 CFU/ml.

Tests were performed at least 1 h after eating and toothbrushing, in a separate dental visit and not during a treatment session; patients on antibiotic therapy during the last month before testing were excluded.

Written parental consent for children participation in the study was first obtained. Children also had the opportunity to accept or refuse participation.

Data privacy as well as the impossible identification of subjects in the case the results are published were ensured.

SPSS 17.0 was used for statistical analysis of data. The statistical significance threshold p was set at 0.05; chi-square test was used for data comparison and correlation analysis was performed using Spearman test.

RESULTS

Evaluation of data obtained after performing bacteriological tests to determine the dental density of *MS* showed that score 3 (that is a density of more than 10^6 CFU/ml) was predominant on both the mesial and occlusal surfaces of teeth 1.6 and 3.6 (tab. I).

Analysis of frequency distribution showed that scores 2 and 3 cumulated were present in more than half of the subjects.

TABLE I

Children distribution (%) depending on *mutans streptococci* scores on dental surfaces

Score	Situs			
	Mesial surface 1.6	***Mesial surface 3.6***	***Occlusal surface 1.6***	***Occlusal surface 3.6***
0	23.52	23.49	23.10	23.04
1	12.44	12.62	12.21	13.14
2	30.76	31.45	24.05	28.42
3	33.28	32.44	40.64	35.40

At the same time, however, it is to note that only 23% of the children included in the study presented a dental density of *MS* of less than 10^4 CFU/ml.

Separate analysis by age group (tab. II) showed that nearly half of the 7-year-old children presented a dental density of *MS* of more than 10^6 CFU/ml.

TABLE II

Distribution (%) of *mutans streptococci* dental scores by age group

Score	Age (years)					
	7	*8*	*9*	*10*	*11*	*12*
0	16.4	17.2	17.8	19.5	21.8	22.1
1	4.7	7.2	17.3	23.6	24.1	26.3
2	36.8	35.2	33.7	29.8	28.2	27.7
3	42.1	40.4	31.2	27.1	25.9	23.9

Score 3 on dental surfaces remained predominant at the age of eight; in children aged 9 and 10 years score 2 became predominant, then the differences between scores attenuated, so at age 12 the four scores presented similar percentages.

Chi-square statistical test indicated that the differences between age groups in the distribution of *MS* dental scores were statistically significant ($p < 0.01$), and Spearman correlation coefficient indicated a weak, inverse correlation: scores decreased with increasing age ($r = -0.20$).

Assessment of *MS* salivary density showed that score 3 was predominant in this case, too (32.54%); as with dental surfaces, scores 2 and 3 cumulated were present in more than half of the investigated subjects (tab. III).

At the same time, however, it is worth mentioning that less than a quarter of the children aged 7-12 years (23.63%) presented a salivary density of *MS* lower than 10^4 CFU/ml.

The age distribution was similar to the one on dental surfaces: at the age of 7, score 3 was predominant (50.1%), as well as at the age of 8 (43.5%); after that, the distribution of scores tended to become uniform, so at the age of 12 years the four scores presented similar percentages.

Statistical analysis revealed statistically significant differences ($p < 0.01$) between age groups regarding the distribution of *MS* salivary scores; Spearman correlation coefficient indicated a weak, inverse correlation between age and the salivary level of *MS* ($r = -0.189$).

TABLE III

Distribution (%) of *mutans streptococci* salivary scores by age group

Score	Age (years)						Total per sample
	7	*8*	*9*	*10*	*11*	*12*	
0	16.3	17	31.5	27.8	25.6	23.5	23.63
1	2.4	6.3	11.3	13.4	19.4	23.5	13.01
2	31.2	33.2	30.9	38.4	28.5	23.4	30.82
3	50.1	43.5	26.3	20.4	26.5	29.6	32.54

DISCUSSION

The role of *mutans streptococci* in initiating carious lesions is very well known today. The predominance of the highest score, meaning a density of more than 10^6 CFU/ml, both on the mesial and occlusal

surfaces of the first permanent molars, is an important factor of an unfavorable distribution of children into caries risk groups.

In addition, score 0, although found in about 23% of the children both in saliva and on dental surfaces, does not always mean that the patient is completely free from *mutans streptococci*, but signifies a density of less than 10^4 CFU/ml. For this reason, the prevalence of *mutans streptococci* can be considered to be high among the examined children.

However, a similar distribution of schoolchildren by the level of *mutans streptococci* was recorded in many other countries. Thus, in Sweden, a prevalence of 76% of this bacterial species was found among children aged 9 years, while in Israel 52.1% of the 12-year-old children presented the highest score for *mutans streptococci*, and only 5.4% the lowest score (1).

Studies in Turkey demonstrated that most of the children aged 7-9 years presented scores 2 and 3 of *mutans streptococci* salivary levels (2).

Data from studies conducted in Italy showed that more than 25% of the children aged 7-9 years presented a salivary density of *mutans streptococci* of at least 10^5 CFU/ml (3), while in India the prevalence of *mutans streptococci* in children aged 13 years was 87.37%, and 33.87% of them presented high levels of this bacteria (4).

One of the important features in *mutans streptococci* ecology is the localized growth manner. While some tooth surfaces may be carriers of *mutans streptococci*, other surfaces, located right in the neighborhood, may not have these microorganisms. Experimental studies have shown that surfaces colonized with *mutans streptococci* are at higher caries risk than non-colonized surfaces. However, their simple presence on a dental surface that does not necessarily mean that caries will develop on that surface, but signifies an increased risk of caries occurence (5).

In this regard, studies have shown a significant correlation between the level of *mutans streptococci* and carious lesions on permanent teeth (6, 7, 8).

Studies that have examined the influence of fluoride mouthrinses on *mutans streptococci* count showed that the use of these solutions clearly determined a decrease of their salivary level (9, 10, 11, 12).

As to the influence of dental sealants, although they obviously reduce the development of fissure caries, their application is not always followed by a reduction in the salivary level of *mutans streptococci*, because the sealant may itself be colonized on the external surface (13).

The literature provides evidence suggesting that in children the risk of caries is greater the earlier the contamination with *mutans streptococci* occurs, although this risk may be partially compensated by other factors, including good oral hygiene and non-cariogenic diet. Moreover, nutrition and oral hygiene can counterbalance each other, so that carious lesions can be controlled even in the situation of a cariogenic diet, with the condition of maintaining a rigorous oral hygiene (14, 15, 16, 17).

CONCLUSION

The results of the present study show a high prevalence of elevated levels of *mutans streptococci* among Iasi schoolchildren, underlining the need for complex prevention programs in children, that should include multiple methods for the primary prevention of dental caries.

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EXTRAHEPATIC MANIFESTATIONS IN CHRONIC HEPATITIS B – THE EXPERIENCE OF THE IASI INFECTIOUS DISEASES HOSPITAL BETWEEN 2012-2014

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EXTRAHEPATIC MANIFESTATIONS IN CHRONIC HEPATITIS B – THE EXPERIENCE OF THE IASI INFECTIOUS DISEASES HOSPITAL BETWEEN 2012-2014 (Abstract): There are numerous extrahepatic syndromes associated with HBV infection: vascular, renal, and cutaneous manifestations, essential mixed cryoglobulinemia, and neurological phenomena. **Aim:** This study proposes an approach to the extrahepatic manifestations of chronic hepatitis B as it has been shown that the persistent production of immune complexes induces extrahepatic lesions. **Material and Methods**: In the interval 2012-2014 180 cases of chronic hepatitis B were admitted to the Iasi Infectious Diseases Hospital. **Results:** The mean age of the study patients was 41 years, most patients being male (65%) and living in urban areas (70%). Cutaneous manifestations of chronic HBV infection were present in 62% of patients and consisted in: rash (25%), Gianotti-Crosti syndrome (15%), cryoglobulinemia (21.11%) and Henoch-Schonlein purpura (12.22%). Panarteritis nodosa (PAN) with a prevalence of 9%, and renal manifestations -glomerulonephritis (2.77%) and nephrotic syndrome- were present. Also reported were neurological manifestations (peripheral neuritis, Guillain-Barre syndrome) in 11.1% of the cases, endocrine manifestations (impaired glucose metabolism, and hypothyroidism) in 19.4%, hematological (thrombocytopenia, hemolytic anemia, aplastic anemia) and cardiovascular manifestations. **Conclusions**: In our group of patients the extrahepatic manifestations often complicated the course of chronic HBV infection with a wide range clinical manifestations involving many organs or systems (skin, joints, nervous, renal, cardiovascular and hematologic systems). **Keywords:** PANARTERIS NODOSA, ESSENTIAL MIXED CRYOGLOBULINEMIA, ACANTHOSIS NIGRANS.

The current importance of acute hepatitis B virus (HBV) infection is due to its widespread around the world, high potential for chronicity and possible progression to liver cirrhosis or hepatocellular carcinoma. Hence the interest of researchers to supplement the data accumulated so far regarding the pathogenesis, clinical and laboratory aspects of HBV infection. Progression to chronic forms is accompanied by a persistent production of immune complexes (which fix and activate the complement) causing extra hepatic lesions. Autoimmune phenomena, with the presence of circulating nonspecific auto antibodies (antinuclear, anti-mitochondrial and anti-albumin) have been

also reported. There are numerous extrahepatic syndromes associated with HBV infection: vascular (vasculitis, panarteritis nodosa), renal cutaneous (papular dermatitis), essential mixed cryoglobulinemia and neurological phenomena (3.4).

MATERIAL AND METHODS

The cases of chronic HBV infection admitted to the Iasi "St. Paraschieva" Infectious Diseases in Iasi in the interval 2012-2014 were retrospectively studied. The 180 patients included in the study were diagnosed by physical examination, liver function, serological, virological and histological tests. Our aim was to determine the prevalence and course of extrahepatic manifestations during the disease in view of adjusting the regimens, on the one hand for treating these complications and, on the other hand, for rethinking the strategy for monitoring and treating the patients with chronic HBV infection (as some therapeutic interventions to manage these complications can negatively influence the course of the underlying disease, as it is the case with panarteritis nodosa (PAN).

RESULTS

The 180 patients included in the study accounted for 8.7% of all chronic hepatitis cases admitted during the study period. The mean age of the study patients was 41 years (range 18-66 years), most patients being male (65%) and living in urban areas (70%). The extra hepatic manifestations encountered during the course of chronic hepatitis C are listed in table I.

TABLE I
Extrahepatic manifestations associated with chronic hepatitis B

System	Manifestations	No.	%
Locomotor	Arthralgia	34	18.9
	Arthritis	3	1.7
Renal	Glomerulonephritis	29	16,11
Cutaneous	Gianotti - Crosti syndrome	6	3,3
	Nonspecific exanthem	45	25
	Raynaud syndrome	46	25,6
	Panarteritis nodosa	9	5
	Essential mixed cryoglobulinemia	38	21
	Henoch-Schonlein purpura	22	12,22
Cardiovascular	Myocarditis	1	0.6
	Pericarditis	0	0
	Hypotension	28	15.6
	Bradycardia	5	2.8
	Tachycardia	8	4.4
Digestive	Gastritis/Duodenitis	44	24.4
	Catarrhal pancreatitis	2	1.1
	Biliary dyskinesia	66	36.7
Nervous	Peripheral neuritis	6	3.3
	Polyneuritis	11	6.1
	Myelitis, extrapyramidal syndromes	2	1.1
Endocrine	Disorders of glucose metabolism	35	19.4
	Hypothyroidism	15	8.3
Hematologic	Bone marrow aplasia	2	1.1
	Thrombocytopenia	95	52.8
	Hemolytic anemia	34	18.9

During the course of chronic HBV infection cutaneous manifestations were present in 62% of patients. *Rash* was encountered in about 25% of patients. *Gianotti-Crosti* syndrome or *"papular acrodermatitis of childhood"*, which is a self-limiting papular or papulovesicular rash, was present in the acute phase of infection in 6 cases, especially among women (15% cases) and young adults, being located in most cases on the distal portion of the limbs and accompanied by pruritus. The recovery occurred spontaneously after 25-45 days and the associated manifestations included fever, diarrhea, and lymphadenopathy.

Cryoglobulinemia was present in 38 (21.1%) cases and *Henoch-Schonlein* purpura in 22 (12.22%) cases. *Essential mixed cryoglobulinemia* is described in association with the HBsAg carrier status; in our study group it manifested by: purpura (14 cases), Raynaud syndrome - 46 (25.6%) cases and arthralgia - 34 (18.9%) cases, triggered by exposure to cold. Fatigue was present in all cases, and renal involvement was found in 29 (16%) cases, manifested by glomerulonephritis (membranoproliferative type with marked monocyte infiltration). Three of the patients also had chronic kidney failure.

Panarteritis nodosa (PAN) is a rare event, but severe in chronic hepatitis B; in our study its prevalence was of 5% (1, 2). Cutaneous PAN was found in 9 (5%) cases, with the following manifestation*: livedo reticularis* and *painful subcutaneous nodules*, ulcerated nodules and distal gangrene manifested by fever, polyarthralgia, myalgia , nettle rash, hypertension, neuritis, soft tissue nodular lesions, *livedo reticularis*, and in severe forms: cutaneous ulcers, distal gangrene acute colecystitis, vascular occlusions. Other encountered cutaneous extrahepatic manifestations were: *nonspecific rash*: maculopapular - 45 (25%) cases, *livedo reticularis* - 3(1.7%) cases, erythema nodosum - 46 (25.6%) cases, stellar angioma -14 (7.77%) cases and *palmar erythrosis* - 6 (3.33%) cases; *acanthosis nigrans* was present in 2(1.1%) cases. Late cutaneous porphyria: dysmetabolic condition associated with uroporphyrin-decarboxylase deficiency has been associated with the presence of anti-HBc antibodies in blood, being reported in one patient. The manifestations at the level of the locomotor system were represented by *arthralgia*-34(18, 9%) cases and *arthritis*-3(1,7%) cases and were clinically characterized by polyarthralgias and arthrites accompanied by joint swelling, the joint involvement being symmetrical, generalized, and affecting especially the small joints of the hand and foot.

The renal extrahepatic manifestations consisted in *glomerulonephritis*, seen in 5 (2.77%) cases, *nephrotic syndrome*, proteinuria and hematuria. Immune complex deposits in patients with viral hepatitis have been detected in the kidneys in the absence of kidney disease and the most common form of glomerulonephritis in chronic hepatitis B was the nephrotic syndrome. *Proteinuria* and *hematuria* were reported in 2 (1, 1%) cases and were accompanied by fever. Neurological manifestations related to the action of HBV or to the induced immune response were present in 20 (11.1%) cases. These were classified as early and late, occurring several weeks after the onset, and included: *peripheral neuritis* (which occurred 4-21 days after the onset) in 6(3.3%) cases; *polyneurites* - 11 (6.1%) cases, *Guillain-Barre syndrome* - 8 (4.4%) cases and *extrapyramidal syndromes* -2 (1.1%) cases. The endocrine events were represented by *disorders of glucose metabolism* -35(9.4%) cases and

hypothyroidism -15(8,3%)cases. The recorded hematologic manifestations in order of their frequency were: *thrombocytopenia* -95(52.7%) cases, *hemolytic anemia* - 34 (18.8%) cases, and phenomena *of aplastic anemia* in 2 (1,1%) cases. Cardiovascular manifestations during chronic hepatitis B were seen in 43 (23.8%) cases, being represented by: *myocarditis*- 1 (0.6%) cases, phenomena of *hypotension* in 28(15.6%) cases, *bradycardia* -5 (2.2%) cases and *tachycardia* in 8 (4.4%) cases. Also recorded were digestive manifestations in 49 cases: *gastritis* 18 (24.4%) cases and *biliary dyskinesia* in 31(17.2%) cases.

DISCUSSION

The pathogenesis of extrahepatic manifestations in chronic hepatitis B is generally immune mediated. Panarteritis nodosa (PAN) is rare, with a prevalence of 1-5%, but a very severe complication of chronic HBV affecting the small and medium-sized vessels. It is more common among patients in North America and Europe but rare in Asians. In our study its prevalence was of 5%, thus concords the values reported in the literature. The incidence of HBV infection in patients with PAN ranges from 30% to 70%. In the US and Europe, where HBV infection is late the association with PAN is prevalent compared to Asia where HBV transmission is predominantly perinatal and the association of PAN has not been reported. Guillevin et al. (7) show that the clinical manifestations of HBV-associated PAN were similar to those of typical PAN except for: gastrointestinal complications (perforation and bleeding, present in 46.3% of cases), malignant hypertension (29.6% cases), renal infarction and orhi-epididymitis (26%). Untreated, the prognosis of HBV-associated PAN is poor, with 30-50% mortality, consequence of vasculitis (3, 4). In glomerulonephritis, the HBsAg-anti-HBs circulating immune complexes were identified in the glomerular basement membrane (5). All three major antigens of HBV (HBsAg, HBeAg and HBcAg) were located in the glomerular capillary wall or mesangium. Deposits of immune complexes in patients with viral hepatitis were detected in the kidney even in the absence of kidney disease. The most common form of glomerulonephritis in chronic hepatitis B was the nephrotic syndrome. Healing is frequently associated with HBsAg seroconversion and only a small proportion of patients' progresses to kidney failure (4).

"Arthritis-dermatitis" syndrome is common among patients with chronic hepatitis B, being present in about 1/3 of patients (6, 7). Joint and skin manifestations are varied and remit spontaneously (8). The association between HBV and essential mixed cryoglobulinemia remains controversial. There are 3 types of cryoglobulins; Type II (monoclonal IgM and polyclonal IgG) and type III (polyclonal IgM and monoclonal IgG) cryoglobulins are classified as essential and are found in patients with chronic HBV infection. The prevalence of cryoglobulinemia ranges from 0% to 15% (9, 10). Purpura and arthralgies are the most frequent manifestations. Cryoglobulinemia is frequently associated with Raynaud's phenomenon, arthritis and Sicca syndrome, which can cause renal, neurological and vascular complications (1, and 10).

CONCLUSIONS

In our study group the extrahepatic manifestations have often complicated the course of chronic hepatitis B taking a wide range of clinical forms and involving many organs or systems (skin, joints, nervous, renal, cardiovascular, and hematologic systems). Their recognition may contribute to

early diagnosis of chronic HBV infection and effective treatment. Although in the literature the incidence of chronic hepatitis B-associated PAN is of 1%-5%, in our study is was of 5%. The importance of diagnosing the extrahepatic manifestations is related to therapy, as corticotherapy can negatively influence the course of chronic HBV.

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ARE THERE ANY PARTICULARITIES IN CROHN'S DISEASE IN NORTH-EASTERN ROMANIA?

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ARE THERE ANY PARTICULARITIES IN CROHN'S DISEASE IN NORTH-EASTERN ROMANIA? (Abstract): Crohn's disease is an inflammatory disease of the gastro-intestinal tract with an evolution marked by activity and remission periods. Lately, the incidence and evolution models of Crohn's disease significantly changed, an increasing number of patients presenting more severe forms. **The aim** of the study is to analyze Crohn's disease in its phenotypic classification and to establish correlation between the localization, the behavior and the activity of Crohn's disease. **Material and methods:** A transversal study on 53 patients with Crohn's disease was conducted. **Results:** from the 53 patients, 56.6% were males, 85% from the urban areas. Under the phenotype aspect, the colonic localization (49.1%) and the inflammatory behavior (52.8%) were predominant. More severe forms of activity were discovered to patients with ileal localization (57.1%) or with penetrant phenotype (33.3%). **Conclusions:** Male patients from urban areas were predominant. The most frequent forms are the colonic and ileocolonic forms. From a behavioral point of view, we observed an obvious predominance of the inflammatory phenotype. The moderate - severe activity was predominant for the ileal localization and the mild - moderate activity for the colonic and ileocolonic localization. A mild-moderate activity is present for the stenosing forms and a moderate -severe one for the penetrant forms. **Keywords:** CROHN'S DISEASE, PHENOTYPE, LOCALIZATION

Crohn's disease (CD) is a chronic inflammatory disease of the gastrointestinal tract of unknown etiology. Both genetic and environmental factors (i.e. smoking) have been incriminated in its pathogenesis (1, 2). Over the last two decades, Crohn's disease incidence model has significantly changed (3,4). A high number of patients have been shown to develop more severe forms of the disease (penetrating or stenosing) in time. Nonetheless, there are still relatively limited data regarding the natural history in population studies.

The classification of Crohn's disease based on phenotypic criteria plays an important role in patient management and could prove to be helpful in determining the patient's clinical course (5).

In 1998, at the International Congress of Gastroenterology in Vienna, a classification of CD was established by taking into account the following factors, as the main

phenotypic elements (6): age (under 40 and over 40 years old), localization of the lesions (L1 terminal ileum, L2 colonic, L3 ileocolonic and L4 upper gastrointestinal tract) and disease behavior (B1 non-stenosing, non-penetrating, B2 stenosing, B3 penetrating).

The reevaluation of the classification from Montreal in 2005 did not change the categories of the classification from Vienna, but it made changes in each of these categories (7). As regards the age of onset, the Montreal classification considers the early onset of the disease for patients aged fewer than 16 as a new category, while the patients aged between 17 and 40 and over 40 years old were placed within categories A2 and A3. This change reflects numerous studies that demonstrate the presence of specific serotypes or genotypes which are more common in patients with an early onset of Crohn's disease (6, 9, 10).

In terms of the localization of lesions, the main problem of the classification from Vienna was the separate classification of the lesions of the upper gastrointestinal tract. Once with the emergence of new methods of investigation (capsule endoscopy), the localizations from the upper gastrointestinal tract were observed to coexist with lesions located at the level of the bowel or colon, therefore, they were no longer excluded from the classification from Montreal. Changes were made in the new classification also as regards the phenotype of the disease based on the observation that the perianal manifestations are not associated only with the penetrating disease. Thus, the authors added a special category for the perianal manifestations that can be added to the other categories of the disease behavior. After establishing the diagnosis and the phenotype, it is essential to monitor the disease in terms of activity. The activity score of CD (CDAI) is the index most used to estimate the severity of CD, widely used in clinical trials for over 25 years. However, CDAI has certain limitations, due to the subjectivity of some parameters such as well-being or pain intensity (11).

Based on these data, our study aims to analyze patients with CD in terms of their phenotypic classification, as well as to establish associations between the localization and behavior of Crohn's disease and its activity.

MATERIAL AND METHODS

A cross-sectional study was conducted on 53 patients with an established diagnosis of CD in the period from October 2011 to April 2013.

A worksheet was compiled for each patient comprising personal data (name, age, sex, place of origin), family and personal history, symptoms and signs of the disease, clinical data established after objective clinical examination, complete biochemical profile.

All patients underwent total colonoscopy, and in cases of suspected small bowel lesion in Crohn's disease, additional tests were requested (capsule endoscopy, computed tomography, magnetic resonance imaging).

The severity of CD was classified according to the activity score of CD (CDAI).

RESULTS

The age of the patient with CD ranged between 19 to 67, with an average value of 39.23 ± 12.64 years old. The average value was slightly higher in females (40.09 ± 9.89 years old) than in males (38.57 ± 14.53 years). Thirty patients were male, representing 56.6% and 23 female (43.4%), resulting a sex ratio of 1.3 / 1. Most pa-

tients came from urban areas (85%).

The data analysis in patients with CD showed that the most common localizations of the disease were the colonic (49.1%) and the ileocolonic (34%). It should be noted that the CD was located in the upper gastrointestinal tract only in 2 patients representing 3.8%. The CD was located in the bowel in 13.2% of patients. From the point of view of the phenotype, there was a clear predominance of the inflammatory phenotype, being present in more than half of the patients (52.8%). The stenosing and penetrating phenotypes were relatively equally represented in a ratio of 24.5% and 22.6% respectively (tab. I).

The case studies on the patients with Crohn's disease showed that moderate-severe forms predominated in patients in which CD was located in L1 (57.1%) and mild-moderate forms in those in which CD was located in L2 and L3 (30.8% and 27.8% respectively), the results providing statistically significant percentage differences (p = 0.04) (tab. II).

TABLE I

Characteristics of the patients with CD

Parameter	*Number of patients*
Age	
A2	29 (54.8%)
A3	24 (45.2%)
Location	
L1	7 (13.2%)
L2	26 (49.1%)
L3	18 (34%)
L4	2 (3.8%)
Behavior	
B1	28 (52.8%)
B2	13 (24.5%)
B3	12 (22.6%)

TABLE II

The association of severity with the location of Crohn's disease

Location	*L1*		*L2*		*L3*		*L4*	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Mild-moderate	2	28.6%	8	30.8%	5	27.8%		
Moderate-severe	4	57.1%	4	15.4%	2	11.1%	1	50.0%
Sever-fulminant			2	7.7%				
Remission	1	14.3%	12	46.2%	11	61.1%	1	50.0%

Significant differences emerged according to the severity of Crohn's disease on the phenotypes of the lesion (p=0.001): the patients in remission predominate regardless of phenotypes; on the other hand, in patients with phenotype B2, mildly-moderate forms predominate (38.5%), while in patients with B3 phenotype, moderately-severe forms predominate (33.3%) (tab. III)

TABLE III

The association of severity with the pattern of Crohn's disease

Severity	*B1*		*B2*		*B3*	
	n	*%*	*n*	*%*	*n*	*%*
Mild-moderate	7	25.0%	5	38.5%	3	25.0%
Moderate-severe	6	21.4%	1	7.7%	4	33.3%
Sever-fulminant	1	3.6%	1	7.7%		
Remission	14	50.0%	6	46.2%	5	41.7%

DISCUSSION

The specialized literature on the distribution of lesions in Crohn's disease is extremely varied. The IBSEN cohort study carried out in Norway on 200 patients showed that in 27% of the cases, the CD was located in the ileum, 45% of the patients had colitis-type CD, 26% ileocolitis-type CD and in 4% of cases the lesions were located in the upper tract (12).

The results were similar in another study in which the incidence of ileitis was of 15%, of isolated colitis of 42%, of ileocolitis of 37% and of the location at the level of the upper gastrointestinal tract was of 6% (13).

On the other hand, slightly contradictory data have been reported in the study carried out by Lewis. In this study, there is a clear predominance of the ileal form in 57% of cases, followed by Crohn's colitis in 27% of cases, ileocolitis in 19% of cases, and locations at the level of the upper tract in only 4% of the cases (14).

As regards the location of the lesions, the results of our study are similar to those reported in the specialized literature: ileal location in 13.2% of the cases, colonic in 49.1%, ileocolonic in 34% and lesions at the level of the gastrointestinal tract in 3.8% of the cases. However, there is a majority of approximately 49% of cases of colitis, a higher percentage than the one reported in the specialized literature, possibly also due to the subdiagnosis of localizations in the small intestine in the context in which colonoscopy is much more accessible than CT enterography or MR enterography investigations or capsule endoscopy exploration of the small intestine or due to regional peculiarities of the disease.

As regards the Montreal classification, there is controversy in the specialized literature in terms of phenotypes. Recent studies have shown that, over time, the phenotype of Crohn's disease can change, the patients with an inflammatory form at the onset being able to develop stenotic or fistulizing complications in the follow-up period of 5, 10 or 20 years (15).

The authors of the Norwegian IBSEN cohort study reported stenosing phenotype in 28% of the patients, fistulizing in 12% and inflammatory in 61% of the patients. After a 5-year follow-up period, the percentages changed significantly, showing an increase in the number of fistulizing and stenosing forms to the detriment of the inflammatory ones. After 5 years of follow-up, 33% of the patients had stenosing phenotype, the fistulizing forms increased to 21%, while only 47% of patients had non-stenosing, non-fistulizing phenotype (12).

In the EC-IBD study, a much higher rate of the inflammatory phenotype, of 78%, was identified in patients with Crohn's disease, while the stenosing and fistulizing forms were diagnosed in much fewer patients, namely 16% and 8% respectively (13).

In a cohort study performed in France on 1199 patients with Crohn's disease, monitored over a period of 8.6 ± 7.9 years, 60% of the patients developed stenosis or fistula. Of these, 13% developed stenosis, 27% developed perianal fistula and 20% intestinal fistulas. The stenosing lesions were associated with ileal and jejunal forms, and the fistulizing ones were associated with colonic localizations (16).

This discrepancy of data from one study to another is also explained by the lack of uniformity in estimating phenotypes in many centers. In some patients, the diagno-

sis of stenosis or fistula was established intraoperatively, without being identified prior to the surgical procedure by means of imaging methods.

In our study, the phenotype analysis highlights a majority of 52.8% of the non-stenosing, non-fistulizing phenotype, the stenosing and fistulizing forms being found in 24.5% and 22.6% of cases. Even though the inflammatory form predominates, there are an increased number of cases of complicated forms, possibly also due to the fact that the study was conducted in a tertiary care center providing treatment for more complex medical cases.

In terms of the association with the disease activity, the moderately severe lesions predominate in patients with ileal localization of the disease and the mildly-moderate forms predominate in patients with colonic and ileocolonic localization. We consider the higher degree of severity of the disease in intestinal forms to be explicable, as these are the localizations most frequently associated with weight loss, palpable mass, abdominal pain, symptoms that fall into the calculation of the CDAI severity score.

Our study shows that the mild-moderate forms predominate in the stenosing phenotype, while the moderately severe forms predominate in patients with penetrating phenotype. We interpret the association between the stenosing phenotype and the mild-moderate activity in the context of systemic manifestations that are less expressed than in the other phenotypes, the symptomatology being insidious until complications such as intestinal obstruction occur. On the other hand, the penetrating phenotype is more frequently accompanied by complications such as fistulas and abscesses having a more visible mark on the classes of symptoms that make up the CDAI score.

CONCLUSIONS

In our study, male patients from the urban areas predominated. The most common localizations involved the colon (colonic and ileocolonic forms). In terms of phenotype, there is a clear predominance of the inflammatory phenotype, which was present in more than half of the patients. Nonetheless, the more complicated forms of the disease (stenosing and penetrating) were identified in an increasing number of patients, largely due to the fact that the study was performed in a referral center. The moderate - severe activity was predominant for the ileal localization and the mild - moderate activity was predominant for the colonic and ileocolonic localization .The stenosing forms were most frequently characterized by mild-moderate activity, while the penetrating forms were most frequently characterized by moderately severe activity.

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ADVERSE REACTIONS OF BIOLOGICAL THERAPY FOR PSORIASIS

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ADVERSE REACTIONS OF BIOLOGICAL THERAPY FOR PSORIASIS (Abstract): **Aim:** To detect in patients with psoriasis the adverse effects during TNF-α inhibitor therapy. **Material and methods:** Fifty-seven patients with psoriasis, aged between 12 and 75 years were analyzed. They were treated with different TNF-α antagonists, the maximum treatment duration being 59 months. All patients were followed monthly after the initiation of therapy by clinical checkup, then every 3 months during the first 6 months of treatment by laboratory screening, and then every 6 month. Chest x-ray and tuberculin intradermal skin test were performed annually or as needed. All symptoms reported by patients were recorded, the treating doctor deciding the need for additional investigations or specialist consult. **Results:** Of the total of 57 patients with psoriasis on biological therapy, 9 patients developed diseases requiring temporary or permanent discontinuation of therapy. The recorded adverse reactions were: infectious (pulmonary tuberculosis, pulmonary empyema), oncologic (rectal cancer, renal cancer), dermatologic (vesiculobullous erythema multiforme major, nodular hypodermtis, secondary erythroderma, and hives) disorders. **Conclusions:** Despite its adverse reactions, biological therapy is safe and is a necessary tool in the treatment of moderate and severe forms of psoriasis unresponsive to other treatments. **Keywords:** PSORIASIS, BIOLOGICAL THERAPY, ANTI-TNF-α.

Psoriasis is a chronic inflammatory disease, affecting the growth and differentiation of the epidermis, with polygenic predisposition and various trigger factors (infections, drugs, physical or emotional trauma, stress) (1, 2). Its prevalence ranges from 0.1 to 11.8% in Europe and the United States, with an average incidence of 2-2.5% and equal gender distribution (3, 4).

Biological therapy in dermatology represented an important step in treating chronic conditions with significant impact on patient quality of life. For the treatment of psoriasis there are already a number of molecules with favorable results, some approved for years, others new, of which we mention the anti-CD11a agents (efalizumab), anti-TNF-alpha agents (etanercept, adalimumab, infliximab), and IL-12/IL-23 antagonists (ustekinumab). The mechanism of action of these agents consists in specific blocking of certain steps in the cascade of molecular events involved in the pathogenesis of this complex disease. Being a

last generation therapy, trials to assess its side effects and compare the effects of these molecules are in progress, and their long-term safety is continuously assessed (3, 4).

MATERIAL AND METHODS

The study group included 57 patients with psoriasis, aged 12 to 75 years, most of them from rural areas (35 patient = 61.4%). They received different TNF alpha antagonists, the maximum treatment duration being 59 months. During treatment, 9 (15.78%) of the 57 patients developed adverse reactions. All patients were followed up monthly after the initiation of therapy by clinical checkup, then every 3 months during the first 6 months of treatment by laboratory screening (blood count, ESR, creatinine, SGOT, SGPT, urine test), and then every 6 months. Chest x-ray and tuberculin intradermal skin test (IDR) were performed annually or as needed, depending on patient complaints and findings of pneumology consultation. All symptoms reported by patients were recorded, the treating doctor deciding the need for additional investigations or specialist consults. The correct administration was rigorously followed no matter of the molecule recommended for treatment.

RESULTS

Of the total of 57 psoriasis patients on biological treatment, 9 (15.78%) patients developed diseases requiring temporary or permanent discontinuation of therapy. The recorded adverse reactions were: infectious (tuberculosis, pulmonary empyema), oncologic (cancer, rectal cancer, renal cancer), dermatologic (vesiculobullous *erythema multiforme* major, nodular hypodermitis, secondary erythroderma, hives).

Of these, 4 presented lung infections: 3 (5.26%) patients developed pulmonary tuberculosis, 1 (1.75%) patient on treatment with infliximab and another (1.75%) patient on adalimumab. Another (1.75%) patient was diagnosed with pulmonary tuberculosis after the switch from infliximab to adalimumab due to lack of efficiency. The fourth patient developed pulmonary empyema after therapy with etanercept (1.75%). The shortest interval between the initiation of biologic therapy and the occurrence of infectious complications was 3 months for infliximab, 5 months for etanercept, and the longest, 59 months, for adalimumab.

In the patient in whom the pathologically confirmed left secondary infiltrative nodular pulmonary occurred short time after the initiating biologic therapy with infliximab, i.e. three months, we suspect a poor screening, resulting in the reactivation of a latent form of tuberculosis. Before the lung disease was detected, treatment with infliximab resulted in a moderate decrease in PASI score (Psoriasis Area Severity Index) from 39.3 to 30.8. After 38 months, following triple TB treatment (isoniazid, rifampicin and pyrazinamide) and confirmation of clinical and bacteriological cure, a switch to adalimumab was initiated. The patient is still on adalimumab treatment, totaling 28 months.

The patient who after 22 months of infliximab treatment was switched to adalimumab due to lack of efficiency (initial PASI = 55.2, PASI at 22 months = 29.4, < 50% of baseline PASI) developed pulmonary tuberculosis 10 months after switching. Incriminated were the socioeconomic status, conditions, underweight, diet low in vitamins and nutrients, and poor rural are he lives in.

The patient on systemic treatment with adalimumab for 57 months, with an obviously favorable course of skin symptoms (PASI score at onset = 26.8, then it gradually decreased to 6.1 at 3 months, 1.2 at 6 months, reaching 0 after 12 months of treatment) was diagnosed with pulmonary tuberculosis, pathologically confirmed by bronchoscope biopsy. The onset form of skin disease was psoriasis vulgaris, but subsequently after applying a topical irritant (Swedish bitters) it turned into a generalized pustular psoriasis treated with long-term systemic corticosteroids. Disease-induced immunosuppression and exacerbated by prolonged systemic corticosteroids probably favored the occurrence of active tuberculosis.

In the study group, malignancies were recorded in 2 (3.50%) patients none of them with a family history of cancer. One of these 2 patients with psoriasis was treated with infliximab for 28 months and due to the lack of response reflected in PASI score = 18.2 (PASI score <50% of baseline PASI = 24.5) he was switched to adalimumab. The course of psoriasis lesions was favorable (PASI at 3 months = 12.4, at 6 months =1.2, and at 12 months =0). But, after 11 months of treatment with adalimumab the presence of a left renal tumor stage cT1bN0M0 was detected, and consequently adalimumab was discontinued. Left radical nephrectomy was performed and pathology findings confirmed the diagnosis.

The second patient was treated with adalimumab for 40 months, with a favorable course of skin lesions and psoriasis-related joint disease (PASI score decreased gradually from 24.2 at baseline to PASI = 8.1 at 3 months, PASI = 0.4 at 6 months, and PASI = 0), until because of fresh blood rectorrhages he was referred to the gastroenterology unit. Colonoscopy revealed a vegetant, friable tumor mass, 10-12 cm from the anus, for which the pathology diagnosis was of infiltrating, moderately differentiated adenocarcinoma. A Hartmann operation with mesorectal excision was performed, the final diagnosis being ampullary mid-rectal cancer.

Adverse dermatological reactions were reported in 4 (7.01%) patients. After 5 months of etanercept, 1 (1.75%) patient presented pleural empyema, so the treatment was discontinued. One month after the last etanercept administration the same patient developed nodular hypodermitis, diagnosis confirmed by pathological examination of skin biopsies. Immediately after this event, the patient received treatment with sulfasalazine for 1 year and for 54 months she is on therapy with adalimumab. After 24 months, due to the severity of joint lesions methotrexate 7.5 mg/week was added at the recommendation of a rheumatologist, with a slowly favorable course, without complete remission of skin lesions, in the context of an unfavorable family climate (husband with lower limb amputation, daughter's death). Initial PASI was 51.2, and decreased gradually during the 54 months to PASI = 4.3.

Another (1.75%) patient developed severe vesiculobullous *erythema multiforme* after 4 weeks of infliximab administration, namely after 2 injections of infliximab, which responded well to short-term average-dose systemic corticosteroids. After the remission of allergy episode the patient resumed the biologic treatment regimen for the treatment of psoriasis with a favorable outcome. After 4 months the patient decided to discontinue the treatment in view of conceiving a child, but the occurrence of

psoriasis lesions within the 16-week free interval required the introduction of a new biological treatment, namely adalimumab, followed for 59 months with a favorable course of psoriasis.

The other two (3.50%) patients, one on etanercept and the other one on infliximab, developed secondary erythroderma after 19 and 30 months, respectively. On therapy, PASI score had an undulating course in both cases, starting from PASI = 10.8 and PASI = 26.2, respectively, with a tendency to decrease and then increase due to lesion accentuation and extension. Additionally, the patient receiving infliximab also presented at the next infusion, after another two months, an episode of acute urticaria, resolved by the administration of antihistamines. In both cases, treatment was discontinued due to its lack of efficiency, PASI score reaching 26.2 and 52.6, respectively at last reassessment.

Three of the patients experiencing adverse reactions to the administered biologic therapies, namely the patient with pulmonary tuberculosis occurred after 3 months of infliximab, the patient with ampullary mid-rectal cancer and the patient with pleural empyema and nodular hypodermitis were also diagnosed with psoriatic arthropathy. These patients have an additional depression of their immune status due to the aggressiveness of the arthro-pathy disease itself.

In our patients the degree of immunosuppression differed, being higher in those who received treatment with methotrexate and general corticotherapy (patient with pulmonary tuberculosis after 57 months of adalimumab treatment), followed by therapy with methotrexate (therapy administered prior to biologic therapy in 6 of the 9 patients), less affected being the only patient on PUVA (Psoralen and ultraviolet A light) therapy prior to biologic therapy (who developed *erythema multiforme* after 2 infliximab infusions, subsequently switched to adalimumab).

DISCUSSION

Before the introduction of mandatory screening for active pulmonary tuberculosis, its rate was high during biologic therapies, accounting for 19/100,000 patients a year (5). Later, when all patients on biologic therapy were screened for tuberculosis, between 2002 and 2006 its incidence has decreased to 7 (5). In case of 100% compliance with the screening protocol tuberculosis rate decreased to 1.8 per 100,000 patients a year but increased to 13 if compliance was below 100% (5).

In our study group the screening for active pulmonary tuberculosis was performed in 100%. Of all 57 patients on biologic therapy, 25 underwent isoniazid chemoprophylaxis as recommended by the pulmonologist. None of the 3 patients who developed pulmonary tuberculosis required prophylactic medication according to the screening performed prior to the initiation of biologic therapy.

Most cases of tuberculosis on adalimumab treatment occurred during the first 8 months of treatment (6). In our study, 1 of the 57 patients developed tuberculosis after 57 months of treatment with adalimumab.

The literature (7) suggests an increased incidence of neoplasms among patients with psoriasis without biologic treatment, compared with the general population. Patients with psoriasis are at a 76% higher risk for developing skin cancer and a 82% higher risk for developing lymphoma compared to subjects in the control group

(without psoriasis), with a statistically significant p value <0.001.

In patients with rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis and Crohn's disease, malignancies, except lymphoma and non-melanoma skin cancer, have occurred at a rate of 0.6 per 100 patient-years versus a rate of 0.4 per 100 patient-years for placebo-treated patients. The mean treatment duration was 5.7 months for adalimumab and 5.5 months in the control group (7). In clinical trials (7), the most common cancers other than lymphoma and non-melanoma skin cancer were breast, colon, prostate, and lung cancers and melanoma. The incidence rate of these cancers is similar to that in general population.

Dermatological adverse reactions reported in the literature (6) were: cellulitis, erysipelas, cutaneous vasculitis, *erythema multiforme*, Stevens-Johnson syndrome, alopecia, worsening of psoriasis or occurrence of other forms of psoriasis in the same patient.

As to etanercept, new cases or reactivation of tuberculosis have been reported, including patients on treatment for latent or active tuberculosis. Studies suggest that the risk of latent tuberculosis reactivation is lower compared with therapy with TNF-alpha blocking monoclonal antibodies (8, 9). Tuberculosis was reported in approximately 0.01% of patients in trials globally. The risk of developing severe infections is higher in those on therapy associated with immunosuppressive drugs such as methotrexate, and patients older than 65 years. As to malignancies, it was found that in patients on treatment with etanercept the risk of developing lymphoma is about 3 times higher than in general population. Cases of acute or chronic leukemia (0.06 cases per 100 patient-years) *versus* 0 in the control group have been reported. Duration of controlled treatment ranged from 3 to 48 months (10). Sixty-seven malignancies other than lymphoma have been reported, among which the most common are colon, breast, lung, and prostate cancers. In patients with psoriasis, 22 of 1261 (1.74%) patients were diagnosed with cancer. Of these, 9 were non-cutaneous solid tumors, 12 non-melanoma tumors (8 basal cells, 5 squamous cells) and 1 patient had Hodgkin lymphoma. Among placebo-treated patients only one developed two squamous cell cancers (10). Of the cutaneous adverse reactions we mention: *lupus erythematosus*, cutaneous vasculitis (including leukocytoclastic vasculitis), *erythema multiforme*, Stevens-Johnson syndrome, Lyell syndrome, subcutaneous nodules, worsening or occurrence of a new type of psoriasis in the same patient (10).

As to infliximab, during clinical trials tuberculosis was reported in 14 patients, of whom four died. Most tuberculosis cases were diagnosed during the first 2 months after the initiation of infliximab therapy (11). In our study group, one patient developed tuberculosis 3 months after the initiation of therapy. Infiximab treatment was discontinued and after 3 years therapy with adalimumab was initiated, the patient still being on this treatment, with a favorable course for 26 months.

Cases of lymphoma, skin, breast, and colorectal cancer, leukemia, Sezary syndrome, Merkel cell carcinoma, hepatosplenic T-cell lymphoma (most often in patients treated with infliximab - 20 compared with one in patients on etanercept, and 2 on adalimumab) have been reported. The risk of developing lymphoma is 3 times higher in those treated with inflixi-

mab compared to general population (12). The following cutaneous side effects have been reported: rash, which is the most common, followed in frequency of pruritus, cellulitis, ulceration, interstitial granulomatous dermatitis, furunculosis, psoriasiform rash, cutaneous vasculitis, erysipelas, atopic dermatitis, necrotizing fasciitis, bullous skin lesions, *tinea corporis*, *varicelliform exanthema*, palmoplantar pustulosis, atypical cutaneous leishmaniasis, *alopecia areata*, *herpes labialis*, cutaneous lymphomas, *erythema multiforme*, seen in patients with rheumatoid arthritis or Crohn's disease. In the interval August 1998 - August 2006, FDA (13) reported 21 severe skin reactions in patients treated with infliximab, of which 15 patients had *erythema multiforme*, 5 Stevens - Johnson syndrome and one case toxic epidermal necrolysis. The mean time to the occurrence of adverse reactions was 28 days after the first infusion, in this case exactly 4 weeks.

Studies comparing the effectiveness, safety and rate of side effects of these three molecules are useful. For example, Sallit et al. (8) in a recent retrospective study compared the infection rate in the same patients before and during treatment with TNF-alpha blockers. The infection rate after biologic treatment initiation was 34.5%, compared to 6.7% before treatment. Etanercept, adalimumab and infliximab were compared. The fewest infectious events were reported in patients on adalimumab (15.3% of patients), followed by etanercept (34.5%) and infliximab with the highest rate of infection (50.5 %). Instead, the rate of serious infections was lowest with etanercept (4%) (8). Other studies (9, 10) have also shown that the risk of developing certain infections is lower when using etanercept.

CONCLUSIONS

Despite the adverse reactions reported in this study, biologic therapy is safe and is a necessary tool in the treatment of moderate and severe psoriasis that do not respond to other therapies. Adverse reactions are usually very rare, but they must be quickly diagnosed and properly monitored. Systematic international reporting on adverse reactions to biologic therapy helps creating a clear image on the safety profile useful to the practitioner.

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NEWS

COMPARISON OF THE EFFECTIVENESS AND ANTIBIOTIC COST AMONG CEFTRIAXONE, ERTAPENEM, AND LEVOFLOXACIN IN TREATMENT OF COMMUNITY-ACQUIRED COMPLICATED URINARY TRACT INFECTIONS

Most of urinary tract infections (UTIs) are uncomplicated for healthy young women that are easily managed with short-term antimicrobial therapy. Complicated UTIs (cUTIs) may occur in both sexes at all age groups and are frequently associated with anatomical or functional urinary tract deficiencies. This research has compared the treatment of urinary tract infections with ceftriaxone, ertapenem and levofloxacin. At the same time, in this study, it was analyzed the quality-price ratio of the three antibiotics. The primary outcome was the time to defervescence after admission and the secondary outcome was duration of hospital stay. The antibiotic cost was calculated as the sum of all antibiotic usage during hospital course. The study compared the clinical characteristics and antibiotic costs in patients with cUTIs receiving three different antibiotics. Compared to patients in the other two groups, ertapenem group had shorter time to defervescence since admission and shorter hospitalization stay. The antibiotic cost was significant lower in ceftriaxone group. This study is subject to several limitations regularly found in retrospective studies. A well-conducted prospective study may be necessary in the future to elucidate the differences in treating cUTIs among the three agents. In conclusion ETP, CRO, and LVX in the treatment of cUTIs had good clinical response. (Lin H-A, Yang Y-S, Wang J-X et al. Comparison of the effectiveness and antibiotic cost among ceftriaxone, ertapenem, and levofloxacin in treatment of community-acquired complicated urinary tract infections. *J Microbiol Immunol Infect,* 2015.

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INCIDENCE OF PRIMARY CARDIOVASCULAR RISK FACTORS IN PATIENTS WITH ERECTILE DYSFUNCTION

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INCIDENCE OF PRIMARY CARDIOVASCULAR RISK FACTORS IN PATIENTS WITH ERECTILE DYSFUNCTION (Abstract): **Aim:** Identification of primary cardiovascular risk factors in patients with erectile dysfunction. **Material and methods:** The study included 308 apparently healthy men, with a mean age of 45±12.1 years. The Sexual Health Inventory for Men (SHIM) questionnaire was used to determine the following indices: BMI, blood pressure, blood glucose, cholesterol, triglycerides, etc. **Results:** The total incidence of cardiovascular risk factors was 56%: 45% in men aged up to 40 years (group I) and 74% in those over 40 years (group II). High blood pressure was found in 9% of the men in group I and 55% in those in group II ($p < 0.05$). Blood glucose levels were recorded in 5% of group I patients 24% in group II patients. Sixty-six percent of the men over 40 years were overweight compared with 16% of men under age 40. Dyslipidemia was present in 65% of group I versus 19% in group II. **Conclusions:** Our study shows that especially in men over 40 years of age erectile dysfunction is a vascular endothelial condition clearly associated with cardiovascular risk factors; the direct association between the severity of erectile dysfunction and increased levels of cardiovascular risk factors reflects their importance in the occurrence and course of the disease, which should be taken into account when diagnosing an erectile dysfunction and selecting the therapeutic approach. **Keywords:** ERECTILE DYSFUNCTION, CARDIOVASCULAR RISK, INCIDENCE.

Erectile dysfunction (ED) is characterized by the inability of a man to achieve and maintain erection sufficient to permit satisfactory sexual intercourse. The worldwide incidence of erectile dysfunction ranges from 19% to 57% and depends on the socioeconomic conditions (2). Linear correlations between ED and cardiovascular diseases with the highest death rate (1) have been demonstrated since the MMAS and Colonge studies conducted in 1994 and 1998. According to the MMAS study, 39% of men treated for a cardiovascular disease have indicated the complete absence of erection (3). In the Colonge study, the prevalence of erectile dysfunction in hypertensive men was 36%, compared with 19% of the control group (4). The risk factors for cardiovascular disease are shared with ED, namely age, atherosclerosis, hypercholesterolemia, dyslipidemia, obesity, hypodynamia, etc.

It is worth mentioning that over 35% of hypertensive men and 66% of men with cardiovascular disease for at least 5 years have ED (5, 6).

ED is largely a consequence of other diseases that can serve as an early marker of cardiovascular risk and an independent prognostic predictor of cardiovascular events in middle-aged and advanced-age men. ED and cardiovascular disease are rarely diagnosed concomitantly, with erectile manifestations occurring 2-3 years earlier. Because penile arteries are smaller in diameter than coronary arteries, atherosclerosis, at a similar degree of endothelial dysfunction, causes a significantly greater and earlier reduction in blood flow in the erectile tissues supplied by the affected vessels.

Currently, it is not known, and it is not supposed that primarily detected ED could be the consequence of other diseases, cardiovascular diseases included, the causes of this disease being often related to age, prostate diseases or some psychological causes.

The presented data demonstrate that the penile vascular bed is a sensitive indicator of systemic vascular diseases (7, 8) which requires the early detection of cardiovascular risk factors that would contribute to the correct diagnosis and initiation of the appropriate treatment.

MATERIAL AND METHODS

This is a prospective study that was conducted between 2010 and 2016 and included 308 patients who sought help for ED in the absence of other previously diagnosed and/or treated conditions. The age of patients ranged from 21 to 73 years, mean age 45 ± 12.1 years. The patients were divided into two groups according to age: ≤ 40 years, 152 patients (Group I) and >40 years, 156 patients (Group II).

To make the diagnosis and determine the degree of ED, we used the SHIM questionnaire. The attitude towards physical activity and smoking was studied. Physical and instrumental examination included: determination of body mass index (BMI), pulse rate and blood pressure. Also determined were: total cholesterol and LDL and HDL fractions, triglycerides and basal glucose level.

The study results are presented in absolute and relative values. For statistical processing, the *Epi Info*^{TM} version 7 (Centers for Disease Control and Prevention, Atlanta, GA, USA) was used.

RESULTS

The presence and degree of ED., Following the analysis of SHIM questionnaires a severe degree of erectile dysfunction was present in 55 patients (18%), including 16 (11%) patients in group I and 39 patients (25%) in group II (tab. I).

TABLE I

Degree of erectile dysfunction severity by study groups (n = 308 patients)

Degree of ED	IIFE5	Group I No %	Group II No %	Total No %
Severe	6.3±2.5	16 (11)	39 (25)	55 (18)
Moderate	13.2±1.8	59 (39)	79 (51)	138 (45)
Mild	17.8±1.9	77 (50)	38 (24)	115 (37)
Total		152 (100%)	156 (100%)	308 (100%)

A tendency for ED to worsen was found with aging, when most patients have moderate and severe forms of ED.

Cardiovascular diseases, arterial hypertension and ED share the same risk factors, namely hypodynamia, obesity, dyslipidemia, hyperglycemia, among others.

Of the 308 enrolled men, only 31% practice daily or regular sports and 29% have a sedentary activity. In people over age 40, sedentary activity is preferred by every other person, and among patients under 40, every fourth individual is physically inactive.

Only 88 patients (29%) said they were non-smokers, the remaining 71% smoked or had previously smoked. By age groups, the percentage of smokers was not significantly different. Differences were evident among non-smokers with ED. Among the young patients, the percentage of non-smokers was 55%, compared with 40% in patients over age 40.

Body Mass Index (BMI) assessment showed that in 180 (58%) patients BMI was less than 26 kg/m2, and 128 (42%) had excess body weight. By age groups there was a statistically significant difference in terms of excess body mass. In group I only 25 men (16%) had a high BMI, and only 3 (2%) were diagnosed with obesity, 127 (84%) being normal weight. Among the patients aged over 40, only 53 (34%) were normal weight, 60 (38%) were overweight and 43 (28%) were obese.

Blood pressure was determined in all patients. In individuals with high blood pressure, we performed repeated resting examinations at 10-minute intervals to exclude the influence of external factors. Repeated blood pressure measurements showed elevated levels in 100 patients (32%), including 14 (9%) group I and 86 (55%) group II patients.

Assessment of lipid profile showed that the level of cholesterol or its LDL fraction was elevated in 130 (42%) patients, with a statistically significant difference between study groups ($p < 0.05$). Thus, 101 (65%) patients belonged to group II and only 29 (19%) to group I. Triglyceride levels greater than 200mg/dl were recorded in 85 (28%) patients. Like previous situations, there was a significant age-related difference. Thus, in group I only 23 patients (15%) presented hypertriglyceridemia, compared to 62 (40%) in group II ($p < 0.05$).

The presented data demonstrate a significant dysregulation of lipid metabolism in over 65% of the patients older than 40 years with primarily detected ED.

TABLE II

Incidence of cardiovascular risk factors primarily detected in patients with erectile dysfunction by age groups

Risk factors	Group I, up to age 40 No %	Group II, over age 40 No %	Total No %
Absence of physical activity	86 (57)	123 (79)	209 (68)
Smoking	69 (45)	94 (60)	163 (53)
BMI >26 kg/m^2	25 (16)	103 (66)	128 (42)
Hypertension	14 (9)	86 (55)	100 (32)
Elevated cholesterol	29 (19)	101 (65)	130 (42)
Elevated triglycerides	23 (15)	62 (40)	85 (28)
Basal blood sugar>6.69	8 (5)	37 (24)	45 (15)

The increased incidence of obesity, dyslipidemia or changes basal blood glucose levels in elderly men is probably explained by the fact that the compensatory reserves of the body begin to deplete at that age.

Two or more cardiovascular risk factors were present in 116 (74%) patients aged over 40, as compared to 68% (45%) of the patients with erectile dysfunction aged up to 40.

Penile pharmaco-Doppler ultrasonography performed to determine blood circulation, systolic velocity, and degree of ED of vasculogenic origin in 95 patients (31%), representing 48 (32%) group I patients and 47 (30%) group II patients is shown in figure 1.

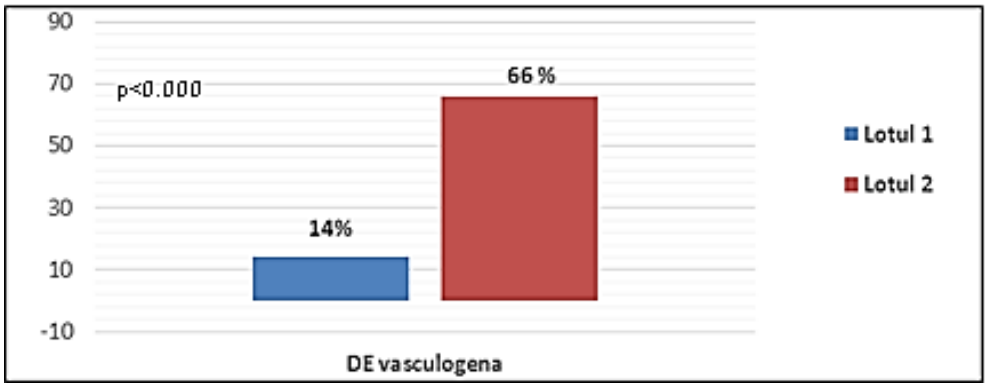

Fig. 1. Presence of erectile dysfunction of vascular origin in study group patients, according to penile pharmaco-Doppler ultrasonography (95 patients).

Determination of fasting basal blood glucose showed levels greater than 6.99 mmol/l in 45 (15%) patients, 8 (5%) in group I and 37 (24%) in group II, respectively. According to the criteria of the American Diabetes Association an increase in basal blood glucose after a single reading in the absence of metabolic decompensation criteria requires repeat testing on different days or a glucose tolerance test (9).

The results of penile Doppler ultrasound (fig. 1) showed a decreased blood flow with a decrease in systolic velocity, respectively the presence of a vasculogenic ED in 7 (14%) group I patients compared to 31 (66%) group II patients ($p < 0.0001$).

DISCUSSION

Cardiovascular diseases are still the leading cause of global mortality, stimulating the search for new directions in the early diagnosis of cardiovascular risk factors. Of major importance are the changes in vascularization at macro- and microcirculatory level induced by ischemia which are strongly dependent on intimal thickening of small intramyocardial arteries and increased coronary tone with diminished coronary blood flow and diminished dilatation of coronary arteries with decreased coronary reserve (10, 11).

ED is considered a derivative of cardiovascular diseases but equally as a possible vascular endothelial disease that is often the first manifestation of cardiovascular diseases. Data from scientific studies have shown that ED in patients over the age of 40 is in most cases associated with a cardiovascular disease not clinically manifest at the time of presentation, or with biochemi-

cal changes associated with increased cardiovascular risk. Montorsi's reference study showed that ED is present in 47% of patients with angina pectoris compared to 24% in healthy males. In patients with acute single-vessel coronary artery disease, the frequency of ED was of 22% and in case of double, or triple-vessel disease of 55%. The duration of ED may have a direct prognostic role for the subsequent risk of cardiovascular events (12).

The third Princeton Consensus Recommendations (13) have considered that ED can be a predictor of clinically nonmanifest cardiovascular diseases. Men who experience signs of ED require complex cardiovascular assessment, with the determination of exercise tolerance and risk of cardiovascular events. In case of a low risk, any treatment for ED may be initiated; the moderate risk involves some additional investigations, and the increased risk requires making a diagnosis and prior treatment of the possible cardiovascular diseases.

Our study aimed at assessing the cardiovascular risk factors in patients with ED who consider themselves healthy. In young patients the absence of cardiovascular risk factors was reported in more than 65%, ED having a rather psychological substrate, while in patients aged over 40 it was reported in only 26%, the organic substrate being the most common.

We have also identified the presence of some risk factors and have diagnosed some diseases in patients who considered themselves healthy. Thus, the incidence of hypertension was of 32%, and of 55% in men over 40 years of age. Fifteen percent of the study patients, 24% of patients aged over 40, respectively, had elevated blood glucose levels. Men with diabetes mellitus are at increased risk of developing ED, which usually occurs about 10 years earlier, compared to men without diabetes. Among the common risk factors, we noticed the high percentage of smokers, 53%. Smoking is one of the unanimously accepted risk factors for developing atherosclerosis and cardiovascular diseases even in the absence of hyperlipidemia and diabetes mellitus by inducing oxidative stress and contributing to the development of atherosclerotic plaques.

In this study, we found a relatively high incidence of obesity and hyperlipidemia, more common in the elderly. Sixty-six percent of elderly men had high BMI, compared with 16% of the younger men. The incidence of dyslipidemia in men older than 40 years with ED was 64%, compared to 19% in men aged up to 40. In some European studies, the incidence of dyslipidemia depending on age, gender and geographical region is 37.2% - 65% (14).

Often physical or sexual activity may be the cause of an acute cardiovascular event (15). Our study shows that patients with ED primarily detected when older than 40 years frequently associate a cardiovascular disease that should be considered when assessing such patients.

CONCLUSIONS

Our study demonstrates that especially in men over 40 years of age erectile dysfunction is a vascular endothelial disease with associated cardiovascular risk factors; the direct dependence between the degree of erectile dysfunction severity and the associated cardiovascular risk factors reflects their importance in the occurrence and course of this disease, and should be taken into account when making the diagnosis and selecting the therapeutic approach.

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PROGNOSTIC FACTORS IN RESECTABLE PANCREATIC CANCER

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PROGNOSTIC FACTORS IN RESECTABLE PANCREATIC CANCER (Abstract): The pancreatic cancer is a disease with exponentially increased incidence, especially over the last decades, being the sixth or even fifth cause of death by cancer in most of the modern societies. Moreover, it is estimated that almost 95% of the patients with this disease are presenting to the doctor in the advanced and unresectable stages. Also, over the past few decades the development and advance of the surgical methods and techniques have improved only morbidity and hospital postoperative mortality, without significant impact on survival. In this way, in the present mini-review we want it to make a short description for some modern aspects regarding the factors implicated in pancreatic cancer survival. **Keywords:** PANCREATIC CANCER, SURVIVAL, MARKER, PROGNOSTIC FACTORS

Lately, the incidence of pancreatic neoplasm has increased steadily, with the pancreatic ductal adenocarcinoma being the most common malignant disease of the pancreas, with the origin in the ductal epithelium and with an incidence of 10-11 per 100,000 inhabitants in the Western countries and also being considered one of the most aggressive human tumors (1).

In addition, this is the fourth cause of death by cancer in North America, having approximately 31,000 new cases per year in Canada and in the United States and, unfortunately, about as many deaths each year, and the also the fifth cause of death by cancer in the Western hemisphere. Moreover, the fatality rate correlates with the age varies between 3.1 and 20.8 per 100 000 inhabitants with a corresponding peak for the patients between 60 and 80 years (2).

Also, surprisingly, despite the numerous studies about pancreas cancer, little is known about this issue. The resections for the cancer of pancreas have entered the therapeutic arsenal for 65 years, but despite the fact that hundreds of articles were written on this subject, there is no unanimity of opinion concerning the efficacy of resection. A number of authors have reported a 5 years survival rate of 30% or even 58%, being supportive of resection (3-5).

Moreover, most of the studies carried out have not been able to identify the significant risk factors for pancreatic cancer. Still, several risk factors were described as being involved: smoking, chronic pancrea-

titis, diabetes mellitus, previous gastrectomy and exposure to radiation or chemicals such as chlorinated hydrocarbon based solvents (6, 7).

Also, the prognosis of the patients with pancreatic cancer and an indication for curative intent resection is determined by lymphatic metastasis, vascular walls and nerve peripancreatic nerve plexuses invasion, and also by the progression of micrometastases in the neighboring tissues and organs.

Unfortunately, 95% of the patients arrive in the advanced, unresectable stages.

Thus, over the past few decades the development and improvement of surgical methods and techniques have improved only the hospital post operatory morbidity and mortality, however without having a significant impact on survival.

In this way, in specialized pancreatic surgery centers the mortality is less than 5%. Also, the long-term 5 years survival after R0 resection is reported in literature as being under 10% per series of screened patients, with no record of recurrence during this period. These results are at odds with other studies which reported the values of the actuarial 5 years survival between 20 to 40% of the resected patients. A possible explanation lies in the fact that the Kaplan-Meier statistical method for assessing the survival is excluding the patients deceased in hospital, as well as those who were lost in the surveillance. Therefore, other methods and criteria should be used to correctly assess the results of the resections, such as the actual survival.

Regarding the adjuvant treatment used for the prolongation of survival after oncologic resection, some studies have suggested the benefit for the application of adjuvant chemotherapy and radio chemotherapy for 6-10 months (even routine application to each patient was suggested!).

A neoadjuvant protocol for pancreatic cancer is reconsidered in recent years, based on the findings of several studies regarding the extension of survival after neoadjuvant radio chemotherapy in 15% of the patients to which the tumor stage has been reduced and followed by oncologic visa resection (8).

Also, approximately 60% of the pancreatic cancers have cephalic localization. Moreover, along with the decreasing of operatory mortality following duodenopancreatectomy, improved survival rates of 30% have been reported (9-12) that are three times higher than the previously published results (13-15).

However, the improvement of early results (decreased mortality, increased rate of resectability) and late results (long-term survival) are two different concerns and, seemingly, without a causal relationship between them.

In this way, some surgeons are not fully confident in these sudden improvements of the survival indices, hence it was suggested by some the requirement of the original diagnosis anatomopathological reconfirmation, and also other authors have proposed a standardized method of evaluation/ reporting of the survival data and identifying the subsets of patients (16).

Thus, in order to find out which subgroup of patients will have greater survival after resection; numerous studies analyzed and confirmed certain factors with prognostic value, such as the size and extension of the tumor, lymph nodes metastasis, the degree of differentiation of tumor cells and tumor involvement of the margins of resection.

Hence, by analyzing the parameters of survival, Han et al. have argued that the statistical evaluation method used is partic-

ularly important: the actual *vs.* the actuarial. In this way, if the data on survival do not contain 'censor date' - patients who have been lost in the follow-up or patients who were alive at the time of the study - there is no difference between actual and actuarial survival. However, this is almost impossible, considering the cases which inevitably are lost in the monitoring (17).

More than that, studies which contain patients who have survived more than five years, may overestimate the results, mainly due to the very nature of the Kaplan-Meier method. As a result, the aforementioned authors have reduced this source of error by selecting patients (n = 123) which have been resected at least 5 years prior to the study and have obtained a cumulative survival at 5 years of 12.1% and actual 5 years survival of 8.9% (11/123).

Comparing the previous data (5 years survival of 7.4%) obtained at their institution in 1986, the actual survival is similar in spite of the significant increase of resecability from 11.0% to 15.6% and the significant decrease of mortality (from 16.7% to 0.8%). Thus, it could not be stated that long-term survival after the resection for pancreatic cancer has dramatically improved (17).

In addition, Welch et al. group have studied the changes in terms of 5 year survival rate for 20 types of solid tumors by using the data from the National Cancer Institute from 1850-1995. They found minimal changes in terms of the 5 years survival rate (from 1% in 1950 to 4% in 1990) and suggested that the improved survival rate can be explained rather through early diagnosis than by reduced mortality by cancer (17).

Also, Yeo et al. conducted a very relevant study which included a group of 242 patients operated for periampullary vaterian adenocarcinoma at The Johns Hopkins Hospital. From these, 149 (62%) were pancreatic tumors, 46 (19%) were ampullary tumors, 30 (12%) distal bile duct cancer and 17 (7%) duodenal cancers. The surgical mortality was 5.3% along the 22-years review with 2% perioperative mortality for the last 100 patients. Also, the 5 years survival by type of tumor was as follows: for pancreatic cancer 15%, ampullary 39%, distal biliary route 27% and duodenal route 59%, while at 10 years survival was 5%, 25%, 21% and 59% respectively.

Moreover, the resected pancreatic tumors had an average diameter of 3 cm, 72% cases with ganglionic MTS and 31% with tumor positive resection margins (usually at the level of hepatic arteries and/or superior mesenteric artery). The average survival was of 12 months with the actual 5 year survival of 15% and actuarial 10 years survival of only 5%. Also, the survival continues to drop after 5 years, the later deaths being directly related to the tumor evolution, suggesting that the 5 years survival for the cancer of the pancreas is definitely not a cure (18).

Wagner et al. group has shown that curative resection is the only significant prognostic factor for survival (19). Moreover, the analysis of 650 consecutive pancreatic resections also showed that curative resection is an important factor influencing survival (20).

Geer et al. have reported the survival at 5 years of 245 from 246 resected patients while no patient of the 653 uneresected patients did survive for more than 5 years. They identified the ganglionic invasion, low tumor differentiation and tumor size > 2.5 cm as predicting factors (21).

Moreover, the group lead by Cameron et al. have reported survival at 5 years of 19% on a group of 89 resected patients and

have shown that the bad prognostic factors are the tumor related: size > 2 cm, presence of tumor adenopathies and vascular invasion (22), while Willet et al. have stressed the importance of performing pancreatic resections obtaining negative tumoral margins, a factor that has led to a 5 years actuarial survival of 22% compared to the 13% overall survival over the entire group (23).

Similar results have been also published by Trede et al. who analyzed the cases of pancreatic resections performed at Mannheim Surgical Clinic between 1972 and 1989. In this retrospective study the actuarial survival at 5 years was 24%. However, for the R0 and R1 resection the actuarial survival increased to 36%, and out of the 11 5-years survivors, 4 of them died due to the base disease (24, 25).

In addition, Baumel et al. conducted a multicenter retrospective study in France in order to analyze the short-term and long-term results of 787 pancreatic resections for cancer which were carried out between 1982 and 1988. The post operatory mortality was 10% and morbidity 35%. Moreover, the age over 70 years and the existence of an associated disease have affected the mortality rate. The median survival for patients who have been discharged was 12.3%, the 5 years actuarial survival was 12% - being lower, 4%, in patients with lymph node metastases as compared to those without ganglionic involvement - 20% (p=0.001).

Also, the surgical mortality was higher after total pancreatectomy than after cephalic duodenopancreatectomy (17% *vs.* 8%, p=0.015), while the median survival and the 5 years survival for total pancreatectomy and for duodeno-pancreat-ectomy was 11 *vs.* 14 months and respectively 3 *vs.* 15 months. Among the studied clinical and anatomopathological factors, the localization to the left of the tumor in the pancreas was strongly correlated with survival.

Duodenopancreatectomy remains the procedure of choice for pancreatic cancer, while total pancreatectomy is indicated only in patients with multifocal carcinoma or in those which cannot undergo a safe anastomosis (22).

Also in this area of research, Richter et al. argued that under the conditions of the resection of a pT1N0M0 tumor, the recurrence will occur, even if later, after 10 years. In fact, it is believed that the cancer of the pancreas is actually the cancer with the lowest 5 years survival rate. Moreover, Richter et al. assessed the long-term results from 194 patients who benefited from the standard Kausch-Whipple resection for pancreatic ductal cephalic adenocarcinoma. In this way, from the 194 patients, 56 patients (28.9%) required extensive venous resection due to the invasion of the portal vein, while 70% patients had a simple, complications free post operatory evolution, with the morbidity of 29.9% (58/194), and post operatory mortality of 3.1% (6/194).

Moreover, the hospital mortality rate was 3.6% (7/194). When confronting the pancreatic cancer patient, the surgeon actually has three options: a therapeutic 'nihilistic attitude', an 'aggressive', or a 'realistic' attitude. The nihilist advocates for a palliative treatment, non-resectable procedures even for resectable lesions, considering that patients will survive longer, with a superior quality of life, after a palliative by-pass than after a resection. Moreover, if taking into account the resections associated morbidity in comparison to that of palliative procedures, the cost-effectiveness ratio is in favor of palliation (23). Also, the aggressive surgical attitude involves a much more extensive, 'super-radical' resection: the total or regional

pancreatectomy with extensive lymphadenectomy regional based on the assumption that pancreatic neoplasm is a surgically curable disease only by extending the field of resection. Those advocating in favor of this attitude suggest even a more expanded lymphadenectomy: superior up the hepatic *hilum* and diaphragmatic hiatus, inferior to the AMI and laterally toward both renal *hila*. Still, until now no study published in literature has confirmed the positive effect of expanded lymphadenectomy on the prolongation of survival (24).

In addition to that, Yeo et al. has demonstrated, however, that expanded lymphadenectomy can be performed with morbidity and mortality similar to standard pancretectomy. However, in this case, the post operatory monitoring of patients from the studied group was too short (2 years) in order to draw meaningful conclusions (25).

Pedrazzoli et al. have confirmed that by extending the lymphadenectomy and the retroperitoneal tissue dissection *versus* standard operation does not increase post operatory morbidity and mortality (26).

Still, an improved effect on survival after extended lymphadenectomy has not been confirmed so far. In this way, by completing a retrospective nonrandom study, Ishikawa et al. compared the standard and the extended technique on 37 consecutive patients and found a year's survival rate of 13% after the standard technique *versus* the 38% following the extended lymphadenectomy (24).

Currently, the surgical mortality in specialized centers has decreased fewer than 5%, even with series of patients without mortality being reported (27, 28). Also, with the increasing number of patients surviving the pancreatic resections, an according increase in the number of the long-term survivors would be expected. However, in most centers the 5 years survival is reported around 5% (tab. I) (29).

TABLE I

5 years survival in some important international research centers

Study group	Institution	Patients (No.)	5-Year Survival (%)
Yeo	Johns Hopkins	201	21.0
Cooperman	Columbia (NY)	70	7.1
Lerut	Insel Spital Bern	25	6.0
Jones	Toronto	28	7.0
Grace	UCLA	37	3.0
Tsuchiya	Collective Japanese series (< 2 cm)	103	30.3
Connolly	Chicago University	89	3.4
Sarr	Mayo Clinic	104	10.0
Baumel	Coll. French series	555	15.0
Fong	Sloan Kettering (70 years)	138	21.0
Klempnauer	Hannover	107	13.8
Trede	Mannheim	122	25.4

Median survival in pancreatic cancer is 20 months for resectable disease, 9 to 12 months for locally advanced (unresectable) disease, and 5 to 7 months with metastatic

disease. Poor prognostic factors include advanced stage, poor performance status and peritoneal disease.

The 5-year survival rate of patient whose tumors were resected is poor; the reported range is 3% to 25%. The 5-year survival is 30% for patients with small tumors (less than 2 cm in diameter), 35% for patients with no residual tumor or for tumors did not require dissection from major vessel, and 55% for patients without lymph node metastasis (30). Performance status and the presence of four symptoms (dyspnea, anorexia, weight loss and xerostomia) appear to influence survival; patients with higher performance status and the lowest number of these symptoms lived longest. In non resectable or metastatic disease, median survival of patients with such disease is 2 to 6 months.

Sener et al. have analyzed the data of 44,438 patients, diagnosed with pancreatic cancer between 1985-1990, by using the national data base from the USA. In this way, the 5 years survival rate on decade of age in patients over 50 years has varied between 7.6 and 4.2% independently of the tumor location in the pancreas.

Also, after resection the 5 years survival was 23.3% for the patients without adjuvant therapy, 17.0% for the cases with resection, radio- and chemo-therapy, and 14.9% for those with pancreatectomy and radiation or chemotherapy.

On the other hand, the patients not receiving a specific treatment for cancer had a survival rate of 5.2%, which is similarly to those who were treated with radio- or chemotherapy, or both, but without resection.

In this way, the results of this study, similar to those of other studies, indicate that despite sophisticated post-operatory management and reduction of mortality for increasingly extended resections, there is no appreciable improvement in overall survival over the past two decades (31-33).

Thus, the general enthusiasm generated by the gastrointestinal tumors study group (GITSG) in 1987 on the use of radiotherapy in combination with 5-FU disappeared rather quickly (34). Also, further studies related to the use of high doses of radio +/- chemotherapy and the use of the neo-adjuvant radio chemotherapy have not demonstrated an increase of the 5 years survival rate, when compared with resection without other associated treatment. However this study has shown that the highest rate survival on tumoral stages was obtained after the tumor resection. Consequently, the resection should be performed when a low rate of complications will be achieved. It is also important that the diagnosis of pancreatic tumors even after resection, to be done with accuracy because the other periampullary tumors have a better prognosis and may alter the statistical results (35).

Nuzhat et al. have analyzed a sample of 125 patients operated for pancreatic ductal adenocarcinoma out of which 116 were completely monitored. The median survival after surgery was 16 months; the 1-, 3-, 5- and 7-year survival rate of for all patients was 60%, 23%, 19%, and 11% respectively. In addition, for the subgroup of resected patients receiving adjuvant therapy the 1-, 3-, 5-and 7-year survival rate was 69%, 28%, 23%, and 18% respectively, as compared to 20% and 0% for the patients not receiving adjuvant therapy ($p < 0.0001$).

Moreover, for the patients who had no tumor adenopathies the 1-, 3-, 5-and 7-year survival rate was 73%, 38%, 26% and 22% *vs.*52%, 14%, 14%, and 9% respectively in

the patients with ganglionic metastasis (p = 0.01). In fact, the multivariate analysis showed that adjuvant therapy was the only factor highly associated with survival (HR=0.26, 95%, CI=0.15-0.44). In this way, the 5 and 7 years overall survival of 19% and 11% highlight the data in the literature that surgical resection may increase long-term survival, particularly if associated with adjuvant chemoradiotherapy (36).

CONCLUSIONS

As mentioned, during the last decades the development and advance of the surgical methods and techniques have improved only morbidity and hospital postoperative mortality, without significant impact on survival. Thus, in the present paper we reviewed some modern aspects regarding the factors implicated in pancreatic cancer survival, in order to increase the awareness regarding this important pathology, which is considered to be the sixth or even fifth cause of death by cancer in most of the modern societies.

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ISCHEMIC MITRAL REGURGITATION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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ECOCARDIOGRAPHIC EVALUATION OF ISCHEMIC MITRAL REGURGITATION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION (Abstract). **Aim of the study:** echocardiographic evaluation of mitral regurgitation (MR) during the evolution of patients with acute myocardial infarction (MI). **Material and methods:** the study included 104 patients (73 males and 31 females), aged between 38-85, diagnosed with acute myocardial infarction (based on clinical, ECG and enzymatic evidences), in order to assess the MR (clinically - a new systolic murmur, and by echocardiography-the severity of MR). Echocardiography was performed upon admission and at 10-30 and 180 days after the onset of acute MI. The evaluation of MR was based on the following parameters: jet area, jet area indexed to left atrium, regurgitated volume, left atrial and left ventricular size, the evaluation of mitral valve apparatus in order to eliminate other possible causes of MR. **Results and discussion:** MR was found in 35 patients from 104 diagnosed with acute MI, as follows: severe in 20 patients (jet area > 8 square cm, jet area indexed to left atrium > 40%, regurgitated volume > 30 mL) and mild in 15 patients (jet area < 4 square cm, jet area indexed to left atrium < 20%, regurgitant volume < 30 mL). In 30 patients MR was produced by the dilatation of mitral annulus (because of the evolution to ischemic dilatative cardiomyopathy), 5 patients developed left ventricular aneurysm; in 3 patients, MR was produced by chordae rupture and in 2 patients we diagnosed an ischemic prolapse of posterior mitral leaflet. In evolution all the patients developed symptoms and signs of heart failure, and 2 patients were referred to surgery. **Conclusions:** The appearance of MR in the evolution of MI is an important sign of bad prognosis by its contribution to the appearance and/ or to the worsening course of heart failure. Mechanisms of this MR are very complex based on the alteration of left ventricular geometry. Echocardiography plays an essential role in the early diagnosis of MR, estimating its severity, the mechanisms and also the prognosis. **Keywords:** MITRAL REGURGITATION, ECHOCARDIOGRAPHY, ACUTE MYOCARDIAL INFARCTION

Chronic ischemic mitral regurgitation (IMR) is a common complication of myocardial infarction which severely affects cardiovascular mortality and morbidity (1, 3, 4). Multiple pathophysiologic mechanisms are involved in generating IMR, each of them having a different importance: left ventricular (LV) remodeling and dysfunction, annular dilation/dysfunction, and mechanical dyssynchrony (2, 3, 5). The most frequent mechanism involved in the occurrence of mitral regurgitation is the presence

of local or global LV remodeling that alters the geometrical relationship between the ventricle and valve apparatus (4, 5, 6, 8). In the wide spectrum of patients with chronic IMR, the assessment of some echocardiographic parameters, such as tethering pattern, leaflet motion, origin and direction of the regurgitant jets, allows one to identify different specific subgroups of patients subjected to different therapeutic approaches (2, 5, 6, 7). The aim of medical and/or surgical therapy is to improve heart failure symptoms, and improve LV remodeling and function and the intermediate/long-term outcome. The targets of surgical MV repair involve annulus, leaflets, chordae and ventricles (9, 10, 11) The restricted annulo-plasty is the most commonly adopted surgical procedure that improves heart failure symptoms but not survival when compared to medical therapy and is also subject to a high incidence of late failure (~30%) (9, 11).

The aim of the study was to evaluate the possible mechanisms involved in the occurrence of mitral regurgitation in patients with acute myocardial infarction.

MATERIAL AND METHODS

The study included 104 patients, 73 (70,19%) males and 31 (29,8%) females, aged between 38-89 years, admitted to the 1st Cardiology Clinic between 2003 and 2012, diagnosed with acute myocardial infarction. All patients were admitted to the emergency unit of the hospital and they were brought by ambulance in most of the cases.

We selected in our study patients who fulfilled diagnostic criteria for acute coronary syndrome:

- clinical symptoms-angina;
- paraclinical tests: electrocardiographic ST changes associated with subendocardial or subepicardial ischemia or new left bundle branch block *(*LBBB) associated with high levels of myocardial cytolytic enzymes (Troponin).

Paraclinical evaluation included:

- evaluation of the risk factors: level of glucose, uric acid, lipid profile, complete blood count, coagulation tests;
- medical tests that evaluate the main organs affected by high blood pressure or/and diabetes;
- 12-lead electrocardiogram - for positive diagnosis and localization of acute coronary syndrome;
- Echocardiogram (2D, M-mode and Doppler) - to assess systolicand diastolic function of LV; regional kinetic changes, the presence of other complications (intracavitary thrombus), and in order to detect mitral regurgitation (the aim of the current study).

RESULTS AND DISCUSSION

The diagnosis of myocardial infarction was established by: clinical typical symptoms, electrocardiographic changes, elevation of myocardial cytolysis enzymes and in few cases-pathological confirmations. Being a major emergency, the first therapeutic measures for all patients were initiated in the emergency care unit concomitant with a cardiologic examination.

The following steps were undertaken: the anamnesis of the patient, with the mention that in the situations in which the condition of the patient did not allow the direct anamnesis, the data was obtained from his relatives or from the previous hospital release documents (patient's previous medical folder respectively).

The following aspects were investigated during the anamnesis:

- patient's cardiovascular antecedents:

high values of blood pressure (HBP), chronic ischemic disease, acute myocardial infarction (IMA), valvular heart diseases, dilated cardiomyopathy (CMD), previously known rhythm and/or conduction disorders, potential surgical interventions at the level of the cardiovascular system;

- the associated risk factors having an impact on the cardiovascular system (smoking, alcohol intake, diabetes);

- associated co morbidities (thyroid gland disorders such as hypo or hyperthyroidism, chronic obstructive bronchopneumopathy (BPCO), stroke);

- treatments followed at home up to the moment of coming to the emergency care unit;

- the emphasis was on identifying the precipitating and aggravating factors of the acute MI: inadequate physical effort, alcohol intake, paroxysmal rhythm disorder, conduction disorder, prolonged treatment discontinuation, excessive salt intake.

The complete examination of the patients involved: assessing the general state, consciousness, pulmonary auscultation, heart and peripheral arteries (carotid) auscultation, checking of blood pressure (BP).

All patients were monitored in the coronary intensive care unit (ECG, breaths/ minute, BP, O_2 sat, diuresis).

Standard 12-lead ECG was performed on all patients immediately after admission to confirm the diagnosis of acute myocardial infarction, the localization of MI and possible arrhythmic complications.

The localization of myocardial infarction in the patients from the group was: anterior - 57 (51.92%) cases; inferior - 23 (22.11%) cases; antero-extensive - 15 (14,42%) cases; infero-lateral and right ventricular infarction - 9 (8,65%) cases (fig.1).

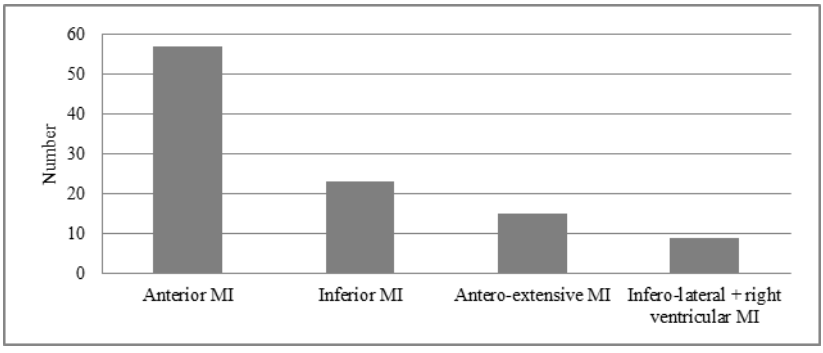

Fig. 1. Localization of myocardial infarction

The analysis of the associated risk factors in the patients from the group revealed the following: of the total number of patients, 31 (29.8%) were smokers, 23 (22.11%) reported chronic alcohol consumption, dyslipidemia was present in 48 (46.15%) patients, 6 (5.76%) of them being under treatment with statin. Diabetes was present in 11 (10.57%) males and 8 (7.69%) females, 5 (26.31%) of them being under treatment with insulin; 3 (2.88%) patients had associated thyroid pathology; 4 (3.84%) of them had antecedents of stroke; 15 (14.42%) had chronic obstructive bronchopneumopathy (BPCO) and 4 (3.84%) presented chronic renal disease (fig. 2).

From all the patients 85 (81.73%) had a history of previous angina.

The clinical examination was focused on the discovery of a mitral regurgitation murmur in patients with acute MI during the evolution.

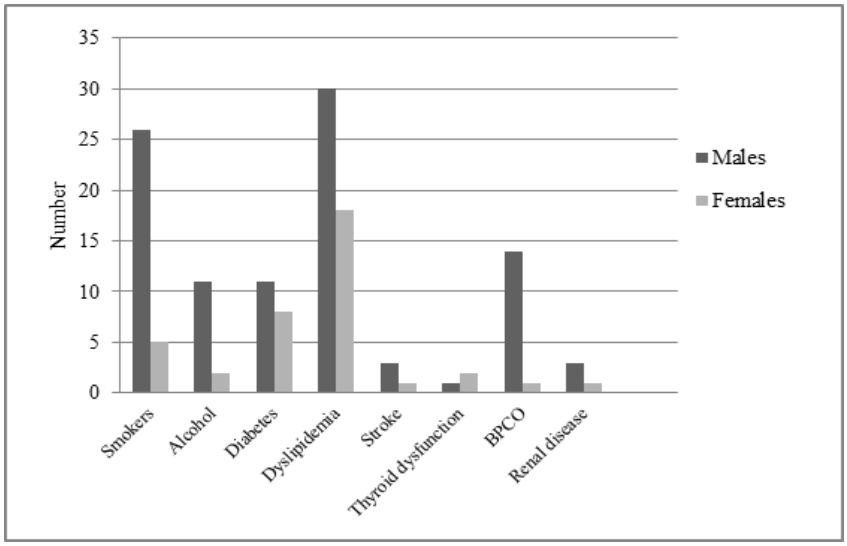

Fig. 2. Risk factors present in the patients diagnosed with acute myocardial infarction.

After confirming mitral regurgitation by echocardiography, the following parameters were determined:

- jet area;
- jet area indexed to left atrium;
- regurgitant volume;
- left atrial and left ventricular size;
- the evaluation of mitral valve apparatus in order to eliminate other possible causes.

Echocardiography 2D, M-mode and Doppler was performed in all patients upon admission and thereafter depending on the situation, 10, 30 and 180 days after onset.

Mitral regurgitation was found in 35 (33.65%) patients from 104 diagnosed with acute MI, as follows: - severe in 20 (19.23%) patients - jet area > 8 square cm jet area indexed to left atrium > 40%, regurgitant volume > 30 mL and mild in 15 patients (14.42%) - jet area < 4 square cm, jet area indexed to left atrium < 20%, regurgitant volume < 30 mL (fig. 3).

The etiology of mitral regurgitation was also estimated by echocardiography as follows:

- in 30 (28.84%) patients echocardiography showed dilatation of mitral annulus (because of the evolution to ischemic dilatative cardiomyopathy), 5 (4.80%) patients developed left ventricular apical aneurysm;
- 3 (2.88%) patients-chordae rupture;
- 2 (1.92%) patients -ischemic prolapse of posterior mitral leaflet (fig. 4).

The localization of the acute MI in patients who develop acute mitral regurgitation was:

- 25 (24%) of patients: anterior myocardial infarction;
- 5 (4.80%) patients: anterior extensive MI;
- 2 (1.92%) patients: inferior MI;
- 3 (2.88%) patients: infero-lateral MI (fig. 5).

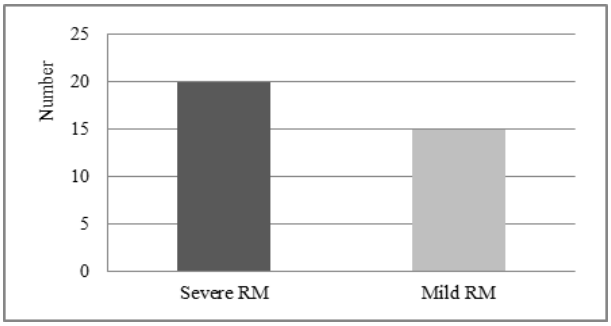

Fig. 3. Classification of mitral regurgitation

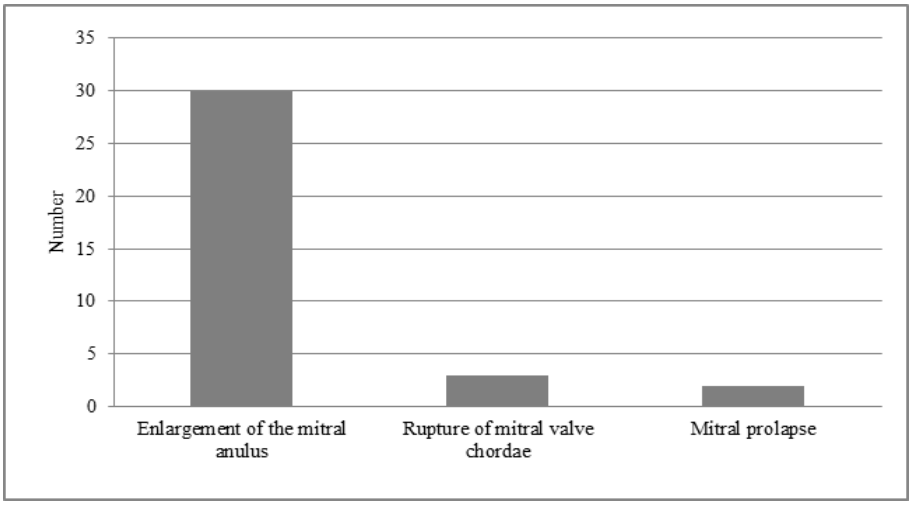

Fig. 4. Causes of mitral regurgitation

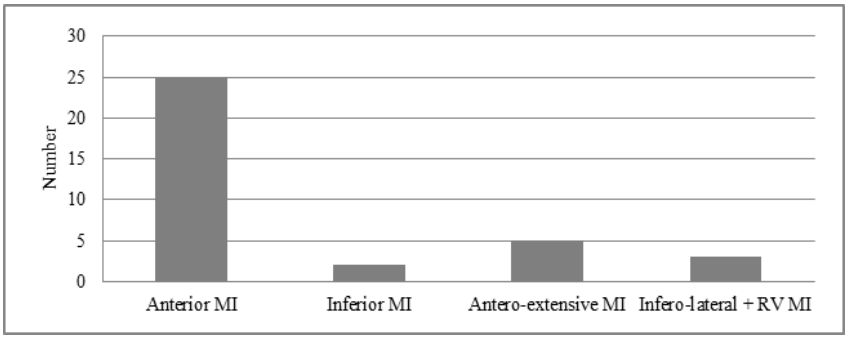

Fig. 5. Localization of acute MI in patients with MR

The evolution of patients from study who developed mitral regurgitation by different mechanisms during evolution of myocardial infarction was:

- all the patients developed symptoms and signs of heart failure;

- 2 (1.92%) patients were directed to cardiac surgery for valvular reconstruction;
- 2 (1.92%) patients (one female and one male) died from cardiogenic shock.

CONCLUSIONS

The appearance of MR in the evolution of myocardial infarction is an important sign of bad prognosis by its contribution to the appearance and/or to the worsening course of heart failure. Mechanisms of this MR are very complex based on the alteration of left ventricular geometry. In our study the principal mechanism was the dilatation of mitral annulus (because of the evolution to ischemic dilatative cardiomyopathy), followed by chordae rupture and ischemic prolapse of posterior mitral leaflet. Echocardiography plays an essential role in the early diagnosis of MR, estimating its severity, the mechanisms and also the prognosis.

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HYPONATREMIA – RISK FACTOR IN PATIENTS WITH CHRONIC HEART FAILURE – CLINICAL, EVOLUTIVE AND THERAPEUTIC IMPLICATIONS

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HYPONATREMIA – RISK FACTOR IN PATIENTS WITH CHRONIC HEART FAILURE – CLINICAL, EVOLUTIVE AND THERAPEUTIC IMPLICATIONS (Abstract): Patients with heart failure are, by definition, hemodynamically unstable. This condition may be accentuated by medication (digitalis, diuretics, antiarrhythmics), so that they become more sensitive to electrolyte disturbances. Hyponatremia is the most common electrolyte disorder, particularly common in the intensive care unit. **Aim:**the evaluation of the incidence of hyponatremia in patients diagnosed with chronic heart failure in order to establish a correlation with the evolution, prognosis and therapeutic implications. **Material and methods:** We analyzed retrospectively 120 patients diagnosed with chronic heart failure NYHA II-IV classes, admitted in the Cardiology Clinic between 2009 and 2013. We analyzed electrolytic disturbances which occurred during different strategies of therapy. **Results:** 120 patients with heart failure were admitted in the Cardiology Clinic between 2009 and 2013, 92 males and 28 females. Diagnosis was established by classical criteria. Evaluation was very complex and included: complete clinical examination, electrocardiogram, echocardiography, chest ray examination and biochemical analyses especially hepatic, renal function and electrolyte status. **Conclusions:** The data obtained showed that electrolyte disturbances are frequent in patients with chronic heart failure, irrespective of NYHA class. Hyponatremia is usually associated with diuretic therapy and may play a very important role in the subsequent development of life-threatening complications. Patients with heart failure who develop hyponatremia during their evolution had a worse prognosis. **Keywords:** ELECTROLYTE DISTURBANCES, HYPONATREMIA, HEART FAILURE

The patient with heart failure (independent of etiology) is, by definition, a hemodynamically unstable patient in which a therapeutic plan, initially beneficial, may have unexpected side effects, some of them life-threatening (1). This type of patient usually has an electrical and hemodynamic instability related to the underlying disease and potentiated by complications and also by medication (diuretics, digitalis, antiarrhythmics) (1, 2, 3). In these conditions, patients become more sensitive to even minor elec-

trolyte balance disturbances, sometimes reacting violently through serious arrhythmias or conduction disturbances. Despite the advanced therapies of heart failure, the significant associated morbidity or mortality due to complex side effects of therapy was not reduced. The medication of the patient with heart failure involves a complex therapeutic schedule. The diuretic medication is essential and it is path physiologically addressed to the salt and water retention (3). Although well tolerated by most patients, diuretic medication acting at renal level disrupts the electrolyte balance so that, depending on the type of mechanism, on the patients' condition at the time, on the associated medication and diet, there may appear other electrolyte and fluid disorders, some of which could be life-threatening (1, 2, 3). Hyponatremia is the most common electrolyte disorder in intensive care units (its frequency is estimated at 30-40% of the hospitalized patients) (4, 5, 6). Hypervolemic hyponatremia, especially, is associated with a bad prognosis in patients with heart failure.

The aim of the study is to evaluate the incidence of hyponatremia encountered in patients with heart failure and to establish a correlation between this disorder and the occurrence of certain complications, and the entailed prognosis and therapeutic implications.

MATERIAL AND METHODS

The study is retrospective and included a group of 120 patients, 92 men (76.66%) and 28 women (23.33%), aged 48-85 years, hospitalized in the 1st Cardiology Clinic between the years 2009-2013 and diagnosed with chronic heart failure of the NYHA II-IV classes.

The classification of patients in the NYHA class of severity of heart failure was as follows:

- NYHA Class II - 19 patients: 10 men, 9 women;
- NYHA class III - 82 patients: 65 men, 17 women
- NYHA Class IV - 19 patients: 14 men, 5 women

The diagnosis of heart failure was based on the known and classical clinical and paraclinical data:

- Symptoms and signs of left ventricular failure (shortness of breath of different degrees, cardiac cough, tachycardia with / without gallop rhythm, presence of organic heart breaths and pulmonary rales stasis);

- Signs of right heart failure: hepatomegaly, peripheral edema (cardiac type), turgid jugular (level I-III), hepatojugular reflux, with or without ascites.

Paraclinical exploration of the patients included:

- Chest radiography - objectified cardiomegaly (ICT over 0.55) with or without signs of pulmonary stasis or pleural effusions.

- Echocardiography (2D, M mode, Doppler) objectified cardiac cavities dilation with decreased contractile function parameters (ejection fraction, shortening fraction), and other aspects: valvular regurgitation, intracardiacthrombus, and presence of spontaneous contrast and signs of hypertension pulmonary artery. Echocardiography was essential for identifying the etiology of heart failure.

- Peripheral venous pressure was elevated in most patients with right heart failure component.

- 12 leads surface ECG, in which these were sought: rhythm or management disturbances, ischemo-lesional changes, overstress of certain cavities, changes due to

electrolyte disturbances.

- Biochemical balance included: hepatic function (transaminases, bilirubin, GGT), renal function (urea, creatinine, uric acid, creatinine clearance, RA), serum electrolytes (Na^+, K^+, $Ca^{+2,}$ Mg^{+2});

- Paraclinical investigation was complemented with complete blood count, spirogram and determination of blood gases and pH.

Patient evaluation was performed first at the time of admission and subsequently, depending on each particular case, was performed daily during hospitalization.

The etiology of heart failure was as follows:

- Ischemic dilated cardiomyopathy - in 30% of cases;

- Ethanolic dilated cardiomyopathy - in 35% of cases;

- Mixed dilated cardiomyopathy (ischemic, toxic, diabetic) - 20% of cases.

- Valvular heart disease - 15% of cases.

Etiology was based on personal history, physical examination and echocardiographic data resulted from examination. In some cases, the presence of the biochemical markers that supported chronic alcoholism was added to these.

Patients' treatment was strictly individualized according to the hemodynamic status at the time of hospitalization, the etiological features, the presence or absence of arrhythmias and/or conduction disturbances and included, besides the appropriate hygienic-dietary measures, the following:

- Diuretic: loop – 15% of patients, spironolactone + loop diuretic combination - 85% of patients;

- Nitrate type vasodilator - 75% of patients;

- Converting enzyme inhibitor (ACEI) - adjusted doses of TA- was administered to 85% of patients (65% received perindopril, 20% received ramipril);

- Beta blocker - 90% of patients (55% carvedilol, bisoprolol 35%);

- Digoxin - 45% of patients;

- Amiodarone - 30% of patients;

- Anticoagulant - 65% of patients;

- Antiplatelet - 75% of patients received aspirin (in 50% of the cases it was associated with an anticoagulant), 28% of patients receiving clopidogrel.

- Dopamine (vasodilator dose) was necessary in 35% of cases because of signs of low cardiac output or renal failure.

Patients received therapeutic regimens that included combinations tailored to each individual case, as follows:

- Loop diuretic + vasodilator (nitrate) + beta blocker + ACE inhibitor - 15% of cases;

- Loop diuretic + spironolactone + ACE inhibitor + beta blocker + vasodilator (nitrate) - 40% of cases;

- Loop diuretic + spironolactone + ACE inhibitor + beta blocker + digoxin + vasodilator - 20% of cases;

- Loop diuretic + ACE inhibitor + amiodarone + vasodilator - 25% of cases.

All patients received an antiplatelet regimen and in 65% of the cases an anticoagulant administration was necessary.

Introducing the anticoagulant regimen was imposed by the presence of echocardiographic intracavitary thrombus or spontaneous contrast, by the presence of dilated right cavities and other clinical signs suggestive of thromboembolism associated phenomena. Throughout the immobilization in bed prophylactic doses of low molecular weight heparinwere also injected.

The nitrate type vasodilator (small doses) was used with preference in patients

with signs of left ventricular failure considered to be due to ischemia and in patients with evident pulmonary stasis with normal values of arterial blood pressure.

RESULTS AND DISCUSSION

120 patients, 92 men and 28 women, aged between 48-85 years and diagnosed with chronic heart failure of the NYHA II-IV classes were hospitalized in the 1st Cardiology Clinic between 2009 and 2013.

Most of the patients were male and were included in NYHA class III - 82 patients (68.33%), (65 men and 17 women). 19 patients (15.83%) were included in NYHA Class IV (14 men, 5 women).

Hyponatremia (serum sodium values between 128-110 mmol/l) was present in all patients with NYHA IV class heart failure receiving diuretic therapy.

From the NYHA III class of heart failure only 35 patients (42.68%) developed hyponatremia after diuretic therapy. Clinically, these patients presented generalized oedema upon admission.

The etiology of heart failure was: dilated cardiomyopathy (ethanolic or ischemic) and valvular heart disease.

All the patients were previously treated with diuretics (loop diuretics alone or in combination with spironolactone). In 5% of cases, hyponatremia persisted after stopping the administration of the diuretic, so it was considered dilution hyponatremia and significantly correlated with increased mortality in this group.

The correction of hyponatremia was difficult, with partial response, limited to fluid restriction and administration of sodium chloride, while continuing the diuretic. In 10% of cases, the patients developed symptoms and signs of brain oedema (disorientation, seizures, confusion, lethargy). Neurological symptoms were correlated with serum sodium values under 120 mmol/l. Gastrointestinal symptoms were present in all the patients: anorexia, nausea, vomiting. In 20% of case signs of neuromuscular damage were present: muscle cramps, generalised weakness. Persistent and refractory hyponatremia was associated in our study with a great incidence of malignant ventricular arrhythmias (ventricular tachycardia and fibrillation). These arrhythmic complications determined the revaluation of the therapeutic plan.

The occurrence of hyponatremia was followed by the impossibility of continuing the administration of diuretic therapy that still remained necessary in the presence of generalized oedema.

In 15% of cases, these electrolyte disturbances have been associated with inflection of the renal function, that is, increased urea and creatinine values understood as renal functional failure and whose main cause was the diuretic abuse with subsequent reduction in pressure renal perfusion. In 7% of cases, renal failure was corrected by temporary withdrawal of the diuretic and proper hydration. In 35% of cases, the administration of vasodilator dose dopamine was necessary (2-3 mcg/kg/min).The final prognosis was worse, 25% of patients died during hospitalization. The main causes of death were: asystole and ventricular malignant arrhythmias.

CONCLUSIONS

Electrolyte disturbances are common in patients with heart failure, regardless of NYHA functional class. In most cases this is due to medication, especially to the diuretic therapy. Hyponatremia is an independent negative prognostic factor especially in patients enrolled in the NYHA IV

functional class and it is often associated with increased mortality. The correction of hyponatremiais very difficult, with partial response, limited to fluid restriction and administration of sodium chloride. The treatment of hyponatremia can lead to potentially dangerous complications. It is essential to monitor the serum sodium level daily in patients with heart failure and a high risk to develop hyponatremia.

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NEWS

NEW TREATMENT FOR MULTIDRUG-RESISTANT TUBERCULOSIS

Tuberculosis (TB) is an infectious disease with high morbidity and mortality. Difficulties related to the diagnosis and treatment of TB have increased incidence of multidrug-resistant tuberculosis (MDR-TB). Management of MDR-TB cases is more difficult because the treatment is longer, less potent, more toxic and more expensive, and determines a cure rate very low and a relapse rate very high. In this situation, the development of novel anti-tubercular drugs has become a necessity. Bedaquiline known as TM207 or R207910 is a diarylquinoline that inhibit synthesis of adenosine triphosphate (ATP) by specific binding to bacterial ATP synthetase. This new compound is active against sensitive and resistant strain of *Mycobacterium*, replicating or dormant bacteria. Clinical trials in MDR-TB patients conducted in South Africa revealed that bedaquiline (400 mg daily for 2 weeks and then 200 mg three times a week, in oral administration) has an early bactericidal activity similar to rifampicin and isoniazid, increased the rate of sputum conversion and also decreased the time for sputum conversion. The same study showed that the most common side effects caused by bedaquiline were: nausea, increase QT interval and the level of transaminases. United States Food and Drug Administration approved bedaquiline for treatment of MDR-TB, when no other drug is available. World Health Organization recommends bedaquiline for adult patients with pulmonary MDR-TB, but also argues the need for the studies about long-term safety (Goel D. Bedaquiline: A novel drug to combat multiple drug-resistant tuberculosis. *J Pharmacol Pharmacothe*r. 2014 Jan;5(1):76-8. doi: 10.4103/0976-500X.124435).

Cătălina Luncă

PSORIATIC RHEUMATISM. INFLAMMATORY ARTHROPATHY

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PSORIATIC RHEUMATISM. INFLAMMATORY ARTHROPATHY (Abstract): Psoriatic rheumatism is an inflammatory arthropathy associated with cutaneous psoriasis that occurs mainly in adults aged between 30 and 45 years. In most cases, cutaneous phenomena precede rheumatic manifestations. Psoriatic rheumatism may begin as a monoarthritis and the diagnosis will be confirmed by the presence of psoriatic plaques or by the personal and family history of psoriasis. As a chronic proliferative epidermal disease, psoriasis is due to a genetic predisposition and it is clinically expressed following the triggering action of several factors. The onset is preceded by various events. Psoriasis is two to three times more common in patients with arthritis and about 10-20% of psoriasis patients show joint damage. **Aim:** The present study aims to demonstrate the clinical and biological efficacy of biotechnology-derived biologic drugs, acting as TNFα antagonists and used as backup therapy, at the same time bringing new hope for broadening and improving therapy in psoriatic arthropathy. **Material and methods:** The study included a total of 21 patients (13 men, 8 women) admitted in the interval 2011-2012 to the I-st Rheumatology Clinic of the Iasi Rehabilitation Hospital. The patients were older than 20 years and had one or more peripheral arthritis conditions, skin and nail lesions. **Results and discussion:** Based on the results, it is recommended that infliximab to be administered in patients who meet CASPAR ((Classification Criteria for Psoriatic Arthritis) diagnostic criteria, have the disease in aggressive stage, and do not respond to treatment with methotrexate (MTX) or other disease-modifying antirheumatic drugs - DMARDs (leflunomide, sulfasalazine) administered for 12 weeks. **Conclusions**: Infliximab is effective in counteracting the potentially harmful elements. It reduces the number of tender and swollen joints, improves the functional capacity and the quality of life, and slows down the radiologic progression. Infliximab is well tolerated, effective, with an optimum safety profile during short-term administration in patients with active forms of psoriatic arthropathy. **Keywords:** INFLAMMATORY ARTHROPATHY, CLINICAL MANIFESTATIONS, EARLY DIAGNOSIS, DIAGNOSTIC CRITERIA.

Psoriatic arthropathy (PA) is a chronic rheumatic inflammatory disease (1) with immunological determinism, causing a combination of two types of damage: *articular* – arthritis (2) and *cutaneous* – vulgar psoriasis (3); the articular damage is of polyarticular type; immunological tests (rheumatoid factor, anti-cyclic citrullinated peptide antibodies - anti-CCP) are usually negative. Prevalence of the disease ranges between 0.1 and 1%. It occurs in about one third of patients affected by cutaneous

psoriasis and is equally distributed between sexes. PA is known to have an erosive and destructive potential in approximately 40-60% of patients (4) and a progressive development even from the first year of diagnosis. Psoriatic arthropathy may cause chronic joint damage, severe functional impairment, leading to significant social and medical costs (5).

The analysis of family distribution (10-20%) reveals the role of heredity, but viral, bacillary, immunological, psychogenic and metabolic factors are also involved. Of the numerous suggested hypotheses, the only universally accepted are the *hereditary* and *metabolic* ones (5, 6).

Monoarticular or polyarticular manifestations of inflammatory nature commonly affect the distal interphalangeal joints of the fingers or toes: their integuments become shiny, look like sausage fingers and the nails show typical lesions, such as penetrated grooved nails and even onycholysis. Misalignment and deformity occur late and cause major functional impairment. This broad clinical spectrum also involves many tissues other than skin and nails, including the synovial membrane, cartilage, bone, entheses, and tendon. The symptoms of the disease can range from minimal entheseal pain to arthritis mutilans, the most destructive form of arthritis.

Besides these peripheral asymmetric articular manifestations affecting both the large joints of the limbs and the medium and small ones, the axial segment may also be affected. There may be also unilateral or asymmetric sacroiliitis or segmental spinal cervical locations and often cervical locations, where ossifications typical to seronegative arthritis coexist with treatment-resistant skin and scalp lesions with poor prognosis. The concomitant involvement of large peripheral joints occurs as asymmetric subacute arthritis, evolving to complete rigidity and stiffness.

Laboratory investigations indicate the absence of rheumatoid factor (which is also a diagnostic criteria), increased ESR and CRP. Major increases of these values characterize the polyarticular forms resembling rheumatoid arthritis, and are associated with the progression of joint disease, early mortality, and erosive and destructive potential (protein blood count, anemia, leukocytosis).

The radiologic exam shows signs of erosive arthritis located at a distal interphalangeal level, with simultaneous presence of proliferation phenomena, bone lysis and ankylosis, unilateral or asymmetric sacroiliiitis and vertebral parasyndesmophyte that affect mostly the upper cervical spine (C2-C3) (6).

Due to its clinical heterogeneity, psoriatic arthritis is sometimes difficult to diagnose, especially in the absence of cutaneous lesions. The clinical course of psoriatic arthropathy may be silent and benign, but many patients may have a severe and sometimes debilitating course that requires early diagnosis.

Our study aims to prove the clinical and biological effectiveness of the therapy with biotechnology-derived drugs that act as cytokine antagonists (TNFα) and are used as backup therapy, but at the same time it brings new hope for broadening and improving therapy in PA.

MATERIAL AND METHODS

The study included total of 21 patients (13 men, 8 women) admitted in the period 2011-2012 to the I-st Rheumatology Clinic of Rehabilitation Hospital Iasi. The patients were aged over 20 years and had one or

more peripheral arthritis conditions, skin and nail lesions.

The following parameters were analyzed: *demographic characteristics* (the mean age of the study group patients was 47.0 ± 2.1 years and the mean duration of articular symptoms 4.2 ± 1.3 years), *personal and family medical history*, *case distribution after the onset of disease*, *clinical manifestations* (axial syndrome, peripheral articular syndrome, enthesopathic syndrome, and extra-articular syndrome), *patient self-assessment* (duration of morning stiffness, pain intensity, and global assessment of disease activity), *evaluation parameters* (joint pain, joint swelling, dactyly, enthesis index, thoracic expansion, Schober's test, finger-ground distance and overall assessment of PA activity), *evaluation of the psoriatic skin lesions* performed by a dermatologist, *laboratory investigations* (hand radiographs, pelvis radiograph for imaging sacroiliac joints, radiograph of cervical dorsal lumbar spine, for assessing the stage of disease), *nonspecific inflammation tests* (ESR by Westergren method, CRP dosage by nephelometry), *assessment of rheumatoid factor* by ELISA and latex assays, *analysis of HLA histocompatibility system*, *immunological abnormalities*, *antibody analysis*, *the type of treatment*, *early clinical and biological evaluation after 6 weeks*.

RESULTS AND DISCUSSION

In our study group, the onset was manifested by psoriatic lesions (72% of cases). The rest of the cases subsequently developed arthritis. Nail damage was present in 50% of cases, as onycholysis, the "oil stain" appearance of the proximity of the nail bed, hyperkeratosis.

Men were mostly affected: 13 men (61.90%) to 8 women (38.09%). The mean age of patients included in the study group was 47.0 ± 2.1 years and the mean duration of articular symptoms was 4.2 ± 1.3 years (fig.1).

There is a familial aggregation of the disease, as it is more common in patients whose blood relatives have psoriatic arthritis.

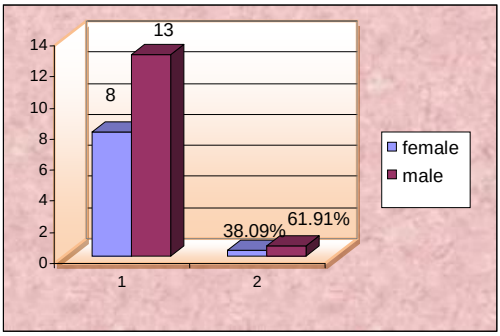

Fig. 1. *Sex distribution of the study group*

The onset was more often in the fourth decade of life, and the peak incidence was in the fifth decade of life, mainly in women.

The conclusion was that the onset of psoriasis can occur at any age, but it was more common in the third and fourth decade of life (fig. 2).

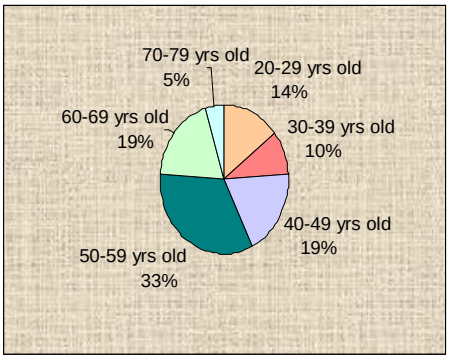

Fig. 2. *Age distribution of the study group*

Psoriatic arthritis has occurred mostly

in rural patients (63%) as compared to urban patients (37%), which can be explained by different education levels and insufficient prevention campaigns (fig. 3).

The onset of the disease may include both characteristic articular manifestations and integumentary lesions. The study group mainly presented psoriatic lesions (62%) and arthritis has occurred subsequently. In 7% of PA patients, the onset of psoriatic lesions was simultaneous with that of arthritis (fig. 4).

HLA B27 antigen was present in a significant proportion of PA patients (45%). HLA B38 and B39 antigens are found in patients with peripheral arthritis, and HLA DR 4 antigen (6%) causes the association of psoriasis with arthritis.

Significant increases in ESR levels are correlated with the progression of articular disease and early mortality. PA caused in two thirds of cases a severe biological inflammatory syndrome (CRP), and in one third of cases the values were moderate and correlated with the erosive and destructive potential, but not with the disease activity.

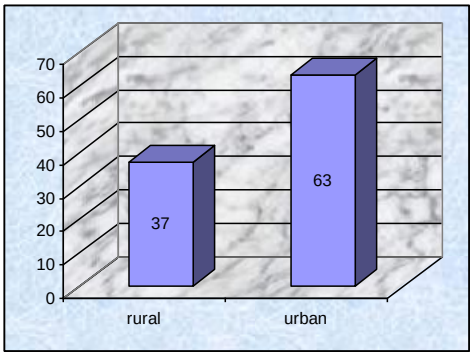

Fig.3. *Area distribution of psoriatic arthropathy study group*

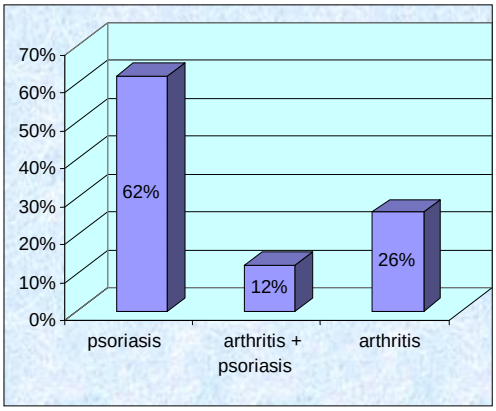

Fig.4. *Onset distribution of the study group*

In our study, PA was the most common joint disorder and manifested as an acute or subacute oligoarthritis affecting large or small joints (hand joints), with inflammatory phenomena in the characteristic subacute forms that are painful and integuments are under tension.

Except for the spine, the inflammatory process affected peripheral joints, periosteum, along the tendons and on the bone insertion of tendons.

Distal interphalangeal joints were mostly damaged. Ankle and knee joints showed asymmetric changes and DIP and PIP joint damage was associated with enthesitis that lead to dactylitis.

The study group had classic lesions of psoriasis vulgaris on the scalp (10%), upper limbs (35%), lower limbs (25%), and trunk (30%). Typical lesions of psoriasis include erythematous plaques that are well-defined, round-to-oval, covered with multi-layered silvery white lamellar scales, that are easy to remove and in a healing process, or covered with thick scales and peeling skin on the damaged areas. Skin erythema and infiltration may also be present (fig. 6).

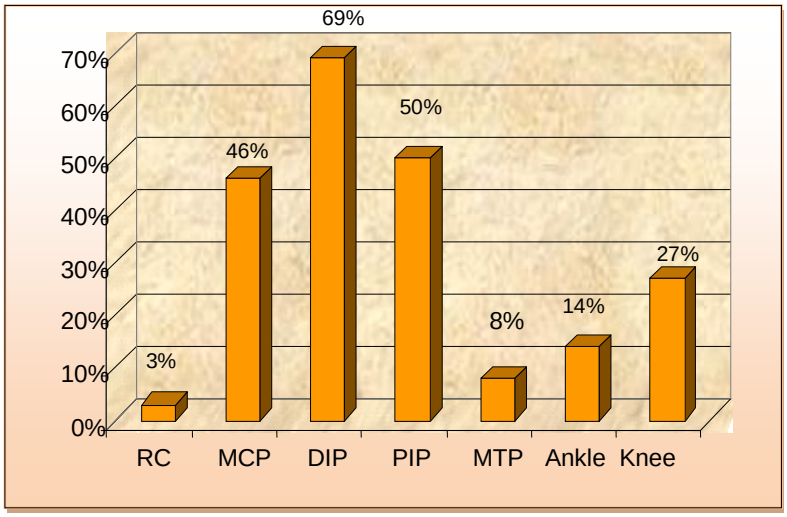

Fig. 5. *Distribution of the type of damaged joints in the study group*

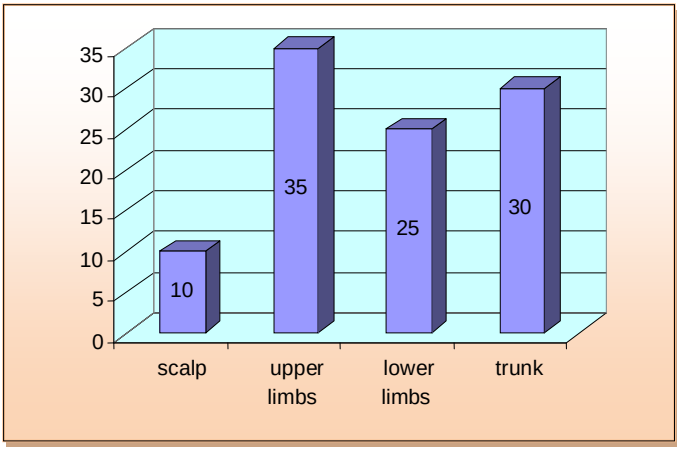

Fig. 6. *Distribution of the psoriatic skin lesions in the study group*

Nail changes were present in 95% of the study patients. These included transverse ridges, punctiform depressions, onycholysis, and nail calcification. The patients with no nail changes were younger or the disease was in an early stage.

Sacroiliitis with axial development occurred mainly in men with HLA 27 antigen (65% of cases), suggesting that the disease has developed for several years.

All patients had morning joint stiffness of variable intensity and duration.

Normal therapeutic course

The effectiveness and safety of infliximab therapy was studied in 21 patients with active polyarticular PA (≥ 5 swollen joints, ≥ 5 tender joints), using 5mg/kbw every 8 weeks. The response to treatment was assessed by measuring changes in the

number of swollen and tender joints, acute phase reactants, the overall assessment of health status by patient and doctor.

The clinical criteria were assessed based on parameters that define the development of the disease (swollen and tender joints, morning stiffness, etc.). An improvement of 75% to 90% was found during treatment.

When a decrease in inflammatory phenomena was achieved, the therapeutic process was supplemented with the interest in the psychological state of the patient, who should be encouraged to reestablish social contacts and to show interest in various activities.

MTX (methotrexate) in association with other drugs is superior to MTX alone. The compliance to polytherapy was optimal, with limited adverse effects, and the functional status of the patients was significantly improved.

Based on the results, it is recommended that infliximab to be administered in patients who meet CASPAR diagnostic criteria (Classification Criteria for Psoriatic Arthritis, developed by W Taylor et al. in 2006), have the disease in aggressive stage, and do not respond to treatment with methotrexate (MTX) or other disease-modifying antirheumatic drugs (DMARDs) administered for 12 weeks.

TABLE I

Effectiveness of therapy with Infliximab + MTX according to ACR criteria

Drug	ACR 20
MTX + Infliximab 3mg /kg	49%
MTX +Infliximab 5mg /kg	53%

CONCLUSIONS

Infliximab is effective in counteracting the potentially harmful elements. It reduces the number of tender and swollen joints, improves the functional capacity and the quality of life, and slows down the radiologic progression. Infliximab is well tolerated, effective, with an optimum safety profile during short-term administration in patients with active forms of psoriatic arthropathy.

The clinical assessment showed that the risk of premature death was closely related to the activity and severity of arthritis and to the presence of radiological erosions and to high levels of ESR.

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NEWS

POSTOPERATIVE INFECTIONS IN PATIENTS WITH TOTAL HIP OR KNEE ARTHROPLASTY - INVOLVEMENT OF ALLOGENEIC BLOOD TRANSFUSIONS

Up to 70% of patients who undergo total hip or total knee arthroplasty receive blood transfusions. A recent study conducted by Friedman and co. investigated whether allogeneic blood transfusion increases the risk of postoperative infection compared with autologous blood transfusion or no transfusion. Using data from more than 12,000 patients assessed in the Phase-III RECORD (Regulation of Coagulation in Orthopedic Surgery to Prevent Deep Venous Thrombosis and Pulmonary Embolism) studies, the authors stratified patients into three groups according to the type of blood transfusion that they received: no transfusion (n = 6313), autologous blood transfusion (n = 1902), and allogeneic blood transfusion with or without autologous blood transfusion (n = 3962). The types of postoperative infection were recorded and included lower or upper respiratory tract and lung infection, bone and joint infection, wound inflammation or infection, urinary tract infection, and other infections. Based on the results of the study the same authors concluded that the rates of any infection, lower or upper respiratory tract and lung infection, and wound inflammation or infection were significantly increased after elective total hip or total knee arthroplasty in patients receiving allogeneic blood transfusion compared with those receiving autologous blood transfusion or no blood transfusion. (Friedman R, Homering M, Holberg G, Berkowitz SD. Allogeneic blood transfusions and postoperative infections after total hip or knee arthroplasty. *J Bone Joint Surg Am.* 2014 Feb 19;96(4):272-8. doi: 10.2106/JBJS.L.01268. PMID: 24553882).

Simona Tucaliuc

RELATIONSHIP BETWEEN KUPPFER CELLS, INFLAMMATION, AND FIBROSIS IN CHRONIC HEPATITIS B AND C

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RELATIONSHIP BETWEEN KUPPFER CELLS, INFLAMMATION, AND FIBROSIS IN CHRONIC HEPATITIS B AND C (Abstract): Kupffer cells are liver parenchymal components of the immune system able of intervention in pathogenic mechanisms involved in viral hepatitis and tumoral lesions. Our study aimed to evaluate the Kupfferian hyperplasia in chronic hepatitis B and C, and to correlate with the severity of liver lesions, focusing on the modality in which Kupffer cells modulate the necroinflammatory events and fibrosis specific for chronic hepatitis morphologic substrate. We investigated 33 cases with chronic hepatitis B and 38 cases with chronic hepatitis C, diagnosed according to Ishak score (in chronic hepatitis B and C) and METAVIR score (in chronic hepatitis C). Kuppfer cells were immunohistochemical labeled, by using an anti-CD68 antibody and heat-induced epitope retrieval (HIER) technique. The sinusoidal reaction expressed by CD68 (+) cells hyperplasia progressively increases along with the intensity of necroinflammatory activity and with the amplitude of fibrosis lesions. Statistically significant differences between Kupffer cells number and the degree of necroinflammatory activitiy and fibrosis, respectively (Ishak score) have been identified in both types of chronic hepatitis. However, no significant differences have been registered when comparing the Kupffer cells number corresponding to each degree of necroinflammatory activity and of fibrosis, in chronic hepatitis B *versus* C, respectively. Our results demonstrate the relationship between Kupffer cells and the severity degree of the disease, without differences between chronic hepatitis B and C. Consequently, we may appreciate that the chronic hepatitis C specific lesions progression, different from that of chronic hepatitis B, is influenced by a different behavior of Kupffer cells, and not by their effective number. **Keywords:** KUPFFER CELLS, CD68, CHRONIC HEPATITIS

A highly represented liver cells population had been firstly described in 1876, by Karl Wilhelm von Kupffer which he had named "Sternzellen" or stellate liver cells corresponding to their shape (1). These cells are considered as an intrinsic part of liver sinusoids endothelial cells and furthermore directly derived from them (1). Tadeusz Browicz had correctly defined the macrophagic characteristics of the stellate liver cells, in 1898; only after two decades researchers had named them as Browicz-Kupffer cells or simply Kupffer cells (2, 3). The blood that transits the liver is exposed

to approximately to $1.2x10^7$ Kupffer cells/tissue grams (4).

Kupffer cells are the main phagocytic cells and APC in liver sinusoid, being at the same time important sources of cytokines exhibiting locally chemotactic action or of stimulatory activity of endothelial and Ito cells. Classic studies have revealed the existence of Kupfferian population heterogeneity and consequently, in a normal liver status, Kupffer cells from the periportal region are larger and more actively involved in phagocytosis, while those from the centrilobular region are smaller and more actively involved in cytokine production and in cytotoxicity (5, 6). A deeper insight into these data have been added by recent immunological studies identifying 2 main types within Kupfferian cell population, namely a proinflammatory phenotype (M1) and another involved in resolution of the inflammatory phenomena in the liver parenchyma (M2) (7, 8).

Classically, light microscopy studies have revealed Kupffer cell hyperplasia in liver sinusoids during the sequence of immunological events characteristics for chronic viral hepatitis (9, 10). Although standard light microscopy reveals the Kupffer cells growth in liver sinusoid and even the „cells crowding" in specific territories, the precise appreciation of the intensity of this phenomenon could be only achieved by supplementary examinations, such as immunohistochemistry.

The purpose of our study is that of evaluation of the Kupfferian hyperplasia in viral chronic hepatitis with B hepatitis virus (HBV) and C hepatitis virus (HVC), respectively, and correlation of Kupfferian hyperplasia with the severity of liver lesions, focusing on the modality in which Kupffer cells modulate the necro-inflammatory events and fibrosis specific for chronic hepatitis morphologic substrate.

MATERIAL AND METHODS

The study group comprised 71 cases of chronic viral hepatitis (subgroup 1 – 33 cases with chronic hepatitis B, subgroup 2 – 38 cases with chronic hepatitis C), diagnosed in the Histopathology Laboratory of the Clinical Hospital of Infectious Diseases "St. Parascheva" Iași.

The cases selection has been achieved starting with the histopathologic diagnosis made according to Ishak score (in chronic hepatitis B and C) and METAVIR score (in chronic hepatitis C), respectively, having as a main criterion the frame into a severity class according to mild, moderate, and severe necroinflammatory activity (NIA). Thus, subgroup 1 included 10 cases characterized by mild necroinflammatory activity (score 1-6), 17 cases with moderate necroinflammatory activity (score 7-10), and 6 cases with severe necroinflammatory activity (score 11-18), and subgroup 2: 11 cases with mild necroinflammatory activity (score 1-6), 18 cases of moderate necroinflammatory activity (score 7-10), and 9 cases of severe necroinflammatory activity (score 11-18).

The studied group has been investigated by immunohistochemistry using an anti-CD68 antibody and heat-induced epitope retrieval (HIER) technique. The working protocol included: blockage of the endogenous peroxidase, incubation with the anti-CD68 antibody (clone KP1, Novocastra, Leica Biosystems) with a dilution ratio of 1:100, amplification with the secondary antibody and the Streptavidin–Biotin-Peroxidase HRP complex (Novocastra Novolink, Leica Biosystems), and counterstain with Mayer's Hematoxylin. The nega-

tive control was also performed.

The quantitative evaluation has been performed by two independent examiners, on 5 microscopic fields/case, using x 200 magnification. After the quantification of CD68 positive cells number for all examined areas, the median value obtained has been associated to the following scoring system (13, 14, 15): CD68 positive cells number < 10 cells – score 0; 10-49 CD68 positive cells number – score 1; 50-99 CD68 positive cells – score 2; ≥100 CD68 positive cells – score 3.

Student's *t*-test was used for all statistical comparisons.

RESULTS

Qualitative evaluation

The qualitative evaluation of CD68 positive cells distribution in sinusoids capillaries allowed the identification of the following aspects:

- the presence of a reduced number of CD68 positive cells, localized mainly in periportal sinusoidal spaces in patients diagnosed with mild activity type of chronic liver disease (fig. 1);

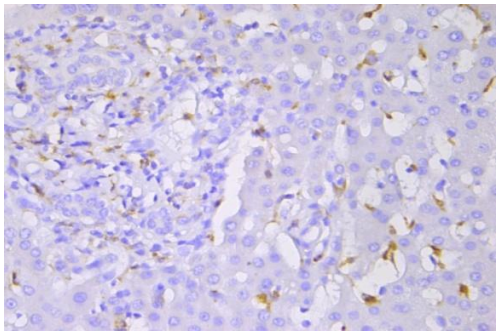

Fig. 1. Few CD68 positive cells in portal space and periportal sinusoids (IHC, x 40)

- the tendency of extension to the entire liver lobule and of CD68 focally concentration in specific "hyperplasia areas" in sinusoids, as closely tighten events to necroinflammatory lesions and portal and intralobular fibrosis enhancement (fig. 2).

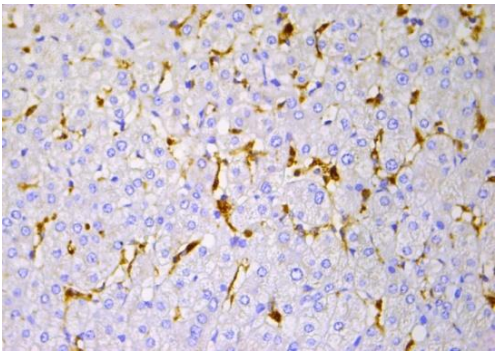

Fig. 2. Numerous intralobular CD68 positive cells, forming hyperplasia areas in sinusoid

Quantitative evaluation

The numerical results of the quantitative evaluation of Kupffer cells (median $\pm$ SD), related to the components of Ishak score (in both types of hepatitis) and of METAVIR score (in chronic hepatitis C), are summarized in table I and II.

Correlated to the numerical value correspondent to median and SD, the correspondent Kupffer cells score had, in both types of hepatitis, an ascendant trend related to the necroinflammatory activity (mild type - score 1 (24.54 $\pm$ 7.94), moderate type - score 2 (83.45 $\pm$ 28.44), and severe type - score 3 (126.45 $\pm$ 8.28)).

Differences between hepatitis B and C have been registered regarding the correlation to fibrosis (considering 1 and 2 score values for mild fibrosis and score values 3 and 4 for severe fibrosis). Thus, in chronic hepatitis B, Kupffer cells score had value 1 (31.96 $\pm$ 14.67) in mild fibrosis and value 3 (107.96 $\pm$ 18.87) in severe fibrosis, while in chronic hepatitis C mild fi-

brosis has been associated to a Kupffer cells score value of 1 (33.51 ± 14.69), and severe fibrosis - with a score of 2 (94.05 ± 31.37).

Related to Metavir score, exclusively applicable in chronic hepatitis C, we have noted the same increase of Kupffer cells number, thus cases with score A1F1/A1F2 being correlated to score 1 (30.64 ± 10.09), for cases with score A2F1/A2F2, Kupffer cells score being 2 (76.89 ± 18.36) and for cases with A3F3 –3 (119.62 ± 15.62).

TABLE I

CD68 positive cells in correlation with the Ishak (NIA, fibrosis) scoring system – chronic hepatitis B

Cases (total #)	NIA	NIA score	Cases (#)	CD68 cells (median ± SD, score)	Fibrosis score	Cases (#)	CD68 cells (median ± SD, score)
10	mild	4	1	24.54 ± 7.94 1	1	1	31.96 ± 14.67 1
		5	6		1	6	
		6	3		1	2	
					2	1	
17	Moderate	7	5	83.45 ± 28.44 2	1	4	
					2	1	
		8	4		3	4	107.96 ± 18.87 3
		9	5		3	5	
		10	3		3	3	
6	severe	11	5	126.45 ± 8.28 3	3	3	
					4	2	
		12	1		3	1	

TABLE II

CD68 positive cells in correlation with the Ishak (NIA, fibrosis) and the METAVIR scoring systems – chronic hepatitis C

Cases (total #)	NIA (score)		Cases (#)	CD68 cells (median ± SD, score)	Fibrosis score	Cases (#)	CD68 cells (median ± SD, score)	Metavir score	Cases (#)	CD68 cells (median ± SD, score)
11	mild	2	1	30.64 ± 10.09 1	1	1	33.51 ± 14.69 1	A1F1	4	30.64 ± 10.09 1
		5	5		1	3				
					2	2				
		6	5		2	2		A1F1	4	
					3	3	94.05 ± 31.37 2	A1F2	3	
18	mod erate	7	5	86.79 ± 21.44 2	2	2	33.51 ± 14.69 1	A2F1	2	76.89 ± 18.36 2
					3	3	94.05 ± 31.37 2	A2F2	3	
		8	5		3	5		A2F2	5	
		9	4		3	4		A2F2	4	
		10	4		4	4		A3F3	4	119.62 ± 15.62 3
9	se- vere	11	5	122.88 ± 16.83 3	4	5		A3F3	9	
		12	4		4	4				

Correlations between Kupffer cells and NIA – Ishak score

Statistically significant differences between Kupffer cells number corresponding to the 3 degrees of necro-inflammatory activities within mild *versus* moderate (p <

0.001), mild *versus* severe ($p < 0.001$), and moderate *versus* severe ($p < 0.001$) have been identified by Student t test, in both types of chronic hepatitis.

However, no statistically significant differences have been identified when comparing the Kupffer cells number corresponding to each degree of necroinflammatory activity and chronic hepatitis B *versus* C.

Correlations between Kupffer cells and fibrosis – Ishak score

In both types of chronic hepatitis, t Student test revealed the existence of significant differences between Kupffer cells number corresponding to the two grades of fibrosis, mild *versus* severe ($p < 0.001$). No statistically significant differences have been identified when comparing the Kupffer cells number corresponding to mild and sever fibrosis, in chronic hepatitis B evaluation versus chronic hepatitis C ($p < 0.001$).

Correlations between Kupffer cells and METAVIR score

Statistical analysis based on Metavir score, applied only in chronic hepatitis C revealed significant differences between Kupffer cells number corresponding to cases evaluated by A1F1/A1F2 score versus A2F1/A2F2 score versus A3F3 score ($p < 0.001$).

DISCUSSION

Kupffer cells and necroinflammatory activity – Ishak score

Kupffer cells are liver parenchyma components of the immune system able of intervention in pathogenic mechanisms involved in viral hepatitis and tumoral lesions, by their capacity to phagocyte antigens and to produce a panel of cytokines (16, 17, 18). They represent 15% of liver cell population and 80-90% of resident macrophages of human body (19).

Literature studies describe two mechanisms responsible for the conservation of Kupffer cells normal number in liver parenchyma in physiological conditions: attraction of new monocytes in liver sinusoids and their transformation into Kupffer cells and the multiplication of already existent Kupffer cells (19, 20).

Kupffer cells exhibit an intense phagocytic activity in liver parenchyma, composing an antigen filter for substances spread by portal vein from the digestive tract. The phagocytic activity is sustained by inflammatory cytokines synthesis, such as: IL-1, IL-6, IL-12, TNF, GM-CSF, CXCL9, CXCL10, MIP-1 (macrophage inflammatory protein 1 alpha), and RANTES (regulated on activation, normal T-cell expressed and secreted) (20, 21, 22). Moreover, activated Kupffer cells express on their surface a series of molecules that provide them similar features with APC, as CD80 and CD40, in viral liver pathology (23).

Nowadays, the idea of cellular heterogeneity within Kupfferian cell population is increasingly supported not only by classic relation between dimension and location in liver sinusoids, as larger and more actively phagocytic periportal Kupffer cells as compared to centrilobular ones, the latter being smaller and especially involved in cytokines synthesis (19, 22, 24). A recent experimental study is registered in this direction, its results identifying at least two Kupfferian subpopulations, as following: CD68 (+) showing an intense phagocytic activity and CD11b (+) exhibiting the capacity of production of immunostimulatory cytokines (such as TNF and IL-12) (22). Supplementary, the hypothesis that CD68

(+) Kupffer cells might basically constitute the resident (fixed) liver Kupfferian cells population, while CD11b (+) cellular subtype might represent a migrated population of macrophage-type cells of spleen and bone marrow origin, mainly in pathologic conditions, such as inflammatory conditions (7, 25) is currently accepted.

Unfortunately, the data regarding these cells roles in viral chronic hepatitis, both with HVB and with HVC are far from being elucidated until present (12, 26).

Within this "collective effort" expressed by a multitude of studies spread by literature concerning complete phenotypic characterization of Kupffer cells, mainly in viral chronic liver pathology, our study focused on CD68 immunostaining of the existent cellular population of liver sinusoids, in 71 cases of chronic viral hepatitis, is currently framed.

Our results based on immuno-histochemical staining and Kupffer cells quantification in chronic hepatitis B and C, respectively, according to the degree of necroinflammatory activity, revealed the statistically significant increase of Kupffer cells number ($p < 0.001$) starting from the mild to moderate, and severe necroinflammatory activity, respectively. It's extremely interesting that the statistical analysis did not reveal significant differences between the numbers of Kupffer cells corresponding to each specific type of necroinflammatory activity (mild, moderate, and severe) analysis, when comparing viral hepatitis B with viral hepatitis C. Considering the pathogenic mechanism, this result may indicate the fact that the different evolution of the two types of hepatitis is not correlated to Kupffer cell number but to their specific behavior of Kupffer cells corresponding to the pathogenic agent.

Our own observations, based on Kupffer cells numerical value and their afferent score, are in agreement to literature data. Qualitative or quantitative researches aimed to highlight the sinusoidal reaction in chronic viral hepatitis, reflected in Kupffer cells number, have pointed out their progressive numerical amplification starting even from mild necroinflammatory activity types (27, 28).

The specific correlation between Kupffer cells and lymphocytes in sinusoids and the direct correlation between their number and the hepatocyte necrosis lesions intensity have been some of the first morphological aspects observed in light microscopy. Thus, McGuiness and al., by comparing CD68 (+) Kupffer cells number in 10 cases of chronic hepatitis, observed an increase of their number associated to a dispersion into the liver lobule, in comparison to control cases (24).

The Kupfferian hyperplasia is far more important as viral chronic hepatitis has been appreciated with a higher aggressiveness. Quantitative analysis of the area occupied in liver parenchyma by immunolabeled Kupffer cells confirms the results of the study. The authors observed a progressive increase in the percentage occupied by Kupffer cells in total area of liver surface correlated to disease severity, ranging from 2.51 ± 0.88 in moderate activity and 4.71 ± 2.23 in severe activity types of chronic hepatitis (29).

The involvement of Kupffer cells in Ito cells activation and in fibrillogenic activity in liver parenchyma have been unanimously recognized (8, 30, 31). However, the molecular aspects of interactions between activated liver macrophages and myofibroblasts are still incompletely known, repre-

senting a point of interest directed toward identification of new possible therapeutic targets in liver fibrosis (32).

Kupffer cells and fibrosis – Ishak score

Recent studies have shown that HVB chronic liver infection even in cases exhibiting a mild necroinflammatory activity is associated to sinusoidal and portal collagen synthesis development. This phenomenon is attributed to Kupffer cells reactivity even in mild aggressiveness hepatitis types to produce a panel of pro-fibrillogenic cytokines, such as TGF-β1 (33).

It is unanimously accepted that patients with HVC have more severe fibrosis lesions compared to HVB chronic hepatitis patients (34). Our results confirmed this assertion.

The evaluation of the fibrosis severity correlated to the necroinflammatory activity has revealed more important lesions in chronic hepatitis C. Compared to chronic hepatitis B, more cases with fibrosis of score 2 and 3 showed mild necroinflammatory activity. Compared to chronic hepatitis B, no case showed score 1 fibrosis and score 4 fibrosis was detected in moderate necroinflammatory activity. All cases showing severe necroinflammatory activity had fibrosis score 4, when compared to chronic hepatitis B.

We have registered statistically significant differences between Kupffer cells number associated to cases with fibrosis score 1 and 2, and score 3 and 4, respectively, in each hepatitis type ($p < 0.001$). We have also noted that the score value correlated to Kupffer cells expression increased from 1 to 3 in chronic hepatitis B and from 1 to 2 in chronic hepatitis C, corresponding to mild and severe fibrosis, respectively. However, despite the above mentioned fibrosis score in the same intensity of the necroinflammatory activity, statistical analysis revealed the lack of statistically significant differences between chronic hepatitis B and C, correlated to Kupffer cells number in fibrosis score 1 and 2 fibrosis score and 3 and 4, respectively.

This negative result supports the idea of fibrosis development as an event not necessarily related to Kupffer cells. The dramatic changes of the last 10-15 years concerning liver fibrosis have been closely correlated to the deciphering of the extracellular matrix dynamic and ultrastructure, to the elucidation of the liver myofibroblasts biology and, not the last, the integration of all these new elements in the complex process nowadays known as "connective repair" (the integrated wound healing response) (35, 36).

Kupffer cells, necroinflammatory activity, and fibrosis – Metavir score

Although Metavir score assures a specific and reproducible evaluation in C viral hepatitis (37), being largely used in histopathologic diagnosis formulation, the mainstream publication review revealed the complete lack of the researches oriented towards the relationship between Kupffer cells and lesions severity, corresponding to Metavir score categories in this type of hepatitis. To our knowledge, there is only a single study that quantifies Kupffer cells and is analyzing their dynamic related to the F (fibrosis) parameter of Metavir score, in the pathologic context of chronic hepatitis induced by chronic alcohol consumption and of fibrosis that develops in the evolution of this disease (38). The mentioned study indicates the lack of a direct correlation between CD68 expression, as a marker

of Kupffer cells, and the degree of severity of the disease, progressively from stage I to III (38).

Within this context we consider that our data have a degree of novelty supported by the differences obtained by comparison of the results obtained by the use of the two scores, Ishak and Metavir. In our opinion, the 3 classes of severity A1F1/A1F2, A2F1/A2F2, and A3F3 of Metavir score more likely reflect the lesions evolution, including the correlation between the necroinflammatory activity and fibrosis than the severity classes separately defined by necroinflammatory activity and fibrosis, respectively, in Ishak score. The most convincing argumentation is provided by the 4 cases that, according to Metavir score, have been framed into a higher category of severity, by A3F3 score. The statistically significant differences between Kupffer cells number (median and SD) in all 3 classes of Metavir severity indicate the involvement of Kupffer cells in enhancement of both inflammation and fibrosis, during the lesions severity evolution in C hepatitis.

In our current activity, the additional use of Ishak score in chronic hepatitis C is justified by the fact that the large numerical value scale correspondent to necroinflammatory and fibrosis activities evaluation offers more precise criteria to evaluate the status of liver parenchyma involvement at the diagnosis moment and METAVIR score, simultaneously applied, allows an evaluation of the general histologic activity, including the interface hepatitis and of associated components of lobular necrosis.

As a consequence of the study oriented towards the quantitative profile of Kupffer cells, we may appreciate that Metavir score offers, by numerical values, an image of the inflammatory process and of fibrillogenesis that includes, as a pathogenic substrate, including Kupffer cells involvement.

CONCLUSIONS

The sinusoidal reaction in chronic liver diseases of viral etiology expressed by CD68 (+) cells hyperplasia progressively increases along with the intensity of necroinflammatory activity and with the amplitude of fibrosis lesions. The classic microscopically findings, the quantitative evaluation, and the statistical analysis indicate the involvement of Kupffer cells, directly correlated to the severity degree of the disease, without differences between B and C chronic hepatitis. Consequently, we may appreciate that the chronic hepatitis C specific lesions progression, different from that of chronic hepatitis B, is influenced by a different behavior of Kupffer cells, and not by their effective number.

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NEWS

SIGNET-RING CELL MELANOMA OF THE GASTROESOPHAGEAL JUNCTION

Malignant melanomas are well-known for demonstrating a wide range of microscopic appearances and, therefore, simulate other tumor types. Consequently, melanomas with unusual phenotypes may present a diagnostic challenge to the surgical pathologist. Signet-ring cell melanoma of the gastroesophageal junction is the first case of probable primary signet-ring cell melanoma at this site and the second case reported in the gastrointestinal tract. Signet-ring cell melanoma is defined as a melanoma with more than 50% of tumor cells having signet-ring–shaped nuclei because of compression by intracytoplasmic globules. Signet-ring cell morphology is extremely rare in melanomas (0.5%) and is one of 5 unusual variants described in a review of diversity in melanomas by Nakhleh et al. Signet-ring cell melanoma was first described in 1988 by Sheibani and Battifora. In 2 reported cases, transmission electron microscopy was used to identify melanosomes, premelanosomes, and discrete aggregates of intermediate filaments distending the cytoplasm. The signet-ring cells in each of these cases strongly expressed S100 protein, HMB-45, and vimentin, confirming the diagnosis. Since the entity was first described, only 19 additional cases have been reported in the literature. Approximately one-half of those cases were metastases or recurrences, with only 8 cases reported as primary lesions. The diagnosis can be confirmed by the presence of immunoreactivity for melanocytic markers, the absence of mucin and immunohistochemical staining for pancytokeratin, and if necessary, examination for ultrastructural features including melanosomes, in signet-ring cell morphology tumors. (Melissa A, Grilliot, R. Goldblum, Xiuli Liu. Signet-Ring Cell Melanoma of the Gastroesophageal Junction. *Arch Pathol Lab Med*. 2012;136:324–328).

Doina Butcovan

THE INCIDENCE OF ESSENTIAL HYPERTENSION IN ELDERLY PATIENTS WITH METABOLIC SYNDROME

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THE INCIDENCE OF ESSENTIAL HYPERTENSION (EHT) IN ELDERLY PATIENTS WITH METABOLIC SYNDROME ASSISTED AT THE REHABILITATION HOSPITAL IASI, DURING OCTOBER 2010-JULY 2012 (Abstract): The arterial hypertension of the elderly represents an important public health issue, with an ever increasing tendency, due to the population ageing process. **Aim:** The aim of this research is to contribute new data related to the incidence of essential hypertension in elderly patients with metabolic syndrome. **Material and methods:** The current study was conducted on a number of 1,832 patients hospitalized in the Cardiovascular Rehabilitation Clinic of Rehabilitation Hospital Iasi, in the period October 2010-July 2012. Of these patients we selected the ones who were 65 years of age or more and who fulfilled at least three of the five criteria that define the metabolic syndrome, using the criteria recommended by AHA/NHLBI. **Results:** The data obtained revealed an increased incidence of the cardiometabolic syndrome in patients over 65 years of age, in a percentage of 73%. The gender repartition revealed a higher frequency in the case of female patients (66%). Of the metabolic syndrome criteria, the highest incidence was registered for the essential hypertension. Most of the hypertensive patients were diagnosed with essential hypertension grade 3 (55%), while the rest had grade 2 (29%) and grade 1 respectively (16%). **Conclusion:** EHT has a significant occurrence in elderly patients. It seems that hypertension of the elderly has a higher occurrence in the case of female patients, due to the lack of hormonal protection. The association with the other risk factors related to the metabolic syndrome makes the elderly more prone to the occurrence and development of cardiovascular diseases. **Keywords**: ESSENTIAL HYPERTENSION, ELDERLY PEOPLE, METABOLIC SYNDROME.

The metabolic syndrome is defined as a group of risk factors leading to cardiovascular disease, whose pathophysiological foundation is related to insulin resistance. Clinical studies have shown that not all patients presenting metabolic syndrome are really insulin resistant, and consequently other cardiovascular risk factors are considered, including essential hypertension, type 2 diabetes mellitus, hyperlipidemia, obesity and others (1, 2). Over the last two decades, the International Health Organization and the National Institutes of Health, together with the American Heart Association have provided several precise definitions of the metabolic syndrome (3, 4).

Arterial hypertension represents the main risk factor in elderly patients, increasing the incidence of coronary and cerebral vascular accidents. Epidemiological studies have shown that most elderly male and female patients have a systolic pressure above 160 mm Hg, while the diastolic pressure is less than 100 mm Hg. As far as patient gender is concerned, it seems that hypertension in elderly patients has a higher occurrence in women (5). Systolic arterial hypertension commonly occurs in elderly patients, but essential forms (systolic-diastolic) can also be detected.

MATERIAL AND METHODS

We investigated the medical records of 1,832 patients hospitalized in the Clinic of Cardiovascular Rehabilitation Clinic of Rehabilitation Hospital Iasi in the period October 2010 – July 2012. We selected the patients over 65 years of age who fulfilled at least three of the five criteria defining the metabolic syndrome. We used the criteria recommended by AHA/NHLBI (American Heart Association). Any three criteria of the five listed establish the metabolic syndrome diagnosis (tab. I).

Of the total of 1,832 patients we selected a number of 239 patients over 65 years of age. The patients were divided according to age groups (patients over 65 years of age and patients under 65 years of age), gender and the presence or absence of the metabolic syndrome as well as taking into account the essential hypertension grade and the number of clinical criteria used in establishing the metabolic syndrome diagnosis (3, 4 or 5 criteria). The frequency of each criterion was then recorded in each subgroup presenting 3, 4 and respectively 5 diagnosis criteria.

TABLE I

Diagnostic criteria for metabolic syndrome (according to AHA / NHLBI)

Clinical criteria (at least 3)	Normal values of the parameters
waist circumference (WC)	≥102 cm in men ≥88 cm in women
serum triglycerides level (TG)	≥150 mg/dl or with treatment for high levels of TG
high-density lipoprotein cholesterol level (HDL)	<40 mg/dl in men <50 mg/dl in women or with treatment for low levels of HDL↓
blood pressure (BP)	≥130 mmHg BP sistolic or ≥85 mmHg BP diastolic with treatment for hypertension
serum glucose level (Glu)	≥100 mg/dl or with treatment for diabetes

RESULTS

From the 1,832 patients hospitalized during that period, only 239 are over 65 years of age. (fig.1). The statistical analysis of the data shows that the number of patients over 65 years of age is statistically significant in comparison with the rest of the patients studied ($p<0.01$).

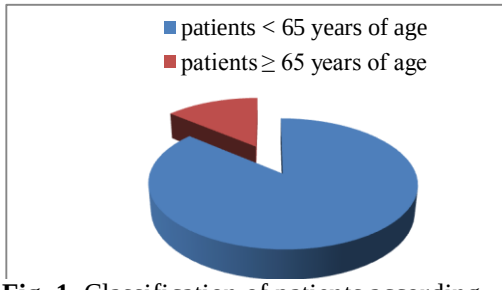

Fig. 1. Classification of patients according to age groups

From the 239 patients who were 65 or older, 174 (73%) were diagnosed with

metabolic syndrome according to the above mentioned criteria (fig. 2). The statistic analysis of the data revealed a significant difference, the value of the p coefficient being under 0.01.

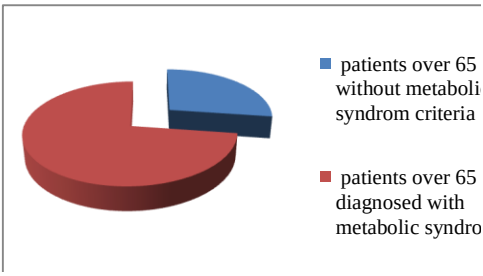

Fig. 2. Classification of patients according to the presence/absence of the metabolic syndrome

From the total number of patients diagnosed with metabolic syndrome, there are 115 female patients and only 59 male patients (fig. 3). The number of female patients is significantly higher than the number of male patients ($p<0.01$).

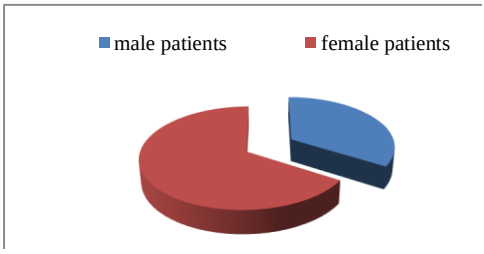

Fig. 3. Classification of patients according to gender

From the 174 patients diagnosed with metabolic syndrome, 104 met just 3 criteria, 50 met 4 criteria while just 20 patients met all 5 criteria of the metabolic syndrome. The statistically significant difference is to be found between the patients meeting 4 or 5 criteria of the metabolic syndrome and the total number of patients included in the study.

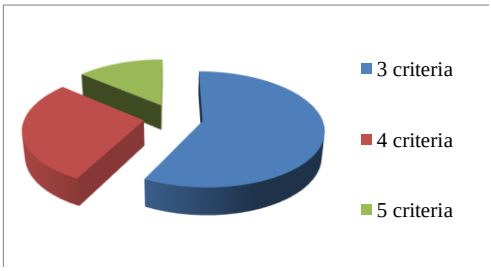

Fig. 4. Classification of patients according to metabolic syndrome criteria

Most of these patients were diagnosed with essential hypertension grade 3 (55%), while the others presented essential hypertension grade 2 (29%) and grade 1 (16%) (fig. 5).

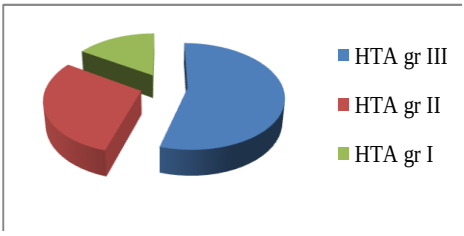

Fig. 5. Classification of patients according to hypertension grade

DISCUSSION

From the total number of 234 patients who fulfilled the age criteria, 73% were diagnosed with metabolic syndrome. The metabolic syndrome incidence was higher in the case of female patients (66%) than in the case of male patients (34%), according to data provided by the literature (6). 59% of the patients had 3 diagnosis criteria, 29% met 4 criteria and only 14% met all 5 criteria. The highest incidence of all criteria used in the metabolic syndrome diagnosis is represented by the essential hyperten-

sion. Most hypertensive patients were diagnosed with essential hypertension grade 3 (55%). In the case of these patients, arterial hypertension may be considered a marker of endothelial dysfunction that leads to the cardiometabolic syndrome. At the same time, endothelial dysfunction is the starting point for the occurrence and development of the atherosclerotic process.

The Framingham study showed that arterial hypertension continues to be a major cardiovascular risk factor that favors atherogenesis. Normal correlations between arterial preessure and age: after the age of 60, systolic blood pressure increases while the diastolic blood pressure decreases (the Framingham study), the changes in arterial pressure having the same meaning for hypertensive patients and patients with normal arterial tension. The isolated arterial hypertension: systolic blood pressure > 140 mm Hg with diastolic blood pressure < 90 mm Hg (implies a pulse pressure above 60 mm Hg). As well as this, the present study provides important prevalent data. Thus, arterial hypertension affects 60-80% of the population over 65 years of age, while isolated systolic hypertension represents 65-75% of the total cases of arterial hypertension in elderly people.

Other epidemiological studies have shown that arterial hypertension has a higher occurrence in overweight people, especially when obesity affects the superior part of the upper body. Obesity represents a risk factor for hypertension as well as for diabetes, cardiovascular disease, osteoarthritis and some forms of cancer (8, 12). Arterial hypertension occurs in 60-70% of diabetics and is at least twice as frequent in this category of patients as compared with non-diabetics. Arterial hypertension increases the cardiovascular risk by micro- and macro- diabetic angiopathy (cardiovascular modifications at the eye level). The association between arterial hypertension and high cholesterol levels increases the cardiovascular risk in diabetics (9).

The prognosis and evolution of arterial hypertension depend on the recorded tension values and the early diagnosis. Untreated arterial hypertension is known as a risk factor that favors the installation and unfavorable evolution of atherosclerosis, leading to cardiac complications (ischemic cardiopathy), stroke, stenosis of the carotid arteries or of the inferior limbs vessels (arthritis). It was noted that 50% of hypertensive patients present ischemic cardiopathy, while the patients with a heart attack have a history of arterial hypertension (10, 11).

The coexistence of other risk factors such as: smoking, obesity, alcohol consumption, sedentary life-style, excess of salt, hyperuricemia (abnormally high level of uric acid in the blood), high blood sugar level and high consumption of animal fat, accelerates the atherosclerosis evolution and leads to arterial hypertension.

The results from the different elderly study populations link the presence of the metabolic syndrome with the development of cardiovascular diseases and functional disability, and further underscore the importance of recognizing and treating its individual components, particularly high blood pressure (12, 13, 14).

CONCLUSIONS

Arterial hypertension in elderly people represents an important public health issue, with an ever increasing frequency, due to the population ageing process. Due to the associated cardiovascular conditions, risk factors and damages to the target organs

that are more serious than in the case of young adult patients, arterial hypertension in elderly patients remains an incompletely defined condition, open to speculations related to its pathogenic mechanisms as well as its treatment possibilities.

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THE ASSESSMENT OF TREATMENT EFFICACY IN AGE RELATED MACULAR DEGENERATION BY EVALUATING THE OXIDATIVE STRESS MARKERS AND OCT MEASUREMENTS

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THE ASSESSMENT OF TREATMENT EFFICACY IN AGE RELATED MACULAR DEGENERATION BY EVALUATING THE OXIDATIVE STRESS MARKERS AND OCT MEASUREMENTS (Abstract): Age related macular degeneration (AMD) is the most frequent cause of blindness in the elderly. **Aim**: to establish the role of oxidative stress in retinal structural lesions in AMD and to monitor the evolution of oxidative stress markers and OCT measurements before and after treatment. **Material and methods**: This is a case-control study that included 19 patients diagnosed with AMD and 40 matched healthy controls. According to the AREDS classification, patients were divided into mild, moderate and severe AMD and received treatment with antioxidants, neurotrophic drugs, intravitreal corticosteroids and/or anti-VEGF. We followed the patients by assessment of visual acuity, optical coherence tomography (OCT) and oxidative stress markers, such as superoxide-dismutase (SOD), thiobarbituric acid reactive substances (TBARS), and C-reactive protein (CRP) from blood samples before and after treatment. **Results**: The OCT showed that the macular edema in patients with severe disease was significantly reduced after treatment, while the cases with normal retinal thickness increased significantly ($p=0.005$). Comparing the mean values of SOD, TBARS and CRP in the two groups, we found that they were significantly higher in the study group compared to controls ($p<0.01$), being higher in patients with severe disease. These values decreased post treatment, but they were still higher than in controls. **Conclusions**: The present research supports the role of oxidative stress and inflammation in AMD and highlights the role of therapy directed against these risk factors. By monitoring the oxidative stress markers in the evolution of the disease, we showed that high values persist after treatment, thus supporting the idea that treatment should be followed for a long time. **Keywords**: AGE RELATED MACULAR DEGENERATION, OXIDATIVE STRESS MARKERS, ANTI-VEGF.

Age related macular degeneration (AMD) is the leading cause of blindness in developed countries. This is a set of degenerative and inflammatory lesions in the macular region, which occurs in previously normal eye and cause a deterioration of central vision and macular function (1). The increasing incidence, the lack of methods of preventing AMD and the negative socio-economic consequences make it a priority among ophthalmic diseases. The current treatment includes systemic treat-

ment with antioxidants and local intravitreal injections with corticosteroids and /or anti-angiogenic factors, photodynamic therapy and macular surgery.

The aim of this study is to assess the role of oxidative stress in AMD lesions and to monitor the evolution of oxidative stress markers pre-and post treatment. We aimed to establish the efficacy of antioxidants, neuroprotectors, intravitreal corticosteroids and anti-VEGF, as well as to establish correlations between functional and structural changes and oxidative stress markers before and after treatment.

MATERIAL AND METHODS

This is a case-control study that included a group of 19 patients diagnosed with AMD, selected from the "N. Oblu" Hospital in Iaşi, and two control groups, one with younger controls and another with controls with similar ages as the cases. Patients with systemic and ocular diseases that could increase oxidative stress, such as diabetes, inflammatory and infectious diseases or vascular occlusions were excluded. According to the law on patients' rights, they signed an informed consent. We explained to them the visual prognosis and the treatment methods.

The follow-up period was for a median of 8 months. Depending on the severity of the diagnosis, patients were divided into 3 groups according to the classification of "Age-Related Eye Disease Study" (AREDS) of the U.S. National Eye Institute: group 1 – mild AMD, group 2 – moderate, atrophic AMD, group 3 – severe neovascular AMD. Patients received treatment with antioxidants, neuroprotectors and intravitreal injections with corticosteroids and/or Avastin. We recorded the visual acuity, anterior pole biomicroscopy, posterior pole appearance (Volk 78 D lens), Optical Coherence Tomography (OCT) and laboratory tests before and after treatment. As regards the laboratory tests, we followed the oxidative stress markers such as thiobarbituric acid reactive substances (TBARS), antioxidant enzymes – superoxide dismutase (SOD), and markers of inflammation – C-reactive protein (CRP). In the study group there were included only patients with severity level 2 and 3. The retinal thickness and retinal nerve fiber layer were measured with Spectral OCT OPTOPOL in 9 quadrants.

SOD activity was determined by xanthine oxidase method, TBARS were quantified by colorimetric method and CRP measurement was made by immune-turbidimetry with calibrator. We assessed the treatment efficacy by studying the evolution of visual acuity, OCT measurements and biomarkers' levels post treatment.

The statistical analysis (performed with the Statistical Programme 7.0) was intended to highlight significant correlations between the structural and functional changes and the results of the blood tests. We also wanted to evaluate the effects of treatment by comparing data obtained before and after treatment. The statistical comparison was made with "Difference tests" from the sub-set of descriptive statistic programs, being significant those differences which stood at a safe threshold of 95% ($p < 0.05$).

RESULTS

We investigated a group of 19 subjects, 8 males (42.11%) and 11 women (57.89%) with a mean age of 72.47 ± 7.74 years (59 to 88). The first control group (elder group) included 11 subjects (3 men and 8 women), with ages ranging between 60 and 85 years.

We used the second control group (younger group), which comprised of 40 subjects (11 men and 29 women), aged between 28 and 60 years, in order to compare the laboratory results.

The patients were treated with local injections with intravitreal Avastin and/or corticosteroids, systemic antioxidants and neuroprotectors, alone or in combination, according to individualized treatment regimens. Patients received an average of 4 injections with corticosteroids and 3 with Avastin.

We analyzed the effects of the treatment in the study group, comparing retinal thickness measurements and the blood results before and after treatment. In patients with the wet form, the mean retinal thickness was 347 273 µ, signifying the presence of macular edema. In patients with dry form, the mean retinal thickness was 257.47µ, falling within normal limits. After treatment, patients, in whom the macular edema reduced, falling within the normal values of macular thickness, were included in severity class 2. We also included in severity class 2 those patients in which the neurosensitive retinal detachment was reduced significantly (fig. 1).

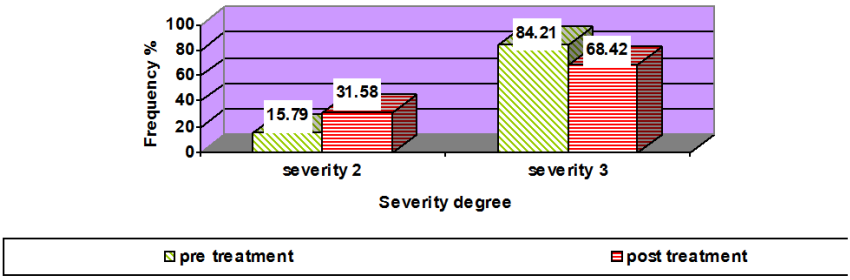

Fig. 1. The frequency of cases before and after treatment

After treatment there was a significant reduction (p = .02) of cases with a visual acuity worse than 0.30. Although not statistically significant, we note that after treatment there was an increase of cases with visual acuity between 0.30 and 0.40.

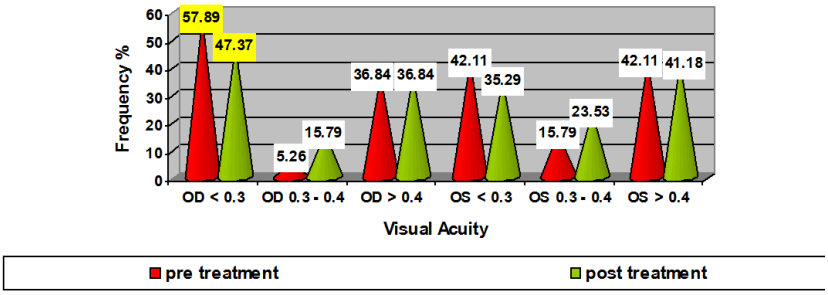

Fig. 2. The evolution of visual acuity before and after treatment

We noticed that after treatment, the incidence of macular edema decreases (fig.3). The frequency of cases with normal retinal thickness increased significantly

from 36.84% to 81.25%.

It appears that the frequency of atrophy after treatment decreased, even if we did not find it to be statistically significant. We measured the levels of oxidative stress and inflammatory markers such as SOD, TBARS and CRP in the study group pre and post treatment and in the control groups at the initial visit. The mean values of SOD, TBARS and CRP in the study group were slightly higher at the first examination compared to the second examination, made after treatment (tab. I).

The mean values of SOD, TBARS and CRP in the study group were significantly higher both before and after treatment compared to the values in the control group. Both before and after treatment, it was found that the mean values of SOD, TBARS and CRP were significantly higher in subjects with a diagnosis of severity level 3 (fig. 4, 5).

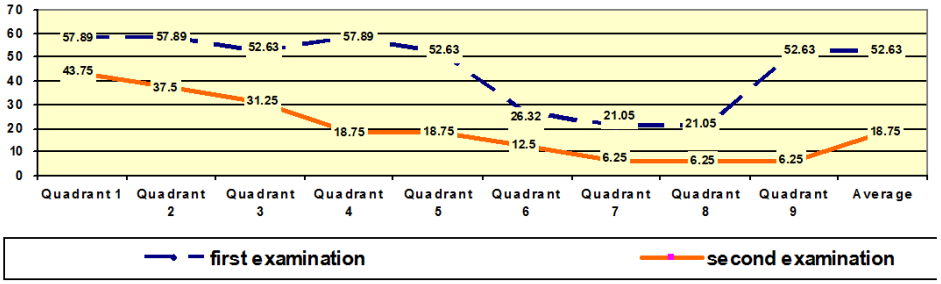

Fig. 3. The frequency of retinal edema before and after treatment

TABLE I

The mean values of the oxidative stress markers

	Number	SOD (U/ml)	TBARS (nmols/ml)	CRP (mg/dl)
Study Group before treatment	19	331.78	4.62	3.25
Study Group after treatment	19	313.15	4.31	2.87
Elderly Control Group	11	269.54	3.90	1.49
Younger Control Group	40	196.05	3.02	0.81

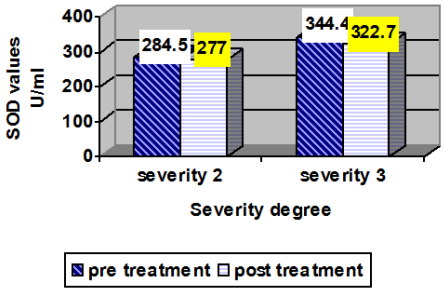

Fig. 4. The mean values of SOD pre and post treatment

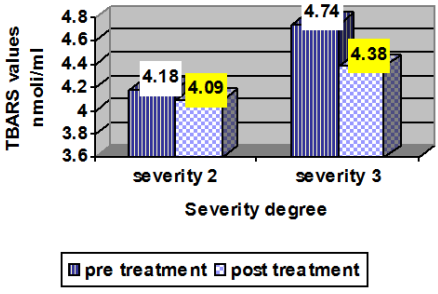

Fig. 5. The mean value of TBARS pre and post treatment

DISCUSSION

In the present study the treatment was performed with intravitreal injections with Avastin and/or corticosteroids, systemic antioxidants and neuroprotectors, alone or in combination, according to individualized treatment regimens. After treatment, the cases with severity level 3 were reduced from 84.21% to 68.42%, increasing the frequency of cases with severity level 2 from 15.79% to 31.58%. In terms of visual acuity, there was a significant reduction in the frequency of cases with a visual acuity below 0.30 from 57.89% before treatment to 47.37% after treatment and an increase in the frequency of cases with visual acuity between 0.30 and 0.40.

We noticed that the frequency of edema decreased significantly from the first examination to the second examination and the frequency of cases with normal retinal thickness significantly increased from 36.84% to 81.25%.

Treatment effects were supported by various studies. A new study showed new ways to treat atrophic and wet AMD. These included neuroprotection, prevention of oxidative stress and suppression of inflammation (2). AREDS study showed a significant benefit of the combination of vitamins and antioxidants, reducing the risk of complications in advanced AMD. The risk of progression was reduced by 25% for the entire study population and by 34% for patients with advanced AMD (3).

Some patients were treated with both Avastin and corticosteroid, and we observed a significant improvement both at the functional level (visual acuity) and at the structural level (assessed by OCT). The close link between the pathogenesis of CNV and VEGF-A expression suggests that VEGF-A inhibition may be a viable treatment in wet AMD. Trials such as VISION (4, 5), MARINA (6), ANCHOR (7), and Pronto (8) showed the benefits of treatment with anti-VEGF. An increase in visual acuity correlated with an improvement on the OCT was observed. CATT 2 study showed good results in maintaining visual acuity both with Avastin and Lucentis injected every 4 weeks (9). A recent study supports the importance of neuroprotective therapy in AMD. There are three neuroprotective-therapy strategies: ciliary neurotrophic factor, Brimonidine and fluorocinolon acetonide (10). The neuroprotective effect of corticosteroids on photoreceptors and RPE was suggested in a study by Glybina and collaborators. They showed that mice treated with fluorocinolon acetonide presented less atrophy and a smaller decline in the ERG b-wave amplitude. Sustained release decreases the retinal inflammation (11).

As regards the antioxidant enzymes and inflammatory markers, the present study showed that the average values of SOD, TBARS and CRP in the study group were significantly higher than those recorded in the control group. The mean values were significantly higher at the first examination compared to the second examination, made after treatment. After treatment, values were still above the normal range, suggesting the need for a long term treatment. The results are consistent with other studies supporting the fact that oxidative stress plays a key role in this disease (12, 13, 14).

CONCLUSIONS

The present research supports the role of oxidative stress and inflammation in AMD, and highlights the role of therapy directed against these risk factors. We found that after treatment, the frequency of

cases with macular edema decreases, while the frequency of cases with normal retinal thickness significantly increases. The mean values of SOD, TBARS and CRP in the study group were significantly higher both pre- and post-treatment, according to disease severity, compared to the values in control group. This suggests that treatment should be followed for a long time. The mean values of blood markers in the study group were slightly higher in the first examination compared with the second examination, after treatment, supporting its beneficial effect.

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IMMUNE REGULATORY PLASMACYTOID DENDRITIC CELLS SELECTIVELY ACCUMULATE IN PERITHYROIDAL LYMPH NODES OF PATIENTS WITH GRAVES DISEASE: IMPLICATIONS FOR THE UNDERSTANDING OF AUTOIMMUNITY

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IMMUNE REGULATORY PLASMACYTOID DENDRITIC CELLS SELECTIVELY ACCUMULATE IN PERITHYROIDAL LYMPH NODES OF PATIENTS WITH GRAVES DISEASE: IMPLICATIONS FOR THE UNDERSTANDING OF AUTOIMMUNITY(Abstract): Endocrine orbitopathy(EO) is the most common extrathyroidal manifestation of Graves disease (GD) but the involvement of the underlying immunological dysregulations remains largely unknown. The major source for IFNα-production, plasmacytoid dendritic cells (PDC) are fundamentally involved in the integration of TH1 and TH2 immune responses but also implicated in the pathogenesis of autoimmune diseases such as lupus. **Aim**: To establish whether PDC may play a role in GD autoimmune reaction and in the pathogenesis of EO. **Material and methods**:In a series of six sequential patients with GD as well as six further patients with multinodular goiter, cervical lymph nodes (LN) were sampled and preserved in the setting of thyroid resection. In parallel, peripheral blood samples were collected. The frequency of PDC from the peripheral blood and lymph nodes were determined. Mononuclear cells (MC) were enriched from lymph nodes and peripheral blood using Fiquoll-hypaque density gradient centrifugation. Mononuclear cells were stained using BDCA-2-PE and CD123-FITC monoclonal antibodies and their frequency subsequently analyzed using fluorescence activated cell sorting (FACS). Dead cells were excluded from analysis by appropriate gating strategies and propidium iodide (PI) staining. **Results**:In all patients with GD (with or without EO), PDC frequency was significantly increased in perithyroidal LN as compared to LN of patient not suffering form autoimmune diseases, e. g. patients with multinodular goiter ($p < 0.01$). The number of PDC infiltrating lymph nodes (LN-PDC) was also higher when compared to peripheral blood PDC (pB-PDC) of patients with multinodular goiter ($p < 0.05$). Finally, LN-PDC counts in perithyroidal lymph nodes of patients with GD were substantially increased when compared to pB-PDC of the same patients ($p < 0.01$) indicating a migration and accumulation of PDC in the draining LN. **Conclusions**: We found evidence that PDC selectively accumulate in perithyoidal LN of patients with GD, but not other thyroid diseases such as multinodular goiter. Based on their central importance in the pathogenesis of autoimmune processes such as in lupus erythematoides, we suggest

that the migration and accumulation of PDC in an anatomical joining point between the thyroid gland and orbita may be of critical importance for the initiation and maintenance of a chronic autoimmune stimulation. This implies a so far unknown role for PDC in GD and as putative cellular targets for new therapeutic approaches. **Keywords**: GRAVES DISEASE, IMMUNEREGULATORY PLASMACYTOID DENDRITIC CELLS, ENDOCRINE ORBITOPATHY, SURGERY

A common part of Graves' disease (GD) clinical picture (90–95 %), the endocrine orbitopathy(EO) appears less frequently in Hashimoto's thyroiditis and rarely unrelated to other thyroid conditions (0,5-5 %). About half of the GD patients have clinically obvious ocular signs, mostly of mild to medium severity, while only 3–5 % of the cases develop a severe EO, with optical nerve compression (1). Typically the EO occurs in the first 6 months from the onset of the thyroid hyperfunction (46-60%) although in 30% of the cases the ocular signs may appear after years, frequently associated with a relapse of the hyperthyroidism. This correlation between the onset of hyperfunction and EO suggests a common pathogenetical mechanism. The pathogenesis of EO comprises an increase of retroocular fibroadipose tissue and swelling of extraocular muscles and orbital connective tissues, due to an infiltration with lymphocytes (T cells), mastocytes and macrophages. A cytokine-related activation of the local connective tissue cells generates an increased production of glycosaminoglycans responsible for the appearance of edema as well as an increased adipogenesis, both resulting in an enlargement of the orbital tissue volume. An overexpression of TSH-R during the differentiation of preadipocytes was noted, underlying the role of TSH-R as an autoantigen in the orbita, which was confirmed by a number of studies on animal models (2).

In contrast to other regions of the body, the orbital fibroblasts express CD40. Normally present on the surface of B-cells, macrophages and dendritic cells, CD40 binds to the CD40-ligands (CD154) activated T-cells and subsequently stimulates the production of cytokines by macrophages. CD40-ligation determines orbital fibroblasts to express IL6 and IL8 (with subsequently enhanced chemotaxis and migration of immune competent cells in the orbita) as well as to activate inflammatory cyclooxygenases (by production of prostaglandine E) and raise the production of hyaluronic acid. Considering that cervico-regional lymph nodes are almost constantly enlarged in cases of Graves' disease, the aim of this pilot study was to investigate the role of the plasmacytoid dendritic cells (PDC) from the perithyroidal lymph nodes, as antigen presenting cells and major IFNα source, in the pathogenesis of autoimmune mechanism of the disease and particularly of EO.

MATERIAL AND METHODS

A series of 10 consecutive patients with GD as well as a series of 10 patients with multinodulareuthyroid goiter were enrolled in this study. In all cases, besides thyroidectomy, perithyroidal cervical lymph nodes (LN) were sampled. In the mean time, peripheral heparine-blood samples (20 ml) were collected. Fiquoll-hypaque density gradient centrifugation was used in order to isolate multinuclear cells (MNC) from LN and blood. To obtain LN tissue, the LNs were previously pressed on ice through a mesh, always on the day of the operation,

to avoid the loss of cells.

By fluorence-activated cell sorter (FACS) following a staining of the PBMC with two monoclonal antibodies (BDCA-2-PE und CD123-FITC), PDC were identified as a clearly defined and highly quantifiable population. The percentage of double positive cells was referred to percentage of MNC. Dead cells were excluded by appropriate gating techniques and propidium iodide (PI) staining. When sufficient material was available, a separation of PDC from MNC was performed by magnetic automated cell sorting (MACS), in order to provide RNA for further genetic testing (cDNA arrays). However, the purity after BDCA-4 MACS was approximately 30 % only. The staining of the MNC followed by monoclonal antibodies as previously mentioned. For immunostaining of TSH receptor in the LN of GD patient samples were fixed in 10% neutral buffered formalin for 24 hours then embedded in paraffin as a routine procedure. Sections were immunostained with a 1:250 diluted polyclonal anti-TSH Ab (Dako, Glostrup, Denmark). Detections were carried out using biotin-streptavidin and counterstained with Hämalaun for 10 sec to visualize nuclei.

RESULTS

Immunohistochemistry showed a powerful expression of TSH-receptors in the perithyroidal LN of the patients with GD and EO (fig. 1).

In all GD patients, irrespective of EO, the frequency of PDC was significantly higher in perithyroidal LN compared to normal cervical LN, sampled from patients with euthyroid goiter ($p < 0.01$).

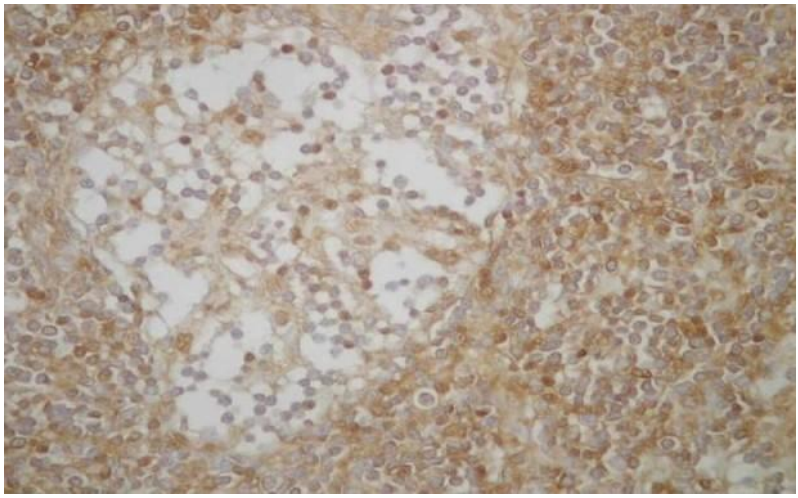

Fig. 1. Positive anti TSH-Receptor immunostain in perithyroidallymphnodes of patients with GD and EO

In a series of healthy patients and normal LN, the initial frequency of PDC was mean 0.4 % of MNC in blood compared to 0.2–0.3 % of MNC in LN. Higher frequencies of lymph nodes-PDC (LN-PDC) were always recorded in GD patients compared to peripheral blood (pB-PDC) ($p < 0.05$). And, above all, LN-PDC was significantly higher than pB-PDC in GD patients ($p < 0.01$)(fig. 2, 3).

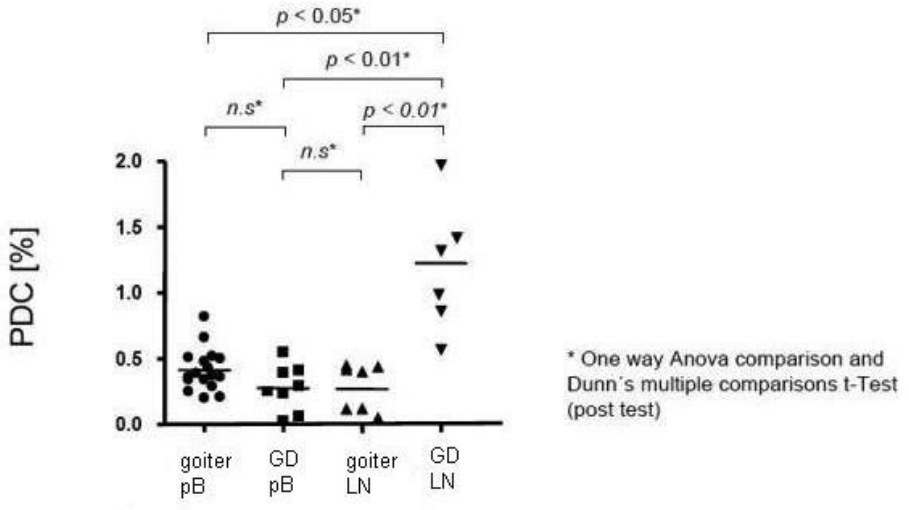

Fig. 2. PDC frequency in perithyroidal LN and peripheral blood of GD patients compared to euthyroid goiter patients

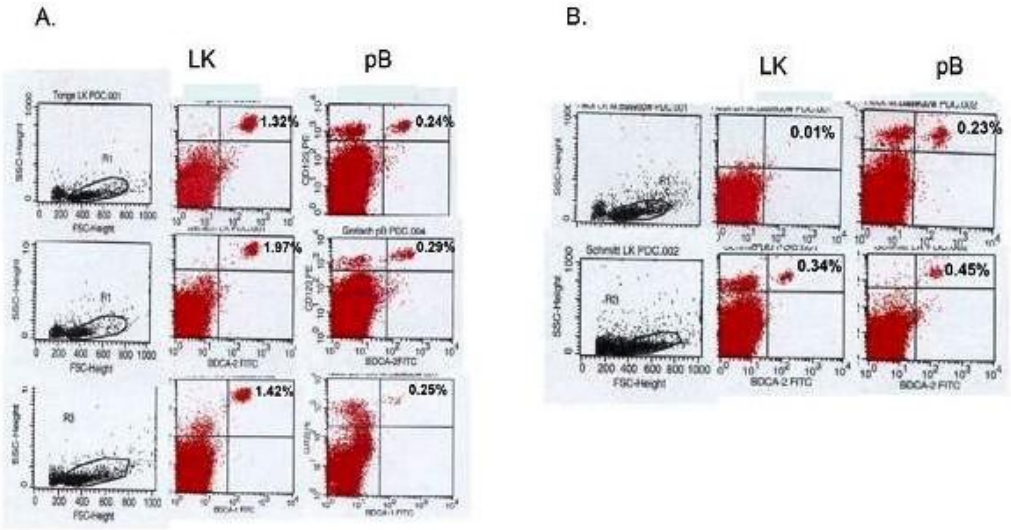

Fig. 3. Imunophenotype-based quantification of PDC in lymphnodes(LN) and peripheral blood (pB). CD123/BDCA-2 positive coloration corresponds to PDC population (right upper quadrant). A) GD patients. B) euthyroid goiter patients

DISCUSSION

As previously noted by Kriss et al. (3), in GD, hyperthyroidism and ocular disease share a common pathogenetical mechanism. They further formulated the hypothesis that regional LN may be the site of antibody presentation and processing. This was supported by the particular observation

of common lymph drainage pathways of thyroid and orbita. In 1970, Kriss and coworkers published the results of a thyroid 99Tcm colloid scintigraphy study, showing that in patients with GD and EO, compared to patients with other forms of hyperthyroidism or nodular thyroid diseases, a rapid and massive fixation of radionuclide occurs in cervical nodes bilaterally. They also proved the communication between the orbita and cervical LN through the inferior orbital fissure, allowing a deposition of antigen structures and the autoimmune reaction in both cervical LN and orbita. A clear correlation was established between the severity and activity of EO and the involvement of cervical LN, which Kriss thought at that time to be favourised by thyroglobuline(TG)(3). The enlargement of cervical LN by patients with GD has been previously noted by surgeons.

A single center two-armed prospective randomized study was published in 1998, comparing the results of subtotal thyroidectomy with or without cervical lymphadenectomy in GD and EO. In a follow-up period of three years, the authors monitored the titer of antibodies, thyroid function and development of EO by orbital MRT. A significant reduction of EO was noted for patients with thyroid resection and lymphadenectomy compared to patients with thyroidectomy only. Objectively assessing the severity of EO the volume of extraocular muscles, significant improvement was observed postoperatively in patients with thyroidectomy and lymphadenectomy ($p<0.001$) compared to patients with thyroidectomy only ($p=0.2$) (4). Moreover, in this study (and the following studies also), a reduction of serum standard immunological parameters to undetectable titers was achieved in patients with lymphadenectomy. It is worth mentioning that, in a previous paper, the same authors reported a significant (but not complete) reduction of TS-Ab after subtotal thyroidectomy, allowing them to state that additional lymphadenectomy generated a drastic reduction of TS-Ab.

Preliminary results of our team showed an intense expression of TSH-receptors in the perithyroidal LN of the patients with GD and EO. This drove us to the assumption that cervical LN plays a significant role in retrobulbar immune processes. Moreover, the confirmed systematical isolation of activated PDC in the LN draining the thyroid and orbita supports our opinion that regional LN are involved in chronic stimulation of immune system, possibly by pleiotrofic effects. It is a known fact that PDC, after initial activation in peripheral blood (e.g. after viral infections), migrate in the LN and present antigens to both B- and T-cells. Immune response may also be augmented by regulation of T-cells. Systemic lupus is probably the most researched autoimmune disease in regard to the role of PDC, which congregate in the skin and generate the autoimmune effect. Therefore, it is a logical assumption that accumulation of PDC in the regional perithyroidal LN is involved in the pathogenesis of the GD. In the view of both literature and our data, we consider that regional LN play a major role in the induction and development of extrathyroidal antigens processing, persistence and autoimmune stimulation in the frame of GD autoimmune hyperthyroidism. For this reason, it is to be proved by further studies if additional cervical modified lymph node dissection should be a standard procedure in the treatment of GD, aiming to significantly improve the outcome of ocular disease.

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NEWS

MICRORNA-27A INHIBITS HEPATITIS C VIRUS REPLICATION

A study by Shirasaki *et al.* on microRNAs expressed in hepatitis B and hepatitis C virus - infected liver, found that miR-27a, which regulates lipid metabolism, is preferentially expressed in HCV-infected liver. miR-27a was found to repress the expression of lipid metabolism-related genes and ApoA1, ApoB100 and ApoE3, essential for production of viral infectious particles. Repression of miR-27a increased the cellular lipid content, viral replication and infectivity, while miR-27a overexpression decreased viral infectivity and enhanced in vitro interferon signaling. The study showed that patients who expressed high levels of miR-27a had a favorable response to pegylated IFN and ribavirin therapy. Expression of miR-27a is up-regulated by HCV infection and lipid overload, and in turn it represses HCV infection and lipid cell storage. The negative feedback contributes to the maintenance of a low viral load and can help the virus to escape the host immune surveillance and cause a persistent chronic infection (Shirasaki T, Honda M, Shimakami T, Horii R, Yamashita T, Sakai Y, *et al*. MicroRNA-27a regulates lipid metabolism and inhibits hepatitis C virus replication in human hepatoma cells. *J Virol*. 2013 Feb 28. [Epub ahead of print]).

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INTERNAL MEDICINE - PEDIATRICS **ORIGINAL PAPER**

MALIGNANT TUMORS OF DIGESTIVE ORGANS AND LIVER METASTASES AT THE TIME OF DIAGNOSIS

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MALIGNANT TUMORS OF DIGESTIVE ORGANS AND LIVER METASTASES AT THE TIME OF DIAGNOSIS (Abstract) **Aim:** Malignant tumors localized in the digestive tract have a tendency to local growth and invasion with lymph node metastasis. Distant metastases through blood with prevalent liver location are detected late in disease progression, in an advanced stage, when therapeutic possibilities are often limited to palliative therapy. **Material and methods:** The study included a series of 139 patients with liver metastases admitted to the Center of Gastroenterology and Hepatology Iasi between January 1 and October 10, 2011 for the identification of primitive tumor. The patients were investigated by endoscopy, imaging, laboratory tests including tumor markers. **Results:** At 99 of the patients (71%) we identified primitive digestive tumors, in 19 patients (13.6%) we found tumors with extradigestive location and in 21 patients (15%) the primitive tumor could not be identified. Primitive tumor was located in various segments of the digestive tract, liver, and pancreas was follows: esophagus 4 - (4%), eso-cardial-tuberositary 2 - (2%), eso-cardial-tuberositary with pancreatic invasion 1 - (1%), stomach 15 - (15%), ileocolon 1 - (1%), colon 19 - (19%), rectum 12 - (12%), liver, multicentric hepatolcellular carcinoma 23 - (23%) billiary tract - cholangiocarcinoma 2 (2%), pancreas 2 - (20%). In a series of 586 patients with malignant tumors of the digestive organs referred to the Iasi Oncology Outpatient Unit between January 1 and September 30, 2011, 132 patients (23%) had liver metastases at the time of diagnosis; the most common locations were the stomach 41% cases (42/119 patients), followed by the pancreas, 35%, and colon 31% patients (28/79 patients and 37/119 patients, respectively). Males were most affected, regardless of primitive tumor. **Conclusions**: Although diagnostic and therapeutic methods have made remarkable progress in recent years, these tumors, by their frequency and advanced stage at diagnosis, remain both an oncologic and public health problem mainly due to the limitations of curative treatment. **Keywords**: DIGESTIVE MALIGNANCIES, SYNCHRONOUS LIVER METASTASES, METACHRONOUS LIVER METASTASES, SMALL HEPATIC LESIONS.

In the last decades, progress in oncology explores both achievements and controversies in the management of malignant digestive tumors. There are continuous changes in

the management of digestive cancers, particularly of those with liver metastases at the time of diagnosis, which in the current debate on neoplastic disease approached at the level of molecular structures, receptors, genetic basis for diagnosis, and biologic therapy keep actual both the topic and its historical value with a role in comparing survival rates or validation of therapeutic success.

In gastroenterology, modern endoscopic techniques introduced and used in current practice, such as echoendoscopy, NBI (narrow band imaging), confocal endoscopy, magnification endoscopy, chromo-endoscopy, video capsule endoscopy, made possible the identification of malignant lesions in all stages of development, regardless of their location in the digestive tube, doubled by curative therapeutic interventions by endoscopic polypecteomy or mucosectomy of early stage neoplastic lesions (1).

However, epidemiological data collected worldwide (GLOBOCAN), in Europe (EUROCARE,), at national, regional, and local level contradict these assertions by the morbidity and mortality rates of advanced digestive cancer with metastases at the time of diagnosis (2, 3).

In this paper we discuss about the evolution of epidemiological trends of digestive cancer with liver metastases from statistical data to prognosis, presenting relevant data from reference studies and also data collected from the patients admitted to the Institute of Gastroenterology and Hepatology, Center of Gastroenterology and Hepatology, and Outpatient Oncology Unit, and particularities found in both research and practice.

Epidemiologically, the "geographic map" of digestive cancer with liver metastases at the time of diagnosis shows a worldwide distribution.

In the US, more than 59,000 patients are diagnosed annually with gastrointestinal tract cancer and liver metastases, and in a series of 150,000 new cases, 25% already had liver metastases and another 20-50% will subsequently develop metastases (4,5). In a meta-analysis of 1260 articles, the prevalence of liver metastases in patients with digestive cancer was found in almost half of the patients with esophageal, colorectal and stomach cancer (6), and the statistics using necropsy data show that incident and the deceased cases overlap (tab. I).

TABLE I
Frequency of digestive cancer with liver MTS. Necropsy studies

Initial diagnosis (%)	Autopsy (%)
Colorectal cancer 20 - 40	100
Esophageal cancer 50	100
Gastric cancer 12	100

Regionally, data centralized in 2001 (source: Iasi Oncology Office) on the frequency of cancer in the Iasi County show that digestive cancer with gastric and colorectal localization is among the most common cancers diagnosed in this geographic area (tab. II)(8).

In a study conducted at the Iasi Clinic of Gastroenterology and Hepatology in 2002 on a series of 119 patients with histological confirmed colorectal cancer with duble Dukes and TNM staging, in 80% of cases the diagnosis was made in a late stage (III and IV). The risk for tumor recurrence and the likelihood of micrometastases (M_x) not assessed by under staging, were poor prognostic factors for these patients, as confirmed by the low 1.6 year-survival rate in the subgroup of patients residing in Iasi town (tab. III) (9).

In 2010, a study conducted at the Iasi Institute of Gastroenterology and Hepatology on a series of 4282 patients evaluated by upper gastrointestinal endoscopy (UGE), 118 cases with malignant lesions (histological confirmed) have been identified, corresponding to a frequency of 2.75% of the endoscopically examined population.

TABLE II

Frequency of cancers according to location in Iaşi County

Cancer - location	**Year**				
	1997	**1998**	**1999**	**2000**	**2001**
bronchopulmonary	273	302	243	281	299
breast	171	164	181	154	174
gastric	**116**	**111**	**130**	**137**	**147**
uterine cervix	90	73	100	74	93
colon	**83**	**85**	**73**	**91**	**74**
bladder	81	60	49	54	55
prostate	77	74	63	53	43
pancreas	**76**	**72**	**75**	**76**	**79**
liver – billiary tract	**70**	**77**	**72**	**84**	**79**

TABLE III

Study series colorectal cancer: TNM/ Dukes staging

TNM stage	**No. cases (%)**	**Dukes stage**	**No. cases (%)**
I	2 - (1.7)	A	-
II	15 - (12.8)	B	5 - (4.2)
III	**25 - (21.3)**	**C**	**13 - (11.2)**
IV	**55 - (47)**	**D**	**2 - (1.7)**

In the interval January-October 2011, 139 patients with liver metastases (study series) were admitted to the Iasi Center of Gastroenterology and Hepatology for the identification of primary tumor. Following assessments by endoscopic methods, imaging – contrast ultrasound, CT, laboratory tests, tumor markers, etc., in 99 patients (71%) primitive digestive tumors and in 19 patients (13.6%) primitive extradigestive tumors were found. In 21 cases (15%) the primitive tumor remained unidentified.

The series of 586 patients with malignant tumors of the digestive organs recorded at the Iasi Outpatient Oncology Unit between January 1 to September 30, which overlaps almost entirely with the above mentioned series, drew attention on the fact that at the time of diagnosis one in four patients (132/586 patients - 23%) also had liver metastases: from a gastric tumor in 41% (42/119 patients), or pancreatic or colon cancer (35% -28/79 patients and 31% -37/119 patients, respectively). Males were prevalently affected regardless of primitive tumor location (tab. IV).

TABLE IV
Malignant tumors of digestive organs (C15-C26) and liver metastases

Malignant tumors	Total	with MTS	Male/Female
Esophagus	20		19/1
Gastric	119	42	83/36
Small intestine and duodenum	4	2	3/1
Colon	119	37	58/61
Rectum -sigmoid	22	5	10/12
Rectum	79	12	49/30
Anal canal	5	1	1/4
Liver	94		69/25
Gallbladder	16	3	6/10
Bile ducts	5	2	2/3
Pancreas	79	28	45/34
Liver MTS	24		15/9

In a WHO report on the countries with highest number of cancer cases, Romania ranks 47 of 50, with countries like Denmark, France, and USA occupying the first 10 positions (10). If we have in view that the diagnosis of cancer means an early stage but also an advanced stage tumor, interpreting the significance of the position in this classification, additional to the presented statistical data, changes the meaning of "position-based advantage" with "outcome nature" given by tumor stage at the time of diagnosis, i.e. late-stage with liver metastasis. Depending on the time of occurrence in relation to primary tumor, two categories of metastases can be distinguished: synchronous, occurring at the same time with the primary tumor or within the first two years after its resection (it is believed that metastases were already present at the time of resection), and metachronous, occurring after more than 2 years after primary tumor resection.

Metachronous metastases may occur after very long time. There are case reports of metastases occurring over 5 years after surgery, the longest interval being 9 years (11). A detailed management image, starting from the incidence of synchronous and metachronous liver metastasis to prognosis, is shown by the epidemiological studies using colorectal cancer as a model. Exemplifying on a French population (Burgundy, 25-year study period), with data collected from cancer registries, synchronous liver metastases were found in 14.5% cases (13,463 patients), while metachronous liver metastases following resection had a 1-year cumulative rate of 4.3% and a 5-year cumulative rate of 16.5%. The approximately eightfold increase in the frequency of liver MTS from stage I to stage III makes that stage at the time of diagnosis to be confirmed as important prognostic factor of increased relative risk (RR) for disease dissemination (tab. V) (12).

TABLE V
Relative risk (RR) for liver MTS frequency

TNM stage	Frequency of liver MTS (%)
Stage I	3.7
Stage II	13.3
Stage III	30.4

What are the therapeutic options in these patients? The evidence gathered so far are powerful arguments and justifications for unfavorable prognosis of these patients despite the combined therapeutic efforts (surgery, systemic or loco-regional chemotherapy, radiation, radio or cryoablation, etc.). Patients with distant metastases are generally not considered curable, palliative treatment being in many cases the only alternative (13). Unfortunately, even in colorectal cancer, where a considerable improvement in the prognosis of liver metastases was recorded, it is accepted that after a longer or shorter period, patients will die from disease recurrence or liver or systemic dissemination. Therefore, given the high incidence of metastases at presentation, guidelines to determine which patients should perform specific tests to identify them are essential.

Therapeutic management indicates that only 10% -15% of the patients with colorectal cancer and liver MTS undergo curative liver resection. Increasing the proportion of patients undergoing timely resection was more important for metachronous liver metastases (16.9%), and only 6.3% for synchronous liver metastases (tab. VII) (14, 15). Even though the main feature of metastasis from colorectal cancer is a relatively slow progression allowing a therapeutic approach that gives the patient a fairly good chance of survival compared with other metastatic cancers, significant therapeutic benefit is reported only in patients undergoing surgery, particularly curative. Also, chemotherapy in unresectable liver metastases from colorectal cancer is a palliative treatment, with poor long-term results given the low response rate not exceeding 20% (16) even though the proportion of patients treated with adjuvant chemotherapy and palliative care grew significantly and faster when synchronous liver metastases were present than in case of metachronous liver metastases.

Regarding the primary cancer, Manfredi draws attention on the ascending trend of palliative resection from 44.3% (1976-1980) to 63.7% (1996-2000) with an average of 60.7% of the cases, but without resection of liver metastases (12). Despite that, 5-year survival rate dropped dramatically regardless of the type of metastases, the differences found being possibly accounted for by local recurrence or systemic dissemination of disease (tab. VI) (16).

More promising approaches put their hope in more effective treatments and mass screening, but until then synchronous and metachronous liver metastases from CRC remain a considerable problem by their frequency and severity.

TABLE VI
Survival rate

Synchronous liver metastases	Metachronous liver metastases
•34.8% at one year •3.3% at 5 years	•37.6% at one year •6.1% at 5 years

This context justifies, based on evidence, the need for new strategies centered on shifting goals towards the early stages of digestive cancer by technological interventions in all cases with premalignant lesions or at risk for digestive cancer. Obviously, the aim is to approach cancer in a therapeutically curable stage, meaning its progression to be stopped by eradication or neutralization of the conditions that favor metastasis.

Benign or malignant in small liver lesion-associated digestive cancer? Another aspect frequently reported in digestive cancer patients is the presence of small liver lesions, the benign or malignant nature of which raises a series of issues, from clinical and morphological differences to diagnostic methods effectiveness. The wider use of staging investigations has further clarified the advantages of introducing new technologies in the diagnosis of cancer. Temporal trend analysis with supporting evidence on stage distribution, as well as temporal trends in survival in relation to the stage at diagnosis have generated an important concept – „stage migration" – which involves the intervention in this migration phenomenon of the new technologies (17, 18).

Thus, patients previously diagnosed with cancer in localized or regional stage are now diagnosed by imaging methods (CT, MRI) with advanced stage cancer. The likely outcome would be to remove low survival patients with previously undetected distant metastases from localized and regional cancers categories and to put them in the advanced cancers category. Therefore, an accurate staging at the time of diagnosis may improve survival rates but not stage progression since the early stage will lose cases surviving shorter than those remaining in this category, while the advanced stage will gain the cases surviving longer than those already in this category. However, Feinstein (1985) stated that overall survival would not change (19). The practical importance of this concept in terms of stage-appropriate therapeutic interventions should be kept in mind.

The clinical importance of using this conceptual model is also supported by the low frequency of small liver lesions (1 cm, <1 cm) reported in 12.7% of the patients, classified in 11.6% of cases as metastases from digestive cancer (colorectal cancer 22 %, stomach cancer 21%), lymphoma - 4% or breast cancer - 14%. Still unresolved remains the benign or malignant nature of the lesions as their histological confirmation is almost impossible because of small size up to 1 cm. The alternative is lesion-size monitoring by CT, MRI, ultrasound so that when an increase in size is detected a biopsy to be taken; time interval is on average 13 months (range 1-37 months). Without treatment, median survival is about 5-l0 months (20, 21). Prognosis is obviously better in patients with single metastases than in those with multiple metastases. In a literature series, 1-year survival was 5.7% in patients with diffuse metastases, compared to 27% in those with metastases in one lobe of liver, and 60% in those with single metastases. Median survival in patients with single metastasis was 25 months (22).

CONCLUSIONS

Despite remarkable progress in diagnostic and therapeutic methods in recent years, these tumors, by their frequency and advanced stage at the time of diagnosis, remains an oncology and public health problem, mainly due to the limitation of curative therapeutic possibilities.

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NEWS

THE EFFECT OF DISINFECTING SOLUTIONS ON THE DIMENSIONAL STABILITY OF DENTAL ALGINATE IMPRESSION MATERIALS

Disinfection of dental materials is necessary to avoid cross contamination with micro-organisms from blood and saliva. One of the available disinfecting methods is the immersion in disinfecting solutions. The main disadvantage of this method is the dimensional changes of the impression materials. Some studies suggest that alginate initially swells and then shrinks, while other studies suggest that alginate swell continuously with time. The situation is currently confused because there are many alginates commercially available and various disinfecting solutions. This study evaluated the dimensional changes of two commercial alginates (Blueprint Cremix and Hydrogum) in distilled water and two disinfecting solutions (Perform ID/sodium hypochlorite), using a traveling microscope, at 5 minutes intervals over a period of 1 hour. The samples were rectangular strips with thickness of 1.5 and 3 mm, respectively. In all cases, both alginates continuously shrank with time, in the three immersion liquids, indicating the transfer of water from the alginate into the disinfecting solution. The $t^{1/2}$ shrinkage plots were generally linear, but with an intercept on the $t^{1/2}$ axis, indicating the possibility of an initial expansion at very short times. In most cases, the ratio of slopes for both thickness were 1.33-1.54. Performed ID gave anomalous results for the 1.5 mm thick samples. At ten minutes the shrinkage was 1.34-1.72%, compared with -0.42% to 0.67% in the other two media. Shrinkage readings at 10 minutes showed much larger differences between 1.5 and 3 mm thickness for Perform ID compared with NaOCl and distilled water. It was suggested that the different behavior exhibited by Perform ID may be due to its higher osmolarity and its lower pH compared with NaOCl. The dependence of shrinkage rates on sample thickness could have clinical implications. An impression of a dentate mouth has an intricate structure with some parts of the impression being thinner than the others. Such regions could shrink more rapidly, indicating the possibility of distortion. The results suggest that manufacturer's guidelines for the time of immersion should be added to the technical info of these disinfectants, in order to minimize the dimensional changes and distortion of alginate impressions (Muzaffar D, Braden M, Parker S, Patel MP. The effect of disinfecting solutions on the dimensional stability of dental alginate impression materials. The effect of disinfecting solutions on the dimensional stability of dental alginate impression. *Dental Materials* 2012; 28: 749-745).

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RARE TYPES OF DIABETES MELLITUS

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RARE TYPES OF DIABETES MELLITUS (Abstract): Diabetes mellitus is a heterogenous disorder characterized by chronic hyperglycemia and induced by a large number of etiopathogenic conditions. Beside type 1 and type 2 diabetes, which account for almost 90% of all cases, practitioners may encounter patients with more infrequent forms of diabetes, as those induced by mutations of a single gene, atypical immune disorders or neonatal diabetes. Monogenic diabetes is represented by genetic disorders in the structure of the β-cell (the MODY syndromes and the mutations of mitochondrial DNA) or in the insulin`s action (type A insulin resistance syndrome, Rabson-Mendenhall syndrome, leprechaunism, lipodystrophies). The rare forms of immune diabetes are determined by antibodies against insulin or insulin receptor or appear as a component of the "Stiff Man Syndrome". Neonatal diabetes is induced by mutations in genes that control β-cell development and function and may have a transient or permanent nature. Knowledge of the uncommon forms of diabetes mellitus enables physicians to apply the optimal treatment, to estimate the evolution of the patient and to apply a complete family screening in order to diagnose all other blood relatives as soon as possible. **Key words:** MONOGENIC DIABETES, INSULIN RESISTANCE, IMMUNE DIABETES, NEONATAL DIABETES.

Diabetes mellitus (DM) is a "syndrome" consisting of a heterogeneous group of disorders, with different etiology and pathogenesis, whose common element is hyperglycemia.

The prevalence of various types of diabetes differs significantly; type 2 DM is the most widely spread, followed by type 1 diabetes. The aim of this paper is to review some of the less common types of diabetes: genetic defects of the β cells, genetic defects in insulin action, some rare types of autoimmune diabetes and the neonatal DM.

1. GENETIC DEFECTS OF THE β CELLS

1.1. MODY Syndromes

In 1960, Fajans and Conn described for the first time a rare form of family inherited, non-insulin-dependent diabetes, occurring mostly in young non-obese Caucasians, with a good response to sulphonylureas. In 1964 Fajans suggested the name of "**M**aturity-**O**nset **D**iabetes of the **Y**oung" (MODY). In 1974 Tattersall published three MODY cases with autosomal dominant inheritance, while in 1975, together

with Fajans, he reported other 26 families with DM with autosomal dominant inheritance, going as far as 3-4 generations. Cases of MODY were identified afterwards on all continents; nowadays 7 types of MODY are described (1, 2).

The MODY syndromes represent 1% of all cases of DM and result from a single gene mutation (monogenic diabetes) which leads to dysfunctional insulin-producing pancreatic cells. The mutation is autosomal dominant inherited. The diabetes occurs in children and teenagers, usually before 25 years of age; although the disease starts during childhood, mild hyperglycemia causes the diagnosis to be delayed till older age. There is no tendency for ketosis.

The diagnosis criteria for MODY syndromes are (2):

1. Diabetes occurrence at a young age (at least one family member has to be diagnosed with DM below the age of 25)

2. There is no need for insulin treatment at least 5 years following the diagnosis;

3. Autosomal dominant inheritance (at least two generations of affected persons, although in most MODY affected families the disease is usually present in more than three generations)

As opposed to type 1 diabetes, MODY syndromes do not present a tendency for ketosis, the autoantibodies are absent (anti-insulin, anti β cell, anti-glutamic acid decarboxylase – anti-GAD) and there is no higher prevalence of HLA DR3 and DR4. As opposed to type 2 diabetes, MODY patients are usually thin, an insulin deficiency is present, but the insulin sensitivity is normal.

TABLE I
Classification of MODY syndromes (2)

Type	Mutant gene / protein
MODY1	Hepatocyte nuclear factor 4α (HNF 4α)
MODY2	Glucokinase
MODY3	Hepatocyte nuclear factor 1α (HNF 1α)
MODY4	Insulin promoter factor 1 (IPF 1)
MODY5	Hepatocyte nuclear factor 1β (HNF 1β)
MODY6	NEUROD1
MODY7	Preproinsulin

The seven MODY types (tab. I) can be grouped in two clinical forms: MODY induced by abnormalities of glucokinase (MODY2) and MODY induced by abnormalities of transcription factors (the remaining 6 forms).

1.1.1. Glucokinase MODY

Glucokinase is an enzyme that catalyses the phosphorylation of glucose to glucose-6-phosphate; in β-cells the glucose-6-phosphate acts as a glucose sensor, determining the regulation of insulin production

in accordance with the glycemic values. Over 200 mutations that lead to the *decrease of the glucokinase function* have been described. In the case of heterozygotes the glycemic level will be regulated at a higher level, but will remain under strict control. In homozygotes the insulin-dependent diabetes occurs in neonatal period (3). There are also mutations that cause an *increase of the glucokinase function*; these mutations cause congenital hyperinsulinism.

The patients present mild hyperglycemia even since birth (usually up to 145-150 mg/dl), these values slightly increasing with age. Postprandial glucose levels, as well as those measured two hours after glucose ingestion in the oral glucose tolerance test (OGTT), are just slightly higher than the fasting level (2, 3). An important increase in the glycemic level indicates that the patient develops type 1 or type 2 diabetes besides the mutation of the glucokinase gene. The value of HbA_{1c} is close to normal (values over 7.5% suggest another type of diabetes). Hyperglycemia is usually asymptomatic and the micro- and macroangiopathic complications of diabetes have an extremely rare occurrence. Differential diagnosis is made with type 1 and type 2 diabetes. As opposed to type 1 DM, in the case of MODY hyperglycemia remains slightly higher, anti β cells antibodies are absent, one of the parents has mild hyperglycemia and the C-peptide remains detectable. The differentiation from type 2 DM is more difficult, as both forms determine slightly higher glycemic levels and there is a family history of diabetes; in the case of MODY, obesity, as well as other insulin resistance markers are usually absent, the OGTT shows slightly higher values of the blood sugar level and hyperglycemia does not progress in time (3).

Due to the fact that the increase in the glycemic level is insignificant and complications are extremely rare, medications that would induce hypoglycemia are not recommended. If the patient is already under treatment, the medication may be ceased when the diagnosis of glucokinase MODY is confirmed. Medication should be stopped gradually, as MODY can also be associated with type 1 or type 2 diabetes (1, 3). Insulin therapy is recommended only during pregnancy, in order to improve the fasting hyperglycemia (4).

1.1.2. Transcription factor MODY

Transcription factors are proteins that bind to the DNA and form part of a complex regulation network that controls the gene expression. Most MODY patients show a heterozygote mutation in the gene of a transcription factor (most frequently the hepatocyte nuclear factors 1α and 4 α). The consequences of this mutation are: a decrease in the insulin secretion in the mature β-cells, a decrease in the development and multiplication of β-cells; apoptosis remains at the same level or is accelerated and the level of certain proteins involved in the carbohydrates metabolism (glucose transporters GLUT-2 or enzymes involved in the glucose mitochondrial metabolism) decreases (3, 5).

The patients are born with normal glucose tolerance; high blood sugar levels and diabetes occur gradually (between 10-30 years of age), as a consequence to the alteration of the β-cell function. The increase of the blood glucose level is age-related and the patients will need treatment (3). OGTT indicates very high glycemic levels two hours after glucose administration, although the fasting glucose levels are just slightly higher (usually under 150 mg/dl)

(3). Patients are usually thin and frequently develop the microvascular complications of diabetes. Besides these characteristics that are common for all types of transcription factors MODY, each type has its own extra-pancreatic manifestations:

MODY3 – Despite the high value of the HDL-cholesterol, the cardiovascular risk is significant (even higher than in the case of type 2 DM); the patients have a low renal threshold for glucose and glycosuria occurs (6, 7).

MODY1 – The patients have high birth weight (macrosomia). The risk of hypoglycemia is higher in the case of a MODY 1 infant (because of the more consistent insulin production *in utero* and during early childhood that eventually leads to a decline in insulin secretion and DM). Other characteristics are a decrease in the HDL-cholesterol values, lower levels of A1 and A2 lipoproteins and higher values of LDL-cholesterol (3, 6).

MODY5 – Renal impairment: renal cysts (66%), single kidney, horseshoe kidney, glomerulocystic kidney disease, renal cystic dysplasia; pancreatic hypoplasia with secondary exocrine dysfunction; low birth weight; genitourinary tract malformations (uterine anomalies, hypospadias); hyperuricemia and gout (3, 5).

As opposed to type 1 DM, in the case of transcription factor MODY one of the parents suffers from DM, the autoantibodies are absent at the time of diabetes diagnosis, ketosis is also absent even if patients do not receive insulin treatment, the C-peptide is detectable in plasma (>8 mmol/l) within 3-5 years following the diagnosis of diabetes, infants have a high birth weight and have a tendency for hypoglycemia, especially in the neonatal period (6). The differential diagnosis also includes type 2 DM; yet in the case of MODY the disease occurs during youth, there is a family history of DM at a young age, obesity, *acanthosis nigricans* or other markers of insulin resistance are usually absent, the lipid profile is characterized by an increase of the HDL-cholesterol, with normal or lower triglyceride values (uncommon for type 2 DM) while the sensitivity to sulphonylurea treatment is high (3, 6).

First intention treatment includes sulphonylurea drugs or prandial insulin releasers (repaglinide). Insulin therapy is considered only in the case of disease progression (7). Due to the high cardiovascular risk in these patients, statins are recommended for all subjects after the age of 40 (3).

1.2. Diabetes caused by mutations in the mitochondrial DNA

Mitochondrial DNA is a circular molecule that contains 37 maternally inherited genes. A punctiform mutation in position 3243 of the tRNA leucine gene (substitution of adenine for guanine) results in the occurrence of DM and sensorineural hearing loss. This type of diabetes remains frequently underdiagnosed. The mutation in position 3243 affects the mitochondrial respiratory chain, leading to cellular energy deficiency. The most frequently affected organs are the high metabolic activity ones: the pancreas, the cochleae, the retina (more seldom), the muscle, the kidney and the brain. The mitochondrial dysfunction in the pancreatic islets results in β-cells dysfunction, which involves the decrease of the β-cell mass and insulin deficiency (insulin sensitivity is usually normal). The mitochondrial DNA is maternally inherited, so that patients have a family history of diabetes in the maternal line; the children of a man carrying this mutation present no risk

of diabetes (8). Most mutation bearers show a high incidence of DM (over 85%) and sensorineural hearing loss (over 75%). Other clinical manifestations are less common: *retinitis pigmentosa*, cardiomyopathy, focal glomerulosclerosis, constipation or myopathy (9, 10).

The diagnosis is confirmed by testing the mutation in position 3243 in mitochondrial DNA in white cells or urine (9).

This type of diabetes requires early insulin treatment. Metformin is to be avoided (due to the risk of lactic acidosis), while coenzyme Q10 supplementation is recommended. The cardiac function must be monitored, especially for the patients with a family history of early cardiomyopathy. Aggressive antihypertensive medication is highly recommended, using inhibitors of angiotensin-converting enzyme in order to prevent renal complications (9). Any factors that would aggravate hearing impairment must be avoided, and ear infections must be treated promptly; when hearing loss occurs, auditory prosthetics and cochlear implants are recommended (3).

1.3. Other types of genetic defects of the β cells

Abnormalities of proinsulin to insulin conversion;

The production of mutant insulin molecules, resulting in an imperfect binding to insulin receptors.

These autosomal dominant inheritance types of diabetes are extremely rare, and their only manifestation is mild hyperglycemia (11).

2. GENETIC DEFECTS IN INSULIN ACTION

A defect in insulin action (or insulin resistance) occurs when a certain quantity of insulin produces a lower-than-usual biological response. The presence of *acanthosis nigricans* (skin indicator of severe insulin resistance) in a thin patient diagnosed with DM should point out to a genetic defect in insulin action (12).

Most often, genetic defects in insulin action are a result of ***mutations in the insulin receptor gene***. Three syndromes related to such mutations are usually described: type A insulin resistance syndrome, Rabson–Mendenhall syndrome and the Donohue syndrome (also known as Leprechaunism). The common characteristics of these syndromes are: hyperinsulinaemia, *acanthosis nigricans*, ovarian hyperandrogenism, glucose tolerance disorders (hypoglycemia and/or impaired glucose tolerance and DM) (13).

Type A insulin resistance syndrome is more common in females and has autosomal dominant inheritance. Patients are usually thin but present severe insulin resistance, *acanthosis nigricans*, signs of virilization (due to high levels of androgen hormones), hirsutism, oligomenorrhea, with frequent association of a polycystic ovarian syndrome (13). *Leprechaunism or the Donohue syndrome* was first described in 1954 by Donohue and Uchida and represents the most severe syndrome caused by the mutations in the insulin receptor gene. The patients present low birth weight, growth deficiency, glucose homeostasis disorders and specific dysmorphic features (large limbs, prominent ears, loss of subcutaneous fat, muscle atrophy, wrinkled skin and hypertrichosis, that make them resemble the Irish mythological Leprechauns (hence the name). The syndrome is characterized by autosomal dominant inheritance; death usually occurs before adulthood (3). *Rabson–Mendenhall syndrome* is also transmitted as an autosomal recessive trait. According to degree of insulin resistance

severity, it can be situated in between leprechaunism and the type A insulin resistance syndrome. Patients display *acanthosis nigricans*, severe growth retardation, dental abnormalities, coarse skin, senile facies, pineal hyperplasia, loss of subcutaneous fat, renal anomalies, abdominal distention and genitals enlargement. At the time of diagnosis patients may display paradoxical hypoglycemias which progress to diabetes. Life expectancy remains low (12, 13).

The clinical signs of these disorders are most often interrelated, and this is the reason for which some authors do not consider them as distinct syndromes, but rather as varied clinical traits of a *continuum* of the severity of the insulin receptor dysfunction (12).

As opposed to type 2 diabetics, patients with mutations in the insulin receptor gene are generally thin, although they display insulin resistance markers, high serum adiponectin levels and normal triglyceride values (14).

Insulin sensitizers (metformin, thiazolidinediones) have limited efficiency for this category of patients. When β-cell function diminishes, insulin therapy (in very high doses) is recommended (12). IGF-1 (*Insulin-like Growth Factor-1*) stimulates cellular glucose uptake and its storage as glycogen. In order to reduce side effects, IGF 1 can be administered together with the IGFBP-3 binding protein (15).

Hereditary lipodystrophies are clinically heterogeneous disorders characterized by loss of adipose tissue; they are associated with severe insulin resistance, DM, *acanthosis nigricans*, dyslipidemia and hepatic steatosis. Female patients can also display hyperandrogenism, oligomenorrhea and polycystic ovarian syndrome (3). There are two types of hereditary lipodystrophies: familial partial lipodystrophy and congenital generalized lipodystrophy.

Familial partial lipodystrophy is an autosomal dominant disease characterized by loss of peripheral adipose tissue from the subcutaneous compartment, associated with muscle hypertrophy, resulting in a "muscular" appearance of arms and lower limbs. Clinically, it is also characterized by loss of subcutaneous adipose tissue from the thorax and abdomen accompanied by an accumulation of adipose tissue in the face, neck and the intra-abdominal area. Diabetes frequently occurs over the course of the disease, especially in the case of female patients. Hypertriglyceridemia provides an explanation for the high incidence of cardiovascular mortality. The diagnosis is based on the "muscular" appearance of the limbs, loss of adipose tissue and diabetes occurrence at an early age in a non-obese patient with hypertriglyceridemia (3, 12).

Congenital generalized lipodystrophy (the Berardinelli-Seip syndrome) is an autosomal recessive disorder characterized by an extreme paucity of fat in the subcutaneous tissues even from birth, as well as by the "muscular" appearance described previously. Functional adipocytes are absent and consequently lipids are stored in metabolically active tissues. Patients display acromegaloid features, hypertrophic cardiomyopathy, intellectual deficits, hypertriglyceridemia, low HDL-cholesterol and hepatic steatosis which can lead to cirrhosis. Childhood growth is accelerated and diabetes appears during adolescence (3, 12).

Treatment for lipodystrophies includes dietary measures (very low content of lipids, less than 15% of total calories), intensification of physical exercise and medication: fibrates and high doses of fish oil for hypertriglyceridemia, metformin, thiazolidinediones and very high doses of insulin

for DM. Recent studies show that leptin administration improves the glycemic control, hypertriglyceridemia and hepatic steatosis (12, 13).

3. RARE TYPES OF IMMUNE DIABETES

Diabetes induced by anti-insulin antibodies: Anti-insulin antibodies may very rarely lead to an extreme decrease of insulinemia, so that they would determine the occurrence of DM.

Diabetes induced by anti-insulin receptor antibodies (type B insulin resistance) occurs in patients suffering from autoimmune diseases (the case of systemic lupus erythematosus, for instance). These patients frequently display *acanthosis nigricans.* The antibodies prevent insulin from binding to receptors at peripheral tissues level, causing hyperglycemia. In some cases, after binding to receptors, these antibodies react as insulin antagonists, leading to hypoglycemia.

Stiff Man Syndrome is a rare autoimmune disease of the central nervous system characterized by the stiffness of axial muscles, with painful spasms caused by unexpected stimuli or physical contact. High titres of anti-GAD antibodies are directed against an enzyme in the nervous system, and consequently the muscle is unable to relax (16). Anti-GAD antibodies also act upon the pancreas (which contains this specific enzyme), so that one third of the patients develop insulin dependent diabetes.

4. NEONATAL DIABETES MELLITUS occurs with a frequency of 1/300000 births up to 1/400000 births. It is the result of mutations of certain genes involved in the development and function of β-cells (17). About half of the cases have spontaneous remission (transient neonatal DM), while the remainder are considered to have permanent neonatal DM.

Permanent neonatal diabetes mellitus occurs most frequently (in about 50% of cases) due to mutation of genes encoding potassium channels (in 85% of cases the mutation arise spontaneously, while some cases are inherited in an autosomal dominant or recessive pattern); less frequently (about 12%) the insulin gene mutation is involved. The diabetes is usually diagnosed within the first 26 weeks of life and is characterized by severe hyperglycemia with ketosis. The C peptide is not detectable, and insular anti-cell antibodies are absent. Infants are smaller than gestational age (because of the reduced fetal insulin secretion) (17, 18). Because potassium channels are also found in the brain, the mutation of the gene encoding them may also determine neurological disorders (muscle weakness and epilepsy).

In the case of diabetes occurring due to mutations of genes encoding potassium levels, the initial treatment is insulin; in 90% of cases insulin can be replaced with sulfonylureas (in higher doses than those recommended for type 2 DM). Sulfonylureas might also improve the neurological symptoms. Diabetes caused by the mutation of the insulin gene requires insulin therapy.

Transient neonatal diabetes mellitus results from abnormalities in q24 region of chromosome 6. The disease occurs in the first week of life, with severe hyperglycemia and dehydration, yet without ketosis. The C-peptide is undetectable, the islet cell antibodies are absent. Newborn babies are smaller according to their gestational age (about 2.1 kg) and may present macroglossia and/or umbilical hernia. The insulin treatment is generally recommended for twelve weeks. In 50-60% of cases, diabetes reoccurs around the age of 14 (3, 18).

CONCLUSIONS

The accurate diagnosis of these types of diabetes can help define the prognosis, establish an efficient treatment and allow screening of patient's family members. Diagnostic testing is to be performed when the clinical picture is uncommon for type 1 or type 2 diabetes, when the anamnesis reveals an autosomal dominant inheritance, when certain characteristic traits of these rare types are detected as well as in all cases of diabetes diagnosed in the first six months of life.

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EVOLUȚIA CLINICO-FUNCȚIONALĂ A BOLNAVULUI CU SPONDILITĂ ANCHILOZANTĂ SUB INFLUENȚA FIZIOKINETOTERAPIEI

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CLINICAL-FUNCTIONAL EVOLUTION OF PATIENTS WITH ANKYLOSING SPONDYLITIS FOLLOWING PHYSIO-KINETOTHERAPHY (Abstract): Ankylosing spondylitis (AS), enigmatic from the etiologic point of view, appears with subjects who are still in school or involved in a productive activity. If detected in its early stages, under complex, constant and long-term treatment, patients have a good evolution. **Aims**: The objectives of the study are to describe the clinical and functional profile of patients with AS, sacroiliitis stage, and the evolution of physical and functional parameters under traditional physio-kinetotherapy. **Material and methods**: This retrospective study was performed on 40 patients with ankylosing spondylitis (AS), who were hospitalized in the Clinic of Rheumatology Iaşi, during 2008-1010, who satisfied the amended New York criteria for this. Subjects underwent an initial evaluation (first admission) and another one at the end of the study (second admission), after approximately 6 months. **Results**: The demographic characteristics, the clinical and functional elements of the study sample have indicated: the average age of 24.83 ± 3.948, predominantly male (82.5%) and 62.5% were from rural areas. Most cases occurred at the age of 19-25 (57.5%), beginning at 18-25 (77.5%), with an average of 19.60 ± 2.318. Following the radiological changes in the various stages of sacroiliitis, stage II prevailed (40%), then stage III (32.5%) and IV (15%). The evaluation of ASAS (Assessment of Spondylo Arthritis International Society) parameters and the respiratory system showed significant improvements of: BASDAI score with 31.53%, BASFI with 37.62%, BASMI with 20.66%, DIE % with 27.53 and of CV with 5.08% as well as a decrease in pain perception measured by VAS scale (p = 0.017). **Conclusions**: In the early stages of the disease, as far as the sample involved in the study is concerned, when the spine and vertebrae joints were not blocked by the evolution of the disease, corrective gymnastics and respiratory exercises, stretching and a good posture are very important, along with other therapies used to prevent axial ankylosis. **Key words**: ANKYLOSING SPONDYLITIS, SACROILIITIS, EVALUATION

Markerul diagnostic al spondilitei anchilozante (SA) este sacroiliita, leziune progresivă, cu patru stadii (Calin). Sacroiliita în marea majoritate a cazurilor este elementul de debut al bolii, manifestările clinice fiind marcate, de regulă, de simptome şi semne lombosacrate; ulterior procesul inflamator se extinde "în pată de ulei" la nivel axial exprimându-se prin lombalgii, dorsalgii de tip inflamator în 80% (1); această evoluție etapizată, trezeşte interesul şi dirijează terapia în direcția aplicării unor

modalităţi de prevenire a fenomenelor anchilozante la nivelul coloanei vertebrale, ce adiţional conduc şi la afectarea unor funcţii viscerale, precum cea pulmonară (2).

Enigmatică sub raport etiologic, SA debutează la subiecţii aflaţi încă în perioada activităţii şcolare sau productive. Depistaţi precoce, sub un tratament complex, insistent şi de lungă durată, bolnavii au o evoluţie favorabilă. Statisticile arată (3) că peste 60% din bolnavi pot duce în aceste condiţii, o viaţă normală, desfăşurându-şi întreaga activitate profesională; 35% - 40%, însă, dezvoltă infirmităţi prin afectarea coloanei vertebrale, cu sau fără prinderea centurilor, a articulaţiilor periferice (mai ales ale membrelor inferioare - 20-30%) şi fenomene viscerale. O analiză recent efectuată de Cochrane (4), concluzionează că fizioterapia (mai ales exerciţiul fizic) este benefică şi, în consecinţă, ea face parte integrantă din managementul SA; aplicarea unui program de exerciţiu fizic personalizat, în regim continuu susţine beneficiul terapeutic, mai ales în fazele precoce de boală.

Studiul nostru a avut două obiective majore: (i) evidenţierea profilului clinico-funcţional al bolnavului cu SA în stadiul de sacroiliită şi (ii) evoluţia parametrilor fizici şi funcţionali în urma aplicării fiziokinetoterapiei tradiţionale.

MATERIAL ŞI METODE

Am efectuat un studiu retrospectiv, prin monitorizarea foilor de observaţie la un lot de 40 de bolnavi cu SA, asistaţi în Clinica de Reumatologie Iaşi, în perioada 2008-2010. Criteriile pentru includerea în lotul de studiu au fost: pacienţi cu sacroiliită, fără afectare axială (clinic şi radiologic), care urmează tratament cu antiinflamatoare nesteroidiene (AINS) şi/sau biologice, fără comorbidităţi de tipul bronhopneumopatie obstructivă cronică (BPOC), diabet zaharat (DZ) dezechilibrat, ischemie coronariană silenţioasă, infarct miocardic, insuficienţă cardiacă sau angină pectorală de repaus sau la efort minim, sechele de rahitism, malformaţii congenitale toracice.

Fişa de evaluare standard, a înregistrat caracteristicile privind distribuţia pe grupuri de vârstă şi sex, după mediul de proveniență, tipul de debut, factorul ereditar, stadiul radiologic al sacroiliitei. Pentru definirea profilului clinico-funcţional al bolnavilor cu SA au fost evaluaţi o serie de indici de durere (VAS - *visual analogue scale*) şi mobilitate (indicii Ott, Schöber, distanţa deget-sol, diferenţa inspir/expir) precum şi scale valorice care au permis calcularea unor scoruri, atât iniţial (la prima internare) cât şi la finalul studiului (a doua internare), la interval de aproximativ 6 luni; au fost urmăriţi şi parametri ASAS (*Assessment of SpondyloArthritis International Society*) (5, 6) şi cel respirator (capacitatea vitală) (7, 2), ce permit evaluarea eficacităţii terapiei tradiţionale ce a fost efectuată instituţionalizat şi la domiciliu la recomandarea specialiştilor din cadrul spitalului şi sub îndrumarea unui cadru de specialitate de kinetoterapie.

REZULTATE ŞI DISCUŢII

1. Caracteristicile demografice ale lotului de studiu

Vârsta pacienţilor cuprinşi în studiu a variat între 18-31 ani, media înregistrată fiind de 24,83 ± 3,948, bărbaţii cu media de vârstă 24,94 ± 3,937 şi femeile cu 24,29 ± 4,271. Aceste valori, subliniază faptul că debutul bolii corespunde datelor din literatura de specialitate (1). În structura lotului, sexul masculin a predominat, 33 cazuri (82,5%) în acord cu datele care menţionează faptul că SA afectează îndeosebi sexul masculin.

Cele mai multe cazuri s-au înregistrat în intervalul de vârstă 19-25 ani (57,5%), urmat de categoria de vârstă 26-32 ani (40%) şi 18 ani (2,5%). Cei mai mulți pacienți (62,5%) au provenit din mediul rural.

2. Elemente clinice şi funcționale

Debutul tipic, s-a observat la vârsta 18-25 ani (77,5%) şi sub 18 ani (17,5%), cu o medie de 19,60 ± 2,318 ani.

Luând în considerare criteriile New York pentru diagnosticul SA (1), am remarcat predominant stadiul II (40%), urmat de III (32,5%), respectiv IV (15%).

Amprenta genetică a fost semnalată (20%) la 8 pacienți de sex masculin şi la 3 de sex feminin; antigenul HLA B27 a fost investigat la un număr redus de pacienți.

În funcție de scala analog vizuală de evaluare a durerii (VAS, 0 - 10 cm; 0 - absența durerii, 10 - durere maximă), am remarcat inițial ponderea semnificativ crescută a pacienților cu o percepție moderată (45%) – severă (40%), în timp ce la evaluarea finală, percepția durerii s-a mutat pe scală către o percepție a durerii uşoară (42%) – moderată (38%) (χ^2=8,20; GL=1; p=0,017). Aşadar, la cea de-a doua evaluare, se remarcă scăderea scorului VAS către o percepție uşoară la 61,1% din pacienții cu percepție moderată (11 / 18 cazuri) şi în proporție de 50% pacienții cu durere severă au scăzut pe scala VAS la durere moderată (8 / 16 cazuri) (tab. I).

TABELUL I

Evoluția percepției durerii în funcție de scorul VAS

VAS		a II-a evaluare									
		1	2	3	4	5	6	7	8	9	10
I evaluare	1										
	2	4									
	3	2									
	4		3								
	5			8							
	6				7						
	7					4					
	8						4				
	9							4			
	10								2	1	1

Durere uşoară scor între 1 şi 3	Durere moderată scor între 4 şi 6	Durere severă scor între 7 şi 10

Aplicând testul ANOVA asupra seriilor înregistrate pentru parametrii funcției fizice şi respiratorii pe parcursul monitorizării s-au constatat că valorile medii ai *indicelui Ott* şi *indicelui diferența inspir/expir (DIE)* (tab. II), au fost semnificativ crescute la reevaluare comparativ cu momentul inițial. *Indicele distanța deget-sol (DDS)* nu a prezentat diferențe semnificative, iar *indicele Schöber* a arătat că valorile medii au

fost semnificativ mai reduse (t=2,76; GL=78; p<0,l01). Valorile medii ale *capacității vitale (CV)*, au crescut uşor la reevaluare, dar nu semnificativ din punct de vedere statistic (t=1,79; GL=78; p>0,05) (tab. II).

TABELUL II

Indicatori statistici ai parametrilor funcționali şi respirator în funcție de momentul evaluării

Parametru	Eval.	Media	Min	Max	Dev.std	Err.std	Varianța	Mediana	p
Schöber (cm.)	I	3,56	1,5	3,5	0,79	0,12	4,69	2,5	**p<0,01**
	II	3,21	1,5	4,5	0,14	0,14	4,36	3,5	
DDS (cm.)	I	17,02	4	30	9,87	1,56	57,99	12	p>0,05
	II	14,13	4	30	9,15	1,45	65,22	11	
I.Ott (cm.)	I	1,99	0	3,5	0,95	0,15	47,73	2,5	**p<0,01**
	II	2,58	1,5	3,5	0,83	0,13	32,17	3,5	
DIE (cm.)	I	3,16	1	5	1,11	0,17	35,13	4,5	**p<0,00 1**
	II	4,03	2,5	6	1,16	0,18	28,57	4,5	
CV (%)	I	104,86	76	138	14,19	2,24	13,53	104,50	p>0,05
	II	110,19	87	138	12,43	1,96	11,28	109,30	

Evaluarea statistică a parametrilor ASAS, a permis aprecierea valorilor medii ale scalelor (9), ce măsoară nivelul de activitate al SA *(BASDAI)*, nivelul abilității funcționale a bolnavului *(BASFI)* şi nivelul mobilității spinale *(BASMI)*. Aplicând testul ANOVA asupra seriilor de valori înregistrate, pe parcursul monitorizării, s-a constatat că toate scalele au prezentat valori medii semnificative mai reduse la a II-a evaluare, la 6 luni, comparativ cu momentul inițial, ceea ce demonstrează o îmbunătățire a calității vieții pacienților cu SA (tab. III).

TABELUL III

Indicatori statistici pentru aprecierea statusului clinico - funcțional în funcție de momentul evaluării

Scala	Eval.	Media	Min	Max	Dev.std	Err.std	Varianța	Mediana	p
BASDAI	I	4,63	1	9	2,29	0,36	49,46	5,00	**p<0,002**
	II	3,17	1	7,9	1,77	0,28	55,84	3,15	
BASFI	I	34,15	4	82	22,07	3,49	64,63	32,0	**p<0,01**
	II	21,30	1	65	19,05	3,01	89,44	19,5	
BASMI	I	3,00	1	5	1,28	0,20	42,67	3	**p<0,05**
	II	2,38	1	5	1,21	0,19	50,84	2	

În ce priveşte consumul de antiinflamatoare nesteroidiene (AINS) şi medicația remisivă, modificatoare de boală DMARDs (*Disease Modifying Anti-Rheumatic Drugs*) nu au prezentat schimbări semnificative la cele două evaluări, pentru lotul studiat.

DISCUȚII

Spondilita anchilozantă are ca suport patogenic procesul inflamator ce se derulează la nivel sinovial (articulații sacroiliace) şi entezic (axial- coloana vertebrală şi periferic- inserțiile fascio-tendinose). Evoluția artritei sacroiliace se derulează în patru stadii ajungându-se la anchiloză (stadiul IV); la nivelul coloanei vertebrale procesul inflamator ascensionează treptat către regiunile înalte ale axisului, lăsând în urmă elemente care blochează sistemul vertebral transformându-l într-o tijă rigidă. Anchiloza articulațiilor interapofizare, costovertebrale şi sindesmofitele deteriorează nu numai mobilitatea axisului ci şi capacitatea respiratorie (1, 2, 8), fapt ce degradează suplimentar indicele de dizabilitate şi calitatea vieții bolnavului. Recunoaşterea bolii în stadiile incipiente ale evoluției sale, singura care poate asigura succesul terapeutic şi implicit un bun prognostic funcțional, depinde de interpretarea interactivă a manifestărilor clinice în contextul unor investigații complementare judicios alese (8).

În stadiile doar cu manifestări sacroiliace, în cazul lotului în studiu, când coloana vertebrală nu a fost blocată de evoluția ascendentă a bolii, fiziokinetoterapia tradițională a determinat evitarea limitării extinderii redorilor şi anchilozelor şi la îmbunătățirea tonusului muscular, necesar menținerii posturii şi a ventilației pulmonare (3).

Pornind de la ideea că o fiziokinetoterapie corectă şi insistentă, într-o fază în care morfologia coloanei nu este afectată (examen radiografic), este foarte utilă pacientului cu sacroiliită, studiul nostru a urmărit evoluția parametrilor clinico-funcționali şi respiratori în două momente situate la interval de 6 luni. Astfel, am constatat îmbunătățiri semnificative cu 29,64% a indicelui Ott, DIE cu 27,53%; a scorului BASDAI cu 31,53 %, BASFI cu 37,62%, BASMI cu 20,66%, iar a CV cu 5,08%, precum şi o scădere a percepției durerii înregistată prin scala VAS. Aşadar, valorile obținute, prelucrate statistic (Testul ANOVA, testul t-Student, Testul χ^2, coeficientul de variație) au demonstrat utilitatea programelor tradiționale de fiziokinetoterapie, în cadrul unui program terapeutic cu o metodologie modernă de susținere funcțională a coloanei vertebrale.

Metoda daneză Heckscher, alcătuită dintr-o suită de exerciții corectoare pentru toate segmentele posibil a fi implicate în actul respirator (10), sugerează că dubla disfuncționalitate din SA (coloana vertebrală şi aparatul respirator) pot deveni obiectivele unor programe speciale de kinetoterapie.

Capacitatea fizică, funcțională a aparatului locomotor şi respirator sunt astăzi cuantificabile prin structuri obiective, validate; evoluția morfologică a bolii, este şi ea cuantifiabilă prin imagistica tradițională şi modernă. Din schema terapeutică pentru SA a EULAR (*European League Against Rheumatism*) se desprinde clar locul fiziokinetoterapiei (5).

CONCLUZII

Din analiza datelor statistice a celor 40 de pacienți cu SA aflați în stadiul de sacroiliită (fără modificări vertebrale), s-a confirmat apariția bolii la vârsta tânără, cu predominanță la sexul masculin.

Îmbunătățirea parametrilor algici, fizici şi funcționali ai bolnavului, exprimată prin scale specifice pentru SA, a scos în evidență contribuția tratamentului complex, cu caracter multidisciplinar, în care cel kinetic, specific sistemului mioneuroartrokinetic, ocupă un loc principal.

În stadiile incipiente ale bolii, când coloana vertebrală şi articulațiile costoverte-

brale nu au fost blocate de evoluţia ascendentă a bolii, kinetoterapia prin mijloacele şi metodele ei, asuplizarea, posturarea sunt foarte importante, alături de celelalte terapii, pentru prevenirea disfuncţiei respiratorii.

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NOUTĂŢI

DISFUNCŢIA DIASTOLICĂ ŞII MORTALITATEA ÎN SEPSISUL SEVER ŞI ŞOCUL SEPTIC

Disfuncţia sistolică a fost prima studiată în cazul şocului septic şi s-a constatat că nu întotdeauna determină agravarea prognosticului. Mai recent, a fost evaluată şi relaţia dintre disfuncţia diastolică şi riscul cadiovascular la pacienţii cu sepsis sever.262 pacienţi internaţi în unitatea de terapie intensivă cu diagnosticul de şoc septic au fost supuşi la 2 ecocardiografii succesive pe parcursul spitalizării, ei fiind evaluaţi şi din punct de vedere clinic şi al datelor de laborator. 36% dintre pacienţi au decedat pe parcursul spitalizării. Scăderea undei e' la nivelul inelului mitral a fost cel mai puternic predictor de mortalitate, şi după ajustarea datelor în funcţie de scăderea debitului urinar şi cardiac sau a saturaţiei oxigenului. Pacienţii cu disfuncţie sistolică şi/sau diastolică au avut creşteri uşoare ale troponinei T şi creşteri importante ale NT-proBNP. Aceste date susţin ideea că disfuncţia diastolică este frecventă şi este un predictor important de mortalitate în şocul septic. (Landesberg G, Gilon D et al. Diastolic dysfunction and mortality in severe sepsis and septic shock. *European Heart Journal 2012*; 33(7): 895-903)

Corina Dima-Cozma

METODE DE CUANTIFICARE A DIZABILITĂŢII ÎN ARTROPATIA PSORIAZICĂ: ANALIZA MERSULUI VICON

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DISABILITY ASSESSMENT TOOLS IN PSORIATIC ARTHRITIS: VICON GAIT ANALYSIS (Abstract) **Aim**: To evaluate health-related quality of life and disability in psoriatic arthritis (PsA) and to perform a complex biomechanical gait analysis in patients with PsA versus healthy controls. **Material and methods**: Preliminary prospective study on 33 consecutive PsA (2006 CASPAR criteria), with active multiple joint involvement disease. Assessments were done according to a predefined protocol and included: rheumatology- clinical evaluation and activity scores, dermatology- Psoriasis Area and Severity Index, PASI-score; functional disability- Health Assessment Questionnaire, HAQ, quality of life score- Short Form36, SF36; biomechanical gait analysis- tridimensional VICON MX optical motion capture system in PsA versus 33 age- and sex-matched healthy controls. *SPSS, EPI-INFO* and *MS EXCEL* were used for statistical analysis. **Results**: While the majority of PsA patients experienced a moderate impact on the quality of life as assessed by SF36 analysis, severe disease had major impact on their physical and emotional health status. The VICON gait analysis proved the statistical significant change ($p<0.05$) on several gait parameters as compared to controls, including: decreased range of motion for flexion/extension of the lower limbs joins and lower cadence, decreased walking speed and step length. Statistically significant correlations (Spearman, $p<0.05$) between changes in PsA gait parameters and SF36 results, but no association with HAQ ($p>0.05$) has been reported. **Conclusions**: The PsA burden is related to both quality of life and functional impairment. Severe changes in gait parameters are essentially reflected in SF36 analysis. **Key words**: PSORIATIC ARTHRITIS, DISABILITY, HEALTH-RELATED QUALITY OF LIFE, VICON GAIT ANALYSIS.

Artropatia Psoriazică (AP), entitate distinctă în cadrul reumatismelor inflamatorii cronice reprezintă o spondilartropatie prezentă la 4-30% din pacienţii diagnosticaţi cu psoriazis cutanat (1, 2, 3). Cu o distribuţie aproximativ egală pe sexe, AP implică atât scheletul axial-coloana vertebrală şi articulaţiile sacro-iliace cât şi scheletul periferic cu tendinţă la afectare asimetrică. Afectarea cutanată precede de obicei afectarea articulară care apare în medie la 10 ani după diagnosticul psoriazisului (4).

Evoluţia cronică, cu perioade de remisiune alternante cu acutizări, determină rapid eroziuni articulare, osteoformare, distrucţii şi anchiloze osoase cu impact

dizabilitant important asupra capacităţii funcţionale şi asupra calităţii vieţii, aspecte evidenţiate de multiple studii (5, 6, 7). Ambele componente ale bolii - cutanată şi articulară acţionează ca şi cumul de factori dizabilitanţi, potenţându-şi reciproc acţiunea.

Capacitatea AP de a genera handicap determină costuri socio-economice înalte, atât directe cât şi indirecte (7,8) similar altor patologii reumatismale considerate prototipuri de boli invalidante - poliartrita reumatoidă şi spondilita anchilozantă (9,10) şi justifică reconsiderarea algoritmului de diagnostic, monitorizare şi managementul terapeutic al AP.

Studiul îşi propune cuantificarea potenţialului dizabilitant al AP prin evaluarea impactului asupra calităţii vieţii, evaluare funcţională şi analiza biomecanică a mersului.

MATERIAL ŞI METODE

S-a realizat un studiu observaţional prospectiv pe un lot de 33 pacienţi spitalizaţi în Clinica de Reumatologie a Spitalului Clinic de Recuperare Iaşi, diagnosticaţi cu AP (criteriile Caspar 2006), cu forme poliarticulare, active de boală, cu implicarea articulaţiilor portante, în diferite stadii de evoluţie, fără alte comorbidităţi, pacienţi aflaţi sub tratament cu medicaţie remisivă *Disease-modifying antirheumatic drugs* (DMARDs) sau terapie biologică – blocanţi ai factorului α de necroză tumorală (a-TNFα).

Studiul a urmat un protocol predefinit care a cuprins cele două domenii respectiv: domeniul reumatologic (parametrii articulari individuali şi paraclinici de activitate ai bolii) şi domeniul dermatologic (evaluare clinică a implicării psoriazice prin scorul de activitate *Psoriasis Area and Severity Index* PASI). Pacienţii au fost evaluaţi funcţional prin chestionarul *Health Assessment Questionnaire* (HAQ), validat pentru AP (5). Impactul asupra calităţii vieţii a fost evidenţiat conform literaturii prin chestionarul *Short Form 36* (SF36). Chestionarul cuprinde 36 de întrebări grupate în opt domenii de interes: statusul fizic, starea de sănătate generală, capacitatea funcţională, durerea, statusul psihic, impactul social, impactul emoţional şi vitalitatea. SF36 oferă două scoruri finale: impactul bolii asupra componentei fizice şi impactul asupra componentei psihice (11, 12).

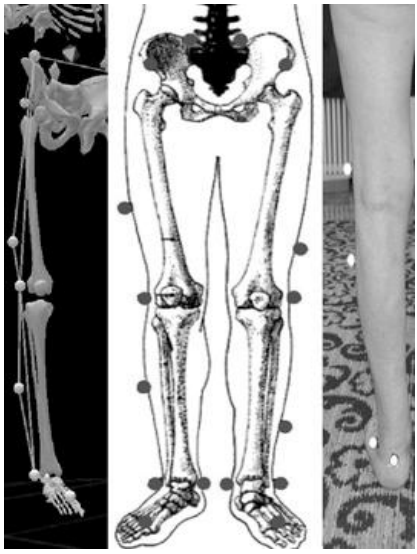

Fig. 1. Modelul *"plug in gait"* de aplicare a markerilor de suprafaţă

Evaluarea mersului s-a realizat cu un sistem optic de analiză tridimensională a mersului VICON (VICON Motion System Ltd., UK). Acesta presupune aplicarea a 16 markeri pasivi pe membrele inferioare ale subiectului care efectuează mai multe cicluri de mers. S-a utilizat modelul clinic predefinit de amplasare a markerilor "plug in gait" (13) (fig. 1). Şase camere de detec-

ţie în infraroşu au recompus prin triangulaţie în ”real time” poziţia spaţială a acestora. Dinamica a fost analizată de softul aparatului. S-au urmărit variabilele biometrice de timp şi spaţiu şi cinematica articulaţiilor coxofemurale şi genunchilor prin comparare cu un lot martor de 33 subiecţi sănătoşi similari pe criteriile vârstă şi sex.

Analiza statistică s-a realizat în programele MS EXCEL, EPI INFO şi SPSSS şi a cuprins analiză descriptivă şi testele: χ-*pătrat*, *t-Student* şi corelaţie Spearman.

REZULTATE

1. Particularităţile lotului: Lotul studiat a cuprins 33 pacienţi cu o repartiţie aproape egală pe sexe Bărbaţi:Femei = 1:1.06. Mediul de proveniență a fost predominant cel urban fapt explicat de accesul uşor la serviciile medicale ale acestei categorii U : R = 26 : 7. Majoritatea subiecţilor au avut nivel educaţional superior 63% - 21 cazuri. Media de vârstă la examinare a fost de 49 ani(32-57, $_{SD}$ 6,4), vârsta de debut fiind în medie de 40 ani (14-44, $_{SD}$8.1). Afectarea cutanată a precedat afectarea articulară în toate cazurile. Durata afectării cutanate a fost în medie de 15,2 ani (8-23, $_{SD}$ 5.8), durata de evoluţie a afectării articulare şi implicit durata AP fiind în medie de 7,5 ani(3-11, $_{SD}$ 2,8). Distribuţia formelor de boală a fost reprezentată de: forma axială 12 cazuri (36,36%), forma poliarticulară 20 cazuri (60,61%) şi un caz (3,03%) de artropatie mutilantă.

2. Domeniul reumatologic: Evaluarea clinică reumatologică a demonstrat forme active de boală cu implicarea unui număr mare de articulaţii tumefiate (NAT) şi articulaţii dureroase (NAD), gradul de activitatea al bolii fiind oglindit şi de tabloul paraclinic-sindrom inflamator prezent la majoritatea cazurilor (tab. I).S-a evidenţiat prezenţa dactilitei la 11 (33.3%) cazuri, eroziunile articulare fiind documetate radiologic la un număr de 19 (57,59%) cazuri; 28 (85%) pacienţi au fost sub terapie DMARDs, 5 (15%) pacienţi în tratament cu a-TNFα.

3. Domeniul dermatologic: S-a evaluat nivelul de activitate al leziunilor cutanate. Un număr de 12 (36,36%) cazuri au prezentat leziuni active cutanat. Scorul PASI a avut o medie de 1,79(1-25,5 $_{SD}$ 4,96); 1 caz cu psoriazis cutanat intens activ, 1 caz cu afectare moderată şi 10 cazuri cu afectare uşoară. S-a detectat afectare ungveală cu leziuni distructive la nivelul patului ungveal la 5(15,1%) pacienţi.

TABELUL I

Domeniul reumatologic: parametrii clinici şi paraclinici

NAD(0-78) medie	16.2 (6-24)
NAT (0-76) medie	**6.9 (3-12)**
Scala analogă vizuală a durerii VAS (0-100mm) medie	**60.48 (40-80)**
Starea de sănătate generală evaluată de pacient GHp (0-100) medie	**6.09 (4-8)**
Starea de sănătate generală evaluată de pacient GHd (0-100) medie	**6.33 (5-8)**
Viteza de sedimentare a hematiilor VSH (<15mm/h) medie	**28 (8-87)**
Proteina C Reactivă CRP (<0.5mg/dl) medie	**3.8 (0.6-12.8)**

4. Analiza capacităţii funcţionale şi a calităţii vieţii: Evaluarea prin chestionarul HAQ a relevat un impact funcţional important înregistrându-se o valoare medie de 1,62 (0,3-2,6 $_{SD}$ 0,61). Analizând pe grupe

de invaliditate s-a remarcat pentru afectarea severă o frecvenţă de 15,1%, afectare moderată prezentă la majoritatea cazurilor (54,5%) şi doar 10 pacienţi cu afectare funcţională uşoară. Scorurile înregistrate prin chestionarul SF36 au fost reorganizate în cele două domenii de evaluare: fizic şi mental. Dacă pentru domeniul mental s-a obţinut o medie de 41,63(15,7-58,4, $_{SD}$8.3), domeniul fizic a înregistrat o medie de 31,54 (20,7-53,6, $_{SD}$8,6). S-a pus în evidenţă, o corelaţie semnificativă statistic între rezultatele SF36 componenta mentală, SF36 componenta fizică şi HAQ :

SF 36 componenta mentală / HAQ r= 0.65, p <0.05 corelaţie Spearman

SF36 componenta fizică / HAQ r =0.64 p <0.05 corelaţie Spearman

5. *Analiza biomecanică a mersului*: Variabilele biometrice de timp şi spaţiu respectiv: viteza de mers (distanţa/timp), cadenţa/ ritmul paşilor (paşi / unitatea de timp), lungimea pasului (distanţa între două contacte succesive cu solul ale aceluiaşi talonului), dublu suport şi suport unipodal (fazele ciclului de mers cu spijin bipodal şi unipodal) au înregistrat valori semnificativ statistice mai mici pentru lotul cu AP faţă de populaţia sănătoasă. Raportul dintre sprijinul unipodal şi cel bipodal la pacientul cu AP atestă inversarea fazelor ciclului de mers, în sensul creşterii fazei de susţinere şi scurtării fazei de oscilaţie. Analiza cinematică s-a realizat prin calcularea intervalului dintre flexia şi extensia maximă în grade definit ca amplitudine de mers (*range of motion* - ROM) pentru articulaţiile portante. Compararea cu lotul martor a atestat valori semnificativ mai mici la pacienţii cu AP (tab. II, fig. 2).

TABELUL II

Variabile biometrice de timp şi spaţiu, date cinematice, analiză comparativă între cele 2 loturi

PARAMETRU	APs Medie DS	Lot control Medie DS	Diferenţa mediilor	Interval încredere diferenţa mediilor	p
Viteza (m/sec)	0,98 (DS 0,28)	1,28 (DS 0,14)	0,30	0,21-0,4	<0,05
Cadenţa (paşi/min)	99 (DS 12)	114 (DS 11)	15	12-18	<0,05
Lungime pas (m)	0,45 (DS 0,08)	0,64 (DS 0,04)	0,19	0,10-0,28	<0,05
Dublu suport (sec)	0,42 (DS 0,2)	0,23 (DS 0,3)	-0,19	-0,25 - -0,14	<0,05
Suport unipodal (sec)	0,49 (DS 0,05)	0,41 (DS 0,04)	-0,8	-1 - -0,6	<0,05
ROM (flx/ext) ŞOLD grade	36 (DS 7)	43 (DS 5)	7	2-8	<0,05
ROM (flx/ext) GENUNCHI grade	47 (DS 8)	56 (DS 3)	9	6-12	<0,05

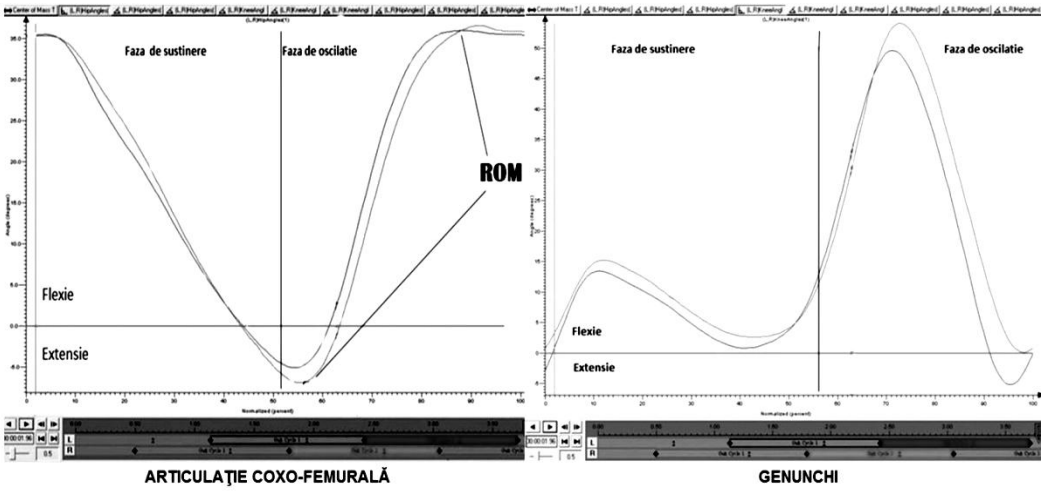

Fig. 2 Cinematica articulaţiilor coxo-femurale şi genunchilor

S-au comparat toţi parametrii de mers cu scorurile HAQ şi nu s-a pus în evidenţă o corelaţie semnificativă statistic (r în intervalul 0,28-0,32). Analiza acestora versus rezultatele SF36 a relevat o asociere semnificativă statistic (r în intervalul 0,59-0,62).

DISCUŢII

Rezultatele studiului atestă capacitatea invalidantă marcantă a AP la pacientul cu o evoluţie a bolii în medie sub 10 ani. Chestionarele HAQ şi SF36 s-au dovedit a fi instrumente valide în evaluarea statusului funcţional şi calităţii vieţii, mai mult, scorurile obţinute au fost în concordanţă cu cele din literatură (5, 6, 11, 12). Evaluarea biomecanică complexă a mersului a pus în evidenţă deficitul motor sever al pacientului cu AP faţă de populaţia sănătoasă. Analiza cinematică s-a corelat cu rezultatele obţinute prin SF36 dar nu şi cu HAQ. Acest aspect poate fi explicat prin faptul că deşi ambele chestionare analizează statusul funcţional global al pacientului, HAQ se concentrează mai mult asupra activităţilor realizate cu membrele superioare. Considerăm în acest sens utilă o analiză biomecanică globală pe un model cu markeri poziţionaţi pe corpul întreg într-un studiu ulterior. Absenţa implicării cutanate la majoritatea pacienţilor studiaţi nu se încadrează în profilul clasic al bolii ci a reprezentat un criteriu de selecţie pentru a pune în evidenţă impactul componentei articulare asupra calităţii vieţii.

CONCLUZII

Artropatia Psoriazică se poate prezenta sub forme invalidante de boală cu impact major asupra calităţii vieţii şi cu deficit funcţional sever al *pattern*-ului de mers atât pentru amplitudinea de mobilizare articulară cât şi pentru parametrii de mers aşa cum o demonstrează analiza VICON. Aceste modificări biomecanice se reflectă în gradele de disfuncţie obţinute prin scorurile SF36, dar o asociere cu evaluarea afectării funcţionale prin HAQ nu a putut fi pusă în evidenţă.

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TRAUMATIC INJURIES CAUSED BY AIRBAGS

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TRAUMATIC INJURIES CAUSED BY AIRBAGS (Abstract): The airbag is a passive life-saving system. In case of car crashes, the airbag inflates in milliseconds, activated by collision sensors and inflates with nitrogen. Since the airbag system became mandatory on automotive industry, the number of injuries associated with it has increased. A study performed by the National Automotive Sampling System (NASS) demonstrated that 66% of passengers suffer skin injuries from airbag. The airbag associated injuries rate is 42% at upper extremities, 41% at the face level, 3% at the neck level, 11% at the chest and abdomen level and 2% at lower extremities level. Though these wounds do not jeopardize the life, about 300000 car passengers suffer airbag related injuries in the USA. In this narrative review paper, we intend to review available literature data regarding airbag related trauma. We want to underline that despite the fact these systems are meant to protect life, they can be harmful or even deadly in certain circumstances. They can induce injuries on a broad range from easy to very dangerous when. **Keywords:** AIRBAG, AIRBAG RELATED TRAUMA.

The increase of the number of cars on the world's roads has brought about a significant increase in car accidents. In developed countries such as the USA and England, the effect has been felt even more strongly due to high accessibility to purchasing vehicles (1). Anyone can be injured because of a car accident either as a driver/car occupant or as a pedestrian. Many such cases have been occurring since the first airbags, so it was necessary to introduce measures for the protection of the road users, either punitive, legal or technological, represented by the belt-type safety systems or the airbag-type system. In the 1950s, the first functional airbag was reported on a car in the USA (1). The first to develop such equipment seems to have been Allen K. Breed. In 1968 he patented an accident and protection detection system. His interest was focused on military life-saving equipment. It was triggered based on sensors and filled with air. He sold his invention to Chrysler. In England, the first airbags were introduced in the late 1980s. Cars began to be extensively equipped with airbags in 1992 on Ford-built cars. In 1995, around 70% of the world's cars were equipped with airbags (1). Even though Breed's invention is a reference in the field of accident protection systems, attempts to patent a similar pro-

tection system have existed since the 1950s - apparently, John W. Hetrick, of Pennsylvania, applied for a patent in August 1952. In Europe, German Walter Linderer and American John Hendrik applied for airbag-related patents in 1951. Hetrick was the first to receive the patent, a few months before Linderer. Linderer's proposal contained a compressed air system that was triggered either by a shock in the car bumper bar, or by the driver.

Based on the information from the automotive industry, statistically, the airbag has saved and still saves lives daily. According to a US Department of Transportation report, between 1987 and 2015 alone, 44869 lives were saved (2). However, airbags seem to have the potential to cause serious and even fatal injuries (1, 3), especially in the case of children, fact made public around 1962. Tests performed by Ford in the Auto Safety Research Office have shown that there are enough forces associated to the airbag that can seriously injure a child. Tests conducted around 1980 continue to support the risk of injuries. Studies conducted over a period of about 40 years have shown that this system designed to protect car occupants can also kill (4, 5).

In 1990, the first reported case of airbag related trauma appeared when a 64-year-old woman died of chest injuries caused by the opening of an airbag. The year 1993 is an unfavorable year in the history of airbags, as the first cases of trauma in which the airbag opening caused the death of 23 children were reported. In 2000, the first fatal case associated with airbags was reported in England (1).

In 1995 appeared the side airbag, designed for lateral impact. In 2001, the knee airbag is launched on the market. The anti-slip (anti-submarine) airbag was designed in 2002, which is included in the seat's backside under the hump, to reduce the forward or backward movement of the basin. In 2005, a new improved dual-chamber airbag, specially created for the front seats, appeared. The two airbag compartments formed a depression in the center at triggering (6) and the "roll over" airbag (7).

In recent years, numerous deaths resulting from airbag injuries have been reported (8). Most accidents occurred at low speeds of the car, speeds at which harms would have not happen to passengers following an impact, no airbag inflates, only car damages. In the United States, NHTSA reported in 1999 146 deaths due to airbag related injuries, of which 84 children. All were front seat passengers. These passengers were either children or adults who were too close to the airbag, and following an impact, the airbag opened at a high speed, hitting the passengers straight in the face and causing, in almost all cases, skull fractures, lesions of the brain and neck blood vessels.

For this reason, it is advisable to keep an acceptable distance from the airbag, at least 20 cm, and to place children in the backseats is possible. At the time, airbags that are adjusted to suit the passenger's position, height and weight, with dual inflation system, which have considerably reduced the number of airbag related injuries, were developed (6).

American studies show that the most common injuries due to airbags are excoriations 64%, followed by contusions (38%), wounds (18%) and burns (8%) (9). The survival chance in car crashes increase if the airbags is doubled by the seat belts (10, 11). It also helps to reduce the risk of

serious injuries by distributing the forces resulting in the impact over the passenger's body.

The front airbags for the driver and front passenger are the most used, followed by the lateral ones for chest protection (12). The latest generation of airbags, already implemented on cars, are activated according to the severity of the collision. If the deceleration detected is below a certain threshold, then the bags are partially inflated. If it is a violent collision, the inflation is performed at full capacity.

The non-slip airbag working principle is like the other airbags. The anti-submarine pad, part of the non-slip bag, may or may not meet the passenger seat. A part of the bag inflates from below the chair or from under the board and hold the car occupants to slip under the steering wheel. At the impact time, it is filled with air limiting sliding or rotation of the pelvis (13).

The airbag device has the following components:

- the bag itself, made of a folded plastic fabric, with a volume of about 60 liters for the driver and 160 liters for the front passenger. Depending on the application, the area where the airbag is placed, the bulk bag volume varies between 30 and 200 liters.
- the cover that holds this bag

The opening of the device is triggered by shock-sensing sensors (which are piezoelectric systems) sensitive to decelerations that send the signal through the electrical contacts to a computerized control module then to the inflator module (electrical priming and gas generator system). If the airbag is activated, an explosive chemical reaction occurs (the most commonly used explosive is sodium azide) and the device is quickly inflated for the individual’s protection. The airbag opens in three phases that are the cutting (the airbag sac case is broken by a combustion material contained by a pyrotechnic device), the catapulting (the airbag is forced by the pressing from the cutting phase, to go out the steering wheel and inflates in about 0.04 seconds) and eventually the explosion (the airbag deflates almost immediately after the catapulting phase, while the gas escapes through special valves).

The lesions caused by the bag of the airbag device differ from those caused by the cover that holds the bag.

There are four types of collision mechanisms in car accidents, namely between the vehicle and other objects, between the vehicle occupant and the interior of the vehicle, between the internal organs and their cavities (the skull, the rib cage) and between the occupants of the vehicle and objects inside the car.

Airbag related injuries

Approximately 2.5% of drivers involved in car accidents suffer different types of airbag related injuries (14). Such lesions can occur at both high and low speed. Also, shorter people, closer to the airbag, can be more easily injured. The same situation includes children and the elderly, persons that could bot prepare the body for the impact, have slow reactions (takes usually longer time to figure out a way to protect in stress situations) (15, 16, 17).

Major types of airbag-related injuries include:

- disfigurement - hard pieces of the steering wheel or the airbag case hit the car occupants faces during the cutting process;
- chemical burns - the inflation gas used by the airbags might burn the face or the body skin when is not going out through

the evacuation valves but through arbitrary airbag crashes;

- fractures – the pressure of the body on the airbag could break upper body bones;
- pulmonary and cardiac complications;
- internal bleeding.

Anatomical formations most often affected by airbags are the face, the chest (exposed directly, closest to the front airbag), the neck (1), and the spine (18).

Lesions due to the cover cutting and bag catapulting

- atlanto-occipital dislocations;
- cervical spine fractures;
- cerebral trunk sections (19);
- cardiac, renal, splenic rupture (20, 21, 22);
- bilateral carotid internal dissection (23). Internal carotid artery lesions are accompanied by typical excoriations at the level of the neck, neurological sequelae, including hemiplegia, and a low Glasgow score, may be associated with a Horner sign (if the sympathetic chain is affected). Internal carotid artery lesions arise from the application of a force in the neck, in our case by the inflated airbag that determines a hyperextension that can cause the dissection of the artery at the junction between the intracranial portion considered immobile, and the extra cranial one, which is relatively mobile. If a patient has been the victim of a road accident involving an airbag, unilateral or bilateral carotid injury should be considered; contrast angiography should be performed if unilateral or bilateral carotid lesions are suspected (24);
- axonal lesions;
- subdural hematomas;
- facial injuries - fractures of the frontal sinus anterior wall, nasal bones, fractures or nasal septum dislocations, orbital wall fractures, temporomandibular joint contusions, dental lesions, breakage of dental prostheses, facial fractures in the form of cranial-facial disjunctions (rare). The airbag causes the temporomandibular joint to suffer, with pain, dislocation of jaw, chewing muscles and occlusion disorders (25).
- fractures of the cervical spine (of one or more vertebrae) by hyperextension;
- anterior and posterior longitudinal spinal ligaments injuries, medullary sections;
- ocular lesions (26), from palpebral hematomas with subconjunctival hemorrhage and corneal abrasions, to retinal detachment and eyeball rupture (27, 28), coexisting with chemical keratitis lesions (29). Other lesions cited in the literature include bullous keratopathy, hyphemia, iris sphincter ruptures, crystalline lens subluxations and dislocations, traumatic cataracts, hemophthalmias, some followed by a permanent decrease in visual acuity or even loss of vision. In the case of people wearing glasses, they break out producing wounds and corneal foreign bodies (30, 31, 32).
- ear lesions - various studies have shown that with the introduction of the side airbags the number of otology lesions associated with the opening of the airbag has increased. Otology lesions depend on the location of the airbag (side), the number of airbags (the more airbags that open, the higher the risk of suffering injuries), the car's interior volume, whether windows are open or not (studies carried out in this respect have shown that in all the cases where there were injuries the windows were closed) (**Error! Reference source not found.**);
- thoracic wall lesions - clavicle fracture, sternum fractures, rib fractures (33, 34, 35);

• open tibia fractures (36).

Lesions due to explosion (slapping)

The most common and frequent lesions are minor ecchymosis and excoriations located in the face, neck and upper limbs.

• chemical burns - chemical keratitis, contact dermatitis in the hands and face might happen when the airbag gas is touching the car occupant's skin depending on how sensitive their skin is to such contact. The children have more sensitive skin than the adults;

• barotrauma: the pressure inside the vehicle suddenly increases when the airbags are inflated. Typical lesions are eardrum perforation (usually unilateral) and bilateral barotraumatic pneumothorax;

• explosive noise with a detonation of over 170 dB can be associated with decreases in unilateral or bilateral auditory acuity, which is accompanied by persistent complaints and equilibrium disturbances;

• chemical pneumonia and asthma-related symptoms (sodium azide forms a white cloud inside the car);

• thermal, usually superficial burns in the hands, face, neck. They are caused by the action of hot gas generated when the airbag is inflated. Inhalation of hot gas can cause burns of the nasal mucosa, pharynx and larynx. The temperature outside of the bag after inflation reaches 80 degrees, sufficient to produce first and second degree burns after contact with the face, neck, and hands. A combination of chemical and thermal burns can cause necrosis of the entire skin layer (37, 38).

Lesions due to the airbag cover

• hands tegument detachment;

• injuries of the hands and forearms - pollex, metacarpal and phalangeal fractures, both bones of the forearm (sometimes bilateral), fingers and hands amputation (39). Open fractures showed severe lesions of muscle, tendons, synovial, vessels, nerves;

• cranial lesions - subdual hematomas, cortical contusions, cranial fractures, atlanto-occipital fractures;

• decapitations (4).

Other lesions

• premature rupture of membranes in a pregnant woman or fetal lesions (40);

• death, especially in children (6).

The spectrum of airbag specific lesions is broad enough to ask ourselves whether this equipment is lifesaving or not. However, from a statistical point of view, its utility has been confirmed, the number of saved lives justifying the use of this type of car accident protection equipment. The safety and security of both the persons inside the car and pedestrians, correlated with sufficiently consistent case studies of injuries caused by contact with airbags, has led to the development of a true industry oriented towards such systems. It has been required to use laboratory tests for safety systems to optimize them, on the one hand (41), also out of the need to reuse the same test equipment configuration in different scenarios. Virtual prototyping and laboratory tests on dummies currently precedes tests on deceased subjects used in car crashes self-controlled in laboratory conditions (42).

Two main potential cause groups of the airbag related injuries were identified. One is related to the airbag system malfunctioning meaning that either the airbag design itself has a technical issue or the system is old, and the maintenance process was not followed up and done properly. On the other hand, the car occupants should be aware that pieces of the airbag case flight

away during the inflate/deflate process, a gas at a certain amount of temperature might touch the car occupants body and burn the skin or just irritate it. Additional education during the driving school must include information about the potential harms the life-saving systems implemented into a car can produce. Even more, the persons with certain medical conditions, such as breathing issue or skin sensitivity should take precautions before driving.

CONCLUSIONS

With the decrease in the number of injuries resulting from road accidents, a new series of lesions, namely those caused by the car's safety systems, has emerged.

Safety systems demonstrated their value every day through the large number of lives saved and will always be one of the most important assets of a car.

Most experts say that an airbag is not advised for pregnant women, but to be as cautious as possible, the pregnant woman should move the seat as far back as possible to create a comfortable distance between her and the dashboard or between the wheel and her.

The solutions to avoid the potential lesions caused by the airbags include head restraint systems, modern safety belts with controlled, continuous tensioning features, intelligent airbags.

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NEWS

CARDIAC ARREST DUE TO VENTRICULAR FIBRILLATION IN A 23-YEAR-OLD WOMAN WITH BROKEN HEART SYNDROME

Broken heart syndrome, also known as takotsubo cardiomyopathy, is a syndrome characterized by a transient regional systolic dysfunction of the left ventricle associated to a psychological stress and therefore constitutes a form of stress cardiomyopathy. Patients with broken heart syndrome are most frequently women, presenting with chest pain and/or shortness of breath within hours or days of an emotional stress, frequently associated with electrocardiographic changes; elevation of markers of myocardial necrosis; large areas of akinetic myocardium involving circumferentially the apex, the base, or the midventricular segments in a noncoronary distribution, generally in the absence of obstructive coronary artery disease. As such, these patients are classically diagnosed with the broken heart syndrome while being evaluated for possible acute myocardial infarction. Less often, however, the diagnosis of broken heart syndrome is less obvious. The authors described the case of a 23-year-old female habitual marijuana user who was resuscitated after cardiac arrest and then diagnosed with midventricular stress cardiomyopathy complicated by subendocardial hemorrhage. They discussed this unique pathological finding, the incidence of arrhythmias in this syndrome, and the possible relation with chronic cannabis and tobacco use. Unfortunately, the patient did not survive, but had she survived, the management of the patient for secondary prevention would have been challenging considering the risk of recurrence with this disease. In conclusion, broken heart syndrome (acute cardiomyopathy due to emotional stress) may cause sudden death due to life-threatening ventricular arrhythmias as the presenting symptom in young women. This may be related to a more severe injury to the heart resulting in subendocardial hemorrhages and may be related to chronic tobacco and marijuana abuse. The optimal acute and long-term treatment of this severe form of stress cardiomyopathy remains unknown (Del Buono MG, O'Quinn MP, Garcia P, Gerszten E, Roberts C, Moeller FG. Cardiac arrest due to ventricular fibrillation in a 23-year-old woman with broken heart syndrome. *Cardiovascular Pathology* 201; 30: 78-81).

Doina Butcovan

AFECTAREA HEPATICĂ ÎN BOALA CELIACĂ LA COPIL

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LIVER INVOLVEMENT IN CELIAC DISEASE IN CHILDREN (Abstract): Celiac disease (CD) is an autoimmune systemic enteropathy triggered by gluten intake in patients with genetic susceptibility, characterized by clinic polymorphism: classic forms, mainly with digestive features, and atypical forms, liver involvement being a part of them. In present, any unknown cytolysis requires screening serologic determinations for CD. **Aims**: to assess the presence of liver manifestations in children diagnosed with CD, the outcome of liver function with gluten-free diet (GFD) and also to emphasize the importance of the immunological screening for CD in patients with unknown etiology liver dysfunctions. **Material and methods:** The trial was formed by 120 patients diagnosed with CD between January 2007 – December 2010 in 2nd and 3rd Pediatric Clinics of "Sf. Maria" Hospital Iaşi; liver function was assessed; there were determined viral hepatitis and autoimmune hepatitis markers; all patients were given GFD, hepatoprotective agents and antivirals specific to each form of hepatitis; the transaminases level variation was followed in time**. Results:** 12 of the CD diagnosed patients (10, 14%) had altered liver function at the onset of disease; the only abnormality was the increased transaminases level in 57, 14% of cases; HBsAg was found positive in 33, 33% (4 cases); liver biopsy in one patient evidenced steatosis. The study has shown that 4% of the patients with cryptogenetic hepatitis have a silent form of CD, the serologic screening for AGA, AEA, ATGA being essential for diagnosis. **Conclusions**: we have to rule out CD in patients with liver disease of unknown etiology, before we consider it as "cryptogenetic"; occurrence of cytolysis in the absence of positive viral markers requires the assessment of screening tests for CD. **Key words**: CELIAC DISEASE, ENTEROPATY, SCREENING SEROLOGIC, LIVER BIOPSY

Boala celiacă (BC) este o enteropatie sistemică autoimună declanşată de ingestia de gluten la pacienții cu predispoziție genetică caracterizată prin polimorfismul manifestărilor clinice: forme clasice, dominate de manifestările digestive şi forme atipice, afectarea hepatică fiind una dintre acestea. În prezent, orice sindrom de citoliză de etiologie neprecizată impune efectuarea testelor serologice de screening pentru BC (1, 2).

Scopul acestui studiu a fost de a evalua prezența manifestărilor hepatice la copiii diagnosticați cu BC. Obiectivele studiului au fost: stabilirea corelațiilor dintre gradul

sindromului de citoliză hepatică şi durata expunerii la gluten, spectrul manifestărilor extra-hepatice asociate şi evoluţia funcţiei hepatice sub regim fără gluten (RFG), precum şi evaluarea importanţei *screening*-ului imunologic pentru BC la pacienţii cu disfuncţii hepatice de etiologie necunoscută.

MATERIAL ŞI METODĂ

Lotul de studiu a cuprins 120 de pacienţi diagnosticaţi cu BC în perioada 1 ianuarie 2007-31 decembrie 2010 în Clinicile II şi III Pediatrie a Spitalului de Copii „Sfânta Maria" din Iaşi. La toţi pacienţii a fost investigată funcţia hepatică prin sindromul de citoliză, bilio-excretor, hepatopriv**,** γGT. În vederea stabilirii etiologiei hepatopatiei am determinat markerii virali – AgHBs, anticorpii (Ac) anti-VHC, anti-Toxoplasma, anti-Epstein Barr, anti-Citomegalovirus, markerii pentru hepatita autoimună. La toţi pacienţii s-a instituit RFG şi tratament hepatoprotector. Pacienţii cu AgHBs prezent au primit şi tratament antiviral specific formei de boală. După instituirea RFG a fost urmărită în dinamică evoluţia sindromului de citoliză.

REZULTATE

Din cei 121 de pacienţi diagnosticaţi cu BC, 10, 14% (12 pacienţi) au prezentat afectarea funcţiei hepatice la debutul bolii.

Vârsta pacienţilor a fost cuprinsă între 19 luni şi 127 luni (vârsta medie 88,5 luni). Repartiţia pe sexe: 7 pacienţi au fost de sex masculin şi 5 pacienţi de sex feminin (fig. 1).

TABELUL I

Valorile medii ale vârstei în relaţie cu sexul pacienţilor

Vârsta (luni)	**Media**	**-95%**	**+95%**	**Dev.std**	**varianţă**	**Er.std**	**Min**	**Max**
Feminin	48,333	-24,952	121,619	29,501	870,333	17,033	19	78
Masculin	61,600	2,359	120,841	47,711	2276,300	21,337	20	127
Total	**56,625**	**23,220**	**90,030**	**39,957**	**1596,554**	**14,127**	**19**	**127**

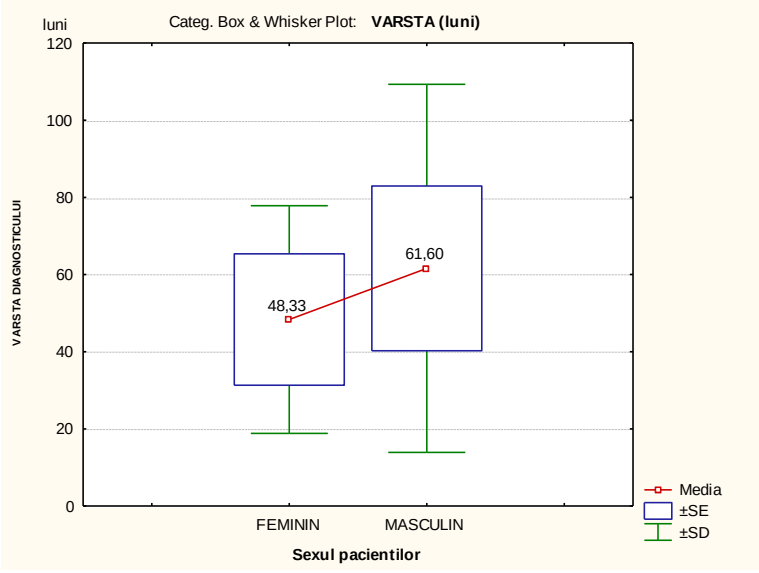

Fig. 1. Valorile medii ale vârstei în relaţie cu sexul pacienţilor

În studiul realizat am observat că nu există o corelație semnificativă statistic între durata expunerii la gluten (intervalul de timp dintre momentul debutului simptomatologiei și momentul diagnosticului BC) și apariția sindromului de citoliză (fig. 2).

	r Coeficient de corelație	p – nivel de semnificație
Interval timp vs. TGP	- 0.6191	0.102

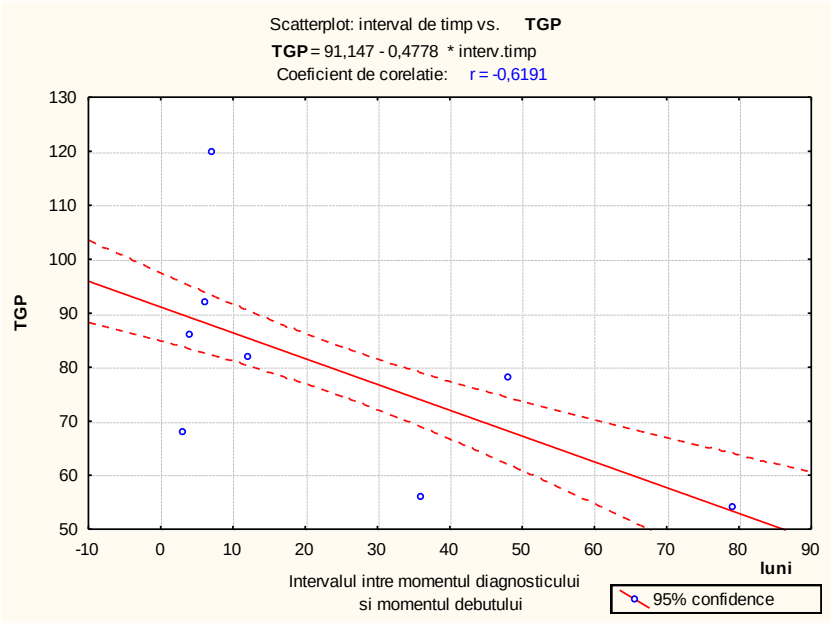

Fig. 2. Dreapta de regresie în corelația dintre intervalul de timp de expunere la gluten și TGP

Nu am întâlnit cazuri în care afectarea hepatică să fie singura manifestare a BC. Tabloul clinic la aceşti pacienți a cuprins: falimentul creşterii (100% cazuri), hepatomegalie (57,14% cazuri), dureri în hipocondrul drept (71,42% cazuri).

Analizând tipul afectării hepatice am observat că singura modificare a fost reprezentată de creşterea transaminazelor în 57,14% cazuri. Serologia pentru identificarea markerilor virali a evidențiat prezența AgHBs în 33,33% din cazuri (4 pacienți). Am observat valori mai mari de 3 ori valoarea normală a transaminazelor la pacienții cu hepatită cronică cu AgHBs (4,36% din cazuri). Fosfataza alcalină a avut valori crescute la un pacient cu nanism şi manifestări de osteoporoză.

Biopsia hepatică efectuată la un pacient a evidențiat prezența steatozei hepatice.

Evoluția transaminazelor după instituirea RFG poate fi observată în graficul 3.

La pacienții cu AgHBs absent s-a observat normalizarea sindromului de citoliză după introducerea RFG la 75% din pacienți (3 cazuri) după prima lună şi la 100% din cazuri după 3 luni de RFG (fig. 3).

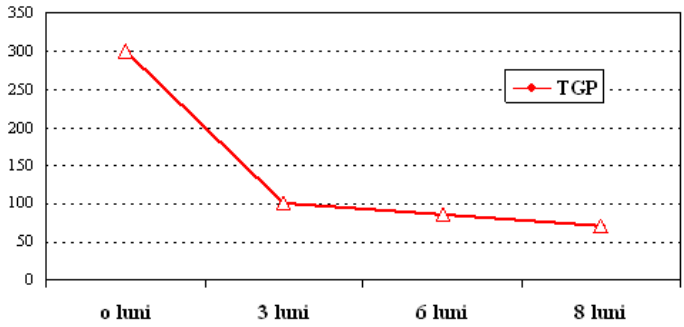

Fig. 3. Evoluția valorii medii a transaminazelor sub RFG

Pacienții cu AgHBs prezent au primit RFG şi tratament antiviral. După 3 luni de tratament transaminazele au revenit la valoarea normală în 2 cazuri. Într-un caz transaminazele s-au menținut la valori crescute după 6 luni de RFG. Clinic, pacientul prezenta persistența manifestărilor BC. Efectuarea biopsiei intestinale de control a evidențiat menținerea leziunilor intestinale de atrofie vilozitară, iar reluarea anamnezei a arătat nerespectarea RFG de către aceşti pacienți.

DISCUȚII

În ultimii ani s-au realizat progrese importante în cunoaşterea spectrului manifestărilor afectării hepatice din BC şi în înțelegerea patogeniei acestor modificări. În prezent se consideră că ficatul reprezintă una din „țintele" toxicității glutenului (3,4). Descoperirea mecanismului autoimun a BC a condus la asocierea sa cu alte boli autoimune, inclusiv cu hepatita autoimună (5,6). Utilizarea markerilor serologici anticorpi anti-gliadină, anti-endomisium, anti-transglutaminază tisulară permite descoperirea formelor atipice ale bolii, printre care şi hepatopatia din BC. Astfel, hipertransaminazemia izolată este recunoscută ca o posibilă formă de manifestare a BC şi poate fi expresia hepatitei „criptogenice" care se remite sub RFG.

În BC se cunosc trei tipuri de afectare hepatică: hepatita reactivă, hepatita cronică şi hepatita autoimună. În lotul studiat nu am întâlnit nici un caz de hepatită autoimună.

În studiul realizat de Bonamico şi colab. autorii au arătat că 54% din copiii cu BC au prezentat în momentul diagnosticului modificarea uşoară a funcției hepatice (creşterea transaminazelor) (1). Efectuarea biopsiei hepatice a evidențiat modificări histologice nespecifice: hiperplazia celulelor Kupffer, infiltrat celular mononuclear, steatoză şi fibroză uşoară (1, 4). Afectarea funcției şi arhitecturii hepatice la pacienții cu BC poate fi consecința unui proces autoimun (6, 7). Prima asociere dintre BC şi afectarea hepatică a fost descrisă în 1978 de Logan et al. (8).

Într-un studiu multicentric efectuat în Italia la copiii cu BC prevalența hepatitei autoimune a fost de 1,1%. Se discută despre rolul transglutaminazei tisulare în declanşarea procesului autoimun din diabetul zaharat, hepatita autoimună, tiroidita autoimună (9, 10). De asemenea, este cunoscut faptul că enteropatia produsă de BC determină malabsorbție şi creşterea permeabilității mucoasei intestinale, iar malnutriția se asociază cu disfuncția hepatică şi poate determina steatoză hepatică.

În prezent există controverse în ceea ce priveşte scăderea riscului de a dezvolta hepatită autoimună sub RFG (5). În studiul realizat hipertransaminazemia a apărut în toate cazurile în timpul expunerii la gluten . Datele din literatură arată rolul posibil al virusului hepatitic C ca trigger al reacției autoimune (6,9). La adultul cu hepatită cronică cu virus hepatitic C prevalența BC a fost de 1,2%. În studiul nostru nu am întâlnit decât asocierea cu virusul hepatitic B în 4,35% din cazuri.

Studiile efectuate au arătat că 4% din pacienții cu hepatită criptogenică prezintă forma silențioasă a BC, screening-ul serologic pentru anticorpii AAG, AAE, ATGt fiind esențial pentru stabilirea diagnosticului (10). Întotdeauna în *screening*-ul serologic trebuie utilizați anti-endomisium, anti-transglutaminază tisulară, valoarea predictivă pozitivă a anticorpilor anti-gliadină fiind redusă (6,8).

CONCLUZII

Investigarea funcției hepatice este obligatorie la toți pacienții cu BC în momentul diagnosticului, incidența sa în lotul studiat fiind de 10,14% din cazuri. Durata crescută de expunere la gluten nu s-a corelat cu apariția manifestărilor hepatice.

Este necesară excluderea BC la pacienții cu hepatopatie de etiologie necunoscută înainte de a fi considera "criptogenică". Prezența sindromului de citoliză în absența markerilor virali impune efectuarea testelor de screening pentru BC.

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CIROZA HEPATICĂ – STARE PROCOAGULANTĂ?

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LIVER CIRRHOSIS - PROCOAGULANT STASIS (Abstract): Abnormal hemostasis tests and bleeding are often associated in liver cirrhosis. In these patients the balance between hypo- and hypercoagulation status is more fragile than in healthy people. In the hemostatic abnormalities associated with chronic liver disease are two main chategory factors: favoring hemorrage and favoring thrombosis. The main factors that favoring hemorrage are: low platelet count, impaired platelet function, decreased levels coagulation factors (II, V, VII, IX, X, XI), quantitative and qualitative abnormalities of fibrinogen, vitamin K defiency, low levels of trombin activable fibrinolisis inhibitor, activat plasminogenic tisular. The factors favoring thrombosis are elevated levels of factors VIII and von Willebrand, decreased levels of protein C, protein S, antithrombin, decreased levels of plasminogen. Traditionally it was thought that arterial and venous thrombosis is rare events in cirrhotic patients but recent studies have indicated that thrombotic complications can paradoxically occur even if clinically an increased risk of hemorrhage is considered. Treatment of venous thrombosis in patients with cirrhosis using routine anticoagulation with heparin and vitamin K antagonists has been described but with a high level of bleeding complications. So, based on the limited data available, AASLD guidelines stated no recommendations for or against the use of anticoagulation in cirrhotic patients with portal thrombosis. Although abnormal hemostasis tests and bleeding are often associated in patients with chronic liver disease it is a relatively poor correlation between hemorrhagic risk and routine diagnostic tests of hemostasis. Management of bleeding complications in liver cirrhosis varies and no general guidelines are available. The main therapeutic strategies are: red cell concentrate, plasma, platelet concentrate, recombinant factor VIIa, factor concentrates, desmopressin, antifibrynolitic agents, thrombopoietin receptor agonists, antibiotics. Clinical studies examining safety and efficacy of the various products for the different bleedeing or trombotic complications of liver cirrhosis need to be initiaded. **Key words**: LIVER CIRRHOSIS, BLEEDING, TROMBOSIS

Caz clinic. Pacienta MM, 54 de ani, din mediul rural s-a prezentat pentru episod de hemoragie digestivă superioară (hematemeză şi melenă). Pacienta este în evidenţa clinicii cu ciroză hepatică virală C decompensată vascular şi parenchimatos de aproximativ 5 ani şi este la primul episod hemoragic. Endoscopia digestivă superioară a evidenţiat varice esofagiene gradul IV cu stigmate de sângerare recen-

tă. Biologic s-a constat anemie uşoară, trombocitopenie (47 000/mm3), prelungirea timpului de protrombină şi a INR-ului. Ecografic s-a evidenţiat tromboză de venă portă; AFP în limite normale şi computer tomografia cu substanţă de contrast nu au identificat elemente sugestive pentru hepatocarcinom. Această situaţie pe care o întâlnim relativ frecvent în practica clinică ilustrează paradoxul anomaliilor de hemostază la pacienţii cu ciroză hepatică: pe de o parte statusul hemoragic (hemoragie digestivă superioară cu scăderea numărului de trombocite, reducerea indicelui de protrombină, creşterea INR – ului) şi pe de altă parte statusul procoagulant exprimat prin tromboză. Care este terapia optimă la un astfel de pacient, dacă medicaţia anticoagulantă este indicată sau nu, rămân întrebări la care datele din literatura actuală nu pot da un răspuns cert.

În ultimii ani viziunea clasică asupra tulburărilor hemostazei din ciroza hepatică s-a modificat semnificativ, cu implicaţii majore atât fiziopatologice cât şi de conduită terapeutică.

Ficatul este locul de sinteză atât al factorilor coagulării cât şi a inhibitorilor lor, cu excepţia factorului von Willebrand (fvW). Având în vedere acest lucru, afectarea hepatică prin boli cronice determină alterarea coagulării, direct proporţional cu scăderea rezervei funcţionale hepatice.

Hemostaza în ciroza hepatică este într-un echilibru fragil între procesele protrombotice şi cele antitrombotice. Cunoaşterea acestor mecanisme care influenţează permanent echilibrul între factorii care favorizează hemoragia şi respectiv tromboza, determină o mai bună înţelegere a fiziopatologiei alterărilor hemostazei în ciroza hepatică şi are multiple aplicaţii terapeutice.

Alterările hemostazei în ciroza hepatică

Trombocitopenia severă şi defectele trombocitare

Anomaliile de număr şi funcţie ale trombocitelor sunt obişnuite în evoluţia bolilor cronice hepatice. Aproximativ 1/3 din pacienţii cu boală cronică hepatică au trombocitopenie (70 000 – 90 000/mm3) care progresează odată cu evoluţia bolii (1).

Trombocitopenia în ciroza hepatică are mecanisme multiple (2,3):

- splenomegalie;
- sechestrare hepatică şi splenică;
- supresie medulară (evidentă în hepatita acută virală C, abuzul de alcool, deficitul de folaţi);
- scăderea producţiei de trombopoietină (funcţia de sinteză a ficatului este absolut necesară pentru producerea de trombocite via trombopoietină - care reglează producţia de plachete în măduvă);
- medicaţie imunsupresoare.

Alterările funcţionale trombocitare sunt consecinţa unor mecanisme multiple (4):

- deficitul sintezei de tromboxan A2;
- deficienţe de stocaj;
- anomalii ale glicoproteinei trombocitare 1b.

1. Scăderea nivelelor factorilor II, V, VII, IX, X, XI, XII

Ficatul este locul de sinteză al fibrinogenului şi al factorilor II, V, VII, IX, X, XI, XII. Factorul von Willebrand (fvW) nu este sintetizat în ficat, ci în endoteliu. Concentraţia plasmatică a fvW este crescută în insuficienţa hepatică acută datorită creşterii sintezei ca proteină de fază acută (răspuns la injuria tisulară) şi secundar injuriei endoteliale, consecinţă a endotoxinemiei.

Factorul VIII este sintetizat în principal de ficat dar şi extrahepatic de celulele endoteliale şi sinusoidale, astfel încât nive-

lul factorului VIII nu descreşte cu agravarea bolii hepatice; nivelul său poate fi chiar crescut întrucât de multe ori boala hepatică asociază inflamaţia. Factorul VIII este crescut în insuficienţa hepatică fulminantă şi scade în coagularea intravasculară diseminată.

3. Vitamina K este cofactor esenţial în producţia de forme biologice active de factori ai coagulării II, VII, IX, X. Alterarea γ carboxilării datorată deficienţei sau antagonismului vitaminei K determină sinteză de precursori inerţi cunoscuţi ca proteine induse de absenţa vitaminei K – PIVKA (Proteins Induced by Vitamine K Absence) care sunt eliberaţi în sânge. Semnificaţia acestor precursori nu este încă clară (1).

4. Fibrinogenul plasmatic în majoritatea cazurilor este normal sau crescut în bolile hepatice cronice. Scăderea fibrinogenului sub 100 mg/dl se întâlneşte în bolile cronice hepatice severe. Fibrinogenul crescut din hepatitele cronice, icterele colestatice, hepatocarcinom este în mare măsură un fibrinogen non-funcţional în 60-70% din cazuri, care are lanţuri alfa şi conţinut crescut de acid sialic, datorită creşterii activităţii sialil transferazei în hepatocitele imature generate în timpul injuriei hepatice. Fibrinogenul scade pe măsură ce boala progresează până la disfibrinogenemie.

5. Alterările sistemului fibrinolitic. Toate proteinele implicate în fibrinoliză cu excepţia tPA şi PAI1 sunt sintetizate în ficat. În ciroza hepatică scad nivelurile de (5):

- plasminogen
- alfa2antiplasmină
- glicoproteină bogată în histidină
- factorul XIII
- trombin activable fibrinolisis inhibitor (TAFI).

Hiperfibrinoliza se corelează cu severitatea disfuncţiei hepatice evaluată prin scorul Child Pough (6).

6. Antitrombina III *(AT III)* este glicoproteină non-vitamina K dependentă sintetizată de ficat şi endoteliu. În bolile hepatice cronice este scăzută datorită reducerii sintezei şi/sau creşterii consumului prin hiperfibrinoliză. Suplereea de ATIII nu corectează fibrinoliza la pacientul cu ciroză hepatică.

7. Plasminogenul leagă fibrina de cheag, iar fibrinoliza este activată prin conversia plasminogenului prin acţiunea tAP (activat plasminogenic tisular) în plasmina; inhibitorul 1 al activatorului plasminogenului este crescut (PAI1). Acesta inhibă tAP şi acest lucru determină o stare de hiperfibrinoliză.

8. Proteina C şi proteina S sunt glicoproteine dependente de vitamina K şi sunt sintetizate în principal în ficat. În afecţiunile hepatice acute sau cronice concentraţia lor scade concomitent cu alţi factori ai coagulării dar nu sub 20%.

Aşadar factorii care favorizează hemoragia în ciroza hepatică sunt: trombocitopenia, anomaliile funcţionale trombocitare, scăderea concentraţiei plasmatice a factorilor de coagulare (II, V, VII, IX, X, XI), anomaliile cantitative şi calitative ale fibrinogenului, nivelurile scăzute de alfa2 antiplasmină, TAFI, tPA în timp ce factorii procoagulanţi sunt: niveluri crescute de fvW şi factor VIII, scăderea proteinei C, proteinei S, antitrombinei, plasminogenului (1).

Semnificaţia prognostică şi evaluarea riscului hemoragic în ciroza hepatică

În general nivelul factorilor de coagulare reprezintă un indicator al funcţiei de sinteză hepatică. Timpul de protrombină face parte din parametrii care indică gradul insuficienţei hepatice conform clasificării

Child Pugh, iar INR-ul este inclus în scorul MELD. Determinarea fiecărui factor de coagulare în parte nu aduce foarte multe informaţii suplimentare. Analiza multivariată a factorilor de prognostic în ciroza hepatică arată că factorul VII este factor independent de predicţie asupra supravieţuirii: factorul VII < 34% este predictiv pentru mortalitate în 93% din cazuri (7).

Pe de altă parte nivelul testelor globale de coagulare nu are valoare predictivă pentru riscul de sângerare (8). În prezent datorită dificultăţilor, inclusiv financiare, în evaluarea activităţii pro şi anticoagulante rolul factorilor coagulării este greu de estimat. Balanţa coagulării în bolile cronice hepatice nu este stabilă comparativ cu subiecţii fără boală hepatică. În consecinţă ea poate fi perturbată, în special dacă coexistă condiţii patologice precipitante care acţionează ca zone trigger: efracţia variceală, infecţiile, insuficienţa renală. Consensul privind hipertensiunea portala (Baveno V) statueaza faptul ca timpul de protrombina/INR-ul nu reprezinta un indicator al statusului coagularii la pacientul cirotic (9).

Trombocitopenia este un marker indirect al prezenţei varicelor eso-gastrice (diametrul splinei/numărul de trombocite se corelează cu prezenţa varicelor) dar nu are valoare prognostică în ceea ce priveşte sângererea variceală sau de la alte nivele. Valoarea trombocitelor se poate corela în schimb cu pierderile de sânge din intervenţiile chirurgicale (10).

Sângerarea prin efracţie variceală în CH este consecinţa hipertensiunii portale. Sunt studii care arată care arată că sângerarea variceală se însoţeşte de anomalii de hemostază mult mai frecvent comparativ cu pacienţii care nu au sângerat. Semnele endoscopice de „nipple" ca marker al sângerării variceale sugerează un rol limitat dar existent al hemostazei la acest nivel.

În timpul procedurilor minim invazive (toracenteză, paracenteză, puncţie lombară) nu există ghiduri care să prevadă valorile optime ale testelor de hemostază, nici tratamentul profilactic sau curativ (11).

Puncţia biopsie hepatică poate fi făcută fără suport de trombocite începând cu valoarea trombocitelor de peste 60 000/mm3; dacă riscul hemoragic este crescut se recomandă realizarea procedurii transjugular.

Trombozele în ciroza hepatică

Clasic se consideră că pacienţii cirotici ar fi protejaţi de tromboze, datorită statusului lor „autoanticoagulant". Date recente din literatură au indicat că tromboza venoasă poate apare la pacienţii cu ciroză hepatică chiar în condiţiile profilaxiei medicamentoase (12).

Hipercoagulabilitatea din CH poate fi sintetic exprimată prin triada Virchoff:

- flux scăzut
- vasculopatie asociată statusului cronic inflamator
- scăderea proteinelor anticoagulante: C, S, antitrombină.

Complicaţiile trombotice pot fi la nivel portal, mezenteric, pe venele hepatice sau periferice, în extremităţi, asociate cu embolii pulmonare.

Tromboza de venă portă

Tromboza de venă portă (TVP) are o prevalenţă între 0.6 şi 26%, funcţiede caracteristicile lotului studiat cât şi de modalitatea de diagnostic (13).

Se cunoaşte că pacienţii cu TVP au evoluţie mai rapidă şi mai gravă (14). Factorii favorizanţi cunoscuţi şi acceptaţi ai TVP sunt (15):

- sexul masculin
- chirurgia anterioară sau tratament intervenţional al HTP

- sângerare anterioară variceală
- scăderea nr de trombocite
- insuficienţa hepatică avansată.

Alături de aceşti factori cunoscuţi în TVP de dată recentă sunt introduşi alţi 2 factori (16):

- mutaţia 20210A a protrombinei
- scăderea sub 15 cm/s a velocităţii fluxului portal măsurat prin ultrasonografie Doppler.

Velocitatea este invers proporţională cu scorul Child Pough: cu cât este mai mică cu atât insuficienţa hepatică este mai severă; sub 10 cm/s poate fi considerată, după unele cercetări factor de risc independent a supravieţuirii (17).

Pentru a avea o concluzie unanim acceptată sunt necesare în continuare studii care să ateste unanim toate aceste date.

Tromboza acută de portă

Este factor de prognostic negativ la pacientul cirotic, atât pentru mortalitate cât şi pentru resângerarea variceală imediată (18).

Diagnosticul de tromboză recentă sau "acută" de portă este dificil şi se poate pune în două situaţii (19-21):

- dacă în urmă cu 60-90 de zile pacientul a avut explorare imagistică negativă pantru acest diagnostic sau
- în cazul simptomelor clinice sugestive pentru acest diagnostic: dureri în etajul abdominal superior, febră, ascită instalata brusc, sângerare variceală etc.

În tromboza acută de portă cercetarile recente recomandă asocierea de activator al plasminogenului tisular cu heparină cu greutate moleculară mică.

Sub acţiunea activatorului plasminogenului tisular, plasminogenul se transformă în plasmină. Are actiune litică numai locală (spre deosebire de urokinază şi streptokinază care induc stare litică sistemică), timp de înjumătaţire de o oră şi 30 minute, cu eliminare a 80% din cantitate în mai putin de 10 minute - ceea ce permite întoarcerea la statusul coagulant anterior. Activatorul plasminogenului tisular se administrează în perfuzie continuă (Actylize – fiole de 20mg sau 50 mg, cu injectomat, 0,025mg/kgc/zi). Totuşi consensul privind hipertensiunea portală (Baveno V) afirmă că, în momentul de faţă, nu sunt suficiente evidenţe pentru a recomanda tromboliza locală în trombozele portale (9).

Heparina cu greutate moleculară mică se administrează subcutanat în funcţie de greutatea corporală: 0,5mlx2/zi la o greutate corporală între 50-59 kg; 0,6 pentru greutate de 60-69 kg şi 0,9 la o greutate peste 90 kg.

Tratamentul se adminstrează 4-7 zile. Pe tot parcursul tratamentului pacienţii sunt monitorizaţi initial şi la fiecare 48 de ore biochimic (INR, fibrinogen, D-dimeri, antitrombina III) şi imagistic. Rezultatele pot fi: remisie completă, parţială sau lipsă de răspuns (22).

Tromboza de arteră hepatică. După transplant hepatic apare mai frecvent la cei care au avut pretransplant TVP, ceea ce denotă o stare de hipercoagulabilitate la aceşti pacienţi (23).

Tromboza ca factor de progresie a fibrozei. S-a observat, în primul rând experimental, prezenţa de microtrombi în vascularizaţia hepatică din ariile de fibroză. Cu alte cuvinte, activarea coagulării în aria vasculară apare ca răspuns la fibroză, determinând extinderea acesteia ca răspuns la ischemie (24).

Tromboza în steatohepatita nonalcoolică. Ciroza hepatică secundară steatohepatitei nonalcoolice (NASH) este în mod particular asociată cu anomalii ale hemostazei ce favorizează statusul protrombotic: nivele crescute de PAI 1, fvW, factor VII,

fibrinogen, precum şi hiperagregabilitate trombocitară şi disfuncţie endotelială (25).

Indicaţii terapeutice

Indicaţiile terapeutice în hemoragiile din CH

În ciuda progreselor efectuate în înţelegerea perturbărilor hemostazei la pacienţii cu ciroză hepatică, tratamentul hemoragiilor nu este standardizat. Intervenţiile terapeutice au ca scop principal oprirea sângerării şi mai puţin corectarea tulburărilor de coagulare.

Administrarea de masă eritrocitară poate fi salvatoare în caz de anemie severă, îmbunătăţeşte funcţia plachetară dar are numeroase efecte nedorite (26).

Plasma înlocuieşte atât pro cât şi anticoagulanţii dar poate prezenta reacţii alergice (1%) precum şi efecte hemodinamice nedorite, la fel ca transfuziile de sânge sau masă eritrocitară (creşterea hipertensiunii portale şi a riscului de sângerare, injurie pulmonară, sindrom circulator de supraîncărcare) (27).

Concentratul plachetar îmbunătăţeşte hemostaza primară; s-a demonstrat o producere adecvată a trombinei la valori peste 50 000/mm3; are efecte secundare similare transfuziilor (28).

Concentratele de factori procoagulanţi au, comparativ cu plasma, avantajul administrării unui volum mic cu concentraţie crescută, ceea ce evită supraîncărcarea volemică. Nu au fost testate în studii randomizate şi, teoretic, au risc de tromboză.

Factorul recombinant VII normalizează timpul de protrombină dar nu şi-a dovedit eficienţa în hemoragia variceală sau în cea secundară intervenţiilor chirurgicale şi este trombogenic (29)

Desmopresina (analog de hormon antidiuretic) îmbunătăţeşte hemostaza primară dar scade eficienţa terlipresinei (30).

Agenţii antifibrinolitici – aprotinina şi acidul tranexamic scad pierderile de sânge în timpul transplantului hepatic. Nu au fost testaţi în sângerarea variceală sau postchirurgicală.

Agoniştii receptorilor de trombopoietină (Eltrombopagul), prin analogie cu hepatita cu virus C, creşte numărul de trombocite (31).

Antibioticele. Până la 2/3 dintre pacienţii cu ciroză hepatică prezintă infecţie activă cu stare de hipocoagulabilitate (dovedită prin trombelastogramă). Administrarea la aceştia de antibiotice scade riscul sângerării, îmbunătăţeşte coagularea şi hemodinamica.

Tratamentul trombozei venose în ciroza hepatică.

Teoretic, ar trebui folosite anticoagulante cu greutate moleculară mică, heparină, antagonişti ai vitaminei K. Complicaţiile care apar în aceste situaţii sunt grave şi numeroase - în 83% din cazuri (32). Mai mult, heparina necesită antitrombină pentru a-şi exercita rolul anticoagulant, iar nivelul antitrombinei este scăzut la aceşti pacienţi.

Folosirea antagoniştilor vitaminei K este şi aceasta dificilă întrucât nu este stabilit un nivel - ţintă pentru INR şi timpul de protrombină (a căror valori de altfel variază între diferite laboratoare).

Conform ghidului AASLD, în TVP la pacienţii cu ciroză hepatică, nu există recomandări ferme, pro sau contra, pentru folosirea de anticoagulante. Sunt necesare studii viitoare care să certifice eficacitatea anticoagulantelor în aceste situaţii.

CONCLUZIE

Coagulopatia pacientului cirotic, cu echilibrul său fragil între factorii procoagulanţi şi cei hemoragici, rămâne încă o provocare pentru medicul practician. Cel mai

recent consens privind hipertensiunea portală (Baveno V) certifică faptul că, pe baza datelor actuale, nu pot fi efectuate recomandari practice privind managementul acestor pacienţi (9). Noile direcţii de cercetare propuse de Baveno V includ nevoile de corectare a tulburărilor de coagulare în ciroza hepatică, precum şi evaluarea semnificaţiei prognostice a coagulopatiei şi trombocitopeniei.

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NOUTĂȚI

MODULAREA EXPRESIEI GENELOR DE VIRULENȚĂ ALE *STAPHYLOCOCCUS AUREUS* DE CĂTRE ANTIBIOTICE BETA-LACTAMICE UTILIZATE ÎN CONCENTRAȚII SUBINHIBITORII

Stimularea expresiei genelor de virulenţă de către concentraţii subinhibitorii ale anumitor antibiotice poate modifica evoluţia infecţiei şi poate face ca terapia antimicrobiană să devină ineficientă. Investigarea efectelor concentraţiilor subinhibitorii ale antibioticelor care inhibă sinteza peretelui bacterian asupra expresiei genelor de virulenţă şi asupra producerii biofilmului la *Staphylococcus aureus* a fost realizată folosind metode difuzimetrice şi prin real time PCR cantitativ. Expresia genelor *spa* (*staphylococcal protein* A) şi *luk*E a fost stimulată de concentraţii subinhibitorii de penicilină şi cefalotin, în timp ce expresia *agr* RNAIII (*accessory gene regulator*) nu a fost afectată. De asemenea, s-a observat producerea unui biofilm mai dens în prezenţa unor concentraţii subinhibitorii de cefalotin. Subrt N, Mesak LR, Davies J. Modulation of virulence gene expression by cell wall active antibiotics in *Staphylococcus aureus*. *J Antimicrob Chemother* 2011; 66: 979-84.

Teodora Vremeră

PERITONITA BACTERIANĂ SPONTANĂ: COMPLICAȚIE SEVERĂ A CIROZEI HEPATICE – STUDIU PROSPECTIV

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SPONTANEOUS BACTERIAL PERITONITIS: SEVERE COMPLICATION OF LIVER CIRRHOSIS (Abstract): **Aim:** To evaluate the prevalence, incidence, pathogens, and clinical outcome of the patients with liver cirrhosis and spontaneous bacterial peritonitis (SBP). **Material and methods:** Prospective study of 34 episodes of SBP occurring in 29 patients (mean age was 56,89±8,79 years) with cirrhosis and ascites admitted to hospital. The diagnosis of SBP was made by a polymorphonuclear cell count in ascitic fluid of >250 cells/mm^3 in the absence of data suggestive of secondary peritonitis. **Results:** In our series SBP occurred more frequently in men, 21 of 29 patients (72,41%). Microorganisms were isolated in 12 episodes (35,29%): gram-negative bacteria in 8 (66,67%), and Gram-positive in 4 (33,33%). The first-line treatment failure and in-hospital mortality rates were 26,47% and 17,64%, respectively. The low in-hospital mortality seems to be related to earlier diagnosis and treatment. In the multivariate analysis, three significant independent predictive factors of in-hospital mortality were identified: a high Model of End-Stage Liver Disease (MELD) score, renal failure, and SBP caused by extended-spectrum β-lactamase-producing organisms. The relapse rate of BSP was 21,73%. **Conclusions:** SBP remains a serious complication, especially in patients with a high MELD score. Ceftazidim represents a good choice in empirical treatment of SBP. **Key words:** LIVER CIRRHOSIS, PERITONITIS, TREATMENT, PROGNOSIS

Termenul de peritonită bacteriană spontană (PBS) a fost introdus în 1971 de către Conn şi Fessel, PBS fiind definită ca infecția spontană a lichidului ascitic în absența identificării oricărei surse infecțioase intraabdominale (1).

Ciroza hepatică (CH) se caracterizează printr-o susceptibilitate crescută la infecții datorită deficiențelor mecanismelor de apărare ale organismului. PBS este cea mai frecventă infecție la bolnavii cirotici, urmată de infecțiile tractului urinar (20%), pneumonie (15%) şi bacteriemie (12%) (2). Recunoscută ca o complicație frecventă şi severă, PBS apare la 8-27% din pacienții cirotici cu ascită spitalizați (3).

Supraviețuirea acestor bolnavi depinde de precocitatea diagnosticului şi tratamentului şi de instituirea profilaxiei secundare a PBS. Având în vedere că PBS poate evolua asimptomatic sau insidios, se recomandă efectuarea paracentezei diagnostice în toate cazurile de CH cu ascită şi când apar manifestări clinice sugestive precum durerile abdominale, febra, encefalopatia hepatică, agravarea disfuncției renale sau hepa-

tice (4). Prezența în lichidul de ascită a peste 250 neutrofile/mm^3 semnifică supra-infecția bacteriană a acestuia (PBS).

Tratamentul antibiotic empiric trebuie început imediat ce diagnosticul a fost stabilit, înainte de a se cunoaşte agentul etiologic şi sensibilitatea *in vitro* (4). Bacteriile cel mai frecvent identificate în 65% din cazurile cu culturi pozitive sunt bacteriile Gram-negative din familia *Enterobacteriaceae*, în special *Escherichia coli* şi *Klebsiella pneumoniae*. Restul agenților microbieni sunt reprezentați de cocii Gram-pozitivi (5). Cefalosporinele de generația a III-a (cefotaxim, ceftriaxona, ceftazidim) şi combinația amoxicilină-acid clavulanic, reprezintă antibioticele de primă intenție în tratamentul PBS.

Prognosticul pe termen lung al bolnavilor cu PBS este nefavorabil. Rata recurenței PBS în primul an este de 40-70%, iar supraviețuirea la 1 an şi la 2 ani, după un episod de PBS este de aproximativ 30-40%, respectiv 20% şi chiar mai scăzută la pacienții cu scor Child-Pugh peste 10 (6). Profilaxia PBS este indicată la următoarele 3 categorii de pacienți cirotici: antecedentele de PBS, bolnavii spitalizați pentru hemoragie digestivă superioară şi cei cu conținut scăzut de proteine în lichidul de ascită. Utilizarea pe termen lung a antibioticelor cu spectru larg poate determina creşterea numărului microorganismelor rezistente la antibiotice cu implicații considerabile asupra managementului şi tratamentului PBS, conducând la necesitatea reevaluării ghidurilor terapeutice actuale (6).

Scopul studiului prezent de a evalua trăsăturile clinice şi prognosticul bolnavilor cu ciroză hepatică complicată cu PBS internați în Clinica de Gastroenterologie a Spitalului Clinic Județean de Urgență ”Sfântul Apostol Andrei” Galați.

MATERIAL ŞI METODĂ

Studiul prospectiv a inclus 122 bolnavi cu CH şi ascită internați în Clinica de Gastroenterologie a Spitalului Clinic Județean de Urgență ”Sfântul Apostol Andrei” Galați în perioada 1 ianuarie 2007-31 decembrie 2007 şi monitorizați pe o perioadă de 36 luni. Diagnosticul CH s-a stabilit pe baza criteriilor clinice, ecografice şi histologice. Severitatea cirozei hepatice s-a apreciat folosind scorurile Child-Pugh şi MELD (model for end-stage liver disease). Scorul MELD s-a calculat utilizând formula: 9,57 x log e (creatinina, mg/dl) + 3,78 x log e (bilirubina, mg/dl) + 11,2 x log e (INR) + 6,43.

Bolnavii au fost evaluați anamnestic, clinic, biologic (teste standard de laborator, incluzând hemoleucograma, teste biochimice hepatice şi renale, indice de protrombină, examen de urină) şi imagistic (ecografie abdominală, radiografie toracică). Paracenteza diagnostică şi cultura lichidului de ascită s-au efectuat la internarea pacienților şi în cazul apariției în cursul spitalizării a simptomelor şi semnelor sugestive pentru PBS (febră, dureri abdominale, alterarea statusului mintal, hipotensiune arterială, deteriorarea funcției renale, leucocitoză) în absența altor factori precipitanți identificabili. Lichidul de ascită s-a recoltat în recipiente sterile, respectând condițiile de asepsie. În fiecare recipient cu mediu de cultură MacConkey agar s-au inoculat cel puțin 10 ml de lichid de ascită, iar bacteriile identificate prin cultură au fost testate pentru sensibilitate la antibiotice prin metoda difuziunii.

Diagnosticul PBS s-a stabilit prin evidențierea în lichidul de ascită a peste 250 PMN /mm^3 în absența semnelor clinice şi radiologice de peritonită bacteriană secundară. Peritonita bacteriană secundară se

defineşte prin identificarea în lichidul de ascită a două sau mai multe microorganisme sau asocierea PMN > 250 /mm^3 lichid ascită cu cel puţin doi din următorii trei parametri: glucoza < 50mg/dl, proteine totale > 1g/dl şi lactat dehidrogenaza (LDH) peste limita superioară a normalului a LDH seric.

Bolnavii cu PBS au primit tratament empiric cu Ceftazidim (cefalosporină de generaţia a III-a) intravenos, 1-2g/12 ore, minimum 5 zile, întrucât aceasta a reprezentat opţiunea terapeutică disponibilă pe perioada spitalizării, studiile indicând o eficienţă similară cu a cefotaximului. Doza antibioticului a fost ajustată conform funcţiei renale pe perioada tratamentului. În cazurile cu eşec terapeutic la antibioticul iniţial, tratamentul a fost adaptat conform antibiogramei sau empiric.

Paracenteza diagnostică s-a repetat după 48 ore de la începerea antibioterapiei pentru stabilirea eficienţei acesteia. Lipsa de răspuns la tratament s-a considerat în cazurile ce au prezentat alterarea stării generale, scăderea PMN < 25% faţă de valoarea iniţială, menţinerea culturilor pozitive după 48 ore de antibioterapie sau care au decedat înaintea repetării paracentezei. Vindecarea PBS s-a apreciat în condiţiile remiterii tuturor semnelor clinice de infecţie, scăderii PMN în lichidul de ascită < 250/ mm^3, normalizării leucocitelor în sângele periferic şi negativării culturilor din lichidul de ascită. Bolnavii care au primit chinolone p.o. mai mult de o lună înaintea episodului de PBS au fost incluşi în grupul cu antibioticoprofilaxie. Mortalitatea intraspitalicească a fost definită ca deces de orice cauză în cursul spitalizării.

Datele au fost exprimate ca medie ± deviaţie standard (DS). Pentru compararea grupurilor s-au utilizat testele *t-Student* (pentru date cantitative) şi χ^2 (pentru date calitative). Prin analiză univariată au fost identificaţi factorii predictivi ai mortalităţii intraspitaliceşti. Variabilele analizate au fost vârsta, sexul, cauza cirozei, PBS, datele clinice şi de laborator efectuate (inclusiv scorurile Child-Pugh şi scorul MELD). Variabilele cu semnificaţie statistică ($p<0,05$) la analiza univariată au fost folosite la analiza multivariată, aplicându-se analiza regresivă logistică binară „stepwise" pentru identificarea factorilor predictivi ai supravieţuirii. În scopul determinării semnificaţiei datelor la analiza multivariată s-a utilizat intervalul de încredere (IC) de 95%, valoarea $p<0,05$ fiind considerată statistic semnificativă. Datele au fost prelucrate folosind *SPSS 17.0*.

REZULTATE

Studiul a inclus 34 episoade de PBS dezvoltate de 29 pacienţi (21 bărbaţi şi 8 femei) din cei 122 bolnavi cu CH şi ascită internaţi în clinică, stabilindu-se o prevalenţă a PBS de 23,77%. Caracteristicile demografice şi clinice ale cazurilor selectate la momentul diagnosticului PBS sunt prezentate în tabelul I. Vârsta medie a fost 56,89±8,79 ani, iar sexul masculin a fost predominant (72,41%). Consumul de alcool a reprezentat cauza cea mai frecventă a CH (68,96%), urmat de infecţia cu virus hepatitic C (34,48%) şi virus hepatitic B (27,58%). Din punct de vedere al clasificării Child-Pugh, a predominat clasa Child C (75,86%), urmată de Child B (24,14%); nici un bolnav nu s-a situat în clasa Child A. Scorul MELD mediu a fost de 21,8 ± 7,4. Majoritatea cazurilor cu PBS au fost simptomatice, febra şi durerile abdominale, fiind cel mai frecvent întâlnite în 18 cazuri (52,94%), respectiv 14 cazuri (41,17%). Encefalopatia hepatică la momen-

tul diagnosticului PBS a fost prezentă în 10 cazuri (29,41%) şi ca simptom unic al PBS în 3 cazuri (8,82%), iar insuficienţa renală în 13 (38,23%) din cazuri. Identificarea factorilor de risc pentru apariţia PBS a fost posibilă în 14 cazuri: 5 cazuri cu antecedente de PBS (4 dintre acestea având antibioticoprofilaxie pentru PBS la momentul internării), 6 cu hemoragie digestivă superioară şi 3 cu proceduri invazive (tab. I).

TABELUL I
Caracteristicile lotului studiat

Vârsta (ani)	56,89±8,79
Bărbaţi/Femei	21/8
Cauza cirozei, n (%)	
Consum de alcool	20 (68,96)
Virus hepatitic C	10 (34,48)
Virus hepatitic B	8 (27,58)
Clasa Child-Pugh (media ± DS)	11,68 ± 2,28
Scorul Child-Pugh, n (%)	
Clasa Child B	7 (24,14)
Clasa Child C	22 (75,86)
Scorul MELD	21,8 ± 7,4
Manifestări clinice, n (%)	
Febră	18 (52,94)
Dureri abdominale	14 (41,17)
Greaţă/vărsături	7 (20,58)
Encefalopatie hepatică	10 (29,41)
Hemoragie gastro-intestinală	6 (17,64)

Cultura pozitivă din lichidul de ascită s-a înregistrat în 12 (35,29%) din cele 34 de cazuri de PBS. Bacteriile Gram-negative (BGN) au reprezentat 66,67% din totalul germenilor identificaţi (8 din 12 cazuri). *Escherichia coli* a fost cel mai frecvent întâlnită (5 cazuri, 41,66%), urmată de *Klebsiella pneumoniae* (3 cazuri, 25%). Cocii Gram-pozitivi au fost prezenţi în 4 cazuri (33,33%) (2 *Streptococcus pneumoniae*, 1 *Staphylococcus aureus* şi 1 *Enterococcus faecalis*). Comparând grupurile de PBS determinate de bacterii Gram-pozitive şi Gram-negative s-a constatat că distribuţia pacienţilor nu a fost semnificativ diferită în ceea ce priveşte scorul Child-Pugh (p = 0,083) şi mortalitatea intraspitalicească (p = 0,246), însă scorul MELD, leucocitele, PMN şi proteinele din lichidul de ascită (LA) au fost semnificativ diferite între cele 2 grupuri (p = 0,042, 0,026, 0,029 şi, respectiv, 0,027). În acelaşi timp, scorurile Child-Pugh şi MELD au prezentat valori mai reduse în cazurile de PBS cu bacterii Gram-pozitive (BGP) (tab II).

Testarea sensibilităţii *in vitro* a bacteriilor a arătat că 5 bacili Gram-negativi (62,5%) au fost rezistenţi la Ampicilină şi 2 (25%) la Ciprofloxacin. Rezistenţa la Ceftazidim s-a înregistrat în 4 cazuri (2 *Escherichia coli*, 1 *Klebsiella pneumoniae* şi 1 *Enterococcus faecalis*), identificate ca fiind bacterii producătoare de beta-lactamază cu spectru extins (BLSE), sensibile, însă, la Imipenem şi Meropenem.

TABELUL II

Caracteristici clinice şi biologice ale pacienților şi bacteriilor izolate

	BGP (n=4)	**BGN (n=8)**	**Valoare p**
Vârsta (ani)	56±6	55±8	0,762
Bărbați (%)	3 (75%)	6 (75%)	0,501
Bolnavi cu scor Child C, n (%)	3 (75%)	5 (62,5%)	0,091
Scor Child-Pugh	10,7±1,2	11,6±1,1	0,083
Scor MELD	16,3±5,1	21,1±8,4	0,042
Leucocite	10247,4±2312,8	8604,5±954,8	0,236
PMN	7880,9±1680,9	4870,9±589,9	0,112
Leucocite în LA	3853,7±1280,3	1816,7±308,6	0,026
PMN în LA	2818,4±1041,5	1265,1±216,2	0,029
Proteine în LA (g/dl)	1,43±0,35	0,87±0,41	0,027
Uree	110,8±32,7	84,7±6,4	0,341
Creatinină	1,8±0,6	1,9±0,4	0,681
Bilirubină	7,6±3,1	6,0±0,9	0,255

Tratamentul empiric cu Ceftazidim s-a început în 26 cazuri (76,47%). În celelalte 8 cazuri, s-a folosit un alt antibiotic întrucât: 6 au dezvoltat PBS în pofida tratamentului cu cefalosporine inițiat în scop profilactic înaintea procedurilor invazive sau pentru HDS), iar 2 pacienți prezentau alergie la cefalosporine. Antibioticele de primă linie au fost administrate timp de 9±4 zile. Răspunsul la tratament s-a constatat în 19 cazuri tratate inițial cu Ceftazidim (73,07%), în timp ce în 5 cazuri (19,23%) a fost necesară schimbarea terapiei empirice datorită scăderii cu < 25% a PMN în lichidul de ascită la paracenteza efectuată după 48 ore de la începerea antibioterapiei. În aceste cazuri, tratamentul antibiotic a fost schimbat cu Imipenem în 4 cazuri şi Ciprofloxacin în 1 caz. În cele 5 cazuri cu eșec terapeutic la Ceftazidim bacteriile identificate au fost fie rezistente la Ceftazidim (4 producătoare de BLSE – 2 *Escherichia coli*, 1 *Klebsiella pneumoniae* şi 1 *Enterococcus faecalis*), fie au avut o sensibilitate redusă la Ceftazidim (*Staphylococcus aureus*). Dintre bolnavii care au primit ca tratament de primă intenție Ceftazidim, 2 au decedat înaintea începerii antibioterapiei de linia a doua, iar doi în decurs de 72 ore după schimbarea tratamentului antibiotic.

Eşecul la tratamentul empiric s-a produs în 9 cazuri (26,47%): 7 cazuri tratate cu Ceftazidim, 1 caz tratat cu Ciprofloxacin şi 1 caz tratat cu Amoxicilină-acid clavulanic. Analiza multivariată a identificat scorul MELD la internare (p = 0,027) şi PBS produse de bacterii producătoare de BLSE (p = 0,004) ca factori predictivi independenți pentru eşecul tratamentului antibiotic empiric. Remiterea PBS s-a obținut în 22 din 26 cazuri tratate inițial cu Ceftazidim (84,61%), 4 bolnavi decedând. Decesul a survenit în 2 din cele 8 cazuri care au primit ca tratament de primă linie un antibiotic diferit de Ceftazidim. Mortalitatea intraspitalicească a fost de 17,64%. Cauzele deceselor au fost reprezentate de: hemoragie digestivă superioară (3 cazuri), sindrom

hepato-renal (2 cazuri) şi encefalopatie hepatică (comă) 1 caz. Pe parcursul perioadei de monitorizare recurenţa PBS s-a produs în 5 cazuri (21,73%) din cei 23 bolnavi ce au supravieţuit primului episod de PBS şi care au beneficiat de profilaxie secundară antibiotică cu chinolone.

La analiza multivariată variabile independente în relaţie cu mortalitatea în PBS au fost insuficienţa renală (creşterea azotului ureic şi/sau creatininei serice peste 30 mg/dl, respectiv, 1,5 mg/dl) (p= 0,038), scorul MELD la internare (p = 0,017) şi PBS cu bacterii producătoare de BLSE (p = 0,026).

DISCUŢII

Scopul studiului prospectiv a fost de a evalua trăsăturile clinice actuale, răspunsul terapeutic şi prognosticul cirozelor hepatice complicate cu PBS internate în Clinica de Gastroenterologie din Spitalul Judeţean Galaţi. Aceasta analiză este justificată de modificările din ultimii ani referitoare la tipul şi etiologia infecţiilor bacteriene în ciroza hepatică (7, 8, 9). Pentru aceasta, studiul a evaluat un grup de pacienţi cu ciroză hepatică cu ascită complicată cu PBS, care au fost monitorizaţi timp de 36 luni.

Rezultatele studiului susţin recomandările ghidurilor actuale privind stabilirea diagnosticul PBS prin numărarea PMN în lichidul de ascită şi iniţierea tratamentul empiric cu cefalosporine de generaţia a III-a (în studiul prezentat Ceftazidim), avându-se în vedere necesitatea schimbării antibioticului doar în 19,23% din cazuri. Ca urmare, Ceftazidimul poate fi considerat ca opţiune terapeutică în PBS, prezentând o eficienţă similară cu a cefotaximului - antibioticul recomandat de ghidurile actuale ca tratament de primă intenţie a PBS.

Cazurile cu eşec terapeutic la Ceftazidim datorat fie bacteriilor producătoare de BLSE (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*), fie sensibilităţii bacteriene reduse la Ceftazidim (*Staphylococcus aureus*) au avut un prognosticul rezervat, cu o rată crescută de mortalitate (50%). Aceste date indică prezenţa bacteriilor producătoare de BLSE ca fiind un factor predictiv independent pentru eşecul terapeutic şi mortalitatea intraspitalicească. Bacteriile producătoare de BLSE au fost rezistente la toate antibioticele, cu excepţia Imipenem şi Meropenem. Ca urmare, în condiţiile identificării prin cultură a bacteriilor producătoare de BLSE sau al eşecului terapeutic la administrarea de cefalosporine de generaţia a III-a, ar trebui avută în vedere înlocuirea antibioticului iniţial cu Imipenem sau Meropenem.

Diagnosticul precoce al PBS, iniţierea tratamentului antibiotic empiric şi adaptarea ulterioară a acestuia conform rezultatelor antibiogramei a permis controlul eficient al infecţiei şi reducerea mortalităţii intraspitaliceşti la 17,64%, rezultatele fiind în concordanţă cu datele din literatura de specialitate (10).

În acest studiu, prezenţa la internare a disfuncţiei renale şi scorul MELD au fost identificaţi ca factori predictivi independenţi ai mortalităţii în PBS, rezultate similare fiind raportate de alte studii (11, 12). Pe de altă parte, aceşti factori se corelează, de obicei, cu prezenţa unei insuficienţe hepatice avansate.

Datele din literatură indică o incidenţă în creştere a bacteriilor Gram-pozitive implicate în etiologia PBS de la 25% în anii 1998-1999 şi la 59% între 2000-2002 (13). În studiul prezentat proporţia cazurilor de PBS cu bacterii Gram-pozitive a fost doar 33,33%. În grupul studiat cazurile de

PBS cu bacterii Gram-pozitive au prezentat valori mai reduse ale scorurilor Child-Pugh şi MELD şi concentraţii superioare ale proteinelor în lichidul de ascită, comparativ cu PBS cu bacterii Gram-negative.

Ghidurile terapeutice actuale recomandă profilaxia pe termen lung prin administrarea zilnică de Norfloxacin la pacienţii care au prezentat un episod de PBS. Însă, profilaxia cu Norfloxacin ar putea contribui la creşterea frecvenţei PBS cu bacterii rezistente la chinolone, existând studii care au raportat dezvoltarea de bacili Gram-negativi rezistenţi la chinolone în flora intestinală a bolnavilor cirotici ce au primit profilaxie pe termen lung cu Norfloxacin (14).

CONCLUZII

Peritonita bacteriană spontană este o complicaţie frecventă şi severă, ce survine în cazurile de ciroză hepatică cu ascită internate în spital.

Paracenteza diagnostică trebuie efectuată în toate cazurile de ciroză hepatică cu ascită şi când apar manifestări clinice sugestive precum durerile abdominale, febra, encefalopatia hepatică, agravarea disfuncţiei renale sau hepatice. În prezenţa a peste 250 PMN /mm^3 în lichidul de ascită se stabileşte diagnosticul PBS (cultura bacteriană pozitivă nu este absolut necesară) şi se iniţiază tratament antibiotic empiric, preferabil cu cefalosporine de generaţia a III-a (Ceftazidim în studiul prezent). În cazurile cu eşec terapeutic la Ceftazidim trebuie avută în vedere schimbarea acestuia cu Imipenem sau Meropenem.

În pofida progreselor diagnostice şi terapeutice recente, PBS încă se asociază cu o mortalitate semnificativă la bolnavii cu ciroză hepatică, în special în cazurile cu scor MELD ridicat.

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NOUTĂȚI

MODULAREA EXPRESIEI GENELOR DE VIRULENTA ALE *STAPHYLOCOCCUS AUREUS* DE CATRE ANTIBIOTICE BETA-LACTAMICE UTILIZATE IN CONCENTRATII SUBINHIBITORII

Stimularea expresiei genelor de virulență de către concentrații subinhibitorii ale anumitor antibiotice poate modifica evoluția infecției și poate face ca terapia antimicrobiană să devină ineficientă. Investigarea efectelor concentrațiilor subinhibitorii ale antibioticelor care inhibă sinteza peretelui bacterian asupra expresiei genelor de virulență și asupra producerii biofilmului la *Staphylococcus aureus* a fost realizată folosind metode difuzimetrice și prin real time PCR cantitativ. Expresia genelor *spa* (*staphylococcal protein* A) și *luk*E a fost stimulată de concentrații subinhibitorii de penicilină și cefalotin, în timp ce expresia *agr* RNAIII (*accessory gene regulator*) nu a fost afectată. De asemenea, s-a observat producerea unui biofilm mai dens în prezența unor concentrații subinhibitorii de cefalotin (Subrt N, Mesak LR, Davies J. Modulation of virulence gene expression by cell wall active antibiotics in *Staphylococcus aureus. J Antimicrob Chemother*. 2011; 66: 979-84).

Teodora Vremera

THE THERAPEUTIC APPROACH TO DEEP VENOUS THROMBOSIS IN THE PATIENT WITH CIRRHOSIS OF THE LIVER

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THE THERAPEUTIC APPROACH TO DEEP VENOUS THROMBOSIS IN THE PATIENT WITH CIRRHOSIS OF THE LIVER (Abstract): Venous thromboembolism is considered a very rare event in patients with liver cirrhosis, thus explaining unpredictable outcome. This may be due to the severe liver disease profoundly affecting coagulation. Patients with cirrhosis also have a reduction in anticoagulant proteins so their coagulative situation is characterized by a new balance of pro- and anticoagulation factors, defined as "rebalanced hemostasis". Starting from the existing data in the literature so far, we studied 8 male patients previously known with cirrhosis, who were hospitalized with deep vein thrombosis (DVT) in the lower limbs in the Cardiology Clinic. The therapeutic approach to deep venous thrombosis associated with liver cirrhosis raises some issues related to the particular bleeding risk in this category of patients. DVT is not a rare condition in cirrhosis. The diagnosis of liver cirrhosis had been previously confirmed and justified by the presence of medical records at admission, and the diagnosis of DVT was based on clinical symptoms of DVT and confirmed using Duplex Doppler ultrasonography criteria for DVT. Viral etiology of cirrhosis was predominant and diabetes mellitus was the only variable that was significantly associated with the presence of DVT. It is important though to consider, that serum albumin level of less than 3 mg/dL tended to be a risk factor. Our results coincide with those reported in the studied literature. The treatment unanimously accepted so far, initially with low-molecular-weight heparin (for 7-10 days) continued with Acenocumarol is adequate, with a low risk of bleeding. The precise mechanisms of hypercoagulability and thrombosis in cirrhosis are not well known and need further investigation. **Keywords:** DEEP VEIN THROMBOSIS, CIRRHOSIS, ANTICOAGULANT.

Currently, it is widely accepted that the liver plays an essential role in the coagulation cascade, which is why the global hemostatic process is deeply influenced by the presence of hepatic disease and its complications (1, 2). Patients with liver cirrhosis, especially those at an advanced stage of disease, have a low synthesis of most factors involved in coagulation and fibrinolysis. This is due to reduced hepatic function and secondary thrombocytopenia of portal hypertension. Severe liver disease profoundly affects coagulation. Impairment of coagulation tests and thrombocytopenia

have previously been associated with an increased risk of bleeding, which is why these patients were "auto-anticoagulated" and therefore "protected" against thrombotic events. This paradigm has been abolished because outside of gastrointestinal bleeding, spontaneous bleeding is not very frequent in cirrhotic. The latest clinical findings support the idea that the cirrhotic patient is exposed to both thrombotic and hemorrhagic events (1, 2).

The therapeutic approach to deep venous thrombosis associated with liver cirrhosis raises some issues related to the particular bleeding risk in this category of patients. The decreased hepatic synthetic function and the hemodynamic alterations due to portal hypertension exert a profound influence on the hemostatic balance. This accounts for the presence of altered coagulative parameters in patients with chronic liver disease. Routine blood tests (like International Normalized Ratio [INR] or Partial Thromboplastin Time [PTT] do not accurately evaluate the coagulative performance in these patients. Thromboelastography is recommended as the most faithful method of assessing coagulation status in the cirrhotic patients (2).

Deep vein thrombosis (DVT) is a serious disorder that often complicates the course of hospitalized patients. Rudolf Virchow was the first who proposed that thrombosis was the result of three underlying pathogenic factors (stasis of blood flow, hypercoagulability and vascular endothelial damage) (1). Many risk factors have been linked to the occurrence of venous thromboembolism (VTE) including age, cancer, acute respiratory failure, congestive heart failure, acute infection, dehydration, rheumatologic disease, etc. (3, 4).

Lesmana *et al*. (1) reported a prevalence of DVT in patients with liver cirrhosis at 4.7%. They considered deep vein thrombosis being a non-rare condition in cirrhotic patients with coagulopathy that warrants further studies on the mechanisms and prevention.

Several studies indicate the incidence of non-portal VTE in patients with chronic liver disease in the range between 0.5% and 6.3% (1, 5-9). Two retrospective studies by Northup *et al*. (8) and Garcia-Fuster *et al*. (9), showed that the risk of developing VTE increases in patients with low serum albumin, but is independent from elevated INR or low platelet count. Serum albumin is a marker of liver synthetic function, and hence, indirectly, of the imbalance of serum levels of anticoagulant factors as antithrombin III, protein C and protein S. This explains the increased risk of VTE in these patients.

A retrospective study by Dabbagh *et al*. (10) confirmed that an elevated INR (even >2.2) does not protect patients with chronic liver disease against the risk of VTE. Other studies showed that not only patients with cirrhosis are not naturally auto-anticoagulated, but that they also seem to have an increased relative risk of venous thromboembolism (both deep vein thrombosis and pulmonary embolism) compared with the control patients (9-12).

Wu *et al*. (13) assessed the incidence of VTE in patients with compensated or decompensated cirrhosis and a random sample of control patients. Interestingly, results indicated that patients younger than 45 years had an increased risk of VTE, either if they were being compensated or decompensated. After 45 years of age, patients with compensated cirrhosis had a lower risk of VTE, while those with decompensated disease had a similar risk compared

with controls. These results may be explained with age-related factors not correlated with liver disease; in younger patients, who normally have low baseline risk of VTE, presence of cirrhosis implies an increased risk of VTE compared with non-cirrhotic patients, whereas in older patients' extrahepatic factors seem to outweigh liver-related risk factors.

Starting from the existing data in the literature so far, we studied during the 2014-2018 period, 8 male patients previously known with cirrhosis were hospitalized with deep vein thrombosis (DVT) in the lower limbs in the Cardiology Clinic. The diagnosis of liver cirrhosis had been previously confirmed and justified by the presence of medical records at admission, and the diagnosis of DVT was based on clinical symptoms of DVT (unilateral swollen or painful leg) and confirmed using Duplex Doppler ultrasonography criteria for DVT. They are the following: no flow signal, direct clot visualization, the absence of spontaneous flow, and the absence of respiration-modulated phasicity of the evaluated veins. Valvular competence was assessed using the Valsalva maneuver.

Patients were aged between 33-63 years and all of them were males. Five of them developed DVT during hospitalization for cirrhosis, which was confirmed clinically by the observation of swollen calf with rash and tenderness, together with Duplex Doppler ultrasonography. Three of the patients were admitted in the Emergency Department. Regarding the location of DVT in 5 cases it was at the level of the popliteal vein (right in 3 cases and left in 1 case), and in 3 cases it was a high DVT of the left ilio-femoral vein. There was no relation between the localization of thrombosis and the biochemical profile in our patients. The platelet count was between 95.000 and 125.000/ mm^3 and no active bleedings were present at the admission.

All patients were treated initially with low-molecular-weight heparin (for 7-10 days) and then with Acenocumarol to maintain INR between 2 and 3. Three of the patients showed ascites on admission and no patient with hepatic encephalopathy was found. One patient was previously diagnosed with diabetes mellitus and he was under insulin therapy. Also, no significant variations in platelet counts and no bleedings occurred during anticoagulation therapy.

Other risk factors, such as smoking, obesity, dyslipidemia, or status of inherited hypercoagulability, were not known. Viral etiology was predominant in the group of cirrhotic patients with DVT (6 patients out of 8), but with no statistical significance. Diabetes mellitus was the only variable that was significantly associated with the presence of DVT. Subsequent multivariate analysis confirmed diabetes mellitus as an independent risk factor for developing DVT in patients with liver cirrhosis with a high odds ratio. However, serum albumin level of less than 3 mg/dL tended to be a risk factor. These results coincide with those reported in the studied literature.

Venous thromboembolism is considered a very rare event in patients with liver cirrhosis, with unpredictable course and unclear mechanism. Viral hepatitis was the predominant cause of cirrhosis in our study (5 cases out of 6) compared with alcoholic and nonalcoholic steatohepatitis in Northup's study (8). Diabetes mellitus was present only in one case and, as we know, it is a risk factor for the occurrence of DVT.

We did not find any association between the clinical factors, such as sex, age,

and laboratory findings, and the presence of DVT in liver cirrhotic patients. Because of the small number of patients, no laboratory marker could be used as a predictor for prothrombotic state, including serum albumin levels. Most of our patients came already with low serum albumin levels (less than the normal cutoff point of 3.0 mg/dL). This data explains why serum albumin levels failed to show significant association with the presence of DVT. Low albumin level may indicate an advanced liver disease in most of our study subjects. In addition, it may be used as an indirect marker for the decreased synthesis of other proteins produced by the liver (7–10). There are other factors, such as protein C, protein S, and antithrombin III, that are involved as natural anticoagulants. During acute or chronic liver disease, their concentrations decrease concomitantly with other coagulation factors, but usually are not lower than 20% of the normal values. Decreased natural anticoagulants, therefore, could be another risk factor for developing DVT.

We identified diabetes mellitus as an independent risk factor for developing DVT. This observation was not surprising, since the risk of VTE is increased in diabetic patients (12). Other features of endocrinological disturbances, such as obesity and dyslipidemia, might be risk factors of DVT; however, a recent study concluded that symptoms of metabolic syndrome were not clinically important risk factors for VTE (14). Further studies are needed to confirm whether metabolic abnormalities, especially those with hepatitis C infection, could be the risk factors of DVT in patients with liver cirrhosis.

There are many risk factors and conditions predisposing to VTE such as age above 40 years, cancer with or without ongoing chemotherapy treatment, history of VTE, prolonged bed immobilization, surgery, trauma, obesity, smoking, and inherited hypercoagulable states (e.g., antithrombin deficiency, protein C and/or protein S deficiency, factor V Leiden, prothrombin gene mutation) (14). Besides, decreased synthesis of the natural anticoagulants, hypercoagulation in liver disease, could also be related to poor flow and vasculopathy associated with a chronic inflammatory state. The potential disease state favoring DVT or PE in patients with liver cirrhosis could be an imbalance in the clotting cascade favoring coagulation, immobilization associated with of end-stage liver disease, infection, and systemic inflammation (11, 12, 13). Our study showed that the age of the patients was between 33-63 years and most of them had chronic hepatic viral infection. Patients with this profile often need hospitalization due to the cirrhosis complications especially ascites. No patient of the group had encephalopathy. Immobilization during hospital stay is known as a risk factor for venous thrombosis due to the stasis of blood flow in the venous system. Therefore, patients with cirrhosis may share the same risk factors of VTE as other hospitalized patients. We did analyze the patients' length of stay before the occurrence of DVT symptoms and we concluded that the risk increased when the length of hospital stay was more than 6 days. No patient of the studied group presented electrocardiographic signs of pulmonary embolism or other arrhythmias (14, 15). As we mentioned, no active bleedings were present at admission, no bleeding during anticoagulation therapy and no significant variations in platelet counts as we would have expected (16). Cancer alone is a known risk factor of thrombosis. We also

analyzed the presence of hepatocellular carcinoma (HCC) and its association with DVT, but the presence of HCC apparently was not a risk factor for developing DVT in our study subjects because HCC was not discovered.

Only one of the patients (40 years old) died 2 months following the diagnosis, due to a respiratory infectious episode. Initial evolution under anticoagulation treatment appeared to be good and no hemorrhagic events were described.

Our study did have several limitations. Firstly, it was not designed prospectively, and it had a small number of patients. Also, DVT was suspected if the patient presented with clinical symptoms/signs suggestive of DVT and further confirmed by Doppler ultrasonography. We did not screen the patients' blood coagulation profile and, consequently, we might have overlooked patients with subclinical DVT.

The principal limitation of the study is related to the small number of patients which did not allow a statistical interpretation.

In our study we cannot estimate the real prevalence of DVT in our group of cirrhotic patients because of the small number of patients included, but, also taking other studies into account, we can appreciate that DVT is not a rare condition in cirrhosis. Diabetes mellitus was the only independent risk factor for developing DVT found in this study.

The treatment unanimously accepted so far, initially with low-molecular-weight heparin (for 7-10 days) continued with Acenocumarol is adequate, with a low risk of bleeding.

The precise mechanisms of hypercoagulability and thrombosis in cirrhosis are not well known and need further investigation.

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NEWS

SERUM CHOLINESTERASE: THE POWER OF PREDICTION

The role of cholinesterase to assess the liver reserves in hepatitis D patients has not been well defined. Therefore, the aim of this study was to determine the capacity of cholinesterase to predict liver function compared to actual synthetic liver function tests and scoring models in patients with hepatitis D and cirrhosis. In this observational, cross-sectional study were included 233 patients with cirrhosis due to chronic hepatitis D. Serum cholinesterase levels were correlated with existing scoring models for chronic liver disease and liver function tests like ascites, Child Turcotte Pugh (CTP), model for end stage liver disease (MELD) score, baseline-event-anticipation (BEA) score for hepatitis D and aspartate transaminase to Platelet Ratio Index (APRI). The results of this study showed well correlation of serum cholinesterase levels with serum albumin, INR and CTP, MELD, BEA and APRI scores and can be considered a prognostic marker of liver reserves in hepatitis D cirrhosis. Thereby, cholinesterase levels should be routinely checked to asses liver function and may be incorporated in MELD scoring. Also, it can be used to follow the staging of liver disease in hepatitis D (Minaam A, Zaigham A. Serum cholinesterase: a predictive biomarker of hepatic reserves in chronic hepatitis D. *World J Hepatol* 2017; 9(22): 953-978).

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EPIDEMIOLOGY AND HISTOLOGY OF MALIGNANT THYROID NODULES IN NORTH EAST REGION OF ROMANIA (MOLDAVIA) BEFORE AND AFTER ALIMENTARY SALT UNIVERSAL IODINATION

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EPIDEMIOLOGY AND HISTOLOGY OF MALIGNANT THYROID NODULES IN NORTH EAST REGION OF ROMANIA (MOLDAVIA) BEFORE AND AFTER ALIMENTARY SALT UNIVERSAL IODINATION (Abstract): Research on the relationship between iodine exposure and thyroid cancer risk is limited and the findings are inconclusive. **Objectives**: Given this molecular data on iodine we decided to evaluate the changes of incidence and histology of thyroid cancer in the North-Eastern region of Romania (Moldavia) after the government decision from 2004 that introduced the universal iodination of alimentary salt. After this decision values of urinary iodine increased from 50 μg/L (2001–2002) to 117 μg/L (2006 -2008). **Material and methods** : We compared the incidence and the histology of thyroid cancer in residents living in an area known as a mild endemic goiter region (Moldavia-Romania) between 2001-2004 with the incidence and the histology of thyroid cancer between 2005-2008 in the same region after the introduction of universal iodization of alimentary salt. **Results:** The number of papillary cancers increased from 125 cases (2001-2004) to 276 cases (2005-2008). The number of follicular cancer decreased from 52 cases (2001-2004) to 27 cases (2005-2008). The ratio between papillary and follicular cancers increased from 4.80 / 1 (2001-2004) at 10.61 / 1 (2005-2008). The number of medullar thyroid carcinoma increased from six cases (2001-2004) to 24 cases (2005-2008). Thyroid anaplastic carcinomas number increased from 7 cases (2001-2004) to 12 cases (2005-2008). The total number of thyroid cancer has increased dramatically after the introduction of universal iodination of alimentary salt with 178% compared to 2001-2004 (from 190 cases in 2001-2004 to 339 cases in 2005-2008), despite the fact that the number thyroidectomies decreased from 1734 (2001-2004) to 1449 (2005-2008). **Conclusion**: After the introduction the universal iodination of alimentary salt starting from 2004 the total number of thyroid cancers increased comparative with the period before universal iodination of alimentary salt. **Key words:** ROMANIA, IODINE SALT PROPHYLAXIS, THYROID CANCER

Before the introduction of salt iodination in 2004 the North-Eastern Region of Romania was known to be a moderate iodine deficiency area. There are some studies documenting the spectrum of TC in iodine-deficient communities (1,2,3,4). Research on the relationship between iodine exposure and thyroid cancer risk is limited and the findings are inconclusive. The molecular mechanism for this relationship of iodine with PTC is unclear. Molecular studies in recent years have identified a T1799A transversion BRAF mutation that occurs in about 45% of PTC and 25% of apparently

PTC-derived anaplastic thyroid cancer but not in any other type of thyroid tumors, such as FTC and benign thyroid tumors. This mutation causes a valine-toglutamic amino acid change in codon 600 of the BRAF protein, resulting in constitutive activation of the BRAF kinase and the Ras 3 Raf 3 MAPK kinase 3 MAPK/ERK pathway. (5) Commencing iodine prophylaxis it has been noted a rise in incidence of PTC relative to the other tumor types but the role of iodine nutrition in the pathogenesis of TC is both complex and controversial (1,3, 6,7,8). Increased exposure to ionizing radiation, changes in iodine nutrition and greater recognition of thyroid neoplasia have all been postulated as contributory (3). The aim of current study was to examine the changes of incidence and histology of thyroid cancer after the government decision from 2004 that introduced the universal iodination of alimentary salt. As a consequence the values of urinary iodine increased from 50 μg/L (2001 – 2002) to 117 μg/L 2006 -2008 (9).

MATERIAL AND METHOD

We reviewed the records of 601 patients diagnosed with thyroid cancer in the Saint Spiridon Hospital Iasi between 2001 and 2008. We excluded cases with clinical features highly suggestive of thyroid cancer, but without histological diagnosis, consequent to refuse of thyroid FNAB or thyroidectomy

In our retrospective study we analyzed the data of patients diagnosed with thyroid cancer in Endocrinology Clinic of Iaşi between 2001 and 2008 and the data obtained from the histological registry of Institute of Pathology Iaşi regarding the same diagnosis in the mentioned period. This Institute examines the excised samples of all operations made in Iasi County. The Endocrinology Clinic and the Institute of Pathology Iaţi have the major role in the management of thyroid cancer cases arisen in Iasi County and in border counties. First of all, as the Endocrinology Clinic is the single institution of this specialty in Iaşi County, it deals with both adult and pediatric endocrine pathology. Secondly, in our county thyroid cancer cases are managed mainly by endocrinologists not by oncologists. Those patients who undergo thyroidectomy without previous endocrine exam, approach the endocrinologist after the intervention, and if not, the cases are registered by the histological exam provided by the Institute of Pathology Iaşi. We compared the incidence and the histology of thyroid cancer in residents living in an area known as a mild endemic goiter region (Moldavia-Romania) between 2001 and 2004 with the incidence and the histology of thyroid cancer between 2005 and 2008, in the same region after the introduction of universal iodization of alimentary salt.

RESULTS

During 2001-2008 a total number of 529 patients from Moldavia were diagnosed histological with thyroid cancer. A trend of impressive increase of incidence of differentiated thyroid cancers especially of papillary cancers but also anaplastic thyroid cancers was noticed between 2005-2008 comparative to 2001-2004. The number of papillary cancers increased from 125 cases (2001-2004) to 276 cases (2005-2008). The number of follicular cancer decreased from 52 cases (2001-2004) to 27 cases (2005-2008). The ratio between papillary and follicular cancers increased from 4.80 / 1 (2001-2004) at 10.61 / 1 (2005-2008). The number of medullar thyroid carcinoma increased from 6 cases (2001-2004) to 24 cases (2005-2008). Thyroid anaplastic thyroid carcinomas number increased from 7 cases (2001-2004) to 12 cases (2005-2008).

The total number of thyroid cancer has increased dramatically after the introduction of universal iodination of alimentary salt

with 178% compared to 2001-2004 (from 190 cases in 2001-2004 to 339 cases in 2005-2008), despite the fact that the number thyroidectomies decreased from 1734 (2001--2004) to 1449 (2005-2008).

DISCUSSION

There was a significant rise in the incidence of papillary, medulary and anaplastic thyroid cancers during the study period despite the fact that the number thyroidectomies decreased . The changes in pathological classification are unlikely to account for the observed changes in TC incidence and spectrum (2,10). Some studies explain as contributory to the rise in TC incidence the increased use of ultrasonography and fine needle biopsy (4,11) but this is unlikely to account for the observed increase in total number of thyroid cancers from our study because the diagnostic methods remained the same reported to the study made in 2001--2004 in the Endocrinology Clinic from Saint Spiridon Hospital Iasi. The role of iodine nutrition in the pathogenesis of TC is controversial (12). Case control studies have also produced contradictory findings, iodine--rich diets having been associated with both a heightened and an attenuated risk of TC (10,13). Despite the conflicting results, a consistent association has been the link between iodine nutrition and tumor histology (11,14). The incidence of FTC is greatest in iodine-deficient populations, whereas the converse is true in regions of iodine sufficiency (1,7,10). Consistent with this observation, iodine prophylaxis is associated with a rise in the proportion of differentiated TC ; so called "papillarization" of TC (15). This is evidenced by a rise in the proportion PTC relative to FTC (3,6). However this does not explain the increasing number of medullar and anaplastic thyroid cancers during 2005--2008 comparative with the period before universal iodination of alimentary salt.

A complex interaction between iodine nutrition and thyroid tumorigenesis may account for our findings.

CONCLUSIONS

There was a significant rise in the incidence of total TC during the study period especially due to an increasing incidence of PTC. Additional studies are required to clarify the relationship between iodine nutrition and thyroid tumorigenesis. In particular, longitudinal data spanning transition from iodine sufficiency to deficiency should establish the contributions of improved diagnostic practice, environmental carcinogens and iodine nutrition to the observed changes in the spectrum of TC.

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SCREENING-UL COLONOSCOPIC LA PERSOANE ASIMPTOMATICE CU ISTORIC FAMILIAL DE CANCER COLORECTAL

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COLONOSCOPIC SCREENING OF ASYMPTOMATIC INDIVIDUALS WITH A FAMILY HISTORY OF COLORECTAL CANCER (Abstract): Background and Aims: First--degree relatives of colorectal cancer (CRC) patiens are at increased risk for developing colorectal neoplasm. The aim of our study was to evaluate the use of total colonoscopy as the screening test in asymptomatic individuals with a family history of colorectal cancer. **Material and method**: Colonoscopy was performed in 125 asymptomatic individuals (64 men and 61 women; mean age 51.7 ± 11.5 years, range 24-77 years) who had one or two first-deegree relatives with CRC. **Results**: Forty-five colorectal lesions were found in 36 subjects (28.8%): one (0.8%) adenocarcinoma, and 44 polyps (31 adenomas; 13 hiperplasic) (28.0%). The cecum was intubated in 96% of cases, and the procedure was generally well tolerated without complications. **Conclusions**: Total colonoscopy is a useful and safe screening procedure for the examinations of asymptomatic individuals with a family history of CRC. **Key words**: COLONOSCOPY, SCREENING, FAMILIAL COLON CANCER, FIRST-DEGREE RELATIVES

Cu peste un milion de cazuri noi şi aproximativ 500.000 de decese anual, cancerul colorectal (CCR) este o problemă majoră de sănătate publică la nivel mondial (1).

În România, asistăm la o creştere îngrijorătoare a prevalenţei CCR, care aproape s-a dublat în decursul unui deceniu (15,28%000 locuitori în 1996, ajungând la 28,6%000 locuitori în 2006) (2).

Aproximativ 20% din totalitatea cancerelor colorectale este reprezentat de CCR familial, care apare la pacienţi cu aglutinări familiale, dar nu respectă un model de transmitere ereditară (3,4). Riscul familial de CCR în prezenţa unei rude de gradul I este de 2-3 ori mai mare decât în populaţia generală şi creşte de 3-4 ori în prezenţa a două rude de gradul I cu CCR sau a unei rude de gradul I diagnosticată până la vârsta de 50 ani (5,6).

Scopul screening-ului este de a reduce mortalitatea prin reducerea incidenţei cazurilor avansate de boală. Screening-ul CCR poate atinge acest obiectiv prin detectarea adenocarcinoamelor într-un stadiu precoce, detectarea şi excizia polipilor adenomatoşi prin polipectomie endoscopică (7,8).

Colonoscopia este considerată cea mai bună metodă de screening pentru CCR pe care ghidurile actuale o recomandă la persoanele cu istoric familial de CCR (9-11).

Scopul studiului nostru a fost evaluarea oportunităţii unui program de screening pentru CCR la rudele de grad I a pacienţilor cu această boală malignă utilizând colonoscopia.

MATERIAL ŞI METODĂ

Studiul s-a desfăşurat în Institutul de Gastroenterologie şi Hepatologie (IGH) Iaşi, în perioada mai 2008 – iunie 2010, iar participanţii la acest studiu au fost rudele de

TABELUL I
Caracteristicile demografice ale lotului de studiu

Nr. de participanți	125
Sex (bărbați:femei)	64:61
Vârsta medie (ani) (limite)	51,7±11,5 (24 -77 ani)
Mediul de proveniență	
Urban	84 (67,2%)
Rural	41 (32,8%)
Județul Iaşi	68 (54.4%)
Alte județe	57 (45,6%)
Rudele afectate cu CCR	
Părinți	84 (67,2%)
Frați/surori	41 (32,8%)
Numărul rudelor afectate	
1 rudă	118 (94,4%)
2 rude	7 (5,6%)

grad I a 460 pacienţi diagnosticaţi cu cancer colorectal. Din ianuarie 2008 am procedat la informarea detaliată a pacienţilor cu CCR privind incidenţa crescută a acestei boli maligne la rudele cu istoric familial şi importanţa diagnosticării acestui tip de cancer într-un stadiu precoce, vindecabil. Pacienţii au fost rugaţi să informeze rudele despre riscul crescut al bolii şi să le invite să participe la acest studiu. De asemenea, au fost trimise invitaţii la rudele pacienţilor, care cuprindeau un scurt material informativ despre riscul crescut în apariţia CCR la persoanele cu istoric familial de CCR şi posibilitatea depistării bolii într-un stadiu iniţial, vindecabil. Persoanele care au răspuns invitaţiei au primit informaţii suplimentare despre importanţa diagnosticului precoce a CCR cu ajutorul colonoscopiei, cu explicaţii detailate asupra procedurii endoscopice şi avantajelor metodei (posibilitatea examinării întregului colon, prelevarea de biopsii şi excizia polipilor depistaţi), fiind de asemenea atenţionaţi asupra discomfortului în timpul examinării şi potenţialele riscuri. După pregătirea corespunzătoare cu preparate orale de tip polietilen glicol (Fortrans) şi obţinerea consimţământului, participanţii au fost investigaţi colonoscopic la IGH Iasi. În studiu au fost incluse persoane fără semne sugestive pentru CCR şi fără comorbidităţi severe care să interfereze cu realizarea colonoscopiei. Costurile au fost suportate de instituţia medicală, iar colonoscopia a fost efectuată de medici gastroenterologi experimentaţi, competenţi în endoscopie digestivă.

Pentru identificarea tipului histologic polipii depistaţi au fost biopsiaţi. Persoanelor cu un rezultat negativ la colonoscopie li s-a recomandat supraveghere colonoscopică la 5 ani.

REZULTATE

Dintre cele 460 persoane invitate, 245 (53,3%) au răspuns invitaţiei şi s-au arătat interesate de studiu, iar 125 (27,1%) s-au prezentat în perioada mai 2008-iunie 2010 la IGH pentru efectuarea colonoscopiei. Participarea la studiu a evidenţiat preponderenţa

TABELUL II
Rezultatele examenului colonoscopic

Rezultat colonoscopie	Nr. pacienți	%	Observații
Colonoscopie normală	48	38,4	
Adenocarcinom	1	0,8	Localizare: rectală Stadiu: Dukes B (T3N0)
Polipi	35	28	Nr. polipi: 44 Localizarea: rect-1; colon stâng-28; colon drept-15 ; Diametru: <10 mm-31 polipi; peste 10 mm-13 polipi Polipectomie: 24 pacienți
Boală hemoroidală	31	24,8	
Diverticuloză	10	8	

TABELUL III
Examinarea histologică a leziunilor depistate

Leziune	Număr = 45	%
Adenocarcinom	1	2,2
Adenoame tubulare	18	40,0
Adenoame tubulo-viloase	8	17,8
Adenoame viloase	5	11,1
Polipi hiperplazici	13	28,9

nesemnificativă a sexului masculin - 64 bărbaţi (51,2%) faţă de 61 femei (48,8%), si a inclus persoane cu vârsta cuprinsă între 24 şi 77 ani (vârsta medie: 51,7 ± 11,5 ani), majoritatea provenind din mediul urban. Caracteristicile demografice ale lotului de studiu sunt prezentate în tabelul I.

În total, au fost identificate 45 leziuni la 36 pacienţi (28,8%): un adenocarcinom (0,8%) şi 44 de polipi (28%) (tab. II). Examinarea histologică a polipilor a confirmat 31 polipi adenomatoşi (18 polipi tubulari, 8 polipi tubulo-viloşi, 5 polipi viloşi) şi 13 polipi hiperplazici. La 14 pacienţi (11,2%) polipii depistaţi reprezentau adenoame avansate (definite cele cu diametrul = 10 mm sau histologic cu displazie de grad înalt sau cu componentă viloasă) (tab. III).

Examinarea completă a colonului s-a reuşit la 120 pacienţi (96%). Procedura a fost bine tolerată, fără complicaţii, sedarea conştientă fiind folosită în rare cazuri.

DISCUŢII

Creşterea incidenţei şi prevalenţei CCR la nivel mondial a trezit un interes sporit în lumea medicală pentru implementarea programelor de screening. CCR îndeplineşte pe deplin criteriile stabilite de OMS pentru instituirea unor astfel de programe: boală cu incidenţă şi mortalitate semnificative, asimptomatică pentru o perioadă îndelungată, dezvoltată din leziuni premaligne, progresie lentă (ani) din stadiile precoce, curabile, la cele avansate, teste de screening accesibile, fără riscuri şi eficiente în reducerea morbidităţii şi mortalităţii (12).

Reducerea incidenţei şi mortalităţii prin CCR în ţările unde s-au aplicat programe de screening a fost atribuită depistării bolii într-un stadiu incipient şi îndepărtarea polipilor adenomatoşi prin polipectomie endoscopică (13-16).

Colonoscopia este considerată de mulţi autori ca „gold standard" în screening-ul CCR, depăşind ca eficacitate FOBT (*fecal occult blood test)* şi sigmoidoscopia flexibilă în detectarea cancerului şi a neoplaziilor avansate (10,14, 17, 18).

Istoricul familial de CCR este un factor de risc bine cunoscut în apariţia bolii, ghidurile actuale recomandând screening-ul colonoscopic la rudele de grad I a pacienţilor diagnosticaţi cu CCR (9-11).

Prin urmare, noi am adoptat colonoscopia ca metodă de screening în studiul nostru şi am avut o rată de participare de 27,1%. Studii similare din străinătate raportează o rată de participare între 30% şi 40% (19, 20). Ruthotto si colab. (21) raportează că mai puţin de 40% din rudele de grad I a pacienţilor cu CCR au acceptat să participe la un program de screening colonoscopic, sugerând că informarea pacienţilor despre riscul potenţial de apariţie a bolii la rude determină creşterea ratei de participare la screening. Studii similare din ţară (22) au demonstrat că screening-ul prin colonoscopie al rudelor pacienţilor cu CCR reprezintă o metodă fezabilă şi sigură pentru depistarea polipilor colorectali şi CCR.

În studiul nostru am depistat 45 de leziuni la 36 pacienţi (28,8%), dintre care adenoame la 20,8% şi polipi non-neoplazici în 7,2% din cazuri; în plus, am depistat un caz de adenocarcinom (0,8%). Constatările noastre confirmă rezultatele altor studii (23--25). Astfel, Pezzoli şi colab. (23) raportează adenoame depistate în 21,7% din cazuri, iar Colombo şi colab. (20) - adenocarcinoame în 0,9%. Pe de altă parte, am găsit un procent ridicat de adenoame avansate – 11,2% dintre cazuri, considerate a fi factori

de risc importanţi în dezvoltarea CCR. Rezultate similare raportează Pariente şi colab. (19) în ce priveşte prevalenţa adenoamelor avansate. Se poate specula că factorii genetici influienţează viteza de creştere a adenomului, mai degrabă decât numărul lor. Această ipoteză este susţinută în studiul publicat de Fossi şi colab. (26) în privinţa timpului de reapariţie a adenoamelor la rudele de grad I a pacienţilor cu CCR.

Intubarea cecală s-a reuşit în 96% din cazuri, atingând aceleaşi cote ca şi în alte centre de endoscopie performante (27).

CONCLUZII

Rudele de grad I a pacienţilor cu CCR sunt mai predispuşi la dezvoltarea adenoamelor avansate decât populaţia generală. Colonoscopia este o metoda eficientă şi fezabilă de screening pentru CCR la rudele pacienţilor cu această boală malignă.

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NOUTĂŢI

Tratamentul HTA are efecte favorabile asupra riscului de fibrilaŢie atrială

Hipertensiunea arterială esenţială acţionează asupra întregului sistem cardiovascular putând induce printre altele o hipertrofie ventriculară stângă ce antrenează o disfuncţie diastolică a ventricolului stâng, asociată unor tulburări de ritm ventriculare şi atriale, astfel încât riscul de fibrilaţie atrială este în general mai crescut la subiecţii hipertensivi. O serie de medicamente antihipertensive acţionând asupra remodelajului cardiovascular, cu precădere asupra celui atrial (de exemplu prin inhibarea sistemului renină-angiotensină-aldosteron), par a împiedica apariţia unei fibrilaţii atriale. Un studiu intracohortă de tip caz-martor, de mare anvergură, aduce argumente în favoarea acestei ipoteze. Datele au fost extrase dint-o bază de date (General Practice Research Database) ce numără teste cinci milioane de observaţii raportate de medicii generalişti englezi. În total, 4 661 de pacienţi hipertensivi având şi fibrilaţie atrială (FA) au fost comparaţi cu 682 993 de pacienţi martori, trataţi pentru hipertensiune arterială (HTA), riscul de FA fiind estimat în funcţie de tratamentul antihipertensiv : betablocante, inhibitori ai enzimei de conversie (IEC), antagonişti ai receptorilor de angiotensină II (ARA II). În grupul martor au fosr reţinuţi pacienţi trataţi cu inhibitori calcici care nu influenţează tulburările de ritm cardiac. De asemenea, au fost excluşi subiecţii ce prezentau factori de risc pentru FA. Analiza comparativă a aratat că riscul relativ de FA (odds ratio – OD) a fost mult mai redus la pacienţii trataţi comparativ cu lotul martor : IEC (OD=0,75), ARA II (OD=0,71), betablocante (OD=0,78) (Schaer BA et al. Risk for incident atrial fibrillation in patients who receive antihypertensive drugs : a nested case–control study. *Ann Intern Med* 2010 ; 152 : 78-84).

Al.D. Ciochină

IMPLICATIONS OF VITAMIN D DEFICIENCY IN INFLAMMATION DUE TO RHEUMATOID ARTHRITIS

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IMPLICATIONS OF VITAMIN D DEFICIENCY IN INFLAMMATION DUE TO RHEUMATOID ARTHRITIS (Abstract): Chronic inflammation due to inflammatory joint diseases, the most important of which is rheumatoid arthritis, is a both topical and overall bone loss risk factor. There have been numerous researches showing that vitamin D plays a major role in inflammation by stimulating innate immunity, and also by boosting its activity and by decreasing adaptive immune activity. In our research, we analyzed the factors involved in bone mass loss in patients suffering from rheumatoid arthritis, as well as the link between the serum level of 25 (OH) vitamin D and the extent of the inflammation. We also tackled the impact of vitamin D supplement delivery on the status of these patients. **Keywords:** RHEUMATOID ARTHRITIS, 25 (OH) VITAMIN D, INFLAMMATION.

Inflammation is closely linked to significant fracture risk (1, 2) in chronic inflammatory conditions, such as rheumatoid arthritis (3, 4) or ankylosing spondylitis, as well as when a low degree of inflammation is detected in apparently healthy individuals.

Rheumatoid arthritis is one of the most important inflammatory conditions since in addition to its triggering local juxta-articular bone impairment, it also entails bone mass loss at considerable distances from the joints (5). In rheumatoid arthritis the bone is a major target for chronic inflammation, this condition being caused by the numerous interactions and interconnections between the bone and the immune system (6). Inflammation occurring in the bone leads to bone resorption increase and it determines local bone formation suppression (7, 8).

Research in this field has shown that juxta-articular bone mass loss and bone inflammation occur during the early stages of the disease and that they precede and are also predictive of erosive bone destruction specific to rheumatoid arthritis (9). A European multi-center study published in 2017 revealed that patients with rheumatoid arthritis usually exhibit vitamin D insufficiency or even deficiency, with significant differences among various coun-

tries. The serum levels of vitamin D in the patients included in this study were inversely proportional to life quality indices, to the stage of the disease and to the degree of disability (10).

MATERIAL AND METHODS

The observational prospective case-control study was carried out for about one year, from January 2017 to January 2018.

The research was aimed at analyzing the factors involved in bone mass loss in patients with rheumatoid arthritis, as well as at determining the connection between the serum level of 25OH vitamin D and the extent of the inflammation, and also the impact of vitamin D supplement delivery on the status of these patients.

There were initially 93 patients with rheumatoid arthritis included in the study, who were selected based on the inclusion criteria set in the study protocol (20 to 65 years of age, confirmed rheumatoid arthritis diagnosis in various disease evolution stages: I, II or III), but also according to the exclusion criteria (less than 20 or more than 65 years of age, related diseases – endocrine or neoplastic conditions). Only 82 patients were left in the study group after reassessment.

Patient data (personal and family medical history), anthropometric indices (weight, height, BMI), data on socio-demographic factors and life style (exercise, active smoking) and data on clinical parameters (number of painful joints, number of swollen joints, morning stiffness) were collected on the initial assessment. Patients were also selected depending on the rheumatoid arthritis stage and maintenance therapy administered before.

A series of biological parameters of inflammation, such as ESR (erythrocyte sedimentation rate) and CRP (C reactive protein), as well as certain biological parameters assessing the possible complications of the basic disease or side effects of the maintenance therapy, among which certain hematological, hepatic or renal factors, were also determined in order to set the stage of the disease. Also, in this study, we dosed 25-OH vitamin D in order to determine whether mild or severe vitamin D deficiency is linked with the extent of bone mass loss, with the level of pain and possibly with the level of disease activity and degree of disability. We calculated internationally acknowledged scores (DAS 28, HAQ, SDAI, CDAI) for all the patients included in the study, in order to be able to assess the level of disease activity and degree of disability. The immunological markers of the disease were determined in all the patients: rheumatoid factor (RF), anti-citrullinated protein antibodies (ACPA), total antinuclear antibodies (ANA), using Elisa-type tests.

On the 6-month follow-up, we analyzed the inflammation parameters again (ESR, CRP), we studied the new values of the immunological markers (rheumatoid factor, ACPA), and we calculated the new internationally-used disease activity scores (DAS 28, CDAI, SDAI, HAQ), in order to be able to monitor disease activity dynamics and its functional impact.

Throughout the study, all the patients underwent classical or biological DMARDs therapy. After their initial assessment, 95% of the patients also received vitamin D supplements and this was decided since we found significant vitamin D deficiency in the patients included in the study.

RESULTS

The number of painful joints was 0 to 24, and the value series median was 6. The results of the Skewness test suggest that

NPJ (number of painful joints) is a continuous variable (p=0.851).

The mean NPJ was 7.46±6.53, i.e. significantly lower than the number of painful joints before the treatment – 8.77±7.67 joints (p=0.001).

The NSJ (number of swollen joints) was 0 to 16, and the value series median was 1, whereas the mean was 2.11, and the results of the Skewness test suggest that NSJ is a discontinuous variable (p=2.488), which restricts the application of parametric significance tests. On reassessment, the mean NSJ was 2.11±0.36, i.e. significantly lower than the number of swollen joints detected before therapy –2.46±0.48 joints (p=0.037).

Pain VAS (visual analogue scale) ranged between 1 and 8. The value series median was 4, i.e. close to the mean value of 4.01. The results of the Skewness test suggest that the VAS value series was homogeneous (p=0.266). On reassessment, the mean VAS was 4.01±2.22, which was significantly lower than the VAS determined at the beginning of the study, which was 4.70±2.44 (p=0.001) (fig. 1).

In the patients included in the research, the disease activity score DAS_{28} had normal distribution (p= -0.026), ranging from 2 to 7 and exhibiting a value series median of 4.03. The second assessment revealed a mean DAS_{28} of 3.97±1.39, which was slightly lower than the score recorded during the first assessment, which was 3.99±1.52 (p=0.813) (fig. 2).

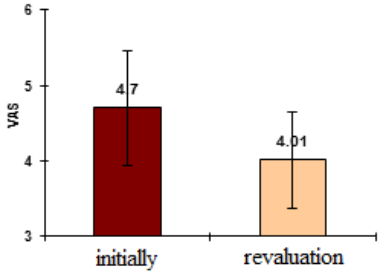

Fig. 1. Evolution of mean VAS

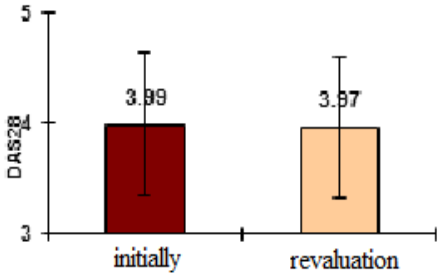

Fig. 2. Evolution of mean DAS_{28}

On reassessment, the ESR ranged from 4 to 115 mm/h, with a homogeneous value series (p=1.259), the mean reading being 34.39±22.25 mm/h, without being significantly different from the initial one of 34.18±22.25 mm/h (p=0.834) (p=0.834).

On reassessment, the CRP showed wide variations within the 0.40-102 mg/dl range, the mean level being 7.28±8.63 mg/dl, i.e. slightly lower than the reading at the beginning of the research 7.55±4.06 mg/dl (p=0.710).

Of all the clinical parameters, only VAS varied significantly depending on Latex rheumatoid factor positivity (4.39 *vs.* 3.39; p=0.046).

The mean level of clinical parameters did not show significant differences depending on Waller Rose rheumatoid factor positivity, with the exception of VAS (4.39 *vs.* 3.39; p=0.046).

The rheumatoid factor tested positive especially in the female sex (p=0.247) and in the urban environment (p=0.582), but the percentage distributions were not statistically significant. 46.9% of the high titre patients and 73.3% of the low titre patients were 60 and older, whereas 63.6% of the

negative titre patients had not reached this age (p=0.039).

Serum calcium and ESR were apparently independent parameters both at the beginning of the study (r= -0.004; p=0.973) and on reassessment (r= -0.068; p=0.546) (fig. 3).

Vitamin D values showed negative correlation with both VAS and HAQ, both on the first assessment and on reassessment.

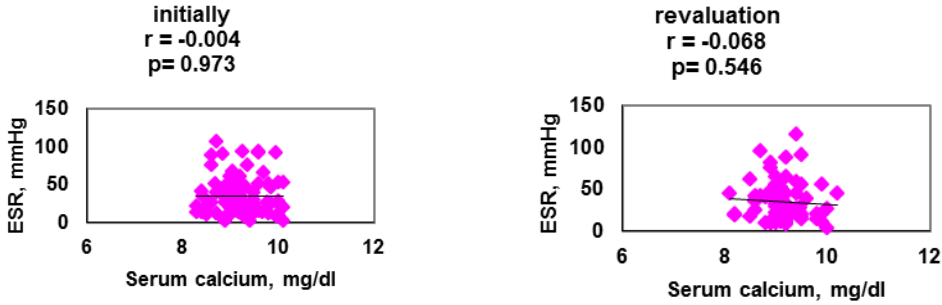

Fig. 3. Serum calcium correlation with ESR

The vitamin D and ESR correlation was indirect and weak, both at the beginning of the study (r= -0.146; p=0.169) and on reassessment (r= -0.169; p=0.130) (fig. 4).

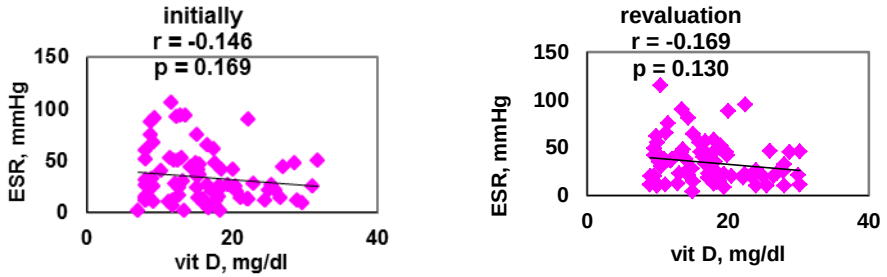

Fig. 4. Vitamin D and ESR correlation

On the first assessment, the lower vitamin D and serum calcium levels were associated with higher ESR values. (fig.5).

On the other hand, on reassessment, the higher vitamin D and serum calcium levels were associated with lower CRP values. (fig.6)

Vitamin D and CRP were also apparently independent parameters both at the beginning of the study (r= -0.017; p=0.881) and on reassessment (r= +0.026; p=0.814) (fig.7).

At the beginning of the study, 21% of the patients had higher serum calcium values and higher white cell counts (r= +0.210; p=0.05), whereas on reassessment only 15.5% of them still exhibited this characteristic (r= +0.155; p=0.165). Nevertheless, these findings cannot be extrapolated to the general population.

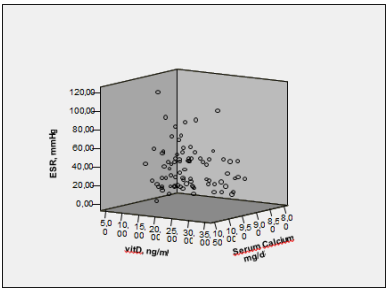

Fig. 5. Serum calcium and vitamin D correlation with ESR

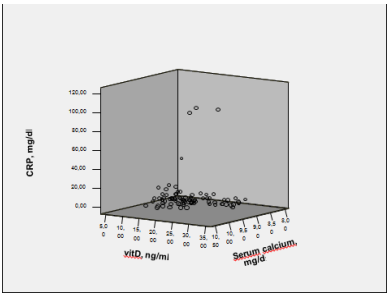

Fig. 6. Serum calcium and vitamin D correlation with CRP

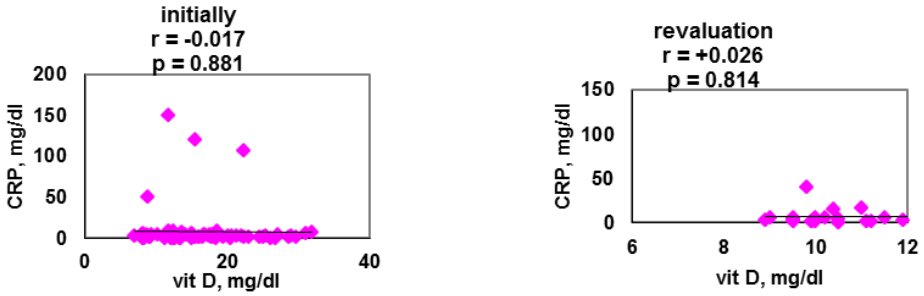

Fig. 7. Vitamin D and CRP correlation

DISCUSSION

A study published in 2018 revealed that low vitamin D values in the serum were associated with a series of autoimmune diseases. This research revealed a negative correlation of serum 25 (OH) vitamin D concentrations with pain and disease activity score in patients with rheumatoid arthritis, yet it was unable to clearly determine the cause-effect relation. The same research found that vitamin D supplements were associated with significant effects on pain and on disease activity in patients suffering from rheumatoid arthritis. It was also found that vitamin D deficiency seems to be tightly connected to pain, whereas high serum concentrations of 25 (OH) vitamin D seem to have immunomodulating effects (11, 12).

On reassessment, the analysis of our group of patients showed a significantly lower mean NPJ level compared to the number of painful joints detected in the patients at the beginning of the research, which proves a positive evolution of pain, correlated with improved 25OH vitamin D levels. This also stands for a mean NSJ level.

On reassessment, the mean VAS level was significantly lower than the VAS determined before the therapy, and it was also correlated with 25 (OH) vitamin D values. 6 months after the beginning of the study, the mean DAS_{28} of these patients was slightly lower than the one determined on the first assessment. The VAS scale on reassessment improved and it was correlated with improved NPJ and NSJ values, as well as with 25 (OH) vitamin D values.

The vitamin D and ESR correlation was indirect and weak, both at the beginning and on reassessment. On the first assessment, the lower vitamin D and serum calci-

um levels were associated with higher ESR values. On reassessment, the higher vitamin D and serum calcium levels were associated with lower CRP values.

Negative correlations of the vitamin D level with the pain and disability scales were detected.

CONCLUSIONS

The assessment of the patients 6 months after the beginning of the study showed a slight improvement of the inflammatory and immunological syndrome, knowing that in addition to their anti-inflammatory maintenance therapy they were also given vitamin D supplements.

Our research showed that strong inflammation was linked to lower vitamin D and serum calcium levels, especially on the first assessment.

The findings of our study are in line with the research in the field, as they reveal that the intake of vitamin D supplements since the onset or since the early stages of rheumatoid arthritis may lead to an improvement of these patients' status, both from the viewpoint of joint and muscle pain and of mobility and stability improvement, and from the viewpoint of bone mass loss slowdown.

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RECONSIDERING STATINS-CURRENT VIEW

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RECONSIDERING STATINS-CURRENT VIEW (Abstract): Statins are widely used in daily clinical practice for the management of dyslipidemia. There are numerous studies that have shown a pleiotropic effect of statins in chronic liver diseases. They increase nitric oxide synthesis, restore endothelial cell function and, by that, increase the number of progenitor endothelial cells which improve endothelial function. Simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension by reduction of hepatic vascular resistance without adverse effects on systemic circulation and with an excellent safety profile. Simvastatin addition to standard therapy in patients with cirrhosis who recover from an acute variceal bleeding episode improves survivability. Statins modulate intrahepatic vascular tone and by that improve liver function and lack of significant hepatotoxic effects. Statins inhibit protease pathway activation, exert anti-inflammatory and immunomodulatory effects, modifying the cell activation cascade. Due to all these mechanisms, statins have antineoplastic effects.
Keywords: STATINS, LIVER CIRRHOSIS, VIRAL HEPATITIS, HEPATOCARCINOMA.

Statins are widely used in daily clinical practice for the management of dyslipidemia. More than half of the patients aged over 75 and one third of the patients aged 40 use that medication to lower their cholesterol level.

On the other hand, it is well known that statins have been associated with potential liver toxicity with increased liver enzymes.

However, evidence suggests a safe and beneficial effect of this "forbidden" drug which became a "magic pill" in almost every liver disease: hepatitis, portal hypertension, cirrhosis, hepatocellular cancer (HCC), nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH) (tab. I).

There are numerous studies that have demonstrated the pleiotropic effects of statins in chronic liver diseases. Statins act by inhibiting the activity of 3-hydroxy-3methyguaryl coenzyme A reductase (2, 3). Secondary to lipid lowering effect, this mechanism has as consequence the decrease of oxidative stress and inflammation by decreasing the activation of inflammatory cells. Consequently, they increase nitric oxide synthesis, restore endothelial cell function and, by that, increase the number of progenitor endothelial cells which improve endothelial function (4, 6).

In vitro studies have also suggested a favorable impact on hepatic inflammation, progression of fibrosis and hepatic vascular tone (7, 8). The effects on fibrosis progression and hepatic decompensation in patients with chronic liver disease reported in some recent studies (9,10) were very variable.

TABLE I
Properties of statins associated with observed benefits in liver disease
Adapted after (1)

LIVER DISEASE	STATIN PROPERTIES
NAFLD/NASH	LDL cholesterol reduction Reduced inflammation Reduced fibrosis Antioxidant properties
Hepatitis C	Reduction of hepatitis C virus replication
Portal hypertension	Increase in NO bioavailability Reduction in collagen production Inhibition of hepatic stellate cell activation
HCC	Inhibition of proliferation Increased apoptosis
Cirrhosis	Reduction in portal hypertension Reduction of collagen production Reduction in inflammation

Statins and portal hypertension in liver cirrhosis

The degree of portal hypertension is determined by hepatic venous pressure gradient (HVPG). Studies and meta-analyses have shown that prognosis of cirrhotic patients improve when HVPG decreases >20% from baseline or to a level lower than 12 mmHg. Less than half of patients receiving β-blockers achieve this target. Moreover, about 15% of patients have contraindications and an additional 15% may not tolerate.

This has prompted an alternative drug for long term treatment of portal hypertension (11, 12).

An ideal drug for portal hypertension was pictured as one that should reduce portal pressure acting as a hepatic vasodilator by decreasing intrahepatic vascular resistance and maintaining or enhancing hepatic blood flow.

Simvastatin and atorvastatin exert their hepatic vasodilatation effect by up-regulating endothelial NO production and act as true liver-selective vasodilators because blood pressure and systemic vascular resistance were unchanged after continuous use. This is totally different from the mechanisms mediating the effects of β-blockers, which reduce HVPG through a decrease in Porto collateral blood flow. The different mechanism of action probably explains the additive effect of simvastatin and β-blockers on HVPG (13, 14).

According to a randomized controlled trial conducted by Abraldes *et al.* (15), simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension.

The study had a randomized two-group design; one group received simvastatin for 1 month, 20 mg/day for the first 15 days, 40 mg thereafter, and the other group received placebo with or without propranolol treatment prior to the study. The authors found that after one-month simvastatin significantly decreased HVPG in patients with cirrhosis and severe portal hypertension. Simvastatin reduced HVPG by 10% or more in 40% of patients, to a value to protect against bleeding in early portal

hypertension (HTP).

In 32% of patients it was recorded a reduction in HVPG of ≥ 20% from baseline. Moreover, the HVPG-decreasing effect of simvastatin is independent of concomitant treatment with β-blockers, because β-blockers determine on average a reduction in HVPG of 10-15%; the additive effect of simvastatin increases the number of patients who are protected effectively against the complications of portal hypertension. In conclusion, the authors summarize that simvastatin lowers portal pressure in patients with cirrhosis and HTP, by reduction of hepatic vascular resistance without adverse effects on systemic circulation and with an excellent safety profile. Another beneficial effect observed in this study was that simvastatin decreases HVPG in patients taking/not taking β-blockers, suggesting that its effect on portal hypertension is additive. Unlike β-blockers, simvastatin improves quantitative liver function test results by enhancing liver perfusion, thus providing additional benefits in cirrhosis. These effects of simvastatin indicate that it has a clear potential for the treatment of HTP in patients with cirrhosis. Adding to the fact that propranolol is contraindicated in severe circulatory dysfunction or severe refractory ascites. Future trials should evaluate whether these critically ill patients are good candidates for statins.

None of the patients in the study who received simvastatin had an increase in liver aminotransferase levels. Moreover, the authors observed a slight improvement in liver function. Statin hepatotoxicity in cirrhotic patients does not seem to be higher than that in the general population.

In conclusion, the study reinforces the promising role of simvastatin in decreasing portal hypertension and improving liver function. Prophylaxis of variceal hemorrhage associated with portal hypertension is a new indication for simvastatin. Critically ill patients (severe circulatory dysfunction or refractory ascites) may be good candidates.

In a multicenter, double-blind, randomized trial (17), addition of simvastatin to standard therapy did not reduce rebleeding but was associated with a survival benefit in patients with Child class A or B cirrhosis. The cirrhotic patients with variceal bleeding receiving simvastatin had a longer survival compared to those receiving placebo. In the cirrhotic patients who recovered from an acute variceal bleeding episode, the addition of simvastatin to standard therapy (β-blockers and ligation) improved survival rate without reducing the rate of other complication. The authors conclude that simvastatin addition to standard therapy in cirrhotic patients who recover from an acute variceal bleeding episode improves survivability. Beneficial effects may be mostly due to the increased hepatic blood flow and consequent improvement in liver function. Although unable to prevent these complications, simvastatin attenuated the deleterious effect of these events and did not increase their rate.

Statins and Hepatitis C compensated cirrhosis

Between 1996-2009, Mohanty *et al.* (18) conducted a cohort study of 40512 patients with HCV-related compensated cirrhosis. They identified 2802 statin users. The study demonstrated a 40% lower risk of decompensation and death in HCV-related compensated cirrhosis in patient who were using statins. The beneficial effects of statins in this study were the reduction of portal pressure in patients with

cirrhosis, improved survival without effect on recurrent bleeding. Beside these, an important effect was that statins modulate intrahepatic vascular tone and by that improve liver function and lack of significant hepatotoxic effects. By these effects, statins reduce the risk of decompensation and death in hepatitis C-related compensated cirrhosis.

Statins and infections in liver cirrhosis

In a study on veterans with cirrhosis, Motzkus-Feagans *et al.* (19) demonstrated that statins provided a protective effect against infections compared with non-users of any cholesterol-lowering medications. Statin use has been associated with a decreased risk of pneumonia and other severe infections, sepsis included. The involved mechanism is explained by the anti-inflammatory properties of statins.

Statins are associated with reduced risk of hepatocellular carcinoma

In a meta-analysis of ten studies including over 1,400,000 patients of which 4298 had hepatocellular carcinoma (HCC), Sigh *et al.* (20) found that statin use is associated with a 37% reduction in the risk of HCC. This effect was more pronounced in the Asian population.

Statins inhibit the activation of protease pathway, exert anti-inflammatory and immunomodulatory effects, modifying the cell activation cascade. Due to all these mechanisms, statins have antineoplastic effects.

There is a difference between Asian and Western population with respect to the chemo preventive effects of statins in HCC. Chronic HVC infection is the dominant risk in Asia and sub-Saharan Africa, whereas in USA and Western countries, alcohol, HCV, NAFLD associated with obesity and metabolic syndrome are responsible for liver cancer. Statins in Asian population exert their effect by antagonizing the oncogenic effect of HBV; the mechanisms by which statins alter the risk of HCC in Western population is not clear (the protective effect may be related to modification of metabolic syndrome insulin-mediated cell proliferation and obesity- associated inflammation).

Additionally, because patients with HCV infection are at higher risk for type 2 diabetes mellitus, statins may exert their antineoplastic effects through anti-infective properties.

The studies have shown that the chemo preventive effects are greater with higher dose. Tsan *et al.* (21) analyzed a cohort of patients with hepatitis B. They found that a high dose duration of statins was associated with greater protective effects on HCC. In line with this study, the study conducted by El Serag *et al.* (22) noticed that statin use for more than 6 months was associated with a significant decrease in the risk of HCC. A significant dose-response relationship between statin use and development of cancer has not been demonstrated yet. Statins have been shown to have a proangiogenic effect at low doses and an antiangiogenic effect at high doses in *in vivo* studies (23).

Lipophilic statins (lovastatin, simvastatin) may have a greater chemo preventive effect than lipophobic statins (pravostatin) due to a greater lipid solubility and membrane permeability (24).

CONCLUSIONS

Our review highlighted the role of statins in in hepatic diseases. Statins lower portal pressure in patients with cirrhosis and portal hypertension. In cirrhotic patients who recover from an acute variceal

bleeding episode statin in addition to standard therapy improve survivability. Due to their anti-inflammatory properties, statins decrease the risk of pneumonia and other severe infections, sepsis included, in liver cirrhosis. Statins improve liver function, lack significant hepatotoxic effects and have antineoplastic effects.

We strongly recommend reconsidering statins use in chronic liver diseases.

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NEWS

SUDDEN UNEXPECTED DEATH IN EARLY PARKINSON'S DISEASE: NEUROGENIC OR CARDIAC DEATH?

The histological hallmark of idiopathic Parkinson's disease (PD) is Lewy pathology (LP), including Lewy bodies in cell somata and thread-like Lewy neurites, both composed primarily of phosphorylated α-synuclein. Recent clinicopathological studies have revealed that individuals with PD show various nonmotor signs. These nonmotor signs are also related to the deposition of phosphorylated α-synuclein and tend to appear earlier than the typical motor signs. An individual with LP who has not yet developed typical motor signs is considered to have premotor PD. The authors presented two cases of sudden unexpected death (SUD) in elderly individuals in whom autopsy unexpectedly showed Lewy pathology, a hallmark of Parkinson's disease. Both individuals were 68-year-old men who were found dead in their homes without lethal trauma or poisoning. Inquests into their deaths suggested that nonmotor cardiovascular signs of Parkinson's disease might have appeared just before their deaths, although few typical motor signs were present. Autopsy showed Lewy pathology in the heart and peripheral autonomic nervous system in addition to lesser involvement of the brainstem that was consistent with Braak stage 3. In case 1, an atrial septal defect of the secundum type with advanced fibrosis of the atrium was present. In case 2, severe stenosis of the atrioventricular node artery with some microscars and diffuse interstitial fibrosis of the basilar ventricular septum were found. These two cases show some premotor Parkinson's disease with a symptom suggestive of autonomic dysregulation which may be a risk of SUD. In addition, coexistence of structural change of the heart with possibly arrhytthmogenic potential may increase the risk of SUD with Parkinson's disease. At present, very few pathological reports have included detailed cardiac pathology of SUD in patients with PD. Here, the authors presented the cases of two elderly men with SUD whose autopsies showed unpredicted LP. So, autonomic dysfunction related to PD has been considered a major contributor to sudden unexplained death (SUD) in patients with PD (Nishida N, Yoshida K, Hata Y. Sudden unexpected death in early Parkinson's disease: neurogenic or cardiac death? *Cardiovascular Pathology*, 2017, 30: 19-22).

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PARTICULARITĂŢI DIAGNOSTICE ŞI TERAPEUTICE ALE HIPERTENSIUNII SISTOLICE IZOLATE

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DIAGNOSIsAND THERAPEUTIC ASPECTS OF ISOLATED SYSTOLIC HYPERTENSION (Abstract): The isolated systolic hypertension is a distinct entity concerning evolving, pathological, diagnosis and treatment particularities. The present study analyzed the behavior of the blood pressure by continuous ambulatory monitoring of a group of patients diagnosed with isolated systolic hypertension by office measurement. **Material and method**: One hundred and twenty nine patients with isolated systolic hypertension at office were included. They were subjected to continuous ambulatory monitoring of blood pressure, then the treatment was started. After six months the patients were reassessed. **Results**: Continuous ambulatory monitoring better indicates the increase of systolic tension and the reduction of the diastolic tension by aging. The pulse pressure is significantly higher at the over sixty-five group. The systolic tension is directly correlated with the pulse pressure and indirectly with the diurnal index. ($r = 0.36$). After six months of treatment the reevaluation shows a statistically significant reduction of the pathological parameters although without achieving tensional control. **Conclusions**: The ambulatory monitoring better highlights the values of hypertension and assesses the LOAD and Day Index values. Achieving the therapeutic goal regarding systolic hypertension proves difficult. **Key words**: ISOLATED SYSTOLIC HIPERTENSION, AMBULATORY BLOOD PRESSURE MONITORING

Hipertensiunea sistolică izolată (HSI) este prevăzută ca entitate patologică distinctă de noile ghiduri, fiind definită ca şi creştere a TA sistolice ≥140 mmHg, pe când TA diastolică este < 90 mmHg (1,2). Gradarea se efectuează identic cu HTA sistolodiastolică, însă se ţine cont numai de valoarea TA sistolice. TA sistolică este cel mai puternic factor de risc cardiovascular la bărbaţi vârstnici şi la femei de orice vârstă.

HSI prezină particularităţi patogenice, evolutive şi terapeutice (3). Istoria naturală, în absenţa intervenţiei terapeutice, predispune la creşterea progresivă a TA sistolice odata cu înaintarea în vârstă şi reducere moderată a TA diastolice, ceea ce accentuează TA diferenţială, adică presiunea pulsului. Acest ultim parametru constituie factor de risc independent la pacienţii peste 65 de ani, conform Ghidului European de Hipertensiune Arterială.

Paternurile hipertensive se modifică odată cu vârsta. Astfel, dacă ne referim la HTA esenţială, la tineri regula o reprezintă hipertensiunea sistolo-diastolică însă, după vârsta de 50 de ani, prevalenţa hipertensiunii sistolice izolate creşte progresiv şi

devine predominantă după vârsta de 65 de ani (4,5).

OBIECTIVELE STUDIULUI

Am efectuat un studiu prospectiv observaţional, care a urmărit comportamentul hipertensiunii arteriale prin monitorizare continuă ambulatorie la un lot de pacienţi diagnosticaţi cu hipertensiune sistolică izolată la determinarea de cabinet.

MATERIAL ŞI METODĂ

Lotul de studiu a fost constituit din 129 de pacienţi monitorizaţi în perioada ianuarie 2007 – octombrie 2008.

Sub aspect terapeutic au fost luaţi în consideraţie pacienţii cu :

1) HTA nou diagnosticată ;

2) HTA cunoscută în antecedente dar netratată ;

3) HTA sub tratament dar necontrolată, fără „perioadă de spălare" (washout) ;

4) Hipotensiunea arterială simptomatică.

Pentru fiecare subiect s-au efectuat anamneză şi examen clinic complet, cu măsurarea TA manual în clino si ortostatism, după care s-a montat sistemul de monitorizare continuă a TA la nivelul membrului nondominant.

Sistemul de monitorizare pe care noi îl utilizăm se numeşte ABPM-04, care corespunde cerinţelor protocolului de validare British Hypertension Society (BHS) referitor la tensiometrele automate. Acest dispozitiv utilizează programul MediBase pentru vizualizare şi pentru prelucrarea statistică a datelor. Aparatul înregistrează tensiunea arterială sistolică, tensiunea arterială diastolică, presiunea medie şi frecvenţa centrală din 15 în 15 minute în intervalul diurn (6 – 22) şi din 30 în 30 de minute în perioada pasivă, conform programării de către medic. Rezultatele sunt afişate ca valori medii, maxime şi minime pe 24 de ore, pe perioada activa şi pe cea pasivă. Softul calculează indicele diurn (ID), indicele de hipertonie (PTE), încărcare hiperbară (LOAD) pentru tensiunea sistolică, diastolică şi medie. Pentru frecvenţa cardiacă şi pentru presiunea pulsului se calculează valorile maxime, minime şi medii pe 24 de ore, ziua şi noaptea.

Metode statistice aplicate au fost : testul t-Student, testul $\chi 2$, coeficientul de corelaţie "Pearson" (r), coeficientul de variaţie (CV%). Datele au fost încărcate şi prelucrate cu ajutorul funcţiilor statistice din EPIINFO şi EXCEL.

REZULTATE

CARACTERISTICI EPIDEMIOLOGICE

Lotul de studiu a fost constituit din 129 pacienţi, 102 femei şi 27 bărbaţi în raportul F/M = 3,37/1. Distribuţia pe sexe a fost în favoarea sexului feminin (79,1%).

Distribuţia pe grupe de vârstă a fost stabilită la data primei investigaţii şi se prezintă astfel : vârsta medie a pacienţilor la intrarea în studiu a fost de 66,23 ± 10,69 ani, variind între 18 şi 93 ani.

Pe sexe, vârsta medie a cazurilor de sex feminin a fost de 66,13 ani cu variaţii de la 18 la 93 ani, iar la sexul masculin vârsta medie a fost de 66,23 cu variaţii de la 51 la 82 ani, fără a se înregistra diferenţe semnificative din punct de vedere statistic între sexe (t = 0,05 ; GL = 127 ; p=0,972).

Se observă o distribuţie crescută a cazurilor la grupa de vârstă 71-80ani (34,9%), urmată de grupele de vârstă 61-70 ani

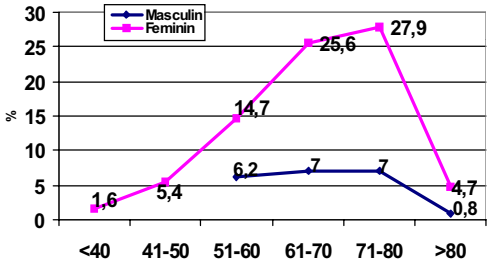

Fig. 1. Distribuţia pe grupe de vârstă şi sex la pacienţii cu tensiune sistolică izolată

TABELUL I
Valori medii ale tensiunii arteriale la lotul de studiu

Tensiune arterială	Cabinet	Medie 24 ore	Minimă	Maximă
TAS	170,64±15,87	152,33±9,83	118,71±13,97	194,29±15,25
TAD	82,475±5,93	72,39±8,74	50,74±8,74	104,40±16,51

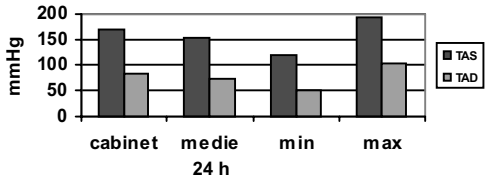

Fig. 2. Valori medii ale tensiunii arteriale la pacienţii cu tensiune sistolică izolată

(32,6%) şi 51-60 ani (20,9%).

Pe grupe de vârstă şi sex, modulul se întâlneşte la sexul feminin la grupa de vârstă 71-80 ani, iar la sexul masculin o distribuţie bimodală la grupele de vârsta 61-70 şi 71-80 ani.

Valorile medii măsurate la cabinet cât şi mediile pe 24 de ore relevă aspectul de hipertensiune sistolică izolată la întreg lotul de studiu.

Pe grupe de vârstă se observă creştere tensiunii arteriale sistolice şi scăderea tensiunii arteriale diastolice odată cu înaintarea în vârstă.

Pe grupe de vârstă nu s-au înregistrat diferenţe semnificative statistic ale tensiunii arteriale sistolice măsurate brahial însă valorile medii măsurate prin monitorizare continuă relevă mai fidel creşterea tensiunii arteriale sistolice şi reducere uşoară a tensiunii diastolice la vârstnici.

PRESIUNEA PULSULUI

Monitorizate pe grupe de vârstă valorile medii depăşesc 50 mmHg la ambele grupe şi sunt semnificativ mai mari la vârste de peste 65 ani ($t = 3,49$; $GL = 127$; $p < 0,001$) :

- ≤ 65 ani : $58,85 \pm 10,66$ mmHg
- > 65 ani : $65,79 \pm 11,72$ mmHg.

Distribuţia de frecvenţe ale pacienţilor cu valorile presiunii pulsului peste 50 mmHg relevă ca fiind factor de risc pentru vârstnici ($\chi^2 = 6,11$; $GL = 1$; $p = 0,013$). Valoarea predictivă ca un pacient cu vârsta peste 65 ani să aibă presiunea pulsului peste 50 mmHg este de 63%.

Indexul diurn s-a monitorizat atât pentru tensiunea arterială sistolică cât şi pentru tensiunea arterială diastolică şi MAP.

Analizând această distribuţie se observă că cele mai mari frecvenţe a pacienţilor revers dipper şi non dipper se întâlnesc la tensiunea arterială sistolică şi de aceea în prelucrare vom ţine cont numai de indexul diurn al TAS.

Indexul diurn nu a diferit semnificativ în funcţie de sex ($\chi^2 = 0,81$; $GL = 3$; $p = 0,847$) însă prezintă diferenţe semnificative pe grupa de vârstă ($\chi^2 = 7,81$; $GL = 3$; $p = 0,05$).

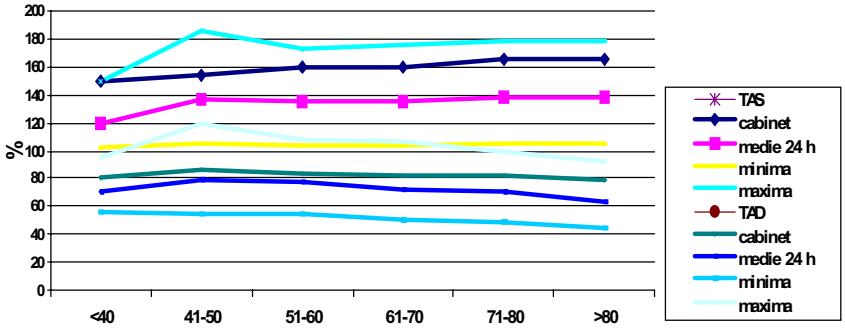

Fig. 3. Distribuţia valorilor tensiunii arteriale pe grupe de vârstă

TABELUL II

Diferenţe ale valorilor medii tensionale în funcţie de grupe de vârstă la pacienţii cu hipertensiune sistolică izolată

Parametru	TAS	
	Cabinet	Medie 24 ore
≤65 ani (n=53)	171,52±14,49	150,52±9,09
>65 ani (n=76)	170,0±16,99	153,63±10,28
Semnificaţie statistică	t = 0,36 p>0,05	t = 1,18 p>0,05
Parametru	TAD	
	Cabinet	Medie 24 ore
≤65 ani (n=53)	82,58±10,66	72,07±9,47
>65 ani (n=76)	81,91±7,63	71,44±9,54
Semnificaţie statistică	t = 0,69 p>0,05	t = 0,03 p>0,05

TABELUL III

Distribuţia de frecvenţe a indexului diurn la pacienţii cu tensiune sistolică izolată

Index diurn	**TAS**		**TAD**		**MAP**	
	N	**%**	**n**	**%**	**n**	**%**
revers dipper	36	27,9	12	9,3	18	14,0
non dipper	61	47,3	51	39,5	61	47,3
dipper	27	20,9	51	39,5	42	32,6
hiper dipper	5	3,9	15	11,6	8	6,1

Indicele de hipertonie (PTE) a fost monitorizat atât pentru tensiunea arterială sistolică cât şi pentru tensiunea arterială diastolică şi MAP. Se observă cele mai mari frecvenţe ale acestui parametru tot pentru TAS.

Valorile medii nu diferă semnificativ între sexe sau grupe de vârstă.

SARCINA DE PRESIUNE (LOAD)

Distribuţia de frecvenţe a sarcinii de presiune analizată în funcţie de sexul (χ^2 = 1,17; GL = 2; p = 0,558) şi vârsta pacientului (χ^2 = 2,74; GL = 2; p = 0,257) nu relevă diferenţe semnificative din punct de vedere statistic.

Lotul de pacienţi cu tensiune sistolică izolată se caracterizează prin corelaţie directă a TAS cu presiunea pulsului, indicele de hipertonie şi suprasarcina de presiune, ceea ce evidenţiază că la valori mari ale TAS cresc în proporţie de 76% presiunea pulsului, PTE 78% şi valorile load în procent de 96%.

Comparaţia dintre TAS cu indexul diurn relevă o corelare indirectă (r=-0,36).

HIPERTENSIUNEA DE HALAT ALB

7 pacienţi (4,3%) din cei 129 investigaţi au prezentat valori tensionale crescute la cabinet, care în decursul monitorizării nu s-au confirmat.

Acest lot se caracterizează prin:

- un număr de 5 femei (3,1%) şi 2 bărbaţi (1,2%), fără a prezenta diferenţe semnificative pe sexe între valorile medii tensionale pe parcursul monitorizării;
- pacienţii cu vârsta sub 65 ani în 4 cazuri (2,5%) şi 2 cazuri peste 65 ani (1,2%).
- corelaţiile TAS cu presiunea pulsului a fost directă (r=0,63), cu suprasarcina de

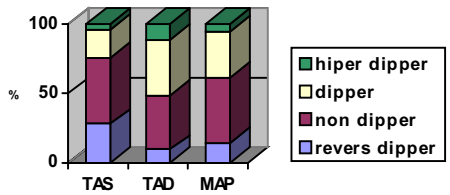

Fig. 4. Indexul diurn în funcţie de tensiunea arterială

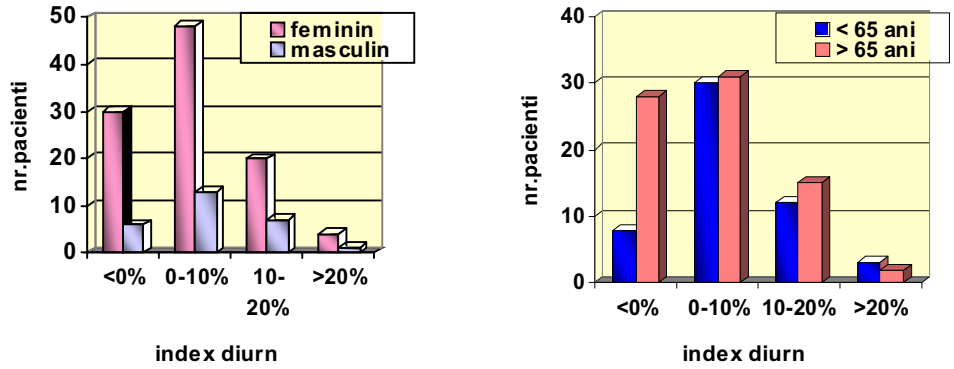

Fig. 5. Indexul diurn în funcţie de sex şi grupa de vârstă PTE

TABELUL IV

Valori medii ale PTE la pacienţii cu tensiune sistolică izolată

Parametru	$\bar{x} \pm \sigma$	Semnificaţie statistică
Sex		
Feminin (n=102)	49,64± 20,02	t = 1,00
Masculin (n=27)	55,58 ± 29,15	p>0,05
Grupa de vârstă		
≤ 65 ani (n=53)	51,38 ± 28,60	t = 0,16
> 65 ani (n=76)	50,53 ± 29,52	p>0,05

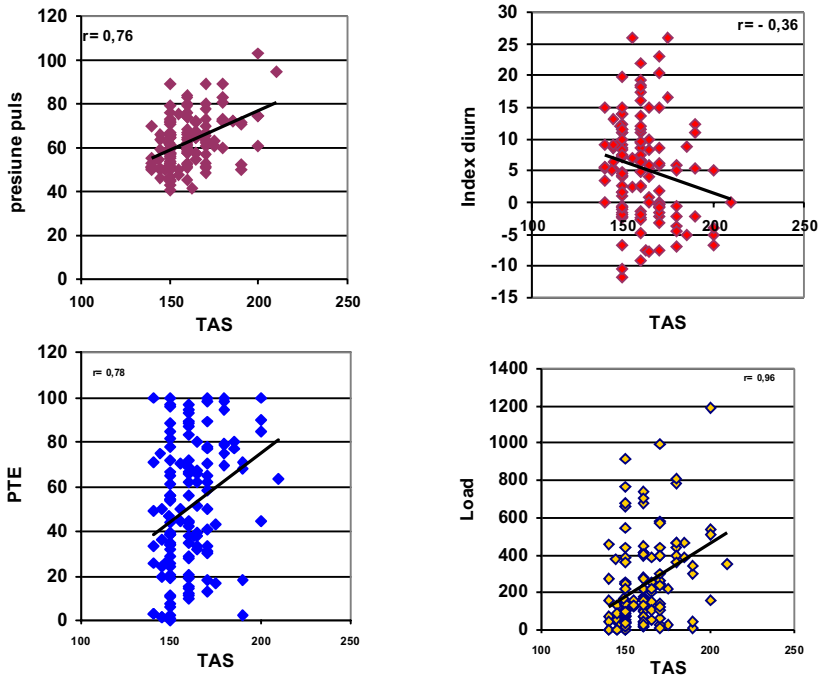

Fig. 6. Corelaţii la lotul cu TAS izolată

presiune (r= -0,41) şi load (r= -0,54) au fost indirecte, iar în comparaţia TAS cu indexul diurn valorile sunt independente (r=0,15).

PARTICULARITĂŢI TERAPEUTICE ALE LOTULUI

Dintre cei 129 de pacienţi, doar 13.7% nu urmau o schemă terapeutică medicamentoasă la intrarea în studiu. Dintre cei 86,3% (111 pacienţi) cu tratament medicamentos, 11,8% (15 pacienţi) urmau monoterapie, 25,4% (33 de pacienţi) urmau tratament cu 2 medicamente antihipertensive, 30,5% (40 de pacienţi) urmau triplă terapie şi 18,7% (24 pacienţi) urmau chiar asocierea a 4 medicamente antihipertensive.

Din întreg lotul, 50 de pacienţi (38,8%) au fost remonitorizaţi la un interval de 6 luni. În această perioadă pacienţii au urmat tratamentul indicat de medic în urma stabilirii diagnosticului. Schemele terapeutice urmate de pacienţii reevaluaţi au fost în principal alcătuite prin asocierea a două (48% dintre cazurile reinvestigate) sau trei medicamente (36%). La 8 pacienţi (16%) s-a practicat monoterapie.

Pe cazuistica studiată se observă că predomină inhibitorii enzimei de conversie a angiotensiunii (ACE-I) în proporţie de 88% dintre cazuri, diureticele tiazidice la 60% dintre pacienţi şi 40% dintre cazuri primesc betablocante.

Ca unic tratament: 12% dintre cazuri au primit numai ACE-I şi 4% betablocante (BB). Cele mai frecvent 2 medicamente asociate au fost ACE-I cu diuretic tiazidic (24%), dar şi ACE-I cu BB (20%). La bolnavii cu infarct miocardic sau insuficienţă cardiacă s-au asociat 3 medicamente, schemele cele mai frecvent utilizate au fost blocantele canalelor de calciu (CCB) + diuretic + ACE-I (16% dintre pacienţii remonitorizaţi) şi ACE-I + diuretic + BB (16%).

Pacienţii au prezentat la 6 luni valori ale tensiunii arteriale sistolice semnificativ mai mici decât la prima investigaţie ($p<0,001$), însă tensiunea arterială diastolică, chiar dacă a prezentat valori medii mai scăzute la remonitorizare, acestea nu au fost semnificative mai mici din punct de vedere statistic ($p>0,05$)

Presiunea pulsului a scăzut semnificativ de la o valoare medie de 89 mmHg la aproximativ 67 mmHg ($p<0,001$).

Indexul diurn, monitorizat Holter 24 de ore a înregistrat valori medii semnificativ mai mari la remonitorizare ($p<0,001$), trecând uşor pragul de 10%.

Monitorizarea Holter evidenţiază, de asemenea, valori medii PTE şi load semnificativ mai reduse la 6 luni ($p<0,001$).

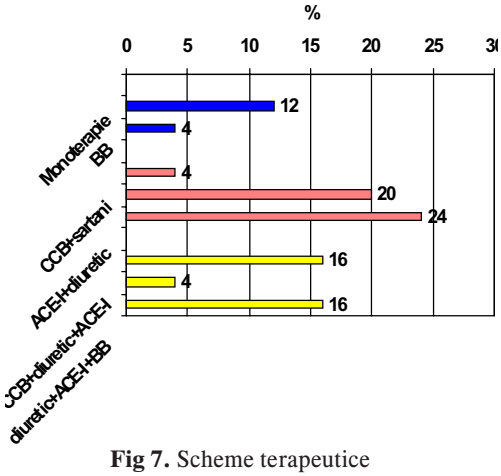

Fig 7. Scheme terapeutice

DISCUŢII

Valorile tensiunii arteriale măsurată la cabinet sunt mai mari decât mediile sistolice şi diastolice măsurate prin monitorizare

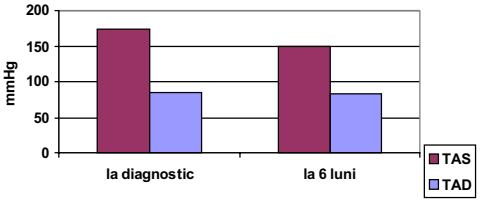

Fig. 8. Valori medii ale tensiunii arteriale la pacienţii cu tensiune sistolică izolată remonitorizaţi

continuă (MCTA), valorile normale pentru fiecare metodă de măsurare fiind precizate de noul Ghid European de Hipertensiune din anul 2007. De aceea O'Brien recomandă MCTA anual la toată populaţia hipertensivă, pentru că reflectă mai fidel valorile tensionale în condiţiile vieţii cotidiene a pacienţilor şi este singura modalitate de apreciere a profilului tensional (4).

Pe grupe de vârstă se observă creşterea TAS şi scăderea TAD odată cu înaintarea în vârstă, expresie a creşterii stiffnes-ului arterial.

Valorile medii tensionale pe grupe de vârstă nu au înregistrat diferenţe semnificative. Aceasta este o caracteristică a lotului, pe care o explicăm prin variabilitatea înaltă a valorilor TAS la vârstnici.

Presiunea pulsului creşte odată cu vârsta şi se corelează direct cu TAS. Valoarea predictivă ca un pacient cu vârsta peste 65 ani să aibă presiunea pulsului peste 50 mmHg este de 63%, de aceea reprezintă factor de risc independent la vârstnici (6) şi constituie ţintă terapeutică.

Predomină profilul nondipper şi reversedipper mai relevant pentru TAS. De asemenea, grupa de vârstă de peste 65 de ani prezintă profil tensional reversedipper în proporţie semnificativ statistic mai crescută. La pacienţii cu HSI analizarea indexului diurn sistolic este mai relevant decât indexul diurn al presiunii medii dar studii mari cu acest obiectiv urmează a fi efectuate.

Valorile cele mai crescute ale sarcinii de presiune (Load) înregistrează frecvenţe mari la TAS : 67,4%.

HTA de halat alb are prevalenţă scăzută în lotul de studiu (4,3%) faţă de datele din literatură (15-30% (7)). Aceasta se explică prin criteriile de selectare a lotului care au înrolat pacienţi vechi hipertensivi în mare proporţie.

Schemele terapeutice de primă alegere bazate pe doze mici de diuretice tiazidice (SHEP) şi blocanţi de calciu cu acţiune lungă (Syst-EUR) au fost completate de utilizarea largă (88% din cazuri) a ACE-I. Aceasta se explică prin prevalenţa crescută a manifestărilor de insuficienţă cardiacă la această categorie de pacienţi, precum şi prin asociere frecventă a tulburărilor de glicoreglare, în particular a diabetului zaharat.

Remonitorizarea continuă a TA după 6 luni de tratament a relevat reducerea semnificativă statistic a tensiunii arteriale medii sistolice, a presiunii pulsului şi a sarcinii de presiune, dar fără a se obţine control tensional. Indexul diurn a fost ameliorat, cu apropiere netă de valoarea normală.

CONCLUZII

Rezultatele obţinute confirmă datele din literatură conform cărora MCTA reflectă cel mai bine comportamentul valorilor presionale, paternurile hipertensive şi profilurile tensionale. Pentru pacienţii cu HSI analizarea parametrilor presionali sistolici definesc mai fidel riscul cardiovascular comparativ cu parametrii presiunii arteriale medii. În practica clinică beneficiul maxim revine pacientului monitorizat, pentru că schema terapautică urmăreşte pe lângă controlul valorilor tensionale şi normalizarea indexului diurn, a sarcinii hipertensive, a presiunii pulsului şi a frecvenţei cardiace.

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NOUTĂŢI

DIAGNOSTICUL PRENATAL ŞI POSTNATAL AL SINDROMULUI DE DELEŢIE 22q11.2

Microdeleţia cromosomului 22q11.2 este cea mai frecventa deleţie întâlnită la om (1 la 4000 până la 1 la 6000 nou născuţi vii). Fenotipul variază de la manifestări severe la indivizi aproape asimptomatici. Manifestările clinice cele mai citate sunt anomaliile conotruncale, hipocalcemia datorată absenţei/hipoplaziei glandelor paratiroide, anomaliile palatine şi deficitele imune secundare hipoplaziei/aplaziei timusului, dar aceste semne şi simptome nu sunt constant întalnite. 93% din cazuri sunt de novo, iar restul sunt moştenite de la unul din părinţi. Autorii au realizat un studiu retrospectiv pe o perioada cuprinsă între ianuarie 1997 şi decembrie 2008 la Timone Children Hospital , Marseille, France. Pacienţii incluşi în studiu au fost cei la care s-au efectuat teste pentru microdeleţie 22q11.2 şi au fost împărţiţi în seria prenatală (169 femei însărcinate) şi seria postnatală (714 cazuri). Prezenţa anomaliilor cardiace fetale a reprezentat principala cauză pentru testarea microdeleţiei cu o rată de detecţie de 5% depinzând de tipul de anomalie. În seria postnatală, prevalenţa defectelor cardiace a fost doar de 49%. Semnele dismorfiei faciale subtile reprezintă cheia diagnosticului la pacienţii care nu au manifestările cardinale ale sindromului. O serie de malformaţii identificabile postnatal (despicătură palatină, malformaţii renale şi de membre, defecte de tub neural sau polihidramnios) au fost asociate cu sindromul deleţiei 22q11.2. Pe baza rezultatelor studiului lor, autorii au propus un algoritm care are rolul de a îmbunătăţi rata detecţiei prenatale a microdeleţiei 22q11.2, dar ţi de a reduce numărul testelor prenatale nejustificate. Primul nivel de investigaţii este reprezentat de echografia fetală şi screeningul aneuploidiilor. În cazul în care se depistează o anomalie conotruncală se caută deleţia 22q11.2. Dacă sunt prezente semne minore (de exemplu anomalii renale, polihidramnios, deficit de creştere, despicătura palatina, polidactilie etc) se verifica ecografic arcul aortic, timusul, lungimea urechii si se face consult genetic cu examinarea părinţilor (întrucât în 7% din cazuri deleţia este moştenită de la unul din părinţi). Semnele ecografice anormale sau prezenta unor anomalii la părinţi impune efectuarea cariotipului fetal şi test pentru microdeleţie 22q11.2. Daca însă echografia (arcul aortic, timusul, lungimea urechii) şi consultul genetic la părinţi sunt normale, nu se impune căutarea microdeleţiei 22q11.2 pe proba fetală. Concluziile studiului au fost că se impune testarea prenatală pentru deleţie 22q11.2 în toate cazurile de depistare a anomaliilor conotruncale la făt, iar examinarea arcului aortic şi evaluarea timică trebuie să se facă echografic. Consultul genetic al părinţilor este necesar înainte de testarea fătului pentru deleţia amintită (Bretelle F et al. Prenatal and postnatal diagnosis of 22q11.2 deletion syndrome. *Eur J Med Gen* 2010, doi : 10.1016/j.ejmg. 2010.07.008).

Lavinia Caba

COMPARISON OF DIFFERENT CLASSIFICATION SYSTEMS IN PREDICTING THE OUTCOME OF DIABETIC FOOT ULCERS – RESULTS OF AN OBSERVATIONAL STUDY

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COMPARISON OF DIFFERENT CLASSIFICATION SYSTEMS IN PREDICTING THE OUTCOME OF DIABETIC FOOT ULCERS - RESULTS OF AN OBSERVATIONAL STUDY (Abstract) **Aim**: To compare three ulcer classification systems as predictors of the outcome of diabetic foot ulcers: University of Texas, PEDIS (perfusion, extent/size, depth/ tissue loss, infection, sensation) and IDSA (Infectious Diseases Society of America) infection grading system. **Material and methods**: Ulcer area, depth, appearance, infection and associated ischemia and neuropathy were recorded in a consecutive series of 150 subjects hospitalized with foot ulcer between January 2014 and June 2016. Presence of osteolysis was assessed by foot X-ray. **Results**: Mean age of the study patients was 60.67 years; 101 (68.2%) were male. Most ulcers (75.7%) were treated conservatively, while 36 (24.3%) subjects underwent amputation. Significant differences between patients with amputation and those conservatively treated were observed with regard to depth ($p<0.001$) and extent of lesions ($p<0.001$) using the PEDIS system, Texas grade ($p=0.005$) and IDSA infection severity ($p<0.001$). All three classification systems predicted ulcer outcome ($p<0.001$). No differences were found between these three classifications in terms of predicting amputations in our study group: PEDIS–ISDA ($p=0.53$), PEDIS–TEXAS ($p=0.78$) IDSA–TEXAS ($p=0.62$). **Conclusions:** All three systems predicted ulcer outcome, without obvious benefits of using a particular classification. **Keywords:** DIABETIC FOOT ULCER, AMPUTATION, OBSERVATIONAL STUDY.

The International Federation of Diabetes estimated in 2007 that one-third of the costs for people with diabetes were caused by foot ulcer. The cost of care for patients with diabetes and foot ulcers is 5.4 times higher than for people with diabetes without foot ulcer. The cost of treatment for diabetics with severe ulcers was eight times higher compared to treatment of superficial ulcers (1).

Neuropathy, angiopathy and immunopathy are the key components responsible for diabetic foot ulcer complications (2). Diabetic foot represents a global term for a number of conditions which have in common the same outcome: infection, ulceration, destruction of deep tissue, osteopenia, peripheral vascular disease and neurological anomalies (3). These factors can cause recurrent injuries of all anatomical components of the lower limb: skin, nails, adipose tissue, muscles, fascia, vessels, nerves, bones and joints (4). The occurrence of the risk factors for the

progression of diabetic foot ulcer depends on the metabolic control (5).

History of foot ulcers increases mortality among individuals with diabetes. A ten-year follow-up of the Nord-Trøndelag Health Study, Norway revealed that having a history of foot ulcer was associated with more than a twofold hazard risk for mortality compared with that of the non-diabetic group (6).

Clinical classification systems of diabetic foot aimed to predict the need of foot amputation. A comparison of these systems is important for clinical practice. The aim of this study is to compare the predictive value of three wide used classification systems of diabetic foot ulcers: The University of Texas Wound Classification, The PEDIS Classification (perfusion, extent/size, depth/tissue loss, infection, sensation) and The IDSA (Infectious Diseases Society of America infection grading system).

MATERIAL AND METHODS

We conducted a cross-sectional study during 2014-2016 on a sample of 148 consecutive hospitalized patients for foot ulcers. We collected information on:

- Demographic data: age, gender, area of residence (urban or rural), and level of education;
- The duration of the disease, the presence of chronic complications of diabetes, the type of treatment: insulin or non-insulin agents;
- Laboratory findings: glycated hemoglobin (Hb A_1c), inflammatory parameters (erythrocyte sedimentation rate - ESR, fibrinogen, white blood cells);
- Presence of foot osteolysis on X-Ray.

The University of Texas classification uses four grades (I-Preulcerative or post ulcerative lesion completely epithelialized, II- Superficial wound, not involving tendon, capsule or bone, III-Wound penetrating to tendon or capsule, IV-Wound penetrating to bone or joint), each of which is modified by the presence of infection (Stage B), ischemia (Stage C), or both (Stage D). This system has been validated and is generally predictive of outcome, since increasing grade and stage of wounds are less likely to heal without revascularization or amputation (7).

PEDIS classification was used to evaluate clinical characteristics of foot ulcer: extent, depth, infection, and perfusion. Perfusion was assessed during physical examination (absence of dorsal pedal or posterior tibial artery pulses of the involved foot) and also by performing non-invasive vascular studies: the ankle-brachial index (ABI). Extent was estimated by evaluating the surface of wound. Depth was evaluated using a sterile blunt nasal probe. The diagnosis of infection was based on the presence or absence of symptoms and signs of inflammation, presence of secretion, the results of laboratory tests. Sensation was evaluated with a 10-g monofilament and/or a 128-Hz tuning fork on one or more sites of the foot (8).

IDSA classification (Infectious Diseases Society of America Diabetic Foot Classifications of Diabetic Foot Infection) has been prospectively validated as predicting the need for hospitalization (3).

Statistical analysis was done with *SPSS* software (Statistical Package for Social Sciences) version 13.0 for Windows (Chicago, IL, USA). To assess the association between variables, Spearman correlation coefficients were determined. Wilcoxon test (data were not normally distributed) was used to calculate if there were significant differences between the values of measurements. Chi-square test was used in order to evaluate the significant difference between two or more samples formed from the frequency data. ROC curve assessed the overall ability of the predictor to discriminate between those individuals with the disease and those without the disease. Comparison between the areas under the curve (AUC) resulted from the analysis of

these three classification systems was realized in MedCalc software.

RESULTS

The studied sample included 148 patients (68.2% male) with diabetic foot ulcer. The mean age was 60.77±12.08 years and the duration of diabetes of 12.05±8.75 years. Almost all patients were previously treated with insulin (81.1%). The average HbA1c was 9.71±1.86. An important proportion of patients (27.8%) had osteolysis on X ray exam. A number of 36 diabetics (24.3%) were transferred to surgery for amputation. The prevalence of complications was the following: diabetic nephropathy 47.3%, neuropathy 77.7%, retinopathy 64.7%, peripheral vascular disease 36.9%.

Patients who suffered amputation were older compared with those with conservative treatment (64.17 *vs.* 59.68 years old, p= 0.027). There wasn't a significant difference related to duration of diabetes (10.83 vs. 12.52 years), gender (68.2% males) or the area of residence (50.7% from rural areas). Retinopathy was more frequent among the patients who were amputated (34.6% of patients with diabetic retinopathy (DR) vs. 18.9% of those without DR, p=0.035). The prevalence of other chronic complications was not associated with the prevalence of amputation in the studied sample. Glycated hemoglobin (Hb A_{1c}) had a higher value in surgically treated patients (9.53±1.84 vs.10.58±1.74, p=0.035).

According to the Texas foot ulcers classification, infection was more frequent than ischemia (67.3% vs. 5.4%) and most patients had preulcerative or superficial wounds (39.5%). According to IDSA classification system most patients had moderate infection (tab. I).

TEXAS classification system was highly associated with the necessity of foot amputation (χ = 23.812, p=0.005) (tab. II).

Severity of infection according to ISDA was associated with limb amputations (χ = 23.087, p < 0.001) (tab. III).

The prevalence of amputation increases with the grade of ulcer extent and depth assessed by PEDIS classification (tab. IV).

All three classifications had enough sensibility to predict foot amputation. Wound penetrating to tendon or capsule or those penetrating to bone or joint with moderate or severe infection, which means deep or extensive lesions associated with ischemia or neuropathy had a prediction value for limb amputation (tab. V).

No significant difference was found with regard to the predictive value of the diabetic foot classification systems (tab. VI).

TABLE I

Severity of foot infection according to University of Texas and IDSA classifications

TEXAS			IDSA		
	No.	%		No.	%
IA	6	4.1	Mild	55	37.2
IB	45	30.6	Moderate	76	51.4
ID	7	4.8	Severe	17	11.5
IIB	25	17.0			
IIC	4	2.7			
IID	11	7.5			
IIIA	5	3.4			
IIIB	29	19.7			
IIIC	4	2.7			
IIID	11	7.5			

TABLE II

Texas classification and the presence of foot amputation

Amputation	IA	IB	ID	IIB	IIC	IID	IIIA	IIIB	IIIC	IIID	Total
Yes %	2.8	5.6	5.6	13.9	2.8	13.9	2.8	33.3	8.3	11.0	100
No %	4.5	38.7	4.5	18	5.77	5.4	13.6	1.53	0.9	6.3	100

TABLE III

Amputation risk and ISDA classification

Amputation	ISDA		
	Mild	Moderate	Severe
Yes %	5.5	30.3	58.8
No %	94.5	69.7	41.2

TABLE IV

Amputation risk and PEDIS classification

		Amputation	$\chi 2$	p
Perfusion	1	20	5.64	0.064
	2	23.9		
	3	47.1		
Extent	1	4.8	18.463	<0.001
	2	24.1		
	3	40.4		
	4	100		
Depth	1	6.7	19.291	<0.001
	2	11.8		
	3	40		
	4	50		
Infection	1	14.3	0.402	0.526
	2	24,8		

TABLE V

Amputation risk and diabetic foot ulcers classifications

	AUC	p	95% CI		Sensitivity (95% CI)	Specificity (95% CI)	Criteria
			Lower Bound	Upper Bound			
PEDIS	.709	.000	.620	.799	88.89 (73.9- 96.9)	44.14 (34.7 - 53.9)	> grade2/score2
TEXAS	.726	.000	.634	.817	72.22 (54.8-85.8)	65.77 (56.2- 74.5)	> II B
IDSA	.743	.000	.655	.831	91.67 (77.5 - 98.2)	45.95 (36.4 - 55.7)	>mild

TABLE VI
Comparison of the predictive value of diabetic foot ulcer classifications

	PEDIS - ISDA	PEDIS - TEXAS	IDSA - TEXAS
Difference between areas	0.0333	0.0161	0.0171
Standard Error	0.0535	0.0597	0.0347
95% Confidence Interval	-0.0717 to 0.138	-0.101 to 0.133	-0.0509 to 0.0852
Z statistic	0.622	0.270	0.494
Significance level (p)	0.5342	0.7870	0.6216

DISCUSSION

In our study the prevalence of amputations for foot ulcers was 24.6%. A meta-analysis conducted by Pengzi Z (9) found a global diabetic foot ulcer prevalence of 6.3% (95% CI: 5.4-7.3%), which was higher in type 2 diabetic patients (6.4%, 95% CI: 4.6-8.1%). The highest prevalence was recorded in North America (13.0%, 95% CI: 10.0-15.9%), while the prevalence in Europe was 5.1% (95% CI: 4.1-6.0%). The higher prevalence of foot ulcers in the diabetics in our study group is possibly explained by the fact that the study was conducted in a facility specialized in foot care.

It was shown that limb ischemia, osteomyelitis, presence of gangrene and ulcer depth were major independent predictors of overall and major amputations. The relationship between major amputation rate and the presence of limb ischemia was very strong. A high Wagner grade was another strong predictor of ulcer outcome. A large observational study conducted by Yesil (10) had shown that several other baseline clinical characteristics such as older age, presence of coronary artery disease. Smoking and ulcer size were associated with either overall or major amputations (11).

Infection was associated with amputation in our patients according to the Texas and ISDA diabetic foot ulcer classifications. Previous studies also showed that limb ischemia and osteomyelitis are associated with an increased risk for amputation (12, 13). Uysal *et al.* (14) found that limb diabetic foot infection and the ulcer duration over 60 days increased the risk for amputation. In patients with diabetic foot, auxiliary methods such as negative pressure contribute in the preservation of the affected limb, after minimal excision (15).

In our study we didn't notice any difference between the type of foot classification in predicting amputation. A study of five classification systems of diabetic foot ulcers (diabetic ulcer severity score (DUSS); University of Texas (UT) diabetic wound classification; Meggitt-Wagner classification; depth of the ulcer, extent of bacterial colonization, phase of ulcer and association etiology (DEPA) scoring system; and site, ischemia, neuropathy, bacterial infection and depth (SINBAD) score) revealed that all five classification systems exhibited high sensitivity, specificity and classification accuracy (16). The Wagner and University of Texas classifications are relatively simple to use to predict the risk for foot amputation. Similar results as those reported by us were found in a prospective study (17). Healing times were not significantly different for each grade of the Wagner or University of Texas systems. There was a significant stepwise increase in healing time with each stage of the UT system, and stage predicted healing similar to that observed in our study patients. In one study, IDSA classification was also associated with limb amputation (3% for no infection, 3% for mild, 46% for moderate and 70% for severe infection) (3).

CONCLUSIONS

In this sample, there weren't notice any difference between the three systems of diabetic foot ulcer classification in predicting amputation. We emphasize that clinical assessment of foot ulcers is important in selecting patients for conservative or for surgical treatment, no matter of the classification system used.

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MALONYL DIALDEHYDE IN ISOPROTERENOL-INDUCED ACUTE MYOCARDIAL INFARCTION

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MALONYL DIALDEHYDE IN ISOPROTERENOL-INDUCED ACUTE MYOCARDIAL INFARCTION (Abstract): The **aim** of this study was to determine the correlation between serum and tissue levels of malonyl dialdehyde (MDA) in experimental myocardial infarction and the assessment of MDA potential in the disease early diagnosis. **Material and methods**: Forty rats were divided into five groups: intact (L1=11), 0.9% NaCl control (L2=11), with experimental acute myocardial infarction (L3=6, L4=6, L5=6) induced by subcutaneous injection of Isoproterenol hydrochloride solution 100 mg/kg and sacrificed at 6 hours, 24 hours and 7 days, respectively. Statistical analysis was performed using the Kruskal-Wallis non-parametric test. The results were expressed as median and interquartile range and the Spearman correlation coefficient was calculated (*SPSS 23.0*). **Results:** Serum MDA levels have shown a steady increase in the experimental groups compared to the intact and control ones. In homogenate, MDA presented a significant decrease in L4, with a return in L5 to in the intact and control groups' levels. **Conclusions**: Our study reveals a high level of reactive oxygen species, thus certifying their role in enhancing lipid peroxidation in ischemia, leading to the formation and accumulation of secondary compounds. The presence and expression of MDA is directly dependent on the degree and extent of oxidative stress and reflects the severity of cardiomyocyte membrane lesions with the loss of heart structural integrity in the reported pathology. **Keywords**: MALONIL DIALDEHYDE, OXIDATIVE STRESS, LIPID PEROXIDATION, ACUTE MYOCARDIAL INFARCTION.

By their severity and spread, cardiovascular diseases (CVDs) have reached epidemic proportions, and human and economic losses are steadily increasing. Identification of the factors that cause or predispose to their occurrence has been and remains the goal of many scientific papers in this field (1, 2, 3).

Ischemic heart disease (IHD), and particularly acute myocardial infarction ranks first according to the number of deaths. Myocardial infarction is usually caused by thrombi in the coronary arteries, which block the blood flow and lead to ischemic myocardial necrosis (3). The key role in the occurrence and development of tissue lesions is played by oxidative stress (OS), induced by myocardial ischemia and accompanied by increased production of the reactive oxygen species (ROS), that can damage DNA (Deoxyribonucleic acid), proteins, carbohydrates, lipids and biologi-

cal macromolecules (4). ROS cause both cellular dysfunction, necrosis and/or apoptosis and specific posttranslational modifications, consisting in dysfunction of cellular proteins and myocardial signaling pathways (5). Lipid peroxidation is a sequence of chain reactions mediated by free radicals, that cause oxidative damage to polyunsaturated lipids (6). The products of lipid peroxidation, the unsaturated aldehydes (including MDA), exert toxic effects, determined by their reactivity with nucleophilic compounds and the formation of DNA (with deoxy guanosine, deoxyadenosine and deoxycytidine) and protein adducts (6). MDA-DNA adducts are biomarkers of endogenous DNA damage, thereby the accurate measurement of plasma MDA levels has a strong prognostic significance of oxidative stress (8). MDA has a pronounced atherogenic potential. Interaction of MDA with lysine radicals generates Lyz-Lyz crosslinks, identified in apoB of oxidized LDL (oxLDL) (5). The formation of DAM-collagen crosslinks contributes to cardiovascular tissue stiffness (6). Increased levels of MDA in acute myocardial infarction (AMI) may cause peroxidative damage of the platelets, followed by their aggregation (9).

Recent studies suggest that MDA could serve as a biomarker for both OS (9) and cell membrane injury (6). The data in the literature on the dynamic changes of MDA in serum and homogenate in myocardial ischemia reviewed by us, are insufficient and often contradictory. There are no edifying studies on a biunivocal relationship between serum and tissue MDA levels in AMI. The above-mentioned shortcomings created the premises for the present study, the results of which probably will find answers to some little studied problems, raised by the daily clinical practice.

The aim of our research was to assay the serum and tissue MDA level in experimental myocardial infarction and to assess the potential role in AMI early diagnosis and prognosis.

MATERIAL AND METHODS

An experimental study was performed on 40 healthy white male rats (*Ratta albicans*) weighing 180-230 grams. Animals were kept at the "Nicolae Testemitanu" State University of Medicine and Pharmacy in standard vivarium conditions: temperature 24±2°C, 12-hours day/night cycle, free access to adequate food and water. Manipulations were carried out at the Laboratory of Biochemistry of the "Nicolae Testemitanu" State University of Medicine and Pharmacy. The study protocol was approved by the Research Ethics Committee of the above-mentioned university (23.03.2015).

AMI was induced by a single subcutaneous injection of 100 mg/kg Isoproterenol hydrochloride (*Sigma Aldrich Chemie GmbH*) dissolved in physiological solution. According to the study design, the animals were randomly divided into 5 groups: intact (L1 = 11) animals kept in standard vivarium conditions; control (L2 = 11) animals to which the physiological solution was administered subcutaneously; experimental (L3 = 6; L4 = 6; L5 = 6) animals receiving 100 mg/kg isoproterenol hydrochloride subcutaneously and sacrificed under sterile conditions and sulfuric ether anesthesia after 6 hours (AMI 6h), 24 hours (AMI 24h) and 7 days (AMI 7d).

Apical cardiac tissue (300mg) was homogenized in ice with 3 mL of 0.25M sucrose buffer (pH 7.4). The obtained homogenate was treated with 30 μL of Triton X-100, placed in the refrigerator for 30

minutes, and centrifuged at 4°C at 3,000 rpm for 10 minutes. The supernatant was stored at -40°C.The collected blood samples were centrifuged for 10 minutes at 1500 rpm. Serum was collected in Eppendorf tubes and kept at -40°C. MDA levels in the biological samples were assessed according to the method modified by Gudumac V *et al.* (10). The MDA content was expressed in µM/L (serum) and µM/g*protein (tissue).

Statistical analysis

The obtained data were statistically processed using the software *SPSS 23.0* version. The descriptive statistics methods were used for median and interquartile range (IQR) calculation. The data distribution was analyzed using the *Kolmogorov-Smirnov* and *Shapiro-Wilk* normality tests.

The homogeneity of variances was evaluated by the *Levene* test. Groups were compared using *Kruskal-Wallis* nonparametric test. The correlation coefficient was calculated by the *Spearman* correlation test. Statistically significant was considered the p value less than 0.05.

RESULTS

In *homogenate MDA values* registered statistically significant variations, initially expressed by slight increase from 4.47 µM/g*protein, IQR 0.78 in intact rats to 4.60 µM/g*protein, IQR 0.78 in AMI6h group, followed by decrease to 3.60 µM/g*protein, IQR 0.47 in AMI24h group (p = 0.002), and return to baseline values of 4.50 µM/g*protein, IQR 0.38 in AMI7d group (p = 0.004) (fig. 1).

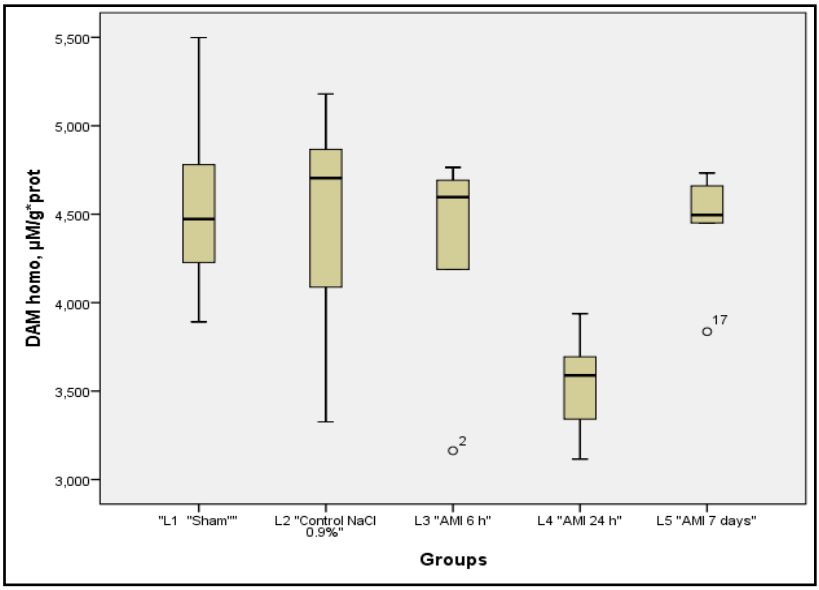

Fig. 1. MDA variations in homogenate

The *modifications of serum* MDA levels were statistically insignificant (fig. 2), with an increase from 19.08 µM/L, IQR 4.76 assessed in the intact group to 20.07 µM/L, IQR 4.02 in the AMI6h group, and to 20.57 µM/L, IQR 5.26 in AMI7d group (p>0.05).

No correlations were found between the serum and homogenate MDA levels.

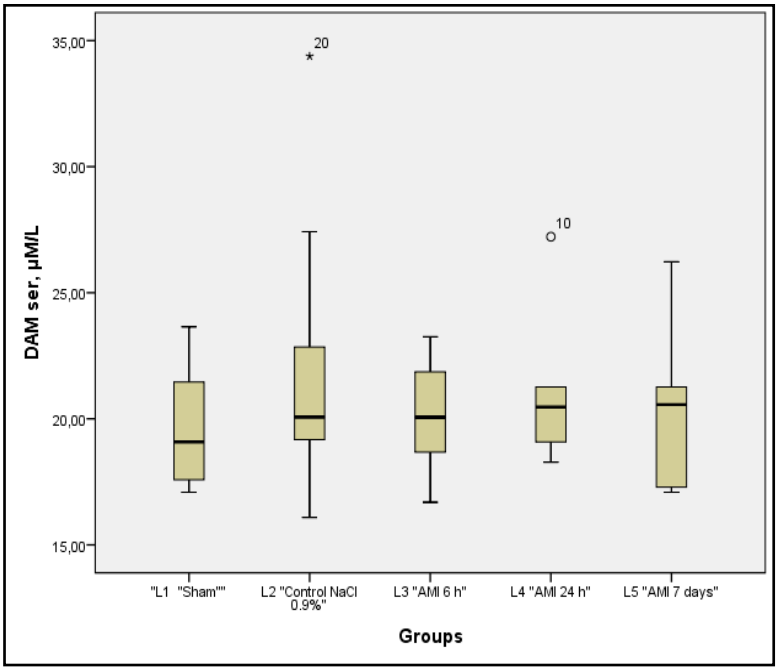

Fig. 2. Serum MDA levels

DISCUSSION

Functional cells permanently generate free radical and reactive oxygen species as part of metabolic processes, the overproduction of which can result in oxidative stress (6). Free radicals are unstable molecules that tend to interact with cell structures (8). ROS can damage fundamental cell biomolecules, including DNA, proteins and lipids (7).

The mechanisms mediated by both free radicals and lipid peroxidation are at the basis of cardiovascular diseases (6). Lipid peroxidation is a chain reaction that accompanies oxidative stress, induces the formation of various active compounds, and results in damage of cell activity (8). The assessment of lipid peroxidation in tissues is a relevant marker to establish the extent of oxidative stress (6). Lipid peroxides derived from polyunsaturated fatty acids are unstable, and quickly break down to secondary compounds (8), that can accumulate at low oxygen pressure in tissue during ischemia (9). MDA is the most commonly known by-product of lipid peroxidation, which can serve as a reliable biomarker of oxidative stress (8), cell membrane injury and indicator of cell and tissue oxidative damage (6).

In our study, MDA content in homogenate initially showed a 3% increase in AMI6h, followed by a 20% decrease in AMI24h, and return to baseline values in AMI7d, that disagrees with the data presented by Kathyaini R. *et al.* (9) in 2017. Possibly the MDA level generated in the cardiac tissue in AMI, characterizes the presence of hypoxic lesions, but it is fast removed from tissue in 24 hours at the same time with the worsening of membrane damage and loss of its integrity. The results reported by Rasool et al. (4) indicate a

significantly higher MDA level in patients with cardiovascular diseases. Singh et al. (8) have experimentally demonstrated that ischemia/ reperfusion induces myocardial formation of MDA: small but detectable amounts are accumulated during ischemia, while marked increase occurs during reperfusion. Studies by Jena et al. (11) and Behera et al. (12) proved the significant increase of serum MDA levels in ischemia. Bastani A. et al. (7) have mentioned the significant elevation of MDA in acute coronary syndrome. The discrepancy in the established MDA content is also the result of both the duration and degree of ischemia in various studies.

Our results are within the ranges reported in the literature and show a 5% increase of *serum MDA* in AMI6h, as well as in AMI24h and AMI7d, exceeding the intact group´s level by 7% and 8%, respectively. The data reported by us confirm that myocardial ischemia is associated with increased generation of ROS, induces oxidative stress and intensifies the lipid peroxidation process, which initially reveals the development of ischemic changes followed by loss of membrane integrity, subsequently leading to necrotic lesions. MDA elevation in AMI indicates an imbalance between oxidative and protective mechanisms, which results in the development of oxidative stress in AMI patients (13).

Significantly elevated MDA levels correspond to the early stage of oxidative stress (6) and confirm the presence of ROS-induced cardiomyocyte membrane lesions (9). Changes that are regularly detected with a certain reproducibility demonstrate an increased sensitivity and specificity with significant potential for AMI identification and prognosis.

CONCLUSIONS

The results of our research reveals a high ROS content, thus certifying their role in exacerbation of lipid peroxidation in ischemia, with the formation and accumulation of secondary compounds. The presence and expression of MDA is directly related with the degree and extent of oxidative stress and reflects the severity of cardiomyocyte membrane changes with loss of heart structural integrity AMI.

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NEWS

EFFICACY OF PIRFENIDONE IN THE CONTEXT OF MULTIPLE DISEASE PROGRESSION EVENTS IN PATIENTS WITH IDIOPATHIC PULMONARY FIBROSIS

Declines in percent predicted FVC (% predicted FVC), declines in 6-min walk distance (6MWD), and respiratory hospitalizations are events associated with disease progression and mortality in idiopathic pulmonary fibrosis. The incidence of multiple events in the same patient over 12 months of pirfenidone treatment is unknown. Patients who received pirfenidone 2,403 mg/d (n = 623) or placebo (n = 624) in the ASCEND (study 016; NCT01366209) and CAPACITY (studies 004 and 006; NCT00287716 and NCT00287729) phase III trials were included in this post hoc analysis. Disease progression events were defined as relative decline in % predicted FVC $\geq$ 10%, absolute decline in 6MWD $\geq$ 50 m, respiratory hospitalization, or death from any cause. The incidence of disease progression events over 12 months was assessed. The most frequent disease progression events were declines in % predicted FVC (pirfenidone vs placebo; 202 *vs.* 304 events) and declines in 6MWD (pirfenidone vs placebo; 265 *vs.* 348 events). Fewer patients who received pirfenidone had more than one progression event compared with placebo (17.0% *vs.* 30.1%; $p < .0001$). Death following one or more progression event occurred less frequently in the pirfenidone group than in the placebo group (2.1% *vs*. 6.3%; $p = .0002$). Pirfenidone significantly reduced the incidence of multiple progression events and death after a progression event over 12 months of treatment compared with placebo. These findings suggest that continued treatment with pirfenidone confers a benefit despite the occurrence of any single disease progression event. (Steven D. Nathan, Ulrich Costabel, Ian Glaspole, Marilyn K. Glassberg, *et al. CHEST* 2019; 155(4): 712-719).

BIOCHEMICAL PARAMETERS IN HEPATITIS C VIRUS INFECTION IN CHILDREN

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BIOCHEMICAL PARAMETERS IN HEPATITIS C VIRUS INFECTION IN CHILDREN (Abstract): Hepatitis C can lead to liver fibrosis. **Aim:** In this study, we evaluated the age, gender, body mass index (BMI), route of transmission and biochemical parameters of children treated with Interferon vs Interferon + Ribavirin and the relationship with fatty liver disease (FLD) and response to treatment. **Material and methods:** This retrospective and prospective study was conducted over an 8-year period (2009-2017) and included 62 children, aged 6-18 years, diagnosed with hepatitis C, admitted to 2nd Clinic of Pediatrics of "Sf. Maria" Children's Emergency Hospital, Iasi. The patients studied were divided into 2 groups: group I - 28 patients who received monotherapy (Interferon) and group 2 - 34 patients who received combination therapy (Interferon + Ribavirin). Liver fibrosis was assessed using FibroScan, and the presence of FLD by abdominal ultrasound. Biochemical parameters were determined at the initiation and at the end of antiviral therapy, 48 weeks. **Results:** In both groups, vertical transmission was the most common route of hepatitis C virus (HCV) infection, but in a large proportion of patients the transmission route could not be identified. The biochemical parameters aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT) were elevated in both group at the initiation of treatment, but decreased during treatment, and the relationship between aminotransferases and FLD was also demonstrated. HCV RNA levels showed a positive correlation with ALT in both study groups (p=.002 in group 1 and p=.036 in group 2). We have also found a negative association of HCV-RNA with bilirubin (r=-.148, p=.000) in group 1. **Conclusions:** The relationship between biochemical parameters and FLD may be a marker of hepatitis progression and of prognosis in these patients. **Keywords:** HEPATITIS C, INTERFERON, RIBAVIRIN, BIOCHEMICAL PARAMETERS.

Hepatitis C virus (HCV) was discovered more than twenty-five years ago (in 1989), but only in recent years the infection caused by this virus has been recognized as a public health problem. Approximately 3% of the world population (180 millions) is infected with HCV (1). Because of the asymptomatic interval of over 2-3 decades, 75% of those infected with HCV are not diagnosed until late, when complications

such as cirrhosis or hepatocellular carcinoma (HCC) are difficult to treat. Between 1996 and 2006, the prevalence of cirrhosis doubled and that of HCC increased twenty times (2).

Hepatitis C virus is an RNA virus that belongs to the family *Flaviviridae*. HCV replicates in the cytoplasm of hepatocytes but is not directly cytopathic. Persistent infection appears to rely on rapid production of virus and continuous cell-to-cell spread, along with a lack of vigorous T-cell immune response to HCV antigens. The HCV turnover rate can be quite high with replication ranging between 1010 to 1012 virions per day, and a predicted viral half-life of 2 to 3 hours. The rapid viral replication and lack of error proofreading by the viral RNA polymerase are reasons why the HCV RNA genome mutates frequently. There are six known genotypes and more than 50 subtypes (3).

High genetic variability of HCV makes it possible to successively infect the host with several viral strains, this being in direct correlation with the severity of disease, the response to antiviral therapy and difficulties in the preparation of effective vaccines.

Children with HCV infection differ from adults in several ways including clearance rate, mode of transmission, progression of fibrosis and duration of potential chronic infection when acquired at birth (4).

In adults in developed countries, the most important route of HCV transmission is intravenous drug use, whereas in resource poor countries, invasive procedures or injection-based therapies with contaminated instruments are the predominant source of new infections (5).

But in children things are different. Vertical transmission of HCV infection is the most common route. In the United States 7,500 new cases are reported each year. In the last decade it is more common in HCV-positive mothers, the risk being 5%, and in HIV/HCV-coinfected mothers the risk increases to 20%. Vertical transmission encompasses several potential transmission routes from an infected woman to her newborn: intrauterine, intrapartum, and postnatal. The pathogenesis of HCV infection during pregnancy is dominated by immunological changes associated with gestation and maternal-fetal tolerance, resulting in increased HCV viral load in the third trimester of pregnancy, which leads to a high risk of HCV transmission to the fetus (5, 6).

Children infected by vertical transmission have a high rate of spontaneous resolution approaching 25-40%. Most of them have spontaneous resolution within the first 2 years, but some of them, 6-12%, may have spontaneous resolution as late as 7 years after infection.

Chronic hepatitis C is associated with a wide variation in serum ALT level, from normal ALT to persistent elevation of ALT. Approximately 25-30% of individuals with chronic HCV infection have persistently normal ALT level (7).

In case of persistent HCV-RNA, the rate of liver fibrosis progression varies widely. There have been extensive studies focusing on the natural course of disease progression from chronic hepatitis C to cirrhosis, HCC, and death. Several studies have shown a significant association between the rate of fibrosis and the age at the time of infection. After determining the estimated duration of HCV infection, the stage of fibrosis was significantly higher in patients infected at an older age (> 40 years

old), than in those infected at younger ages, like children (8).

There have been significant advances in the understanding the virology and natural history of chronic HCV infection in children. In addition, there are now several treatment options for children with chronic HCV infection and many new therapies on the horizon (9).

The aim of this study was to evaluate the age, gender, route of transmission, BMI, response to treatment and identify the biochemical changes in patients with hepatitis C prior to and during antiviral treatment comparing the two groups of patients, group 1 who received Interferon vs group 2 who received Interferon+ Ribavirin, and the relationship with fatty liver disease (FLD).

MATERIAL AND METHODS

We conducted a retrospective and prospective study of 62 children, aged 6-18 years, diagnosed with viral hepatitis C and admitted to the 2nd Clinic of Pediatrics of "Sf. Maria" Children's Emergency Hospital, Iasi, Romania between 2009 and 2017. The study included 2 groups of patients:

- group 1: 28 patients who received monotherapy (Interferon)
- group 2: 34 patients who received combination therapy (Interferon + Ribavirin).

Inclusion criteria were positive diagnosis of hepatitis C and detectable HCV RNA, age over 6 years, liver fibrosis > F2, and parental consent. Informed consent was obtained from the parents, containing permission to do laboratory tests, abdominal ultrasound and FibroScan. The study was approved by the Ethics Committee of the "Sf. Maria" Children's Emergency Hospital Iasi.

Patients who were included in group 1 were selected after the presence of anemia and thyroid dysfunction that contraindicates the administration of Ribavirin and the chronological criterion, meaning that in 2009 the only therapeutic option in children was represented by Interferon in patients with HCV infection.

The biochemical parameters (AST, ALT, GGT, bilirubin, and ALP) and HCV-RNA were also determined at the initiation (AST1, ALT1, GGT1, BILIRUBIN1, ALP1 and HCV-RNA1) and at the end of antiviral therapy, 48 weeks (AST4, ALT4, GGT4, BILIRUBIN4, ALP4 and HCV-RNA4). Liver fibrosis was assessed by FibroScan, and the presence of FLD by abdominal ultrasound.

Viral factors like HCV-RNA were correlated with biochemical parameters such as ALT and bilirubin level, and the ROC curve showed us the predictability of FLD risk.

In the study, the statistical analysis and graphical representations were performed in the *SPSS* program, where correlation coefficient r Pearson, measure degree of connection between variables and p represents a measure of statistical significance.

RESULTS

In group 1, mean age was 11.85 years, and 53.5% were male. The risk factors with the highest frequency among these patients were: vertical transmission (28.5%), blood transfusions (7.14%) and dental treatments (3.57%) (fig. 1). Analyzing the weight of the patients I found that 46.63% of the patients were overweight, with a BMI of 22.58 ± 4.9 kg/m².

According to FibroScan, 92.86% of the patients had a METAVIR score F2 and 7.14% F3. All patients were evaluated by

abdominal ultrasound and 60.71% of them had FLD.

Evaluation of the therapeutic response showed that 64.29% of the patients had sustained virological response (SVR) at 24 weeks after the completion of the antiviral therapy.

In group 2, mean age was 11.5 years, and 61.7 % of the patients were female. The proportion of cases in which we have identified the existence of risk factors for HCV infection was of 58.9%, in 26.4% of them vertical transmission, followed by dental treatment, 11.76%, blood transfusion and surgery in 8.82% of patients, and 2.94% by tattoos (fig. 1). In both study groups the transmission route has not been identified in a significant percentage of patients. In the group 2, 42.94% of the patients were overweight, with a BMI of 23.34 ± 3.2 kg/m² before starting antiviral therapy.

All group 2 patients (100%) had a F2 METAVIR score, and 61.76% presented FLD (tab. I). At 24 weeks after completion of therapy 88.2% of patients had SVR.

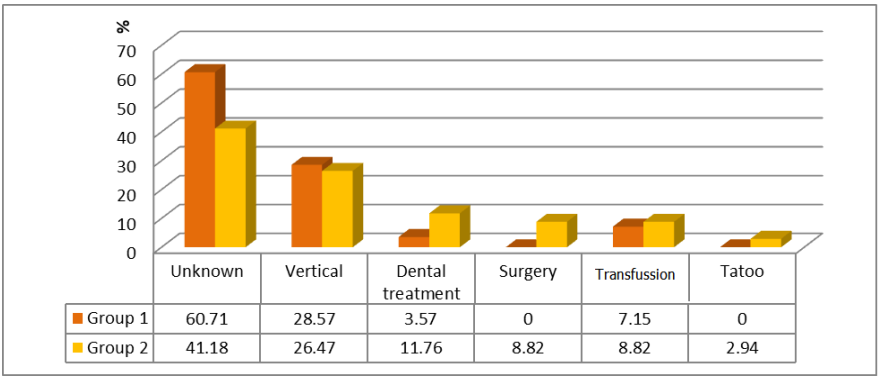

Fig. 1. Routes of transmission

TABLE I

Demographic characteristics and assessment of FLD and fibrosis (before treatment)

	Group 1 Mean (SD)/ Number (%)	Group 2 Mean (SD)/ Number (%)	p value
Total	28	34	p=.040
Age	11.85±3.65	11.5 ±3.1	p=.002
Girls	13 (46.4%)	21 (61.7%)	p=.001
Boys	15 (53.5%)	13 (38.2%)	p=.601
FibroScan (METAVIR score)			
F2	26 (92.86%)	34 (100%)	
F3	2 (7.14%)	-	
F4	-	-	
Abdominal ultrasound			
FLD	17 (60.71%	21 (61.76)	

In group 1 mean serum AST and ALT levels at initiation of Interferon therapy were 88.11 UI/mL ±57.25 and 92.35 UI/mL ± 53.40, respectively. Mean bilirubin, ALP and HCV-RNA levels were 1.38±0.70 mg/dL, 453.03 ± 195.76 UI/mL and 1,419,083.89 UI/mL ± 2140692.14, respectively, with a positive correlation between ALT and HCV- RNA (p=.002) and a negative correlation between bilirubin and HCV-RNA (r =.-148, p=.000) (tab. II).

TABLE II

Biochemical parameters

Biochemical markers	Group 1	Group 2
AST1 (UI/mL)	88.11±57.25	91.41 ± 82.40
AST4 (UI/mL)	43.39 ±19.40	39.58±18.08
ALT1 (UI/mL)	92.35±53.40	103.44± 59.60
ALT4 (UI/mL)	40.2 ±21.45	41.35±23.34
GGT1 (U/L)	47.07 U/L ±23.01	46.35±22.81
GGT4 (U/L)	35.01 U/L ±8.40	34.82 U/L±8.60
BILIRUBIN (mg/dL)	1.38±0.70	1.01±0.69
ALP (UI/mL)	453.03±195.76	430.14±19.7
ARN-VHC1 (UI/mL)	1419083.89±2140692.14	1039838.67 ±1796386.77
ARN-VHC4 (UI/mL)	179714.28±341911.46	322640.70±877730.75

At the end of 48 weeks of treatment, mean serum AST and ALT levels were 43.39 UI/ mL ± 19.40 and 40.2 UI/mL ± 21.45, respectively (tab. II). Most of the patients showed normal values at the end of therapy.

Mean serum GGT level at therapy initiation was 47.07 U/L ± 23.01, and at the end of therapy 35.01 U/L ± 8.40.

The ROC curve revealed that ALT (68.7%) and AST (61.7%) show good predictability for FLD in this group of patients (fig. 2, tab. III).

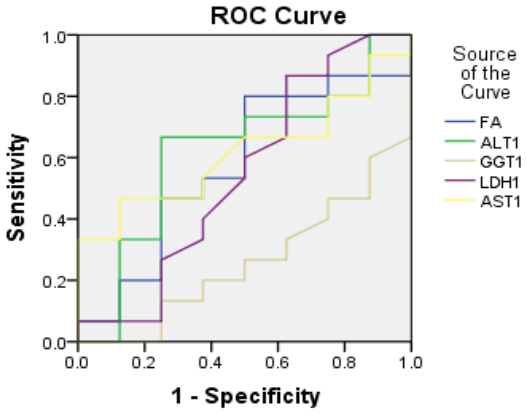

Fig. 2. ROC curve for the predictability of FLD risk

TABLE III
Biochemical markers for the predictability of FLD risk

Test Result Variable(s)	Area
ALP	.575
ALT1	.687
GGT1	.258
LDH1	.546
AST1	.617

In group 2, mean serum AST and ALT levels at initiation of Interferon and Ribavirin therapy were 91.41 UI/mL ± 82.40 and 103.44 UI/mL ± 59.60, respectively (tab. II).

Mean serum bilirubin, ALP and HCV-RNA were 1.01 ± 0.69 mg/dL, 430.14 ± 19.7 UI/mL and 1039838.67 UI/mL ± 1796386.77, respectively, with a positive correlation between ALT and HCV-RNA (p=.036). At the end of the 48 weeks of treatment, mean serum AST and ALT levels were 39.58 UI/mL ± 18.08 and 41.35 UI/mL ± 23.34, respectively (tab. II).

We've found that ALT levels were more elevated in boys than in girls in both study group.

Mean serum GGT level at therapy initiation was 46.35 U/L ±22.81, and at the end of therapy 34.82 U/L ± 8.60.

The ROC curve revealed that ALT (56.6%) and AST (54.9%) show good predictability for FLD in this group of patients (fig. 3, tab. IV). We found that the correlation between HCV-RNA and AST, ALP and GGT was not significant.

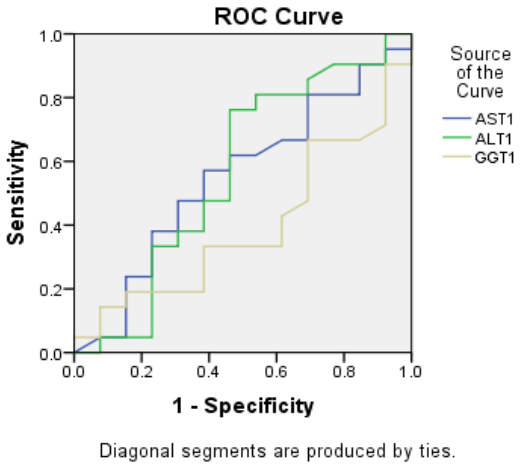

Fig. 3. ROC curve for the predictability of FLD risk

TABLE IV
Biochemical markers for the predictability of FLD risk

Test Result Variable(s)	Area
AST1	.549
ALT1	.566
GGT1	.396

DISCUSSION

Since the introduction of Interferon for the treatment of hepatitis C, the definition of what constitutes a response to therapy has evolved considerably. Therefore, serum ALT has been used as a surrogate marker of HCV-RNA, and the purpose of antiviral therapy is to achieve both a biochemical

and virological response, especially since AST and ALT are closely related to the occurrence of FLD, which in turn leads to the progression of hepatitis C to cirrhosis (10, 11).

Many patients with chronic hepatitis C have FLD. FLD is defined as excessive lipid accumulation within the hepatocyte cytoplasm and has been more recently recognized as a significant cause for cirrhosis in the United States. There are two forms of FLD in patients with hepatitis C, specifically metabolic statuses and HCV-induced statuses (12). Metabolic statuses is a process which occurs in the setting of obesity, hyperlipidemia, and elevated serum AST and ALT and insulin resistance. The other type of steatosis found in patients with HCV is fatty infiltration that is directly elicited by the virus (13).

This study included 2 groups of patients diagnosed with hepatitis C. Group 1 included 28 children with a male predominance (53.5%), and group 2 included 34 children with female predominance (61.7%). In both study groups, the main transmission route was the vertical one, which means a longer infectious period during which FLD and fibrosis may occur. In our study the primary route of transmission was vertical, in agreement with the findings of other authors (14).

Over 50% of our study patients were also diagnosed with FLD by abdominal ultrasound. Obesity play an important role in the appearance of FLD therefore the BMI is an important variable in the analysis of patients with HCV infection (16). In our study 46.63% of patients in group 1 and 42.94% of patients in group 2 were overweight before beginning antiviral therapy. This may be related to the presence of FLD in the patients we followed. Elevated aminotransferase levels act as an indicators of cell injury. Our study showed a favorable evolution of aminotransferases during antiviral therapy in both groups of patients and we found a positive correlation between ALT with HCV-RNA in both study groups. In a study from India conducted by R. Abraham et al. in 105 patients it was found that serum ALT level correlated poorly with HCV-RNA. Other studies had found correlation between serum ALT and HCV-RNA (17).

Bilirubin and ALP levels were followed, and we found a negative correlation between bilirubin and RNA-HCV in group 1. Elevated bilirubin level is usually associated with liver injury and can be useful to determine the optimal dose of Interferon (18).

Mean AST, ALT and GGT levels at initiation of antiviral therapy were above the upper limit of normal, but their values showed a downward curve in patients treated both with monotherapy and combination therapy.

In both study groups the ROC curve was performed to test the biochemical markers for the predictability of the FLD risk, which revealed that AST and ALT show good predictability, data also reported by other authors (19, 20).

CONCLUSIONS

Our study shows that in patients with HCV infection which are overweight AST and ALT levels during antiviral therapy have a strong relationship with HCV-RNA and FLD, therefore decreased of BMI should be considered in this group of patients. Aminotransferases showed a significant decrease in both study groups during antiviral therapy therefore, the normalization of serum aminotransferases leads to improved prognosis of patients with hepatitis C.

This may lead to the conclusion that these biochemical parameters and BMI may be used as prognostic factors at patients with HCV infection.

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CORELAŢII ENDO-HISTOLOGICE ÎN GASTRITA CRONICĂ DETERMINATĂ DE *HELICOBACTER PYLORI*

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ENDO-HISTOLOGICAL CORRELATION IN CHRONIC GASTRITIS BY *HELICOBACTER PYLORI* (Abstract): In this study, we analysed the correlations between endoscopic aspects and histopathological changes at the patients with dispeptic simptoms and anti-Helicobacter pylori antibodies, investigated with upper endoscopy. **Material and methods**: Our study comprised of 418 patients from Bacău Hospital, in period October 2005 - October 2008. Histopathological diagnosis was correlated with endoscopic aspects. **Results**: Average age was 55 years, with predominance of woman and rural people, without statistical significance. Histopathological diagnosis of nonatrophic gastritis was found at 211 (50%) patients, atrophic gastritis at 192 (46%) patients, intestinal metaplasia at 108 (26%), low grad intraepithelial neoplasia at 70 (17%), high grad intraepithelial neoplasia, at 4 (1%) patients, neoplasia at 28 (7%). The most severe diagnostic was atrophic gastritis with intraepithelial neoplasia. Our analysis has found nonatrophic gastritis in 211 patients (50%), atrophic gastritis in 192 (46%), intestinal metaplasia in 108 (26%), low grade intraepithelial neoplasia in 70 (17%), high grade intraepithelial neoplasia in 4 (1%), superficial gastric carcinoma in 27 (7%) and one MALT lymphoma. **Conclusion**: There was no correlation between the endoscopical aspects and the histological diagnosis. All histological changes were strongly related to *Helicobacter pylori* infection. Prospective studies are needed to evaluate whether the eradication of *Helicobacter pylori* will indeed reduce the risk of gastric cancer. **Key words**: GASTRITIS, HELICOBACTER PYLORI, INTESTINAL META-PLASIA, INTRAEPITHELIAL NEOPLASIA

CHRONIC HEPATITIS C VIRUS INFECTION AND PERIPHERAL NEUROPATHY

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CHRONIC HEPATITIS C VIRUS INFECTION AND PERIPHERAL NEUROPATHY (Abstract): Hepatitis C virus infection has been associated along time with non-liver manifestations, including cardiovascular, renal, metabolic, immune-related, and central and peripheral nervous system damage. The **aim** of this study was screening for chronic, distal and symmetrical peripheral neuropathy (PN) in a group of patients treated with interferon -free regiments and to determine the influence of sustained viral response (SVR) on the clinical symptomatology. **Material and methods:** The study involved 132 patients, with different stages of liver fibrosis (F1-F2) and (F3-F4) treated with interferon-free regiments, which achieved SVR. We screened patients using clinical examination and neuropathy disability score (NDS) for chronic, distal and symmetrical PN and follow its evolution or remission after SVR. All patients were evaluated in three different moments: at baseline, after achieving SVR and more that 6 months after SVR. **Results:** Out of 132 patients, 36 had been identified with PN, a higher incidence in the subgroup with advanced liver fibrosis was found. After SVR an clinical improvement in PN was seen in both groups of liver fibrosis, more pronounced near complete remission in the F1-F2 fibrosis group. NDS score improved also in > 90 % of patients with early/ significant fibrosis and decreased over time in the F3-F4 group, the results had statistical significance (p = 002*). **Conclusions:** After interferon-free therapy serious improvement is seen in HCV infected population regarding the severity of the polyneuropathy but also in clinical symptomatology. **Keywords:** CHRONIC HEPATITIS C VIRUS INFECTION, PERIPHERAL NEUROPATHY, INTERFERON-FREE REGIMENS.

Hepatitis C virus (HCV) infection is a major health problem affecting about 71 million people all over the world and is a leading cause of cirrhosis and hepatocellular carcinoma (1). On the other hand, there are several reported non - liver HCV- associated disorders, including cardiovascular (ischemic heart disease), renal (glomerulonephritis, renal insufficiency), metabolic (insulin resistance, type 2 diabetes), immune-related (mixed cryoglobulinemia, sicca syndrome, immune thrombocytope-

nia), central and peripheral nervous systems (PNS) (2). HCV infection increases mortality from booth hepatic and extrahepatic diseases (3, 4). Among the neurological extrahepatic manifestations of chronic HCV infection, peripheral neuropathy (PN) is one of the most frequently associated in variable proportions from 10% (5) to 50% (6).

Since the discovery of interferon treatment, researchers studied the long-term effects of sustained virologic response (SVR) on liver disease and non-liver manifestations in order to observe its effect of the all-cause mortality. The conclusion of many studies was that SVR may decrees overall mortality from both causes, either hepatic also extrahepatic manifestations of HCV infection (7, 8).

Combination therapy with interferon pegylated or standard and ribavirin may achieve clinical symptomatology resolution in patients with cryoglobulinemic related polyneuropathies (9, 10). In patients with PN and negative cryoglobulinemic status the influence of SVR is still unclear, although cases of clinical resolution of PN in these patients are cited in the literature (11).

The current treatment of HCV infection with interferon - free regiments is highly effective with rates of SVR more than 95 %, fare tolerance and few side effects (12). It has been expected that such effective treatment will reduce morbidity and mortality of HCV liver related complications as well as extrahepatic manifestations (8). There are several studies which reported improvement in patients with PN associated with cryoglobulinemia after interferon free regiments (13, 14). However, studies regarding use of interferon-free regiments in PN without cryoglobulinemia are lacking.

MATERIAL AND METHODS

This is a prospective observational study of 132 patients with chronic HCV infection who obtained SVR after therapy with based combinations of Paritaprevir / Ombitasvir / Ritonavir and Dasabuvir or Sofosbuvir / Ledipasvir for 12 weeks, between 2015-2019 at Institute of Gastroenterology and Hepatology, Iasi, Romania. All patients were evaluated for inclusion in the antiviral treatment for HCV. All patients included in the study accomplished the criteria for antiviral treatment recommended by the Romanian health protocol. Patients included in this study were screened for polyneuropathy in three different moments: at the baseline, after achieving SVR and after more than six months after SVR. All patients were evaluated according to the protocol and liver fibrosis was assessed by Fibroscan or Fibromax measurements. The study group was divided in patients with early/significant liver fibrosis (F1-F2) and advanced liver fibrosis (F3) and cirrhosis (F4).

Diagnosis for polyneuropathy was established clinically by symptomatology and neuropathy disability score (NDS) (tab. I). Paresthesia was the dominant symptom mainly described as tingling, burning sensation, a feeling of constriction, buzzing or stinging.

Statistical analysis. The statistical analysis was carried out using *SPSS 24.0* software. Continuous data were expressed + SD and categorical data as percentage. We used Mann Whitney U test to compare groups based on the degree of fibrosis, and *Chi square* for categorical variables.

TABLE I

Neuropathy disability score

	Right	Left
Pin prick	0 = present 1 = absent	0 = present 1 = absent
Vibration perception threshold (normal = can distinguish vibrating/non vibrating)	0 = present 1 = absent	0 = present 1 = absent
Temperature perception	0 = present 1 = absent	0 = present 1 = absent
Achillean reflex	0 = present 1 = present with reinforcement 2= absent	0 = present 1 = present with reinforcement 2= absent
Interpretation	0 -1 normal 2 -6 = mild modifications 6 –9 = abnormal scoring with high severity risk for neuropathy ≥ 9 - 10 = complete loss of sensation	

RESULTS

Baseline demographics, clinical and characteristics of the study lot are presented in the second table. In this study we subdivided the patients includes using two groups of fibrosis based on the results of the Fibroscan and Fibromax (tab. III).

Polyneuropathy was found in 36 patients. Polyneuropathy had a higher incidence in the subgroup with advanced liver fibrosis (F3-F4) - 32 patients (29.1%) compared with those with early/significant liver fibrosis (F1-F2)- 4 patients (18.2 %) (fig.1). These finding highlight that not only the presence of the HCV itself is a cause of incident polyneuropathy but also the duration of the chronic disease. We found a clinical regression of the polyneuropathy after achieving SVR from 29.1 % to 23.6% in the advanced fibrosis group (fig.1). We noticed that in this stage of evaluation an improvement of the polyneuropathy is seen in patients with advanced fibrosis and no modification in severity in the early/significant fibrosis group.

TABLE II

Baseline biochemical and demographic characteristics of the study lot

Parameter	HCV group n= 132
Age (yrs.) (mean + SD)	50.90± 19.2
Rural/ Urban area	75/57
Sex (M/F)	47/85
Hemoglobin (g/dL)	11.78±2.21
Platelets (x10^9/mmc) (mean ± SD)	181±17.22
Albumin (g/dL)	2.89±1.22
Age < 65 (yrs.)	67
Age > 65 (yrs.)	51

TABLE III
Subdivided groups based on the stage of liver fibrosis

Stages of fibrosis	Patients (n)	Percent (%)
F1	4	3.0
F2	18	13.6
F3	24	18.2
F4	86	65.2
Total	132	100.0

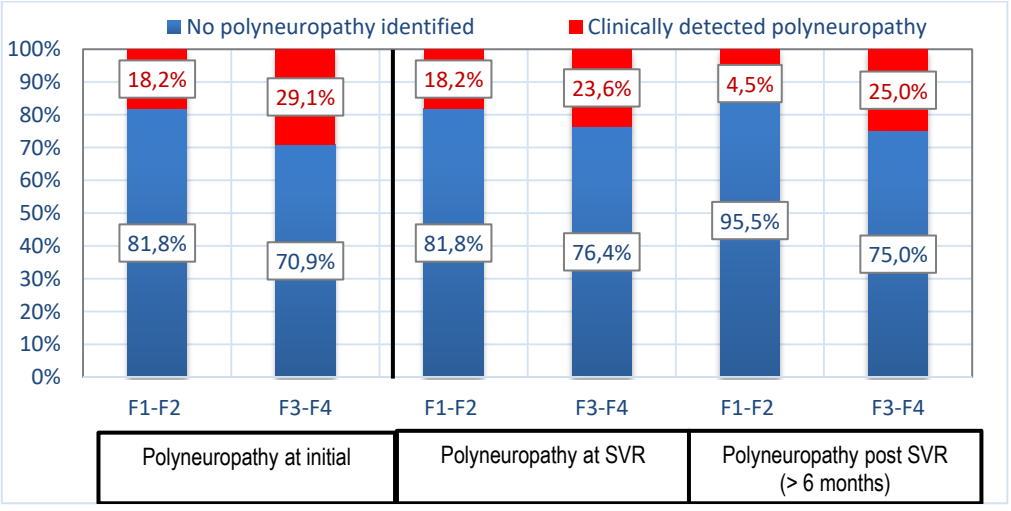

Fig. 1. Incidence of polyneuropathy screened in three study periods

An important finding was seen in the subgroup of patients with F1-F2 fibrosis where out of 4 patients (18.2%) the reduction on the NDS was seen in more than 90% of the cases, still remaining one patient (4.5%) with PN. The difference between these two subgroups had no statistical significance (p= 1.56).

After assessing the incidence in the study lot based on the stages of liver fibrosis, we characterized the evolution in time of polyneuropathies quantified by NDS. In patients with F1-F2 fibrosis, mild polyneuropathy with 3 points NDS (9.1%) predominates, with two cases (9%) being registered with neuropathy of 2 points and 4 respectively, whereas in patients with advanced fibrosis, predominance of polyneuropathy with 2 (13.6%) points NDS, although it also observes a relatively close percentage of mild polyneuropathies with 3 (11.8%) points degree. However, the differences recorded between the two groups are not statistically significant (fig. 2).

When evaluating polyneuropathies at the time of SVR, it is found that most patients have 1 (9.8%) point NDS or 2 (10.6%) points. All patients with F1-F2

fibrosis in whom polyneuropathies were registered have this condition with the 2 points on the NDS, whereas in patients with advanced fibrosis there is a slightly more frequent case of polyneuropathies with 1 (11.8%) point NDS, followed by those with 2 (9.1%) and 3 (2.7%) respectively (fig 2). In this case, also, the differences recorded are not statistically significant (p= 0.195). After SVR it is seen a improvement in the severity of polyneuropathies compared with the previous evaluation. The majority of the cases had polyneuropathy with 1(11.3%) point on the NDS, 2 points is seen in three patients and 3 in four patients (7.7%). Only one case with 4 points NDS polyneuropathy was found. In comparison with previous evaluation we observe that in the subgroup of patients with advanced fibrosis that presented mild polyneuropathies six (11.5%) had 1-point NDS and four (7.7%) presented 3 points , other three patients had 3 (3.8%) and 4(1.9%) points NDS (fig.2). We observe that the severity of the polyneuropathies decreased over time after SVR. The difference observed has statistical significance (p= 002*).

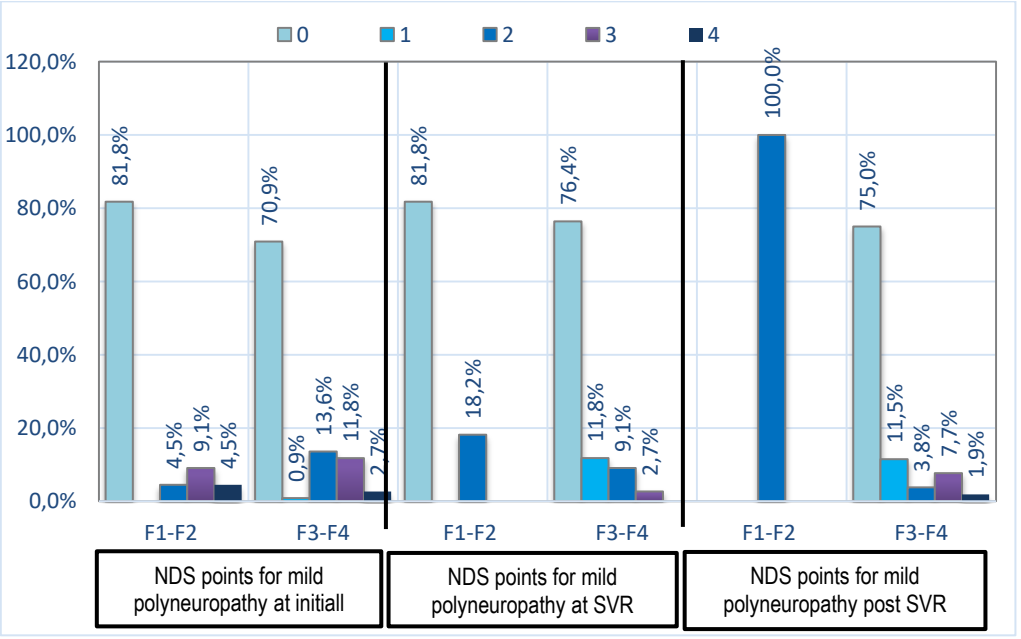

Fig. 2. NDS points of mild polyneuropathy identified in three moment of evaluation in the study group.

DISCUSSION

HCV has been associated along the time with multiple extrahepatic manifestations, the mechanism responsible for these been still partially unknown. It is known that immune response, production of cryoglobulins and antibodies can precipitate CNS and PNS damage. Central lesions are frequently determined by the chemokines and cytokine's that penetrate the hemato-encephalic barrier, affecting the nervous system (15).

The nervous system is not to be consid-

ered a replication site for HCV, very few studies shown in postmortem tissue sample from patients infected with HCV traces of ARN HCV (16, 17, 18). This should bring an inquiry of multiple sites of viral replication.

Clinical signs spectrum of chronic HCV infection is unspecific with few symptoms such as fatigue are present. When the virus involves other systems such as PNS, myalgia and sensorial neuropathies can occur. Axonal lesions and demyelination modulate the clinical spectrum varying from subclinical forms exposed only after electrophysiological studies, to sensitive and motor polyneuropathies. An important percentage varying from 40-70% of the patients with HCV chronic infection experience peripheral neurological damage such as polyneuropathy (19).

In our study we screened patients for PN, polyneuropathy being the neurological extrahepatic manifestation in our lot. The percentage of patients with polyneuropathy and HCV chronic infection was 27.3%. Similar incidence was found in the literature. Thus, Zaltron *et. al* reported an incidence of 30 % (20). A higher incidence in polyneuropathy was seen in those patients with cryoglobulinemia. Migliaresi *et al.* reported 80% of the patients with mixed cryoglobulinemia had polyneuropathy in the absence of metabolic risk factors (21).

Subdividing the study lot based on the degree of fibrosis was necessary in order to verify if other factors except cryoglobulinemia may influence the incidence and the severity of polyneuropathy. No real improvement is seen after 12 weeks of treatment in the group with F1-F2 liver fibrosis, in comparison with the advanced fibrosis group where is seen a regression of clinical symptoms in 5.5% of the patients. Other studies in the literature tried to check for regression of neurological symptoms in other HCV related extrahepatic manifestations. One of these is brain metabolism disfunction, considered as a result of HCV activity. It was observed in a small group of patients treated with Sofosbuvir and Ledipasvir that treatment completion and achieving SVR restored to normal the levels in the brain of N- acetyl aspartate, an important indicator of neuronal dysfunction recovery (21).

Other types of improvement in neurological brain disfunction were identified after achieving SVR after interferon -free regiments. A double-blind placebo randomized clinical trial that included 750 patients with HCV treated with Sofosbuvir and Ledipasvir. An improvement of neurological symptomatology such as fatigue was seen after four weeks of treatment (23). In our study a significant improvement was seen in the period of follow up after achieving SVR. Not only that polyneuropathy decreased in the severity from 4 points NDS to 1 point, but in the early/significant fibrosis group an important reduction (> 90%) of clinical symptomology can be noticed.

It is proven in studies that patients with HCV- related PN and circulating cryoglobulins have a high rate of clinical remission after interferon- free antiviral treatment. This remissible condition, considered to be the main cause of polyneuropathies in these patients has also proven to reaper shortly after the end of the antiviral treatment (24).

CONCLUSIONS

Beyond the liver, patients with HCV chronic infection are exposed to multisys-

temic damage due to serious extrahepatic manifestations. Polyneuropathy remains a concern in these patients influencing negatively the quality of life. After interferon-free therapy serious improvement is seen in HCV infected population regarding the severity of the polyneuropathy but also in clinical symptomatology.

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NEWS

DEVELOPMENT OF EFFECTIVE VACCINES FOR ENTEROTOXIGENIC *ESCHERICHIA COLI*

Diarrhea in children is caused mostly by enterotoxigenic *Escherichia coli* (ETEC). Vaccination could be an effective way to prevent ETEC diarrhea. A specific vaccine was not developed yet due to ETEC strains heterogeneity. In The Lancet Infectious Diseases, Firdausi Qadri and colleagues examined the safety and immunogenicity of enterotoxigenic *E. coli* vaccine (ETVAX). ETVAX is an oral, inactivated, vaccine which contains four *E. coli* strains that overexpress adhesins CFA/I, CS3, CS5, and CS6, mixed with a recombinant protein, LCTBA. It previously shown to be safe and highly immunogenic in Swedish and Bangladeshi adults. The study was based on a randomized phase 1/2 trial in children and infants. They showed that ETVAX was safe and well tolerated by children. At a quarter of an adult dose, adverse events like mild vomiting or fever were rare. They observed that the immunogenicity of ETVAX was age-dependent: children aged 12–59 months had significant increases in IgA antibodies in lymphocyte secretions (ALS) targeting all five vaccine antigens, but children aged 6–11 months had significant ALS and fecal IgA responses to only three antigens (CFA/I, CS3, and LTB). The accomplishment of ETVAX in ETEC-endemic regions it's shaded by some concerns like the multitude of ETEC adhesins that are not targeted in the vaccine, or the immunity to the B subunits of heat-labile toxin, but not to the toxic heat-labile A subunit, nor to the heat-stable enterotoxin (Qadri F, Akhtar M, Bhuiyan T, *et al*. Safety and immunogenicity of the oral, inactivated, enterotoxigenic Escherichia coli vaccine ETVAX in Bangladeshi children and infants: a double-blind, randomized, placebo-controlled phase 1/2 trial. *Lancet Infect Dis* 2019; https://doi.org/10.1016/S1473-3099(19)30571-7).

THERAPEUTIC CHALLENGES AND PROGNOSIS INFLUENCE OF CARDIOVASCULAR COMORBIDITIES IN CIRRHOTIC PATIENTS

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THERAPEUTIC CHALLENGES AND PROGNOSIS INFLUENCE OF CARDIOVASCULAR COMORBIDITIES IN CIRRHOTIC PATIENTS (Abstract). The effects of advanced liver diseases on heart function, like cirrhotic cardiomyopathy, hepato-pulmonary syndrome or porto-pulmonary arterial hypertension, are well known today. Cardiovascular Diseases (CVD) may affect the liver or increase a pre-existed liver disease in acute or chronic heart failure. The aim of this study was to evaluate the frequency of CV comorbidities in cirrhotic patients and to establish the influence on patients' outcome. **Material and methods**: The current prospective study was conducted over a 16-month period and included 105 patients diagnosed with liver cirrhosis (LC). Patients were extensively analyzed for the presence of CVD, both clinical and echo cardio graphic. **Results**: The main etiology of LC was viral C infection, followed by alcoholic exposure. Dilatative cardiomyopathy, high blood pressure, heart failure with reduced ejection fraction, arrhythmias and deep vein thrombosis were more prevalent in patients with viral liver disease. The valvular diseases were more frequent diagnosed in patients with a worse prognosis (31.4% *vs.* 25.7%, p =0.001), though there were no difference between patients with viral or alcoholic etiology. **Conclusions**: Our study confirmed that there is a high prevalence of different CVD in patients with LC, mostly in patients with viral etiology, and there is a great need for standardized protocols and guidelines for the initial approach, treatment and follow-up of the CV dysfunction in LC patients. **Keywords:** LIVER CIRRHOSIS, CARDIOVASCULAR DISEASES, VIRAL LIVER DISEASE.

Liver cirrhosis (LC) defines the histological reshaping of the liver secondary to a chronic liver injury with the development of regenerative nodules surrounded by fibrous tissue that leads to portal hypertension and end-stage liver disease. Due to its numerous and severe complications, LC represents one of the major causes of morbidity and mortality with a heavy burden on the health care system. The Global Burden of Disease reported an increase in mortality due to cirrhosis complications in the last years with a total of 1 million deaths in 2010 and 1 million due to viral hepatitis

and hepatocellular carcinoma (1). Concerning the cardiovascular diseases (CVD), it is still the first cause of mortality with 17.9 million deaths annually, 31% of all global deaths (2). Taking into consideration the close connections and interactions between liver and heart functions, it is important to routinely assess the CV system on cirrhotic patients, aiming to improve the overall treatment results. It is well known the effect of advanced liver disease on heart function, like cirrhotic cardiomyopathy, hepatic-pulmonary syndrome and porto-pulmonary arterial hypertension. Also, CVD may affect the liver or worsen a pre-existed liver disease in acute or chronic heart failure, either by arterial hypo perfusion in the left-sided heart failure or by passive congestion secondary to increased systemic venous pressure in the right-sided heart failure (3).

CV comorbidities in liver disease represent any simultaneous pathological findings that are neither causes nor consequences of LC but can increase the mortality and have a negative influence on therapeutic outcome. Moreover, the clinical relevance of studying these comorbidities comes from the risks of diagnosis errors that may appear when the two pathologies interfere. Furthermore, the coexisting heart diseases pose important treatment challenges, either by drug interference (i.g.in patients with viral hepatitis C following interferon-free treatment) or drug-induced hepatotoxicity (side effects of CV medication). Given the epidemiological significance, there were conceived comorbidity score systems (CirCom score) that evaluate the total burden of comorbidities on LC patients with a greater mortality prediction(4).

The aims of the current study are to evaluate the frequency of CV comorbidities in LC patients and to establish the influence on patients' outcome.

MATERIAL AND METHODS

The current study was conducted over a 16-month period and included 105 patients (74 men and 31 women), admitted in the Gastroenterology Clinic of "Sf. Spiridon" County Clinical Emergency Hospital (60 patients) and in Infectious Diseases Hospital (45 patients) for LC of various etiologies. All patients were supplementary evaluated in the Cardiology Clinic, where a detailed medical history, physical examination, blood pressure measuring, electrocardiogram recording, and echocardiography evaluation were performed. The data were collected from patients'personal files where they have signed an informed consent regarding the current study. The diagnosis of LC was established based on clinical manifestations and biological, endoscopic and ultrasound changes suggestive for advanced liver disease and portal hypertension and confirmed by a non-invasive method Fibromax or Fibro Scan. Liver function was quantified by Child-Pugh and Model of End-Stage Liver Disease (MELD) scores.

The exclusion criteria were the refusal of patients, patients with pathological conditions that prevented a correct echocardiography examination (thoracic surgery, extreme obesity, severe osteo-articular chest pain), patients with neuropsychiatric pathologies or special socio-cultural beliefs who refused a physical exam or who did not sign the written consent.

The study was conducted in accordance with the Helsinki Declaration and all patients were consecutively enrolled in the study after providing written informed consent.

We assessed the patients using a GE Vivid V7 echocardiograph (2D-echo, M-Mode, pulsed-wave Doppler (PW), continuous-wave Doppler (CW), tissue Doppler (TDI), color Doppler, the parameters being described as follows: (a) Systolic function using ejection fraction (EF) of the left ventricle (LV); (b) diastolic dysfunction (impaired relaxation: E/A<1, E/e' ratio<9, pseudo-normal: E/A > 1 with E/A ratio<1 after Valsalva maneuver and a TDI determined e'/a'<1, E/e'=9-15; restrictive pattern-E/A>2, E/e' ratio >15); (c) Valvopathies visual assessment, color flows, velocities and pressure gradients; (d) dilated cardiomyopathy: LV diameter > 57 mm (or >112% of maximum physiological diameter corrected with age and body surface area) with global heart dilatation and systolic dysfunction (EF<45% and/or fractional shortening (FS)<25%).

All data was statistically analyzed using the *SPSS 20.0*, *Microsoft Excel* 2010 and *EpiInfo* 7®. A p value <0.05 was considered statistically significant.

RESULTS

The patients mean age was 54.6 years and 70.47% were males. We divided our patients into two groups based on age for comparative analysis: aged ≥ 60 years (31.42%) and patients < 60 years (68.58%).

Regarding the etiology of LC, the viral etiology was present in 83 cases (56 men and 27 women, p=0.19) while the alcoholic and mixed (viral and alcoholic) etiology was found only in males (12, respectively 4 cases). Autoimmune origin was more common in females than in males (4 cases compared to 1 case, p= 0.01). We observed an important association between alcoholic etiology of the cirrhosis and male sex (p= 0.01) while the autoimmune etiology was specifically encountered in women (p= 0.01).

As for the relationship between the incidence of specific CV comorbidities and the etiology of LC, dilated cardiomyopathy (DCM) was statistically significant correlated with alcoholic etiology (p= 0.01),high blood pressure (p= 0.01) and heart failure with reduced ejection fraction (p= 0.01). Moreover, there was a statistically significant correlation between viral etiology and the presence of arrhythmias (p= 0.04) and, respectively, deep venous thrombosis/ chronic venous insufficiency (p= 0.04). More detailed information can be found in first table.

TABLE I

Correlations between various cardiac comorbidities and the etiology of cirrhosis

Cardiac pathology	Viral etiology	Toxic etiology	Total	p value
DCM	9 (9.47%)	5 (5.26%)	14 (14.73%)	0.004
HBP	35 (36.84%)	10(10.52%)	45 (47.36%)	0.007
IHD	13 (13.68%)	1 (1.05%)	14 (14.73%)	0.503
HFrEF	10 (10.52%)	5 (5.26%)	15 (15.78%)	0.008
HFpEF	20 (21.04%)	2 (2.1%)	22 (22.14%)	0.564
Valvular diseases	52 (54.73%)	8 (8.42%)	60 (63.15%)	0.787
Arrhythmias	31 (32.63%)	1(1.05%)	32 (33.68%)	0.046
DVT	21 (22.09%)	0	21 (22.09%)	0.048

DCM: dilated cardiomyopathy; HBP: high blood pressure; IHD: ischemic heart disease; HFrEF: heart failure with reduced ejection fraction; HFpEF: heart failure with preserved ejection fraction; DVT: deep vein thrombosis

Analyzing the associated risk factors for CVD, there were important differences between patients with viral etiology versus patients with toxic LC. Metabolic risk factors were highly associated with toxic etiology in terms of increased body mass index (18.07% *vs.* 66.66%, p< 0.01) and the presence of diabetes mellitus (7.22% *vs.* 33.33%, p=0.05). However, the diagnosis of dyslipidemia could not be associated with a specific etiology of cirrhosis (p=0.79). Anemia was common in cirrhotic patients and it also represented a common cause for heart failure decompensation, being significantly correlated with a viral origin of cirrhosis (p= 0.04). Another important CV risk factor was the advanced age, the presence of a toxic LC being highly associated with patients aged over 55 years (p= 0.02).

Following the echocardiography assessment of the diastolic function, the negative modification of this parameter was highly associated with toxic etiology (83.33% with toxic LC *vs.* 53.01% with viral etiology, p= 0.04). The global systolic function of the left ventricle was also modified in patients with toxic LC (p = 0.04) while regarding the pathologies of the aortic or mitral valve there was not significantly correlation with a specific etiology (p= 0.24).

There were certain differences between men and women concerning the CV comorbidities thus pointing out the importance of an individualized approach for every patient. Thus, high blood pressure was more common in cirrhotic females (61.29%in women *vs.*35.13%in men, p = 0.01), as well as the valvular pathology (74.19%in women *vs.*50%in men, p =0.02). On the other hand, deep vein thrombosis was significantly associated with male sex (25.67% in men *vs.* 6.45%in women, p= 0.02). Chronic virus C hepatitis remained the etiology of LC in the majority of patients (36 men and 20 women) without significant difference regarding the gender (p= 0.20). Also, we found no statistically relevant differences between men and women for chronic virus B hepatitis etiology (p= 0.31) or for the ones with a mixed virus B and virus D etiology of LC (p= 0.45).

Regarding the evolution of the patients, 41.90% presented a severe prognosis. Over 1-year monitoring, 9 patients died while out of 34 patients on the liver transplant waiting list, 5 patients have been transplanted (tab. II).

TABLE II

Evolution of the cirrhotic patients according to their cardiac pathology

Cardiac pathology	Deceased/transplant list patients (41.90%)	Stable patients (58.10%)	Total (100%)	p value
DCM	6 (5.71%)	8 (7.62%)	14 (13.33%)	0.938
HBP	14 (13.33%)	31 (29.52%)	45 (42.85%)	0.05
IHD	6(5.71%)	8(7.62%)	14 (13.33%)	0.938
HFrEF	6(5.71%)	9 (8.57%)	15 (15.78%)	0.871
HFpEF	9(8.57%)	13 (12.38%)	22 (20.95%)	0.915
Valvular diseases	33 (31.42%)	27 (25.71%)	60 (57.14%)	0.001
Arrhythmias	10 (9.52%)	22 (23.15%)	32 (32.67%)	0.142
DVT	11 (10.47%)	10(9.52%)	21 (20%)	0.276

DCM: dilated cardiomyopathy; HBP: high blood pressure; IHD: ischemic heart disease: atherosclerosis; HFrEF: heart failure with reduced ejection fraction; HFpEF: heart failure with preserved ejection fraction; DVT: deep vein thrombosis

Negative long-term prognosis was associated with the presence of valvular diseases ($p<0.01$) while an increased blood pressure at the initial examination was a significant marker for a more stable evolution of LC ($p=0.05$). Other CV comorbidities did not present any significant correlations with the outcome of the patients. By analyzing the CV comorbidities among the deceased patients, we found a statistically significant correlation only with the presence of deep vein thrombosis ($p=0.05$).

DISCUSSION

It is known for years that heart and liver are intimately related existing a broad spectrum of acute and chronic CV pathologies that can influence the treatment and outcome of cirrhotic patients. On the other hand, chronic liver injuries can affect heart function in the absence of other CVD or, at least, they may accelerate the natural progression of pre-existing heart diseases. The epidemiological data regarding the etiology of LC and gender distribution revealed similarities with other international studies, but also some particular aspects. Thus, in our study, the vast majority of patients presented a viral etiology (79.04%), followed by a toxic one (11.42%), very similar to the results obtained by Wang *et al.* on a large-scale study (80.62% and 5.68%, respectively) that included 8080 cirrhotic patients (5)highlighting and confirming that emerging countries like China and Romania are areas with a high prevalence of chronic viral hepatitis, opposed to the trend revealed by a study conducted in Germany by Sivanathan V. where the most frequent etiology was related to alcohol consumption (52%) (6).

In this prospective and observational study, we found a very high rate of CVD among cirrhotic patients (80%), many of them being unaware about these diagnoses. More than half of the patients (56.83%) presented diastolic disfunction, an early marker of cardiac dysfunction in the presence of LC. These findings are similar to the ones found by Wong et al (7), its pathophysiological foundation being the initial hypertrophy (due to hyperdynamic circulation) and subsequent fibrosis and myocardial stiffness that alter the left ventricle filling pressures. However, diastolic dysfunction cannot be attributed only to cirrhosis *per se*, but also to DCM, especially the one caused by alcohol abuse. In fact, alcohol may represent a “bridge” between cardiac and hepatic pathology, being an exponent of how diastolic dysfunction can occur via two different pathways.

In our study DCM was highly associated with alcoholic etiology of the cirrhosis ($p=0.04$), further confirming this bidirectional relationship between diastolic dysfunction and liver injury via retrograde congestion. Similar to previous studies demonstrating that diastolic dysfunction precedes systolic dysfunction (8) we observed that only 20% of patients presented an ejection fraction (EF) <50%, being well known that cirrhotic patients generally have a preserved contractility. More interesting, patients with impaired EF were significantly associated with a toxic etiology of cirrhosis ($p=0.042$), basically diagnosed with DCM, but being unmasked in situations with an increased demand for cardiac output associated to impaired myocardial contractility such as in conditions of hemodynamic stress - infections, physical effort, certain medication or surgery (especially liver transplant) (9).

Summarizing the above information, a significant proportion of cirrhotic patients (37.2% of total cases) presented a degree of

heart failure (HF), either with or without systolic dysfunction. Even if toxic etiology of LC was highly associated with DC Mand with HF with reduced EF (p=0.08), viral etiology of the cirrhosis and especially the one caused by hepatic C virus (HCV) presents high interest from the therapeutic point of view. Several studies have previously confirmed an association between hepatitis C virus infection and cardiomyopathy, but none could really establish a pathogenic relationship (10). These issues have arisen because the interferon (IFN)-based therapy for HCV infection has severe cardiac side-effects, thus limiting its use in patients with advanced HF. Promising preliminary results are presented by Poller *et al.* (11) who observed a positive cardiac effect of the new IFN-free regimens in patients with advanced HF (NYHA III-IV classes). They reported a case of a patient with NYHA IV scheduled for heart transplant who entered in NYHA II class at the end of the treatment with an IFN-free medication and heart transplant being no longer considered. The explanation was that HCV was etiopathogenetically involved in myocardial dysfunction for at least two reasons: the IFN-free therapy does not eliminate any other cardiotropic virus (e.g. Coxsackie B3) nor does it have any antifibrotic or cardiac anti remodeling action.

Patients with high BP may develop normal BP during the progression of liver fibrosis due to vasodilatation with low overall systemic vascular resistance and high arterial compliance. In our study 42.8% of patients presented BP>140/90 mmHg at the initial check, more common in women than in men (61.29% *vs.* 35.13%, p= 0.01) and, interestingly, being associated with a better outcome (p=0.05). On the other hand, high BP was incriminated as an independent risk factor for non-alcoholic steatohepatitis, that can progress to cirrhosis, a direct correlation being already proved in multiple studies (12, 13).

Valvular pathology, whether aortic or mitral, is associated with an increased risk in cirrhotic patients, especially in those on transplant awaiting list. If a valve replacement is urgently required (due to acute heart failure or increased risk of sudden death), interventional, minim invasive techniques are preferred, with high advantages in terms of mortality compared to classic surgery (14), mainly because of the high bleeding risk of cirrhotic patients.

Diabetes is probably the most studied comorbidity in cirrhosis, being highly associated with the etiologies related to alcohol abuse and non-alcoholic fatty liver disease. Literature data have shown that diabetes is a predictor of fibrosis, accelerated fibrosis progression, increased liver-related mortality and development of hepatocarcinoma (15). It was also highlighted the synergy between a high alcohol intake and the exacerbated effect of diabetes in cirrhotic patients (16). On12976 Danish cirrhotic patients included in the CirCom study, diabetes without complications was not associated with mortality whereas diabetes with complications did increase mortality (17).Other studies revealed that insulin use was associated with increased risk of hepatocarcinoma and increased mortality, while the use of oral drugs like metformin or thiazolidinediones was linked to a decreased risk (18).Definitely, there are other researches that infirmed the link between diabetes and mortality: a study from the Netherlands including 226 patients diagnosed with cirrhosis in 2001-2011 found that diabetes was not associated with all-cause and liver-related mortality (19). We recorded a similar result in our cohort of 105 patients, diabetes

not being associated to the overall mortality rates.

The limitations of the current study include a small number of analyzed patients, lack of details given specific alcohol intake or exposure to other toxic, the absence of full medical data records to attest liver disease duration, unspecified CV comorbidities or other previous treatments.

CONCLUSIONS

Our study confirmed that there is a high prevalence of different CVD in patients with LC, mostly in patients with viral etiology. New large-scale prospective studies are needed to identify more specific aspects and standardize protocols or guidelines for the initial approach, treatment and follow-up of CV dysfunction in cirrhotic patients.

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NEWS

CONTRAST ENHANCED DIGITAL MAMMOGRAPHY

With the adoption of digital mammography as a replacement for film mammography, work began in the early 2000's to develop a technique that would allow digital mammography to be used with contrast enhancement to depict cancers that would otherwise be occult on standard unenhanced mammography. Those efforts resulted in the development and clinical testing of contrast-enhanced digital mammography (CEDM) using the dual energy subtraction technique. Today CEDM is available commercially for clinical use. It is estimated that over 200,000 CEDM examinations have been performed to date in both research and clinical settings. To perform a CEDM examination, an intravenous is placed in the forearm or antecubital vein and a low osmolar iodinated contrast agent is administered at approximately 3ml/s using a power injector. Contrast agents with iodine concentration between 300 mg/ml and 370 mg/ml are typically used. The volume of contrast is similar to that used for a CT scan, approximately 1.5 ml/kg body weight, typically around 90- 150 ml. After a delay of at least 90 seconds from the end of the injection, the patient is positioned for 2 standard mammography views (craniocaudal and mediolateral oblique) of each breast. Rather than a standard single energy mammogram, however, the CEM device acquires dual-energy image pairs in each projection. Since there is less than 1 second between the low-energy and high energy images, the imaging time is the same as that needed for a standard mammogram. Additional projections may be obtained since optimally enhanced images can typically be obtained up to 7-10 minutes following injection. Following image acquisition, contrast-enhanced subtraction images are produced using a weighted logarithmic subtraction of the low energy image from the high energy image. (John Lewin, Maxine Jochelson, Contrast enhanced digital mammography. https://www.sbi-online. org/ RESOURCES/ WhitePapers/ TabId/595/ ArtMID/1617/ArticleID/601/Contrast-Enhanced-Digital-Mammography.aspx).

PULMONARY ARTERIAL PRESSURE IN LIVER TRANSPLANT CANDIDATES. A CENTRE EXPERIENCE AND REVIEW OF LITERATURE

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PULMONARY ARTERIAL PRESSURE IN LIVER TRANSPLANT CANDIDATES. A CENTRE EXPERIENCE AND REVIEW OF LITERATURE (Abstract): Porto-pulmonary hypertension (POPH), characterized by portal hypertension associated with high pulmonary vascular resistance (PVR), represents an important complication in liver cirrhotic (LC) patients. Our study aimed to determine the association between biological profile and pulmonary hypertension in a group of patients on the liver transplant awaiting list. **Material and methods**: The current 3-year prospective study analyzed 60 LC patients admitted for liver transplant evaluation and regular follow-up. All patients underwent biochemical evaluation and LC severity was evaluated by using MELD score and Child-Pugh class. Pulmonary hypertension was determined by standardized echocardiographic evaluation, most important markers being mean and systolic pulmonary artery pressure (mPAP, sPAP). **Results**: Mean age was 50.55 ± 8.3 years, 83.3% males. sPAP was significantly higher in women (24.08±7.76 *vs*. 27.33±13.7 mmHg, p=0.040). The presence of ascites was directly correlated with the severity of pulmonary hypertension (p=0.033) while mPAP was higher in patients with autoimmune LC. mPAP correlated with MELD score (r=0.832, p=0.030), fibrinogen level (r=0.887, p=0.021) and bilirubin level (r=0.758, p=0.045). During the follow-up period, mPAP decreased significantly in patients that received liver transplant. However, mPAP was not a mortality risk factor for the awaiting liver transplant patients. **Conclusions**: Our study revealed that fibrinogen and total bilirubin levels, as parameters of cirrhosis severity, are in direct correlation with mPAP. Furthermore, refractory ascites in end-stage cirrhosis patients could be associated with a higher risk of pulmonary hypertension. Further larger studies on POPH incidence and follow-up in liver cirrhosis are warranted. **Keywords**: PULMONARY HYPERTENSION, LIVER TRANSPLANT, LIVER CIRRHOSIS, ASCITES.

Pulmonary hypertension, characterized as mean pulmonary arterial pressure (mPAP) more than 25 mmHg at rest or more than 30 mmHg during exercise, is often characterized by a progressive and sustained increase in pulmonary vascular resistance that eventually may lead to right ventricular failure (1).

Liver cirrhosis (LC) is one of the numerous conditions that can cause pulmonary hypertension. The association between liver cirrhosis and pulmonary disease consists of a series of vascular changes in the pulmonary vessels due to alterations in distal pulmonary arteries with intimal proliferation, medial hypertrophy, fibrotic changes, plexiform lesions and in situ thrombosis (2). The pathogenesis is still unclear although some of the mechanisms were identified as increasing nitric oxide (NO) levels which determine excessive pulmonary vascular dilatation with secondary ventilation perfusion mismatch (V/Q ratio), intrapulmonary arterio-venous shunt or limited oxygen diffusion (3). There are no medical effective therapies for porto-pulmonary hypertension (POPH), the only reliable treatment being the liver transplant. In the absence of liver transplant the median 24-month survival rate is 23% (4).

POPH represents a form of pulmonary hypertension that is associated with portal hypertension characterized by high pulmonary vascular resistance (PVR) as a consequence of pulmonary vasoconstriction and remodeling. The POPH prevalence in cirrhotic patients is 2-14% (5, 6). The diagnosis is made by right cardiac catheterization by determining the mPAP, the left-sided filling pressures and elevated PVR (>250 dyne/s/cm-5) in patients with cirrhosis and portal hypertension (7, 8). The severity of POPH can be stratified in different risk classes, depending on the mPAP: mild form 25 - 35 mmHg; moderate form 35 - 45 mmHg; severe form >45 mmHg (9). mPAP between 35 and 50 mmHg is associated with an increase in mortality of 50% after liver transplant10. Other studies suggest that in patients with severe form of POPH the mortality is estimated to almost 100% (11). Considering this, liver transplant is indicated only to those who respond to vasodilatory medical treatment with the aim of lowering mPAP below 35 mmHg and a PVR below 400 dyne/s/cm-5 (9). After liver transplant, the pulmonary hypertension is resolved within 6 months at which time medication may be interrupted. In the severe form, mortality after liver transplant is estimated at 42% at 9 months and 71% at 36 months (12, 14). Pulmonary hypertension and cirrhosis are more common among women and patients who also suffer from autoimmune hepatitis and about 5% of the patients with end-stage liver disease will develop pulmonary hypertension (12, 15, 16).

There are no clear clinical factors that determine the risk of POPH in patients with advanced liver disease. Also, the predictors or biological mechanisms responsible for the development of POPH in these patients remain unknown (9, 16, 17). Therefore, the aim of the study was to determine the frequency of POPH in patients with LC on the liver transplant awaiting list and to analyses the patients' biochemical profile in order to identify the relations between biological factors and the severity of the pulmonary hypertension.

MATERIAL AND METHODS

In the current study we have analyzed 60 cirrhotic patients admitted in our institution for liver transplant evaluation and regular follow-up. Patients with other conditions that could lead to portal or pulmonary hypertension or with any pre-existing cardiovascular abnormalities were excluded from the study. The study was conducted as a cross-sectional analysis for a period of three years. The local ethical committee approved the study, and a written informed

consent was obtained from all the patients. The past medical history, clinical examination and laboratory details were obtained from the medical records.

Diagnosis of POPH and patient evaluation

PVR, measured traditionally by right heart catheterization, is an essential parameter of the pulmonary hypertension. However, Doppler echocardiography is an accepted method to evaluate the pulmonary pressure with comparable results. In cirrhotic patients in evaluation for liver transplant this echocardiographic method decreases the requirement of extensive invasive measurements. Our protocol study involved the measurement of the pulmonary pressure by the peak systolic velocity of the tricuspid regurgitation flow and the acceleration time of the pulmonary systolic flow. The current guidelines were used to evaluate all cavities and cardiac volumes. All echocardiographic measurements were repeated three times by the same cardiologist expert. The patients were considered to have pulmonary hypertension by sPAP > 40 mmHg. mPAP was calculated from sPAP (mPAP = 0.61 sPAP + 2mmHg) (18).

All patients were evaluated by abdominal ultrasound and upper digestive endoscopy.

Liver cirrhosis was diagnosed based on clinical signs, biological, ultrasound and endoscopical changes suggestive for portal hypertension and advanced liver fibrosis. MELD score and Child-Pugh score were used to evaluate LC severity.

Biochemical investigations included complete blood count, serum bilirubin, albumin, total protein, prothrombin time/international normalized ratio, liver enzymes (alkaline phosphatase, aspartate transaminase, alanine transaminase, gamma glutamyl transpeptidase), lipid panel, renal function, inflammatory marks such as C reactive protein and fibrinogen.

Statistical analysis

Statistical analysis was performed by using the *SPSS version 21.0* software (SPSS Inc., Chicago, IL, USA). Continuous variables are expressed as mean ± SD and categorical variables as percentages. Biochemical parameters in cirrhotic patients with and without POPH were compared. Quantitative variables with normal distribution were compared using the *Student's t* test. For non-parametric variables, we have used *Mann-Whitney* test. The *Kolmogorov-Smirnov* test was used to check the normality of the data distributions. Chi square test (*Fisher* exact test for small samples) for categorical data was exploited. *Spearman's* correlation coefficient was used to investigate possible correlations between the POPH and different clinical and biological parameters. Statistical significance was considered as p-value ≤ 0.05.

RESULTS

Mean age was 50.55 ± 8.3 years (range 26-63 years), most of them males 83.3%. The first three etiologies of LC were viral B infection (45%), viral C infection (26.7%) and chronic alcohol consumption (16.7%). All patients diagnosed with alcoholic LC had more than 6 months of alcohol abstinence. The majority of patients were classified as Child-Pugh class B (43.3%) with a mean MELD score of 16.9 ± 5.5.

Out of all patients, 5 (8.3%) of them were diagnosed with POPH. In the POPH group 3 were males (60%), viral B infection and autoimmune hepatitis being the most frequent etiologies while the severity

of liver disease was classified in Child-Pugh class B and with mean MELD score 19.9 ± 1.8. The baseline characteristics of the patients included in the study are presented in first table. Alcoholic cirrhosis patients did not present POPH. Among the POPH patients, sPAP values varied from 42 to 55 mmHg.

TABLE I
Baseline characteristics

Parameter	All patients (n = 60)	POPH group (n = 5)	Control group (n = 55)	p-value
Gender, male, n (%)	50 (83.3)	3 (60)	47 (85.4)	0.144
Age, years, mean ± SD	50.55±8.35	54.4±6.22	50.20±8.47	0.641
Etiology of cirrhosis, n (%) HBV HCV Alcohol Autoimmune Other	 27 (45) 16 (26.7) 10 (16.7) 4 (6.7) 3 (5)	 2 (40) 0 (0) 1 (20) 2 (40) 0 (0)	 25 (45.4) 16 (29.1) 9 (16.3) 2 (3.6) 3 (5.6)	 0.246
Child-Pugh class A/B/C, n	12/26/22	0/3/2	12/23/20	0.483
Child-Pugh score, mean ± SD	8.68±2.11	9.40±0.54	8.62±2.19	**0.015**
MELD score, mean ± SD	16.96±5.5	19.0±1.82	16.8±0.78	**0.044**
Creatinine (mg/dl), mean ± SD	0.97±0.54	0.71±0.08	0.99±0.56	0.202
Albumin (g/l), mean ± SD	3.24±0.74	3.33±0.61	3.23±0.75	0.457
Total Bilirubin (mg/dl), mean ± SD	3.37±3.29	3.66±1.47	3.35±3.43	0.365
Fibrinogen (mg/dl), mean ± SD	271.8±92.1	341.2±93.2	264.7±89.9	0.077
ALT (UI/L), mean ± SD	59.6±51.3	34.0±13.6	62.2±53.1	0.090
AST (UI/L), mean ± SD	81.9±55.1	74.6±44.9	82.7±56.3	0.418
GGT (UI/L), mean ± SD	96.9±84.4	81.2±71.9	98.6±86.1	0.518
Ascites, n (%)	39 (65)	5 (100)	34 (61.8)	**0.033**
HRS, n (%)	7 (11.7)	1 (20)	6 (10.9)	0.396
Encephalopathy, n (%)	15 (25)	2 (40)	13 (23.6)	0.418
Hepatocellular carcinoma, n(%)	15 (25)	0 (0)	15 (27.3)	0.173

There was a statistically significant difference between sPAP in women compared with males (24.08±7.76 *vs*. 27.33±13.7, p=0.040) although this difference has not reach the statistical significance for calculated mPAP (16.91±5.29 *vs.* 19.42±8.1, p=0.140).

Regarding ascites, there has been found a direct correlation between the presence of ascites and the severity of pulmonary hypertension (p=0.033) (fig. 1). There was no difference between the LC etiology and the risk of POPH, although mPAP was higher in patients with autoimmune LC.

Regarding the severity of hepatic cirrhosis assessed by Child-Pugh functional classification, 19.7 % of the patients were in class A, in class B 39.3 % and in class C 36.1 %. A correlation between the severity of pulmonary hypertension (reflected by

the value of sPAP) and Child-Pugh class was investigated. The results revealed that sPAP values were not significantly correlated with Child-Pugh class (p = 0.840).

Regarding all biochemical parameters, there was significant direct correlation between mPAP and MELD score (r=0.832, p=0.030), fibrinogen level (r=0.887, p=0.021) and bilirubin level (r=0.758, p=0.045).

During the follow-up period, 20 patients (33.3%) died, and 10 patients (16.7%) received a liver transplant. Increased mPAP was not a risk factor for mortality on the awaiting liver transplant list.

DISCUSSION

POPH is a recognized subtype of pulmonary arterial hypertension and a complication of LC. However, the majority of the cirrhotic patients did not associate this complication. In fact, even though this complication is in direct relation with portal hypertension, not all the cirrhotic develop POPH (5, 19). In our study, 8.3% of the patients with portal hypertension developed POPH, data that is within the prevalence range reported in the literature (9). Although in our study there were more males with cirrhosis that developed POPH, there was no significant difference observed across gender in the incidence of POPH (p=0.144). However, a study performed in the United States reported a greater incidence of pulmonary hypertension in cirrhotic women11. In our study we found no relation between gender, age, LC etiology and the diagnosis of POPH, results that are rather similar with other reported data (8, 9). However, our result is different with results from other studies that suggested that females are at higher risk of developing POPH (16).

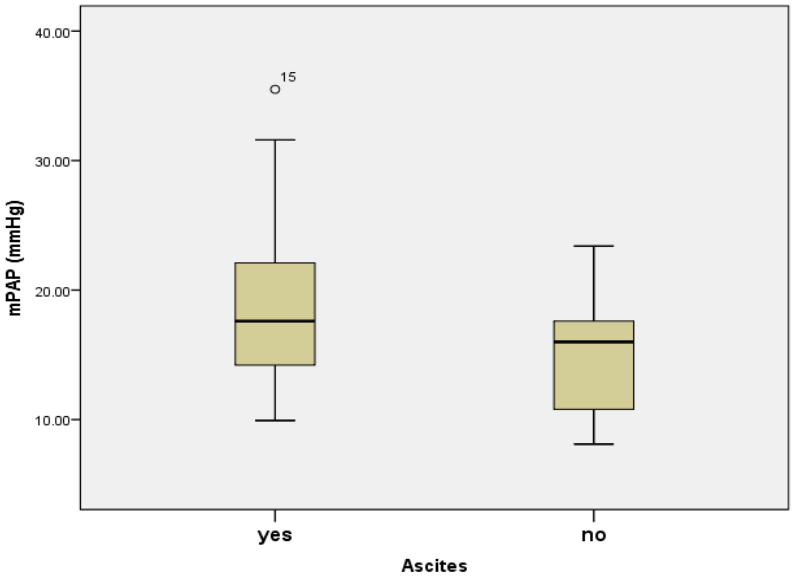

Fig. 1. The relationship between ascites and mPAP

Our study results demonstrated that the severity of LC is related with the development of POPH. We showed that the patients with POPH had a higher Child-Pugh class

and we reported a direct positive correlation between mPAP and the MELD score.

Ascites represents a marker of severity of portal hypertension. In our study, ascites was present in 65% of the patients and had a direct relationship with the severity of the pulmonary hypertension. Other studies showed no correlation but suggested that POPH was more common in patients with refractory ascites and LC possibly due to endothelin-1 excess in the pulmonary circulation (20).

Regarding female patients with POPH and autoimmune cirrhosis, the pulmonary pressure normalized 1 week after the liver transplant and remained normal at 1 month suggesting a good outcome and prognosis. The mechanism of these findings is not fully understood. In many studies, a strong association has been found between portal hypertension and pulmonary hypertension, whether or not liver disease was present. This led to the idea that normalizing the portal pressure could help lowering the pulmonary pressure. Findings in other studies are in concordance with this hypothesis showing improvements and even normalization of the pulmonary and portal pressure after liver transplant. The anatomopathological abnormalities in POPH are rather similar with those described in idiopathic PAH. The most important histological abnormality includes proliferative arteriopathy and necrotizing arteritis, occlusion of the vascular lumen by the smooth-muscle cells or the endothelial cells, and in-situ thrombi formation secondary to a systemic inflammation. The porto-systemic shunts could determine the shunting of the vasoactive substances (endothelin-1, serotonin, interleukin 1, glucagon and secretin) from the splanchnic to the pulmonary circulation. These vasoactive mediators are bypassing the liver metabolism and negatively influence the pulmonary vasculature (21, 22). We demonstrated a direct strong correlation between fibrinogen level as a marker of systemic inflammation and the mPAP. These mechanisms suggest that reversing the portal hypertension could lead to a decrease in pulmonary pressure.

Among the examined laboratory parameters, we found a direct correlation between mPAP and the severity of liver disease assessed by MELD score, fibrinogen level and total bilirubin level. Other studies suggest a correlation between hemoglobin and POPH with a lower value of hemoglobin in the POPH group compared to the non-POPH group (20). This study suggests that hemoglobin level is an independent risk factor for the development of POPH. A decreased hemoglobin leads to a significant cardiac output increase that exacerbates the hyperdynamic splanchnic circulation which is known to be a major contributor to portal hypertension (10). However, in our study no correlation was found between the hemoglobin value and POPH.

Our study has some strengths and also several limitations. Thus, it is one of the few studies examining the incidence of POPH in liver transplant patients. However, the relatively small sample size and lack of long-term evaluation represent limitations to our data.

CONCLUSIONS

In our study, we found no association between gender, age, etiology of the hepatic disease and the development of pulmonary hypertension. The severity of the hepatic disease, fibrinogen level and total bilirubin level are in direct correlation with mPAP in cirrhotic patients. Refractory ascites in end-stage cirrhosis patients could be associated with a higher risk of pulmonary hypertension. Further larger studies on

POPH incidence and follow-up in liver cirrhosis are warranted.

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NEWS

RECENT PROGRESS AND CHALLENGES IN *SCREENING* AND CHARACTERIZATION OF UGT1A1 INHIBITORS

Uridine-diphosphate glucuronosyltransferase 1A1 (UGT1A1) is an important conjugative enzyme in mammals that is responsible for the conjugation and detoxification of both endogenous and xenobiotic compounds. Strong inhibition of UGT1A1 may trigger adverse drug/herb-drug interactions, or result in metabolic disorders of endobiotic metabolism. Therefore, both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have recommended assaying the inhibitory potential of drugs under development on the human UGT1A1 prior to approval. Among the human UGTs, UGT1A1 is of particular clinical significance due to its unique activity in the conjugative detoxification of bilirubin, the endogenous by-product of heme metabolism. Also, UGT1A1 plays a pivotal role in endobiotic homeostasis, in addition to its contribution to xenobiotic disposition and detoxification. Small-molecule inhibitors of UGT1A1 may profoundly influence the catalytic activity of UGT1A1, thereby triggering undesirable effects, like drug/herb-drug interactions (D/HDI) or drug/herb endobiotic interactions: protease inhibitors- indinavir, nilotinib, sorafenib, atazanavir, saquinavir, lopinavir, ritonavir, nelfinavir, tyrosine kinase inhibitors nilotinib, regorafenib, sorafenib, pazopanib, lapatinib, erlotinib, gefitinib and icotinib have been implicated in the development of hyperbilirubinemia, for which UGT1A1 inhibition is the most likely cause. Other drugs levothyroxine, ketoconazole, vitamin A, diclofenac, canagliflozin, diflunisal, gossypol, P-Cresol, herbal extract milk thistle, saw palmetto, echinacea, green tea, epigallocatechin gallate, garlic, ginseng, black cohosh, and valerian, fatty acids, flavonoids, quinine or lignans. Unfortunately, currently commercially available substrates for UGT1A1 (such as bilirubin, estradiol, and etoposide) have different limitations, such as poor selectivity, poor chemical stability, or their use for high-throughput screening (HTS) is unfeasible. Notably, the design principles are already available and experience in the design and development of specific fluorescent substrates for UGT1A1 will surely assist us and other researchers in developing new specific fluorescent substrates for other UGTs. For instance, 1-naphthol was found to be a good fluorescent substrate for UGT1A6, which can be used for HTS of UGT1A6 inhibitors using recombinant enzyme as an enzyme source. However, 1-naphthol can be conjugated by other human UGTs as well, limiting its applications to the recombinant enzyme, rather than use in more complex system such as HLM. Very recently, a set of fluorescent 7-hydroxycoumarine derivatives have been developed as specific substrates for UGT1A10 (an extrahepatic UGT) and at least two of them, appear to work well in tissue preparations. All these findings are very helpful for the design and development of fluorescent probes for different human UGTs, and we hope that more practical fluorescent probes for human UGT enzymes will be successfully developed and used in the near future (Xia LV, Yangliu XIA, Moshe Finel, Jingiing Wu, Guangbo GE, Ling Yang. *Acta Pharmaceutica Sinica B* 2019; 9(2): 258-278).

BETWEEN ETHICS AND UTILITY OF ^{99M}TC HDP BONE SCAN IN CANCER. A RETROSPECTIVE STUDY ON 5,670 PATIENTS

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BETWEEN ETHICS AND UTILITY OF ^{99m}Tc HDP BONE SCAN IN CANCER. A RETROSPECTIVE STUDY ON 5670 PATIENTS (Abstract) **Aim:** This paper aims to emphasize the importance of nuclear imaging for cancer patients evolution and personalized treatment. **Material and method:** A retrospective study comprising 5,670 patients, who performed bone scans, between 2003-2015, at the "Sf. Spiridon" County Clinical Emergency Hospital Iași Nuclear Medicine Laboratory. Studied parameters were demographic data, referral diagnosis, staging, tumor markers, bone metastases and patient follow-up. **Results:** Of all, 3,960 were oncological patients. They performed in evolution 3,846 bone scans, between two (21.01%) and 11 (0.05%) explorations. Patients' age varied between 2 and 97 years (with two peaks, at 55 and 65). Gender distribution showed female (61.73%) over male (38.27%) predominance, especially in multiple scans (73.32%). For half of the patients the bone scan showed evolution (worsening), meaning the treatment was only partially effective and required modifications. Ethically, for these patients bone scan was irreplaceable. Its absence would have meant undergoing a partially/inefficient treatment, a reserved prognosis and a shorter survival period. The benefits outweigh the risks in terms of radiation exposure. **Conclusions**: Ethics in oncological nuclear medicine is complex, following the balance between risk and benefit, in the interest of a personalized management. As a result, ethically it is equivalent to maximizing the benefits of this minimally invasive functional investigation, scintigraphy, which is essential for treatment modulation, in order to improve the disease prognosis and increase the patients' survival rate, for precision medicine in cancer. **Keywords**: BONE SCINTIGRAPHY, ETHICS, NEOPLASM, PERSONALIZED TREATMENT, PRECISION MEDICINE.

The ethical issue of using radiation for beneficial purposes, for the patient, was, and still is, under debate in literature (1, 2).

These silent enemies, radiations, that we cannot see, smell, or feel by our senses, with no visible clue of their actual existence, may either be the worst enemy a human may have or the best friend to a patient. As to this point, we can say that the ethical approach in nuclear medicine is equivalent to the risk and benefit balance for a patient (3, 4, 5). The annual effective dose limit for the general population is 1 mSv/year, with an increase of up to 5 mSv/year (special cir-

cumstances) provided as the mean value for 5 consecutive years does not exceed 1 mSv/year. In case of patients, however, the annual dose will be higher compared to the general population, being recommended not to exceed the maxim dosage mentioned by the guidelines (6).

Considering that the value for this category of people is slightly relative, then it assumed to be relative in relation to the diagnosis.

This paper addresses a certain category of patients, diagnosed with osteophilic neoplasms, in which the percentage of primary cancerous tumor dissemination throughout the body at bone level is very high. For this type of patients therapy and evolution depend on patients clinical status, tumor markers, pursuit of bone metastasis and the dissemination degree, and algorithm of diagnosis (3, 4).

What is ethical for the oncologic patient? To do what must be done to ensure a higher survival rate for the patient (5).

OBJECTIVES

This paper aims to support, through a retrospective study, that, for oncological patients, especially patients with osteophilic cancers, in nuclear imaging, ethic means that it is irreplaceable in patients' personalized evolution approach.

MATERIAL AND METHODS

This is a retrospective study conducted over the course of 12 years (between the 1st of January 2003 to the 31st of December 2015) on the patients that had undergone ^{99m}Tc HDP bone scintigraphy and are registered in the archive of the Nuclear Medicine Laboratory "Prof. Dr. Valeriu Rusu" from "Sf. Spiridon" County Clinical Emergency Hospital, from Iasi. The inclusion criteria in this study were the referral diagnosis of neoplasm.

Patients were explored according to EANM guidelines (7), with a dose of 9.6 MBq/kg for adult patients and by using Websters rule for pediatric patients (adult dose x [(age + 1) / (age + 7)]). All well hydrated, according to the guidelines, and voided their bladder immediately before the bone scan. Images were acquired at 2 hours after the radiotracer administration using a dual-head Gamma Camera equipped with low energy high resolution collimators. Usually, whole body images were acquired (acquisition type: anterior/posterior; matrix: 256x1024; zoom: 1.00; speed: 12-14cm/min) and, in relation to the referral diagnosis, patients history and complains, early images, late static images (acquisition type: static; matrix: 256x256; zoom: 1.00; duration:10 minutes) or SPECT images (matrix: 128x128; 180° rotation; zoom: 1.00; 64 view:15 second/view) were also performed. Images were reconstructed by using filtered back projection, Butterwoth filter (cutoff 0.4 cycles/pixels, power 7) or iterative.

Other studied parameters of the patients referred to demographic data, referral diagnosis, staging, tumor markers, follow-up of bone metastases and the clinical/biomarkers evolution of the patient since the last scintigraphy.

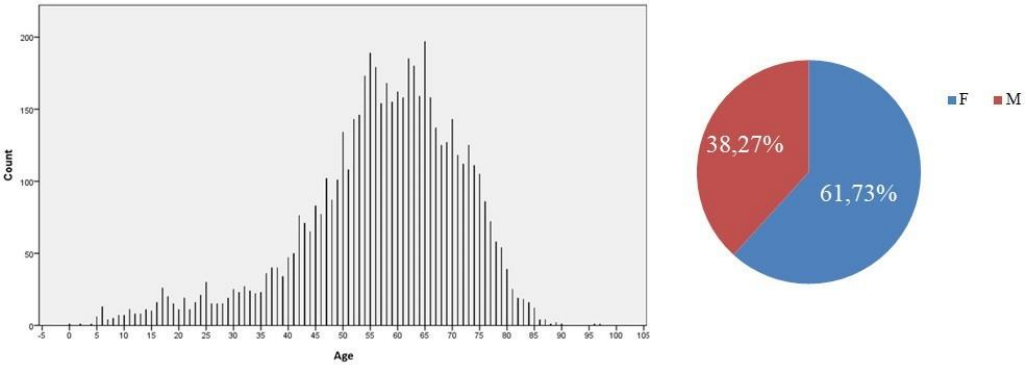
In order to quantify more accurately the evolution of the patients from one scintigraphy to another, an index system was developed, to evaluate the changes: "-2" for patients who had a clearly positive evolution (disappearance of metastases); "-1" for patients with a favorable evolution (incomplete disappearance of metastases); "0" for stable evolution compared to the last investigation; "+1" for patients with new secondary bone

dissemination sites; "+2" for patients with new sites, a negative evolution and an increased degree of radiotracer uptake.

RESULTS

Out of the 5,670 patients examined with bone scan in our laboratory in this period (an average of 454 patients with bone scan per year), approximately 70% (3,960 patients) were oncological patients. 3,846 of them performed in evolution bone scans, between two (21.01%) and 11 (0.05%) explorations. Patients age varied between 2 and 97 years old (with two peaks, at 55 and 65 years). Gender distribution showed female (61.73%) over male (38.27%) predominance, especially in multiple scans cases (73.32%) (fig. 1).

The quantification system makes evidence of differences related with the number of bone scans and from one investigation to another.



a b

Fig. 1. Distribution of age (a) and gender (b) for the patients examined with bone scans

48 patients have undergone at least 5 bone scans, between 2003-2015. In these patients that had undergone at least 5 bone scans, 81.25% females, we determined that the evolution of the primary pathology was negative in half of them, out of which most of them were also females (fig. 2).

By comparison, most of the studied patients had undergone only one bone scan. The percentage of patients that had more than 5 investigations, was diminishing the higher we got (fig. 3). To that end we only found two patients that had the highest number or investigations (11 bone scans).

Comparative analysis of scintigraphic results of patients with multiple presentations determined that the ratio between stationary and evolving cases is 50:50.

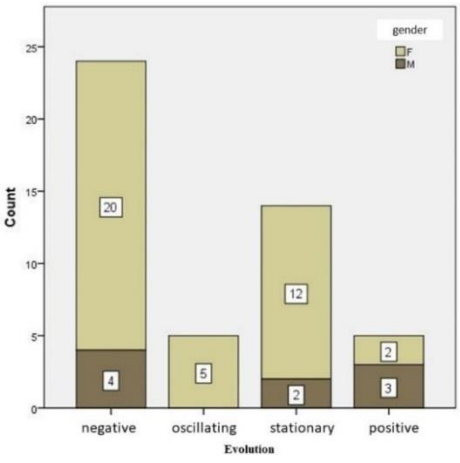


Fig. 2. Evolution of the 48 de patients that had undergone at least 5 bone scans

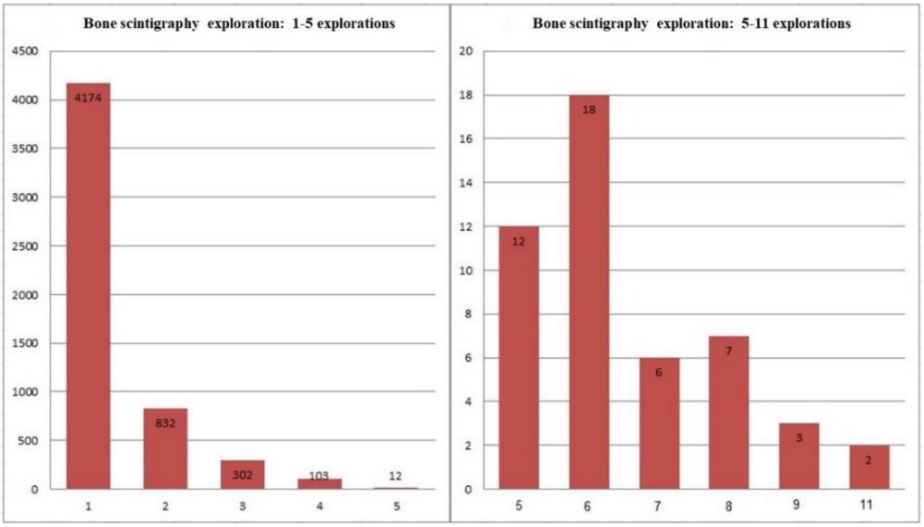


Fig. 3. Number of patients in relation to number of bone scans per patient

Considering the referral diagnoses, almost 40% of cases that we analyzed were with breast cancer and only 14% with prostate cancer. Other referral diagnosis included bronchopulmonary neoplasm, uterine, rectum and bone metastasis. By taking all the referral diagnosis into consideration we concluded that approximately 44.32% of all the investigated cases were breast, ovary and uterine neoplasms (fig. 4).

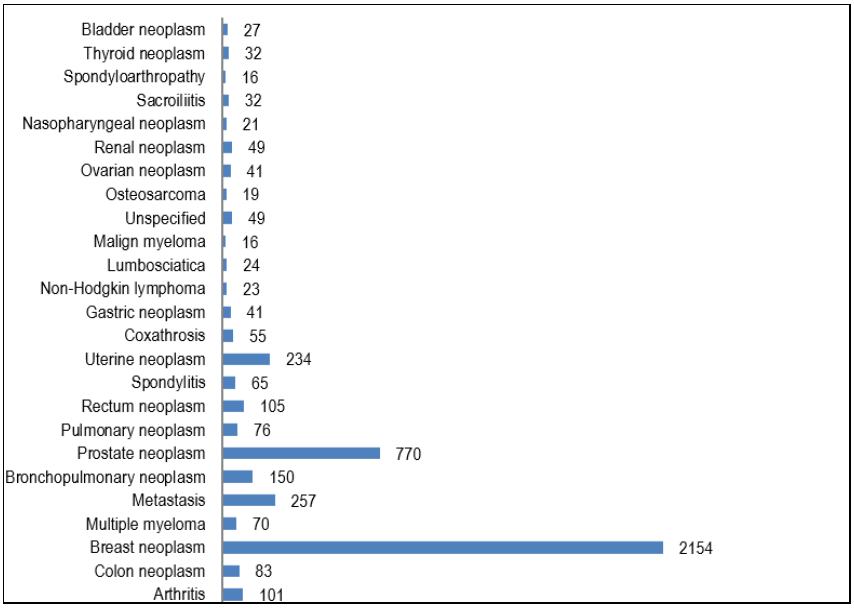


Fig. 4. Patients distribution according to referral diagnosis
(only diagnosis with more than 10 cases were represented)

DISCUSSION

Bone scintigraphy is a noninvasive imaging investigation that studies the biodistribution of the radiotracer at the skeletal level by using bisphosphonates (methylene diphosphonates – MDP; hydroxy ethylene diphosphonate – HDP; hydroxy methylene diphosphonate – HMDP; 2,3 – dicarboxypropane – 1,1 – diphosphonate – DPD; pyrophosphate – PYP) (8,9). Due to the radiotracer uptake mechanism, dependent on local bone vascularization and osteoblastic activity, consisting in the absorption of the radiotracer at the surface of the hydroxyapatite crystals (9), the positive metastases image is evident up to six months earlier on bone scan, in comparison with a correspondent positive image on radiology (where X rays images are related to the tissular densities differences).

This investigation can be indicated for the diagnosis of bone diseases in oncology, rheumatology, bone and joint infections, orthopedics, sports and traumatology, metabolic bone disease, pediatrics (7). The irradiation dose is between 2.9-4.0mSv (300-740MBq) (10), dependent on the administered dose to the patient (9.6MBq/kg). Standard protocol, essentially with whole body delayed images, is generally used, but personalized protocol, with additional images, is possible without other supplementary radiation risks, due to the particularities of imaging in nuclear medicine (the patient is the only source of gamma radiation, after intravenous administration of the radiotracer). The result can give to the practitioner a positive (stationary, non-evolutive cancer - efficient treatment) or negative (evolutive cancer - inefficient treatment, partially or totally) feedback, essential for a personalized treatment approach. The absence of scintigraphy in the latter situation would have led to a bad prognosis and a lower surviving rate. For this category of patients, bone scintigraphy represented a must.

In comparison, in radiology, the X-ray beam is directed toward the patients' body and will be mitigated depending on the tissue density. Bone density modifications will determine positive images on the X ray. However, a whole-body examination is not indicated due to the high irradiation doses to which the patient would be exposed (9). Furthermore, the obtained images offer only structural details but no functional ones.

The MRI can evaluate the structure and the function of the patient's body, by using a whole-body examination which is possible without any kind of radiation (9). Although this is true, the MRI also presents a few limitations such as a high price or limited access to the investigation, in some cases.

Information obtained through X-ray and/or MRI cannot replace but are complementary to the information obtained through bone scintigraphy.

CONCLUSIONS

The ethical approach in oncological nuclear medicine is complex, being equivalent, practically, to following the balance between risk and benefit in the interest of personalizing the patient's treatment. As a result, ethically it is equivalent to maximizing the benefits of this minimally invasive functional investigation (scintigraphy) which is essential for treatment modulation in order to improve the prognosis of the disease and increase the patient's survival rate.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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NEWS

RISK OF COVID-19 IN PATIENTS WITH AUTOIMMUNE INFLAMMATORY RHEUMATIC DISEASES

There is a continuous debate whether patients with autoimmune inflammatory rheumatic diseases (AIRD) treated with disease-modifying anti-rheumatic drugs (DMARDs) are more susceptible to COVID-19 infection. In their prospective observational study, Nuñez *et al* described patients with AIRD from a Madrid hospital who were positive to COVID-19 during the pandemic peak. The results are reassuring: the use of DMARDs does not seem to be followed by a higher hospital admission related to COVID-19, so they do not pose a risk of contracting the virus. Patients admitted more frequently were naïve to anti-TNF-alpha agents; in this respect, the IL-6 inhibitors (tocilizumab, sarilumab) have proven beneficial in patients with SARS-CoV-2. On the other hand, the data showed that older age, comorbidities and the presence of AIRD increased the risk of hospitalization compared to the non-rheumatic population. (Nuñez DD, Leon L, Mucientes A, *et al.* Risk factors for hospital admissions related to COVID-19 in patients with autoimmune inflammatory rheumatic diseases. *Annals of the Rheumatic Diseases* 2020; 79(11): 1393-1399).

RISK FACTORS OF INFLUENZA A AND B VIRUS INFECTION IN CHILDREN POPULATION FROM SOUTH- EASTERN AREA OF ROMANIA

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RISK FACTORS OF INFLUENZA A AND B VIRUS INFECTION IN CHILDREN POPULATION FROM SOUTH-EASTERN AREA OF ROMANIA (Abstract): The objective of this study was to identify clinical and paraclinical characteristics along with risk factors in patients with influenza A and B virus infection. **Material and methods:** This retrospective study included children diagnosed with influenza, admitted to the pediatric department of Constanta Infectious Diseases Clinical Hospital, from Romania, during the 2015-2019 influenza cold seasons. Data regarding clinical characteristics, laboratory tests (fibrinogen, white blood cells (WBC), hemoglobin (Hb), platelets (PLT), erythrocyte sedimentation rate (ESR), creatine-kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and C-reactive protein (CRP), and management were collected. **Results:** Over the study period, 265 patients with influenza A and 83 patients with influenza B were investigated. The mean age of patients with influenza A was higher than of patients with influenza B (6.48 vs 5.9 years). It has been showed that cough was statistically different in both types ($p=0.0094$). The same statistically significant values were seen for rhinorrhea ($p=0.0035$), vomiting/nausea ($p=0.0362$), dyspnea ($p=0.0449$), myalgias ($p=0.0412$) and pneumonia ($p=0.0077$). At admission, the laboratory tests showed higher values of fibrinogen in A influenza and higher values of WBCs in B influenza. Hemoglobin, PLT, CK, and AST shows a significant difference for B influenza ($p<0.001$). In contrast, CRP shows significant differences of values for A influenza ($p=0.001$). Moreover, the early antiviral treatment was almost similar for both influenzas early from the onset ($p=0.0418$). **Conclusions:** Although we have found the predominance of clinical characteristics and non-specific inflammation markers as risk factors in patients with influenza A virus, the predisposing to influenza B should be paid also attention. This information's should be used in order to optimize its utilization for early effective and preventive management strategies in pediatric population.
Keywords: INFLUENZA A VIRUS, INFLUENZA B VIRUS, CLINICAL, LABORATORY, CHILDREN.

The influenza ribonucleic acid viruses showed to have an important impact not only for seasonal endemic infections but also for pandemic outbreaks (1, 2). These types of viruses present significant immunogenic proteins on their surface. Due to their segmented genome, it allows these viruses to make antigenic shift changes, which leads to weakening of the adaptive immune system for a range of avian, mammalian species, and humans (1). In humans, there are three specifically types of influenza strains: A, B and C (3, 4). The higher incidence of influenza A and B was seen in major of children around the world (5, 6). Concerning influenza B virus, some studies have sustained the fact that this virus can lead to some limited respiratory infections (7), and sometimes being even similar with the symptomatology of influenza A virus, considering the same rate of hospitalization and death rates (8, 9). The variations of specific viruses type in pediatric population shows a direct relationship with child's age (10). Interestingly, a higher attention was seen for influenza A virus in comparison with B influenza, noticed the fact that B virus could be less virulent with a lower pandemic potential (11, 12). Considering this task, in the last decades, some studies have been reported more clinical features between A and B influenza, in which B virus presenting only a limited higher level of severity (13, 14). The objective of this study was to identify clinical and paraclinical characteristics as risk factors in the patients with influenza A and B virus infection admitted to the pediatric infectious diseases department for early preventive management strategies.

MATERIAL AND METHODS

This retrospective study included 348 children treated for A (n=265) or B (n=83) influenza in the infectious diseases children department of Constanta Clinical Infectious Diseases Hospital from Constanta, Romania with mean aged of 6.48 for A influenza and 5.9 for B influenza, which required hospitalization.

Eligibility criteria were children's aged ≤16 years diagnosed with laboratory confirmed influenza A or B during the epidemic season, between January 2015 and May 2019. Patients with unidentified flu represented the exclusion criteria.

The clinical characteristics related to fever, cough, rhinorrhea, vomiting/nausea, diarrhea, dyspnea, myalgia, pneumonia, or bronchiolitis were collected. The diagnosis of pneumonia was established by acquiring abnormalities data from chest X-ray or pulmonary computed tomography (CT) scan.

The laboratory tests collected included fibrinogen (normal range (nr) = 150-400 mg/dl), white blood cells (WBCs, nr = 6-17/10*9/L), hemoglobin (Hb, nr = 11-14 g/dL), platelet (PLT, nr = 150-400/10*9/L), erythrocyte sedimentation rate (ESR, nr = 3-9 mm/1 h), creatine-kinase (CK, nr = 24-195 U/L), alanine aminotransferase level (ALT, nr = 0-40 U/L), aspartate aminotransferase level (AST, nr = 0-37 U/L) and C-reactive protein (CRP, nr = 0-5 mg/L).

The indication for hospitalization included fever, and respiratory symptoms (i.e., cough or pneumonia). Oseltamivir as antiviral agents were generally prescribed in the cases where children had positive PCR for influenza A or B virus. All patients recovered without complications.

Agreement of Ethic Committee from Constanta Infectious Diseases Clinical Hospital from Romania and informed con-

sent of patients and parents were obtained.

Statistical analysis

Comparison of proportions were performed by chi-squared test for the comparison of two proportion (from independent samples), expressed as percentage, and the comparation of mean values of continuous variables were performed by independent samples t-test.

When the calculated p value is less than p= <0.05 the conclusion is that the 2 proportions are significantly different, respectively the two means do differ significantly.

RESULTS

A number of 265 patients with influenza A and 83 patients with influenza B viruses were included in the study (fig. 1), in which patients with unidentified flu were excluded.

The mean age of the patients with influenza A was higher than that of patients with influenza B (6.4 *vs.* 5.9 years), the majority being found in 2016 (January-March) and 2019 (January- May).

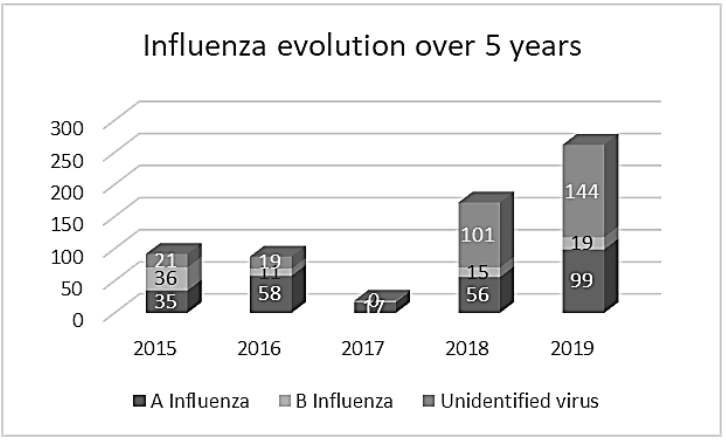

Fig. 1. The evolution of influenza A, B, and unidentified flu in pediatric patients.

Clinical manifestation like fever was found predominantly in influenza B virus and vomiting/nausea, diarrhea, dyspnea, myalgia, pneumonia and bronchiolitis in influenza A (tab. I).

It has been showed that cough was statistically different in both types (i.e., 63.39% in influenza A *vs.* 79.51% in influenza B, p=0.0094). The same statistically significant values were seen for rhinorrhea (i.e., 73.2% *vs.* 55.42%, p=0.0035), vomiting / nausea (i.e., 77.35% *vs.* 65.06%, p=0.0362), dyspnea (i.e., 18.49% *vs.* 8.43%, p=0.0449), myalgia (i.e., 46.03% *vs.* 32.53%, p=0.0412) and pneumonia (i.e., 67.54% *vs.* 50.6%, p=0.0077). The laboratory tests showed higher values of fibrinogen in A influenza case and higher values of WBCs for B influenza (308.82 *vs.* 298.95 and 3,857.28 *vs.* 4,068.23). Hemoglobin (i.e., 10.96 *vs.* 12.16), PLT (i.e.,182846.73 *vs.* 215480.95), CK (i.e., 316.01 *vs.* 408.31), and AST (i.e., 45.61 *vs.* 51.66), shows a significant difference for B influenza comparing with A influenza (p<0.001). In contrast, CRP shows significant differences of values for A influenza (i.e., 7.05 *vs.* 5.53, p=0.001). Moreover, ESR values showed similar values for both A and B influenza (tab. II).

TABLE I

Clinical characteristics and diagnosis by influenza type

Clinical characteristics	Influenza type		χ^2_{calc}	*p value*
	A (n = 265)	B (n = 83)		
Fever	198 (74.71%)	69 (83.13%)	2.059	0.1513
Cough	168 (63.39%)	66 (79.51%)	6.742	0.0094
Rhinorrhea	194 (73.2%)	46 (55.42%)	8.522	0.0035
Vomiting / Nausea	205 (77.35%)	54 (65.06%)	4.39	0.0362
Diarrhea	51 (19.24%)	11 (13.25%)	1.167	0.2800
Dyspnea	49 (18.49%)	7 (8.43%)	4.022	0.0449
Myalgias	122 (46.03%)	27 (32.53%)	4.170	0.0412
Pneumonia	179 (67.54%)	42 (50.6%)	7.112	0.0077
Bronchiolitis	27 (10.18%)	5 (6.02%)	0.86	0.3538

TABLE II

Laboratory results of patients by influenza type

Test	Influenza type		p value
	A (n = 265)	B (n = 83)	
Fibrinogen (mg/dL)	308.82	298.95	0.110
WBC (/10*9/L)	3,857.28	4,068.23	0.115
Hb (g/dL)	10.96	12.16	<0.001
PLT (/10*9/L)	182,846.73	215,480.95	<0.001
ESR (mm/1 hr)	13.87	13.53	0.605
CK (U/L)	316.01	408.31	<0.001
ALT (U/L)	45.54	47.72	0.148
AST (U/L)	45.61	51.66	<0.001
CRP (mg/l)	7.05	5.53	0.001

Values are presented as mean values. WBC = white blood cells; Hb = hemoglobin; PLT = platelets, ESR= erythrocyte sedimentation rate; CK = creatine- kinase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CRP = C-reactive protein.

Approximately the same number of patients from both influenza types received prescription for early antiviral treatment with Oseltamivir (60.37% for influenza A *vs.* 73.49% for influenza B (tab. III). Furthermore, about 35.09% from influenza A and 49.39% from influenza B received antibiotics (p=0.0273) and 30.18% from influenza A and 18.07% from influenza B, received corticosteroids (p=0.0434).

TABLE III

Management of patients with A and B influenza

Management	Influenza type		χ^2_{calc}	*p value*
	A (n = 265)	B (n = 83)		
Early antiviral treatment (<48 hours from the onset of symptoms)	160 (60.37%)	61 (73.49%)	4.145	0.0418
Antibiotics	93 (35.09%)	41 (49.39%)	4.871	0.0273
Corticosteroid therapy	80 (30.18%)	15 (18.07%)	4.081	0.0434

DISCUSSION

Influenza showed to be a self-limited disease in children, being associated with higher mortality rates, especially school aged children, considered that the prevalence of A influenza increased according to age similar with other studies and noticed in our previous studies also (15, 16, 17).

Regarding the influenza spreading, in temperate area the influenza appears in winter contrary to other disease like Boutonneuse fever, which is a seasonality of disease with a peak during the summer (18).

The most common months with the largest number of severe influenza virus infection cases in our study were from January to March, and just in year 2019 we noticed cases also in April and May.

Moreover, the presence of a greater increase in both laboratory tests and early treated patients was seen for influenza B virus, contrary to that of influenza A which is associated with more severe outcomes (11, 19). This could be explained by the fact that patients with influenza A were older than patients with influenza B (6.48 *vs.* 5.9), which is in accordance with the results of other studies (14, 20, 21). Another study sustains that patient much younger (i.e., less than 6 months) had a higher mortality rates than those aged between 13 and 17 years (22). The value of liver aminotransferases, AST and ALT were slightly elevated like in many infectious diseases with systemic involvement (23) but not elevated like in an acute viral hepatitis caused by A hepatitis virus (24). The findings from our study and previous studies from the same region confirm a high burden of acute disease in Romania and provide useful data to support the implementation of vaccination (25).

In our study, the predominance of influenza was A from our region, which is in accordance with other studies which show that influenza A was the predominant type of influenza in both Australia and the United States (22, 26).

Usually, the complications of influenza A and B are characteristics for acute respiratory distress syndrome, being accompanied with death (27, 28). In our study, although the majority of clinical parameters presented higher values for influenza A, it seems that influenza B showed to have a higher impact on the health of our pediatric population sustained by the laboratory test results.

In the case of influenza B infection, some authors have been showed from CT features bilateral diffuse ground-glass opacities besides lung pathology of diffuse alveolar damage (29), changes similar to other pulmonary disease like tuberculosis (30, 31) pulmonary hypertension (32) or cancer (33). However, in our study the most common chest X-ray changes were

like in an interstitial pneumonia suggestive for features of influenza.

Another study sustained the fact that myalgia is a symptom that belongs more to influenza B virus (34). Although there have been few studies which reported important results regarding muscle-derived enzymes (35), together with neurological exam, urine myoglobin, localized myalgia in legs, and/or renal function, our study shows just a slightly increase in CK levels among patients with important myalgias. Moreover, clinical features like myalgia were seen more predominantly in influenza A virus.

The antiviral treatment was almost similar early administered in less than 48 hours (i.e., 60.37% for influenza A and 73.49% for influenza B), considering the fact that in children infection is nonspecific, being difficult to differentiate from other respiratory infection. However, the early administered treatment showed to have many benefits on symptoms (36, 37, 38).

The antibiotics were given in approximately 35.09% in influenza A and 49.39% in influenza B patients and corticosteroid treatment in 30.17% for influenza A and 18.07% for influenza B patients. Therefore, pediatric clinicians should pay more attention on these patients, giving them an early treatment for influenza for those suspected. The initiation of corticosteroid therapy still remains controversial. Although some studies sustained the fact that corticosteroids can decrease mortality (39, 40), other studies have been found that this treatment can lead to poor outcome (41-43). In our study corticosteroids were recommended in patients with flu complicated with bronchiolitis and myositis. Although there were registered different complication like bacterial pneumonia, bronchiolitis or myositis evolution was favorable in all cases, no death being registered.

CONCLUSIONS

Early recognition of clinical symptoms and laboratory features as risk factors of both influenza A and B, as in our present study, could provide an effective management and outcomes in pediatric population. Although we have found the predominance of both clinical characteristics and nonspecific inflammation markers in influenza A virus patients, the predisposing to influenza B should be paid also attention. Since influenza can be prevented by vaccine it should be routine encouraged in children like in adults in order to prevent future hospitalization cases.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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UTILITY OF THE INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE IN PATIENTS WITH HEART FAILURE - THE EXPERIENCE OF A CARDIOVASCULAR REHABILITATION CLINIC

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UTILITY OF THE INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE IN PATIENTS WITH HEART FAILURE - THE EXPERIENCE OF A CARDIOVASCULAR REHABILITATION CLINIC (Abstract): Cardiac rehabilitation is a multidisciplinary program that improves health-related quality of life and exercise capacity in patients with heart failure. The International Physical Activity Questionnaire - Long Form (IPAQ-L) is an instrument for monitoring population levels of physical activity. The aim of our study was to evaluate the utility of IPAQ-L in rehabilitation of patients with heart failure. **Material and methods:** We performed a single-center cross-sectional study on 119 patients in which we evaluated anthropometric parameters and cardiovascular risk factors such as smoking, alcohol consumption, blood pressure profile, glycemic, and lipid profile. The physical activity level was determinate by applying IPAQ-L. **Results:** Factors that correlate with better levels of physical activity were younger age ($p = 0.013$), weight status ($p = 0.028$), and abdominal circumference ($p = 0.007$). The application of the IPAQ questionnaire showed that 79.8% of the subjects performed physical activity at a level of at least 1,500 MET-minutes/week. **Conclusions:** Within a cardiac rehabilitation program, IPAQ-L can be used to assess physical activity, being an instrument with reasonable measurement properties for national monitoring. **Keywords:** CARDIAC REHABILITATION, HEART FAILURE, IPAQ, PHYSICAL ACTIVITY.

Cardiac rehabilitation is a therapeutic option for patients suffering from a variety of heart conditions. Although the cardiovascular rehabilitation program was initially targeted for patients with coronary artery disease, the range of cardiovascular and metabolic pathologies that benefited from it, grew significantly over time, with an improvement of the patient's quality of life, including increased exercise capacity and physical activity. Its prescription in these situations is backed up by large evidence

that shows improved clinical results. Despite this data, cardiac rehabilitation referral and attendance remain low, requiring the development of actions to promote its practice (1).

According to a 2020 study, quantification of cardiac rehabilitation can be made using International Physical Activity Questionnaire (IPAQ). The variability in moderate to vigorous physical activity and sedentary behavior as measured by IPAQ or accelerometer, can objectify a community's health status (2).

The patient's ability to evoke and identify their recent PA as light, moderate or vigorous is a fundamental limitation of self-report surveys. The IPAQ questionnaire has been verified in 14 locations across 12 nations and offers consistent PA measurements. IPAQ is available in two versions. While the IPAQ Short Form (IPAQ-S) is advised for ample prevalence studies, the IPAQ Long Form (IPAQ-L) allows for a more extensive study of PA across four domains: occupation, transportation, home, and leisure time (3).

The ***aim*** of our study was to evaluate the usefulness of IPAQ-L questionnaire in patients with heart failure included in a cardiac rehabilitation program. Also, we wanted to assess the link between the IPAQ-L questionnaire items and the patients' clinical, biochemical, and paraclinical variables and the impact of cardiovascular comorbidities such as obesity, hypertension, and type 2 diabetes on IPAQ-L outcomes.

MATERIAL AND METHODS

We performed a single-center cross-sectional study that lasted 12 months (1 January 2017 - 31 December 2017), including patients with various cardiovascular pathologies, who had previously undergone cardiovascular recovery programs and received advice on lifestyle changes and control of risk factors. The patients included in the study were at least at the second hospitalization in the Cardiovascular Rehabilitation Clinic from the Clinical Rehabilitation Hospital, Iasi, Romania. Anthropometric parameters, cardiovascular risk factors (smoking, alcohol consumption, blood pressure profile, glycemic and lipid profile), exercise capacity (exercise test), quality, and quantity of physical activity performed by applying the International Physical Activity Questionnaire have been assessed.

The local Ethics Committee for Scientific Research of the "Grigore T. Popa" University of Medicine and Pharmacy Iasi (certificate of approval: 5483/8 March 2017) and the Ethics Committee for Scientific Research of the Rehabilitation Clinic Hospital (certificate of approval from 27 January 2017) gave their approval for the clinical investigation to be performed. All the patients signed an informed consent form.

RESULTS

The study included 119 patients, with a mean age of 57.9 years (minimum 34 years, maximum 82 years), the gender ratio being balanced (50.4% men). All of the descriptive statistics can be observed in first table. The application of the IPAQ questionnaire showed that 79.8% of the subjects performed physical activity at a level of at least 1,500 MET-minutes/week.

Factors that correlate with better levels of physical activity were younger age ($r = -0.222$, $p = 0.013$), weight status (weight $r = 0.202$, $p = 0.028$), and abdominal circumference ($r = 0.245$, $p = 0.007$). Physical activity did not correlate with the effort

capacity evaluated objectively by exercise test (r = 0.053, p = 0.569). No significant differences were found, in terms of the level of physical activity performed, for different associated cardiovascular risk factors: hypertension (p = 0.259), diabetes (p = 0.483), alcohol consumption (p = 0.286). No significant differences were found between the genders in terms of physical activity (p = 0.559).

TABLE I

Descriptive statistics of the studied population

	Total	Males	Females	p-value
Number	119	60 (50.4%)	59 (49.6%)	
Age (years)	57.9 (± 7.5)	58.2 (± 8.3)	57.7 (± 6.7)	0.707
Weight (kg)	83.5 (± 12.4)	88.0 (± 12.0)	78.9 (± 11.2)	<0.001
BMI (kg/m^2)	30.2 (± 4.7)	29.6 (±5.0)	30.7 (± 4.4)	0.211
AC (cm)	98 (± 12.9)	101.1 (± 14)	94.8 (± 10.9)	0.007
SBP (mmHg)	134.1 (± 15.5)	132.4 (± 17.5)	135.9 (± 12.9)	0.213
DBP (mmHg)	83.5 (± 10.4)	83.6 (± 10.0)	83.5 (± 10.8)	0.955
Glycemia (mg/dL)	116.9 (± 39.0)	124.8 (± 43.2)	108.6 (± 32.2)	0.022
TC (mg/dL)	200.1 (± 42.7)	191.4 (± 45.2)	208.9 (± 38.3)	0.024
HDL-C (mg/dL)	48.3 (± 13.3)	45.5 (± 12.3)	51.2 (± 13.7)	0.020
LDL-C (mg/dL)	121.8 (± 37.5)	116.3 (± 39.0)	127.5 (± 35.4)	0.120
Non-HDL-C (mg/dL)	151.5 (± 40.4)	146.0 (± 43.0)	157.3 (± 37.0)	0.127
TG (mg/dL)	163.1 (± 113.2)	169.9 (± 133.7)	156.3 (± 88.1)	0.515
Hypertension	95 (79.8%)	43 (36.1%)	52 (43.7%)	0.021
Diabetes	36 (30.2%)	25 (21%)	11 (9.2%)	0.005
Alcohol	12 (9.2%)	11 (8.4%)	1 (0.8%)	0.005
Smoking	26 (21.8%)	23 (19.3%)	3 (2.5%)	<0.001
LVMI (g/m^2)	119.4 (± 36.0)	128.4 (± 35.2)	108.4 (± 33.4)	0.006
%HR (%)	74.8 (± 12.4)	72.4 (± 11.4)	77.2 (± 13.0)	0.033
Exercise resistance (W)	89.4 (± 30.0)	103.1 (± 30.3)	75.4 (± 22.4)	<0.001
Workload (METS)	4.8 (± 1.3)	5.1 (± 1.4)	4.54 (± 1.2)	0.026
MET-min/week	10193 (± 11165)	10790 (± 12119)	9586 (± 10171)	0.558

BMI: body mass index, AC: abdominal circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TG: triglyceride, LVMI: left ventricular mass index, MET: metabolic equivalent of task

From the point of view of physical activity, no statistically significant differences were observed between patients who consumed alcohol (13,630 ± 13,697 MET) and those who did not (9,843 ± 10,854 MET) (fig. 1). In addition, there was no

statistical significance when talking about the association of physical activity with the ones diagnosed with diabetes mellitus (11,422 ± 13,404 MET) and the ones that do not associate this disease (9,660 ± 10,087 MET) (fig. 2).

Similar findings without statistically significant differences were observed between patients diagnosed with hypertension (10,663 ± 11,812 MET) and those without hypertension (8,332 ± 8,049 MET) when performing physical activity (fig. 3).

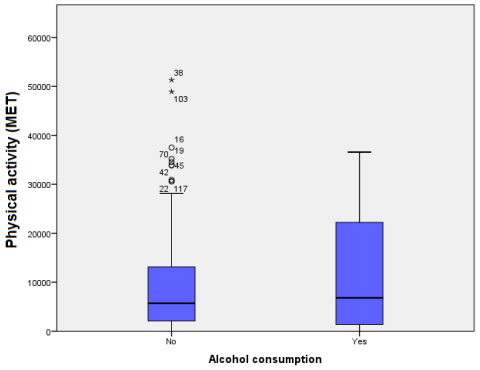

Fig. 1. The correlation between alcohol consumption and physical activity (MET)

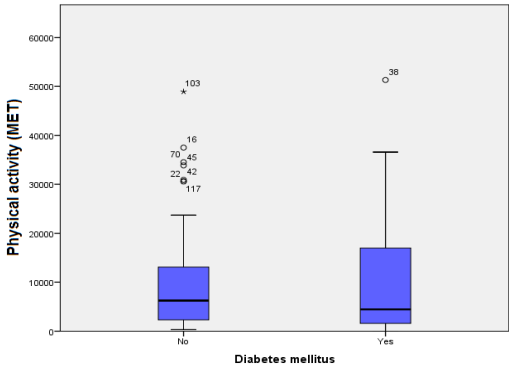

Fig. 2. The correlation between diabetes mellitus and physical activity (MET)

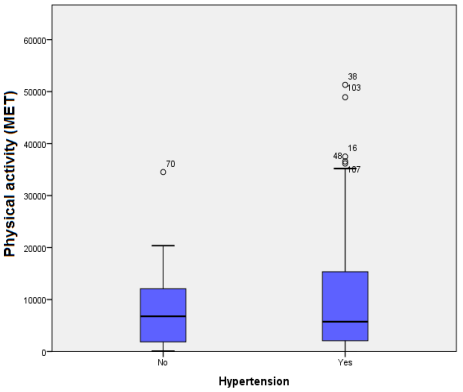

Fig. 3. The correlation between hypertension and physical activity (MET)

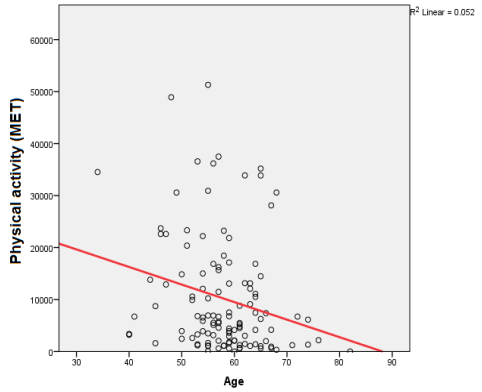

Fig. 4. The correlation between age and physical activity (MET)

We can observe a negative statistically significant correlation between age and physical activity, this being a well-known fact that once the patients get older, they have a lower capacity of performing the same amount of physical activity compared to a young adult (fig. 4).

A positive statistically significant correlation was found between physical activity and waist circumference and weight, respectively (figs. 5, 6). Basically, the patients with overweight and obesity spent

more time performing intensive physical activity, this happening because they are enrolled in a cardiac rehabilitation program.

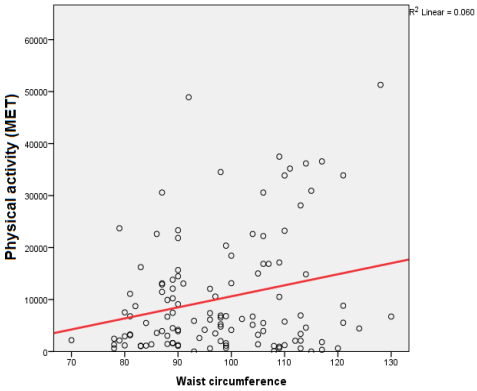

Fig. 5. The correlation between waist circumference and physical activity (MET)

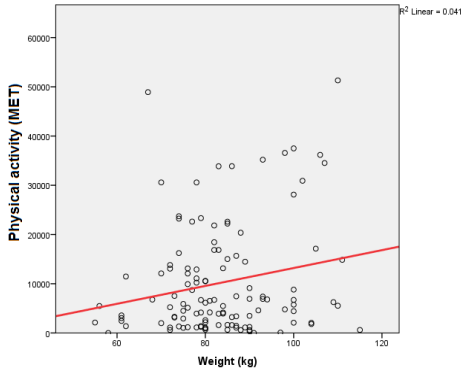

Fig. 6. The correlation between weight and physical activity (MET)

DISCUSSION

The IPAQ questionnaires were investigated in both developed and developing countries' urban populations. Therefore, attention is suggested when analyzing IPAQ data from rural or low-literacy areas in underdeveloped nations (4). Despite the fact that the IPAQ-L has been validated for PA assessment in individuals over 18 years of age (4), it loses reliability, like most self-reported techniques of detecting PA levels, since patients usually exaggerate their PA levels (5). For example, the IPAQ-L overestimates moderate to vigorous PA while underestimating the sitting time (6, 7). Therefore, we opted to adopt this strategy in our analysis because recent studies found that subjects interviewed by a professionally trained person, reported PA levels that were significantly closer to the true ones than those who filled out the IPAQ questionnaire on their own (6).

Even though IPAQ-L has been linked to an exaggeration of overall physical activity in comparison with the IPAQ Short Form (3), its benefit lies in its ability to distinguish domain-related physical activity, providing a complete analysis of the research group's activity patterns. Such information could be valuable in developing successful sedentary behavior intervention strategies (8).We found no significant link between BMI or AC and weekly PA in the investigated domains, despite previous publications reporting a negative correlation among levels of physical activity and BMI (9).

Men engage in more moderate to vigorous daily effort (primarily occupational and leisure-related) than women, according to a study published in 2019 (9). Women spend little time practicing PA, which is mostly in household-related domains. Despite the fact that gender has been shown to have a significant impact on the amount and type of physical activity, both men and women in our study participants expended equivalent physical effort, as measured by the IPAQ questionnaire. In the household and garden domains, the difference in the amount of vigorous physical activity con-

ducted by men versus women approached borderline correlation.

Even if prior research has demonstrated an age-related decline in physical activity (10, 11), our study only identified a moderate relationship. Although many researchers have found an inverse relationship between physical activity and excess weight (12, 13), other studies have found an unusual positive relationship between total physical activity and body mass index (particularly among women), which could be described by changing lifestyles or noticeable determination (14, 15, 16).

In a study published in 2016 (17), the authors investigated the use of IPAQ-L in persons with type 2 diabetes, including both the ones who have been diagnosed with peripheral neuropathy but also the ones who do not have this disease. Individuals with type 2 diabetes diagnosed with peripheral neuropathy were found to be considerably less active compared to those with type 2 diabetes alone ($p = 0.04$), according to the researchers. A study published by Mynarski *et al.* that compared the direct physical activity measurement with IPAQ versus indirect physical activity evaluation with an accelerometer in type 2 diabetes patients found no significant difference between the two approaches ($p < 0.05$). As an outcome, their results suggest that the IPAQ could be used to evaluate PA in individuals with type 2 diabetes (18). Our results are not in concordance with previous studies, being without statistical significance.

In a study published in 2019 (19), the authors found limited concordance among self-reporting adherence to physical activity in the clinical context and IPAQ interviews in individuals with high blood pressure. Furthermore, the authors pointed out that the physical activity suggestion has a low correlation with blood pressure control in the clinical situation, implying that physician advice alone is insufficient to transmit the benefit of supervised physical activity that is proven in the clinical trials to clinical practice. Bravo *et al.* (20) found that self-reported physical activity using IPAQ was mostly related to domestic ($p = 0.018$) and work activities ($p = 0.001$) at moderate intensity in sedentary hypertensive females.

CONCLUSIONS

Within a cardiac rehabilitation program, IPAQ-L can be used to assess individual physical activity levels. IPAQ-L is an inexpensive tool for measuring physical activity in order to produce public health policy proposals or to improve treatment strategies. Health care services are currently under a lot of strain because of the present pandemic situation. The IPAQ-L has reasonable measurement properties for suggesting it as an instrument for national monitoring.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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BURNOUT AND PSYCHOLOGICAL REACTIVITY TO STRESS OF MEDICAL STAFF IN THE CONTEXT OF THE COVID-19 PANDEMIC

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BURNOUT AND PSYCHOLOGICAL REACTIVITY TO STRESS OF MEDICAL STAFF IN THE CONTEXT OF THE COVID-19 PANDEMIC (Abstract). The new pandemic context increases the mental reactivity to stress of health representatives by summing up the conditions of overwork, measures to prevent the spread of COVID-19 and excessive media coverage. **Aim**: To monitor the frequency of manifestation of mental symptoms of stress in medical staff, during the COVID-19 pandemic. **Material and methods**: The prospective study, from which we present preliminary data, was conducted on a group of 147 subjects, medical staff and support staff from COVID and non-COVID hospitals, who gave their free consent to answer questions referring to the development of reactive symptoms caused by the COVID-19 pandemic. The questionnaire used was adapted, with the author's consent, from the original published in 1993 in "In the Tiger's Mouth: An Empowerment Guide for Social Action", specifically intended to investigate the burnout syndrome. **Results**: The most frequently to answer were nurses (42.2%) and the resident doctors (29.3%). Moderate psychological impairment was the most frequent symptom (57.1%) and was more frequently mentioned by subjects from non-COVID hospitals (> 50%, p = 0.046). Severe impairment of mental function was more frequently mentioned by subjects from hospitals with COVID profile (40.7%). **Conclusions**: The results of this study confirm the prognostic value of psychological stress symptoms in the onset of the burnout syndrome. The current pandemic context favors a different reaction to stress, especially on medical staff exposed to multiple consumptive factors. **Keywords**: BURNOUT SYNDROME, PSYCHOLOGICAL STRESS SYMPTOMS, MEDICAL STAFF.

The burnout syndrome has been a problem of global interest in work fields for a long time. In 2016, Maslach and Leiter defined it as a delayed response to interpersonal stressors at work. The three dimensions subsidiary to stress reactivity were considered to be exhaustion, cynicism and inefficiency at work (1).

There are a multitude of variables associated with burnout in medical staff. The pandemic context overlaps with them, becoming a factor in increasing the level of a priori stress.

As of January 25, 2020, in the new pandemic COVID-19 context, more than forty thousand medical staff from other provinces have been deployed in Hubei Province in an attempt to prevent an increase in the

number of fatal consequences. At the same time, medical personnel from all over China have begun to fight this highly infectious disease.

However, at that time, anticipations of possible psychological disorders in the context of the growing epidemic and uncertainty about the lack of medical supplies, especially personal protective equipment, were premature (2).

In that point of solidarity, were recorded the first concrete stressors of the epidemic, predictors of worldwide psychological devastation, especially among those working in the medical field: fear of infection and feelings of insecurity, maintained by the widespread media broadcasting (3).

A multicenter survey investigating psychological disorders in medical staff working in different positions showed statistically significant differences between groups, finding a significant increase in total scores for anxiety, depression, sleep quality and stress in participants at high risk of COVID-19 exposure ($p < 0.05$). Subsequent post-hoc analysis indicated that medical staff sent to Hubei had higher scores of anxieties, depression, sleep quality and stress (adjusted $p < 0.05$). The combined prevalence of anxiety, depression and insomnia, for these medical workers, reached 38% (3).

The burnout syndrome is defined, in the narrowest sense, as an accumulation of fatigue over long periods of time, with decreased motivation, interest and productivity at work. It is currently being studied in close connection with predictability with stressors such as those mentioned for the current pandemic context (fear of infection, feeling insecure about protective equipment), but also with the frequency of mental states such as anxiety, depression, insomnia or exhaustion (4, 5).

Psychological interventions are recommended in the early stages of burnout. Their advantages are the improvement in quality of personal and professional life. The disadvantages are the worsening of symptoms, the onset of burnout and decreased efficiency at the work (6). Recommendations for the support of medical staff during the pandemic are theorized, through interventions both in the personal sphere and at the level of the health system. Most studies focus on psychological support (7, 8, 9). Individual resilience, however, plays a key role in managing stress reactivity (9).

MATERIAL AND METHODS

We present a prospective study that began in January 2021, from which we highlight the preliminary data on a group consisting of 147 subjects, medical staff and support staff from several COVID-19 and non-COVID-19 hospitals from Iasi, Romania, who gave their free consent to answer questions regarding the occurrence of reactive symptoms caused by the COVID-19 pandemic.

The questionnaire we used was adapted, with the author's consent, after the one published in 1993 in "In the Tiger's Mouth: An Empowerment Guide for Social Action" by Katrina Shields, intended to investigate the burnout syndrome. The distribution of the questionnaires was initiated after acquiring the acceptance of the Ethics Commission. Completion of the questionnaires was done after obtaining the informed consent of all participants. In processing the reactions, the principle of scores was used, applied individually, but which varied by framing the answers in a qualitative value, designated by reference to the percentiles. We will present the results of the psycho-

logical manifestations within the burnout syndrome on the studied cases.

Data were collected by a team of physicians and analyzed using *SPSS 18.0* software. The ANOVA test, the F test $_{(ANOVA)}$, the multivariate analysis, the ROC curve, the χ^2 test and the t-Student test, the Kruskall-Wallis and Pearson correlation were applied in the quantitative interpretation of the data. Statistical significance was defined in the 95% confidence interval ($p < 0.05$).

RESULTS

Depending on the occupation of the respondents, most frequently answered: nurses (42.2%), representing 45.9% of the staff working in hospitals and the resident doctors (29.3%), 27.9% of the staff working in hospitals (fig.1, tab. I).

TABLE I

Lot structure according to occupation

Occupation	Hospital (n=122)	Ambulatory (n=25)	Total (n=147)
Specialist / Senior physician	13 (10.7%)	10 (40.0%)	23 (15.6%)
Resident physician	34 (27.9%)	9 (36.0%)	43 (29.3%)
Nurse	56 (45.9%)	6 (24.0%)	62 (42.2%)
Other staff	7 (5.7%)	-	7 (4.8%)
Orderly	12 (9.8%)	-	12 (8.2%)

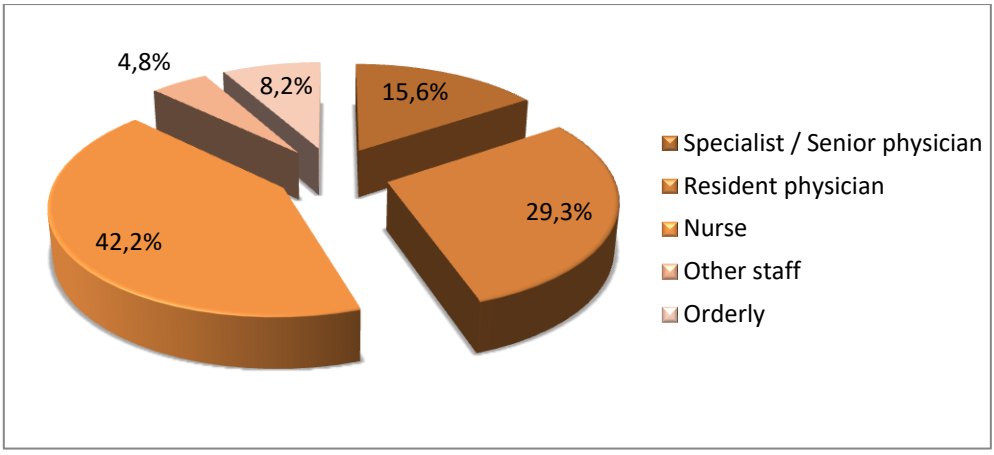

Fig. 1. Distribution of subjects according to occupation

Some of the items provided significant results:

Item 5. *Do you feel tired because your sleep is:*

- insufficient sleep - was reported "often" (26.5%) and "very often" (16.3%) by about 50% of the subjects;

- restless sleep, more frequently found as "sometimes" in 32.7% of subjects;

- sleep full of nightmares was noticed in about 25% of the subjects (27.8%), "often" only 5.4% and "very often" 2%.

Item 6. *Do you feel more:*

Emotional impairment showed the fol-

lowing percentage distributions (fig.3):

- irritability was reported "often" (17%) and "very often" (10.2%) by approximately 50% of the subjects;

- anxiety was more frequently reported by 38.8% of the subjects, "often" and "very often" being noticed in 21.1% of the subjects;

- emotional instability was "often" noticed only in 7.5% of the subjects and "very often" in 4.8%, but 51% of the respondents did not declare this manifestation.

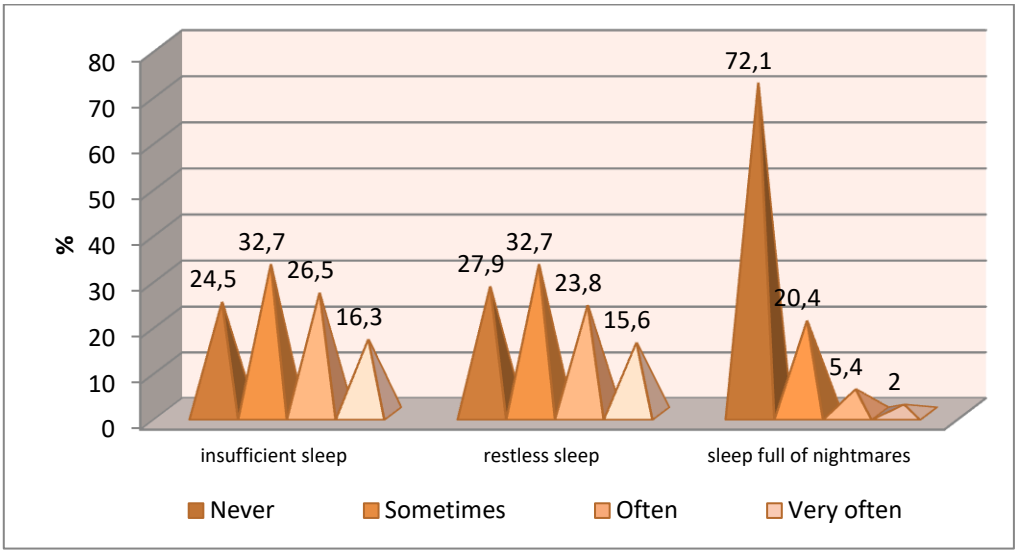

Fig. 2. Results to item 5 *"Do you feel tired because your sleep **is**"*

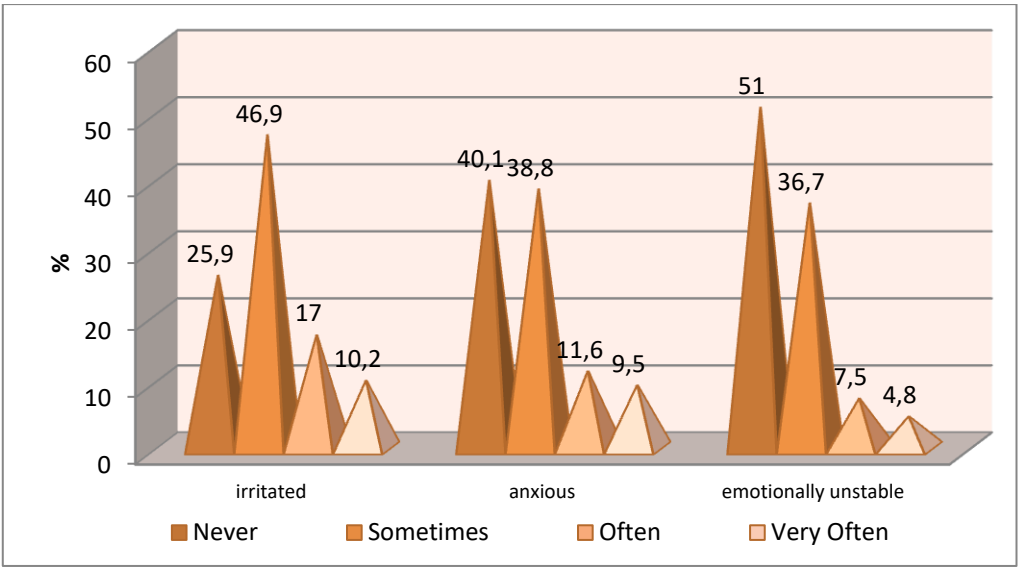

Fig. 3. Results to Item 6 "*Do you feel emotionally affected*"

Item 7. *Are you afraid of:*

The fear of infection did not exist in 36.1% of the subjects, but 46.3% of the respondents sometimes had this fear. Fear of infection was "often" and "very often" reported by 17.7% of subjects. The share of cases according to the fear of transmitting the disease to others was relatively balanced among respondents: 25.2% never had this fear, but 27.2% answered "often" (fig. 4).

Item 8. *Do you feel lately that coming to work no longer pleases you?*

The discomfort of going to work was declared "often" (12.9%) and "very often" (17%) by about one third of the subjects, but 42.2% of the respondents still had the pleasure of going to work (fig. 5).

Item 9. *Do you feel more:*

Less than 10% of the subjects stated that in the last period they were "very often" sad (9.5%), lacking the desire to communicate (6.1%) and lacking optimism (8.2%) (fig. 6).

Item 10. *Since the onset of the COVID pandemic, have you feel like drinking alcohol?*

Alcohol consumption during the pandemic was reduced: "often" (1.4%) and "very often" (2.7%), and 78.9% of respondents did not change their behavior (fig. 7).

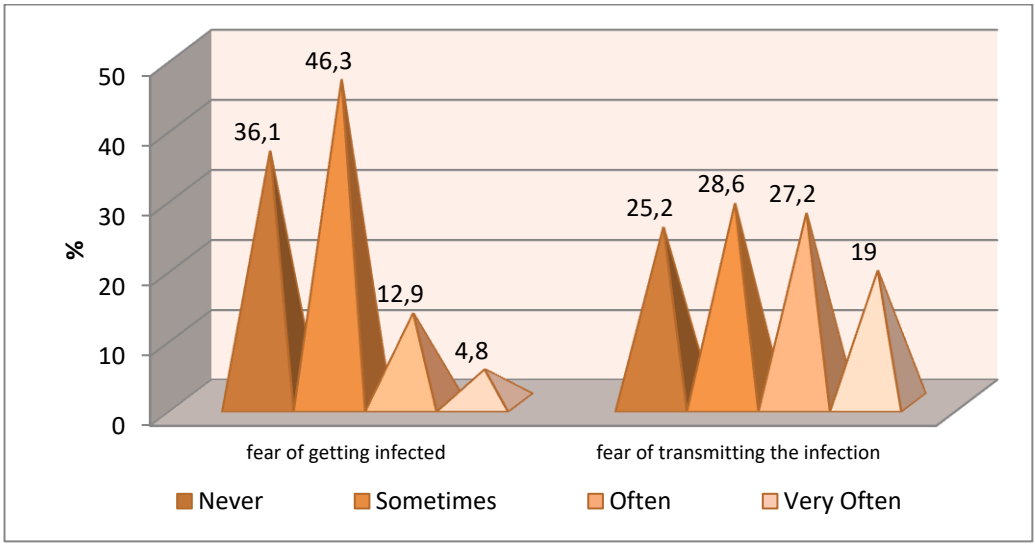

Fig. 4. Results to Item 7 *"Do you have fears"*

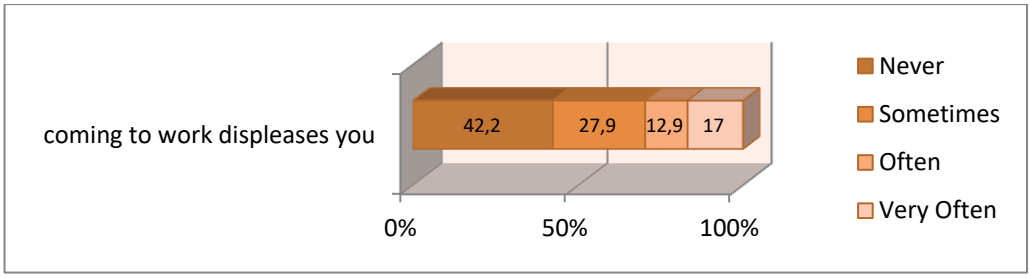

Fig. 5. Results to Item 8 *"Do you feel lately that coming to work no longer pleases you"*

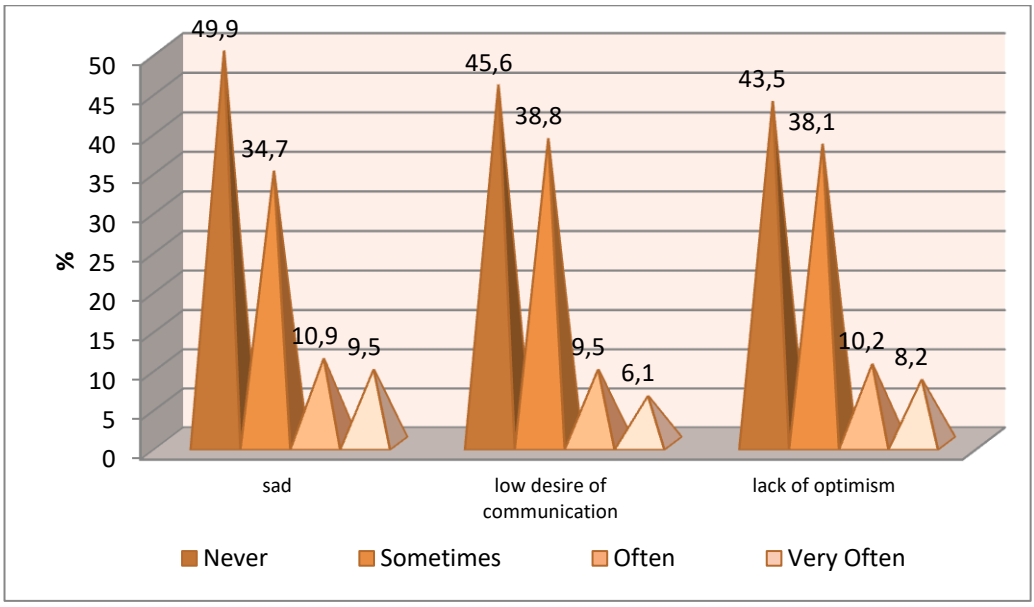

Fig. 6. Results to Item 9 *"Do you feel emotionally affected"*

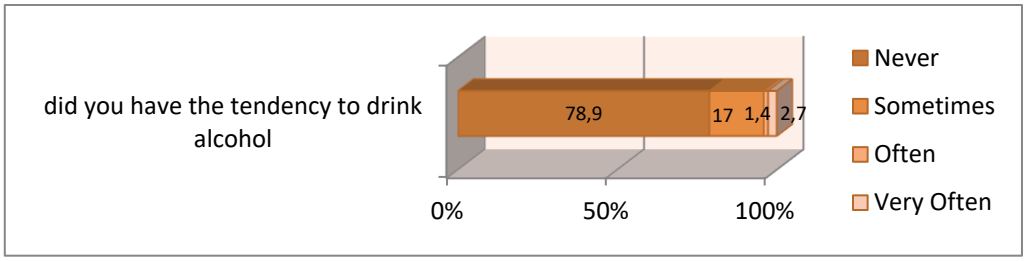

Fig. 7. Results to Item 10 *"Did you have the tendency to alcohol consumption"*

Item 11. *Recovery measures*

The proposed recovery measures showed the following percentage distributions (fig. 8):

- more free time was mentioned "often" (33.3%) and "very often" (23.1%) by about 50% of the subjects;

- qualified help at work (psychotherapy) was "often" and "very often" requested by 25.1% of the subjects, but not mentioned by 46.9% of the subjects

- financial supplementation, as a recovery measure, was mentioned "often" (28.6%) and "very often" (29.3%), but 17% of respondents did not state this solution.

Within the studied groups, a share of 57.1% of subjects with moderate, 19.7% mild and 23.1% with severe impairment of psychological manifestations was noticed (fig. 9).

Specialist and senior physicians (73.9%), resident physicians (62.8%) and nurses (56.5%) more frequently mentioned moderate mental impairment, while 57.1% of the other staff more frequently mentioned mild mental impairment, and 50% of nurses, severe impairment ($p = 0.05$) (fig. 10).

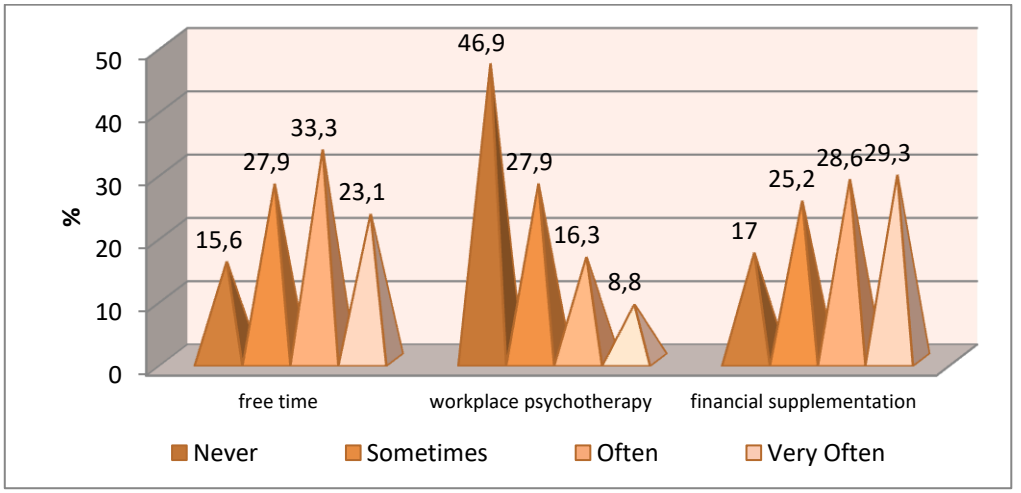

Fig. 8. Results to Item 11 *"Recovery measures"*

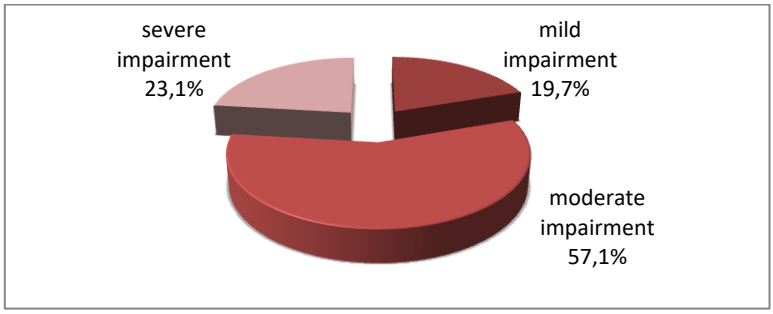

Fig. 9. Distribution of cases according to the overall score for psychological manifestations

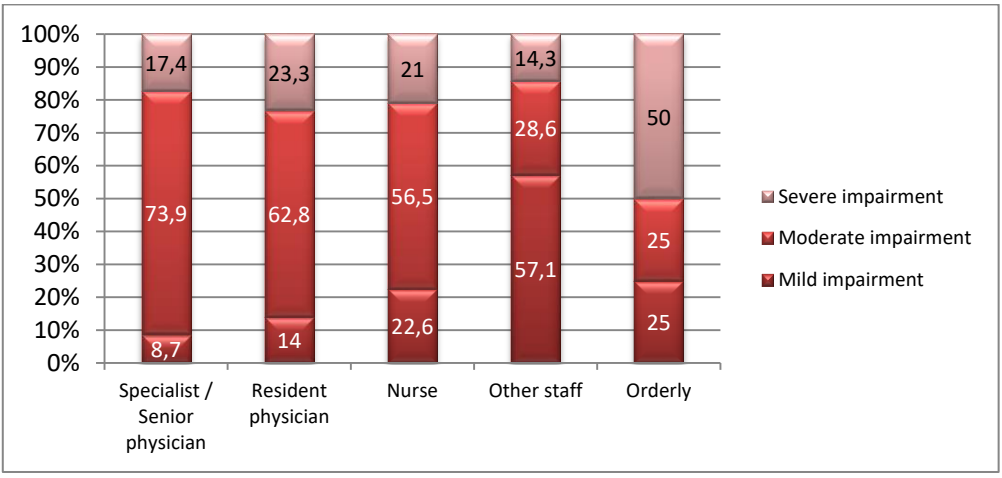

Fig. 10. Distribution of cases according to occupation and overall score for psychological manifestations

The gender distribution of the overall score for mental manifestations showed moderate impairment in both sexes: 69.6% of men and 54.8% of women, but the slightly higher frequency of severe impairment in women should be noted (8.7% *vs.* 25 .8%; p = 0.146) (fig. 11).

Both in the age group under 40 and in those over 40, the degree of mental impairment was moderate (56.3% vs 58.8%; p = 0.741) (fig. 11).

In patients from urban areas, but especially in those from rural areas, the degree of mental impairment was moderate (48.6% *vs.* 85.7%; p = 0.033) (fig. 11).

The distribution of scores for psychological manifestations according to marital status showed moderate impairment in both categories: 63.6% of subjects without a partner (unmarried, divorced, widowed) and 55.8% of subjects with a partner, but the percentage differences were not significant from a statistical point of view (p = 0.174) (fig. 11).

It should be noted that about 1/4 of the subjects had severe mental manifestations, with the exception of males and patients from rural areas.

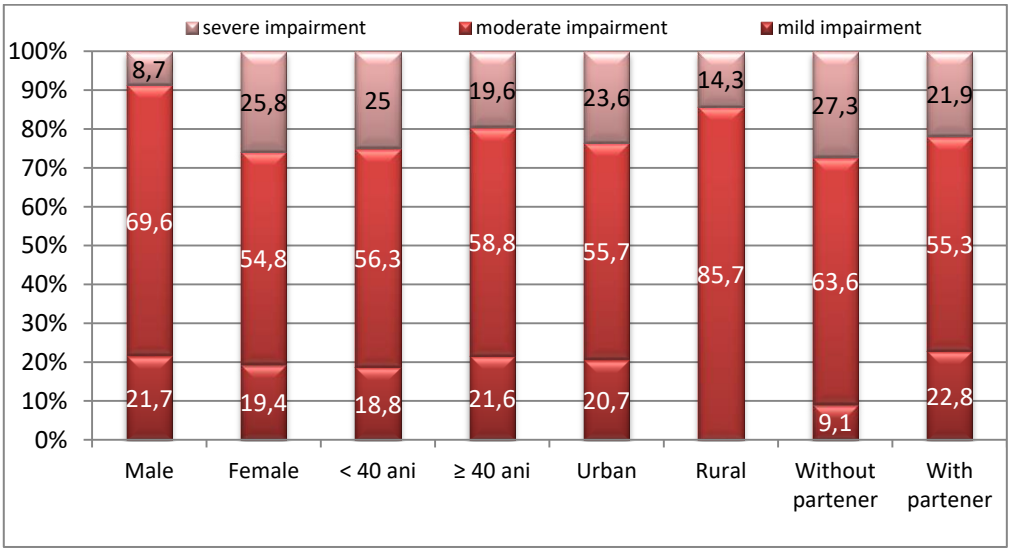

Fig.12. Distribution of cases by gender, age groups, background and marital status according to the overall score for mental manifestations

DISCUSSION

According to our results, over half of the subjects expressed fear of becoming infected and transmitting the disease. The current literature confirms this. The prevalence of psychosomatic and psychological symptoms recorded in medical personnel during the COVID-19 pandemic was predominantly represented by the fear of transmitting the infection to their families and contracting the disease (7, 10, 11).

Fear of infection was also associated with the scores of exhaustions, depression, anxiety, and stress (p <0.05), and physicians and nurses working in direct contact with infected patients recorded increased levels of exhaustion, depression, stress at work and lack of job satisfaction, compared

to those least exposed (12, 13, 14).

The rate of depression is higher in those with a higher degree of exposure to the disease, both during the COVID-19 pandemic and during the SARS pandemic (6, 15, 16). Our data are statistically significant from this point of view, as the participants from hospitals with COVID profile were the majority in terms of severity of psychological symptoms, compared to the non-COVID medical units.

According to our results, 1/3 of the respondents said they lost the satisfaction of going to work. All medical staff (treating physicians, resident physicians, nurses) suffer from the impact of following new circuits, being subjected to a limited range of interaction with patients and the brevity of the anamnestic and clinical examination, sometimes with the risk of truncating fundamental elements. In addition, the stress of the program and the modified shifts, the prolonged wearing of equipment and protective masks, lack of food, hydration and rest were acutely experienced. The stress that occurs during the equipment undressing process, whose strict protocol ensures the chance of effective protection, but in which a minimal error can be a circumstance of individual infection and or of those around (13, 14, 17) was also frequently reported.

According to our results, the psychological impairment in the context of stress reactivity was moderate in over half of the doctors and nurses, being mild in other staff. The most severely affected were hospital attendants, in a statistically significant proportion. The literature shows a tendency to severe mental symptoms in nurses, by comparison them being more affected than doctors (18, 19, 20).

Regarding the ways of recovery proposed by the subjects, financial supplementation and free hours registered the highest rate.

There are current directions regarding how to prevent the burnout syndrome, including the optimization of the staff schedule, the number of patients taken over by each, the improvement of protective equipment and operating circuits, the assurance of special transport and accommodation when necessary (21, 22, 23). An online education and counselling platform for medical staff (Battle Buddy), with an interest in specific steps for launching psychological resilience interventions (24, 25, 26) are also considered.

CONCLUSIONS

Our data show that the predictors of severe mental manifestations of burnout are fear of infection, fear of transmitting the disease and insufficient sleep. The overall score of mental manifestations showed a moderate mental impairment in over half of the participants; the severity of mental symptoms is predominant in those most exposed to the disease, especially in nurses and medical assistants.

The assessment of psychological symptoms in the case of burnout must be related to all dimensions of the social life, including contemporary values, economic aspects, geographical area and cultural characteristics, independent of the pandemic crisis, as the initial psychological background can determine how to respond to the crisis.

There are substantial overlaps between the clinical samples of burnout and non-burnout patients. The use of screening tools without a rigorous medical history also creates the risk of both false positive and negative results.

Current issues of particular relevance to the medical world include the possible links between the burnout syndrome and mental illnesses, with differential diagnosis being necessary and difficult to perform in certain clinical circumstances.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

UVC LIGHT EFFICIENTLY KILLS SARS-COV-2

A direct approach to limit airborne viral transmissions is to inactivate them within a short time of their production. Far-UVC light (207-222 nm) efficiently kills pathogens potentially without harm to exposed human tissues. As all human coronaviruses have similar genomic sizes, far-UVC light would be expected to show similar inactivation efficiency against other human coronaviruses including SARS-CoV-2. Continuous far-UVC exposure in occupied public locations at the current regulatory exposure limit would result in viral inactivation. Low-dose-rate far-UVC exposure can potentially safely provide a major reduction in the ambient level of airborne coronaviruses in occupied public locations. Thus far-UVC light potentially has about the same highly effective germicidal properties of UV light, but without the associated human health risks. (Buonanno M, Welch D, Shuryak I *et al.* Far-UVC light (222 nm) efficiently and safely inactivates airborne human coronaviruses. *Sci Rep* 2020; 10: 10285).

PALLIATIVE CARE IN NEOPLASTIC PATIENTS WITH *CLOSTRIDIOIDES DIFFICILE* ASSOCIATED INFECTION - THE EXPERIENCE OF A UNIVERSITY HOSPITAL FROM NORTH-EAST ROMANIA

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PALLIATIVE CARE IN NEOPLASTIC PATIENTS WITH *CLOSTRIDIOIDES DIFFICILE* ASSOCIATED INFECTION - THE EXPERIENCE OF A UNIVERSITY HOSPITAL FROM NORTHEAST ROMANIA (Abstract): *Clostridioides difficile* infection (CDI) has become an important public health problem over the last decade, especially in developed countries. Both the incidence of infection and the severe forms recorded significant increase. At the same time, *Clostridioides difficile* is the main cause of diarrhea associated with medical care and, in particular, antibiotic use. The **aim** of this study was to measure the impact of CDI on neoplasic patients. **Material and methods:** We have quantified in this prospective observational study symptoms, the effects of hospitalism and antibiotic use at 132 patients diagnosed with *Clostridioides difficile* infection in "Sf. Parascheva" Infectious Diseases Clinical Hospital, between 2017-2019. **Results:** Most of the patients experienced delays in diagnosis and treatment; almost 35% waited 6-21 days and 15% over 21 days. The delayed diagnosis brought further complications; 65% did not experience symptom resolution within seven days after initiating the treatment. We have noticed that after CDI, patients' priorities have changed; they started to be more concerned about the healthcare system, particularly time to diagnosis and treatment, antibiotic usage, alimentation and needs from a healthcare provider. **Conclusions:** The main issues are the management of symptoms and the need to use strategies that will allow the patients to control their illness and treatment better. **Keywords:** PALLIATIVE MEDICINE, QUALITY OF LIFE, MULTIDISCIPLINARY APPROACH, NEOPLASTIC PATIENT, CARE PREVENTION.

Clostridioides difficile infection (CDI) is an important public health problem worldwide, associated with significant morbidity and mortality, correlated with antibiotic use, and prolonged hospitalizations (1-5). *Clostridioides difficile* is a Gram-positive anaerobic, sporulated bacillus of the order *Clostridiales*, family *Peptostreptococcaceae*, genus *Clostridioides*, first described in 1935, recognized in 1978 as the etiological agent of pseudomembranous colitis (6). It's found in humans, animal feces, water, and soils. Pathogenic strains produce multiple toxins (7), the most common are enterotoxin (*Clostridioides difficile* toxin A) and cytotoxin (*Clos-*

tridioides difficile toxin B). Both toxins may produce diarrhea and inflammation in infected patients. It's responsible for 15-25% of cases of post-antibiotic diarrhea and all cases of pseudomembranous colitis (8, 9).

Clostridium difficile is a major emerging human pathogen found in the human colon without causing disease (10). Several risk factors are involved in the development of CDI, including admission to healthcare facilities, comorbidities, advanced age, and exposure to antibacterial agents (11). Studies indicate that *C. difficile* is normally present in 2-5% of the adult population; (12) colonization is frequently associated with a history of unrelated diarrheal diseases, such as food poisoning or laxative abuse. (13) It is the main cause of hospital-acquired infectious diarrhea in adults, with rising infection rates since 1999 (14, 15). People with no history of gastrointestinal symptoms are unlikely to become asymptomatic carriers. These individuals are a major reservoir of infection. (16) Disruption of host-microbiota, most commonly by antibiotic treatment, increases susceptibility to infection. (17,18) Almost 20% of adults over the age of 65 are asymptomatically colonized by *Clostridioides difficile*, which increases the chance of CDI and can be a source of transmission in a medical facility (19,20)

Clinically, CDI ranges from uncomplicated diarrhea to fulminant and sometimes fatal, pseudomembranous colitis (associated with severe inflammation of the colon and the formation of pseudomembranous on the surface of the intestinal mucosal) (12).

The estimated all-cause 30-day mortality rate among patients who acquire CDI in healthcare facilities is 3-30% (21, 22).

MATERIAL AND METHODS

This prospective observational study is performed in the "Sf. Parascheva" Hospital of Infectious Diseases Iasi, Romania. The recruitment period was three years, 2017, 2018, 2019. The patients were selected using following criteria: adult (age > 18 years old), history of neoplasia, patients presenting bacteriological evidence of CDI during hospitalization, after giving informed consent.

The exclusion criteria were patients involved in an ongoing study, patients who could not fill out a questionnaire for any cognitive reasons, and the patients with chronic inflammatory bowel disease.

All relevant clinical data were collected from the patient's records: gender, age, comorbidity, symptoms, length of stay, ICU (Intensive Care Unit) hospitalization, immunosuppression, presence of any CDI episode within the previous 2-year period, treatment, outcome. Informed consent was obtained from all subjects involved in the study.

RESULTS

A total of 132 patients were included. Four patients died during hospital stay (for causes other than CDI) after completing the survey, and their data were included in the analysis.

The ages were between 30 and 99 years old with a median of 64.5 years, 38.64% were female, and 61.36% were men. The majority (78.05%) were between 50 and 79 years old (tab. I).

The CDI was coded as primary diagnosis in 89% of cases indicating that it was

the reason for admission to the hospital. The four most frequent primary diagnoses were sepsis, urinary infection, endocarditis, and viral encephalitis.

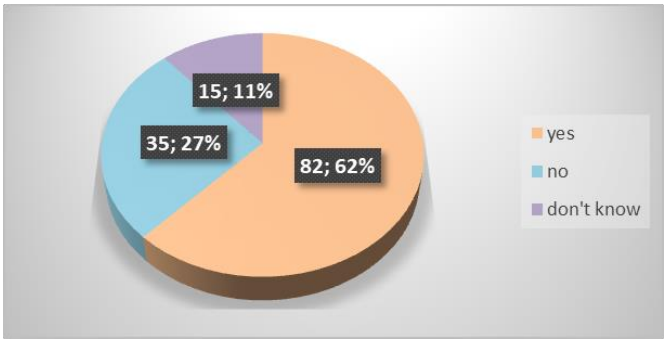

Fig. 1. Distribution of patients that took antibiotics before diagnosis.

TABLE I.
The patients distribution by gender and age.

Gender	N	%
Female	51	38.64
Male	81	61.36
Age (yo)	N	%
18-29	0	0
30-39	3	2.27
40-49	6	4.54
50-59	18	13.66
60-69	45	34.09
70-79	40	30.3
80-89	18	13.63
90-99	2	1.51
Median age	60-69	

CDI has been most commonly correlated with exposure to antibiotics and prior hospitalization for non-CDI-related reasons. Almost 62% of the patients indicated taking antibiotics three months before being diagnosed (fig. 1). Of these, 20.45% had colon cancer, 13.63% pulmonary cancer, 12.87% prostate cancer, 12.87% liver cancer (fig. 2).

Almost 38% indicated their symptoms began in the hospital, 31% indicated that the symptoms started in the first six days of admission, 25% indicated 1-2 weeks, 26% indicated 3-4 weeks, and 18% reported between 6 weeks and six months (fig. 3).

Almost 61% of the patients indicated that their symptoms began within hospitalization. Only 10% indicated they were at home when the symptoms started, and 24% indicated living in a long-term care home (fig. 4).

CDI can be a severe infection, especially in immunosuppressed patients. The most common symptoms were watery severe diarrhea (72%), common diarrhea (65%), fatigue (68%), loss of appetite (59%), and

weight loss (53%) (fig. 5). Most respondents experienced two or more symptoms, with the majority reporting between four and seven.

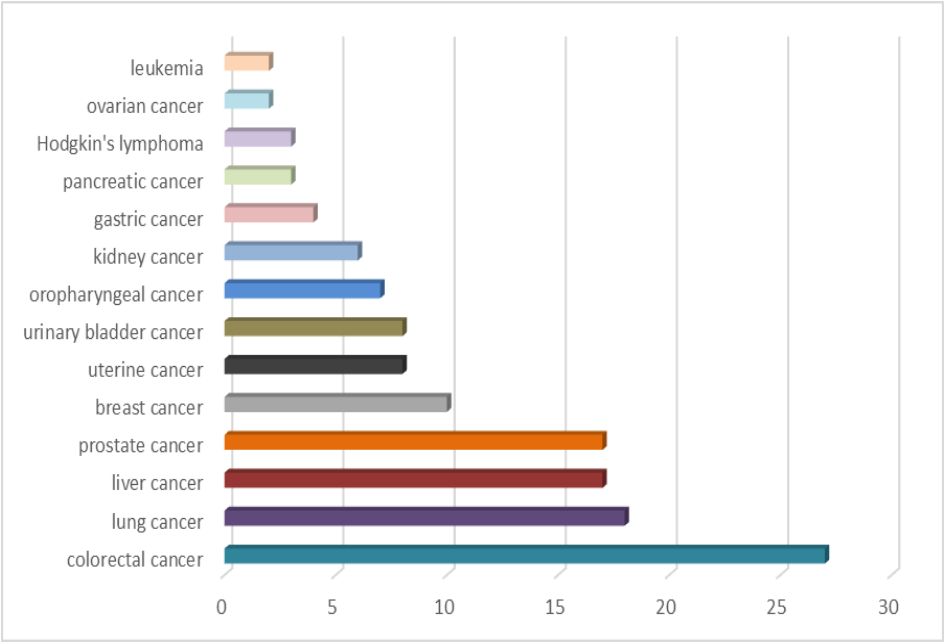

Fig. 2. Distribution of patients according to their history conditions.

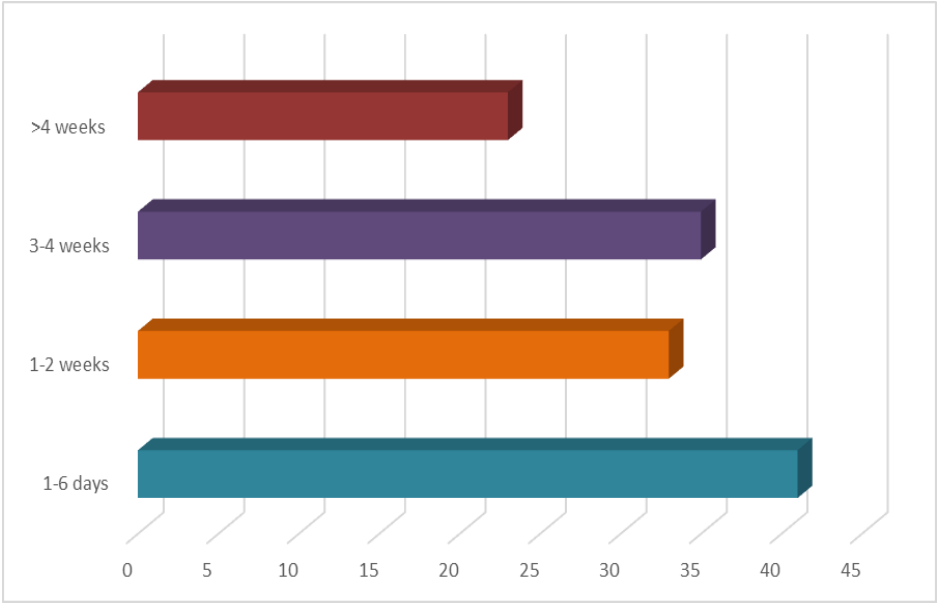

Fig. 3. Development of first symptoms.

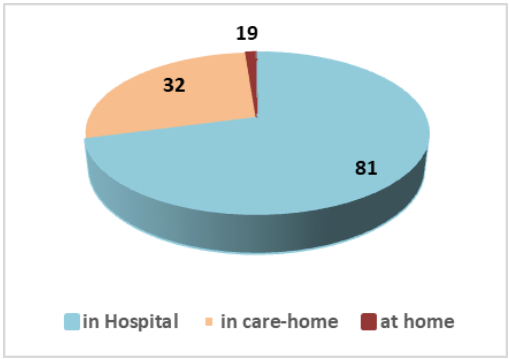

Fig. 4. Exposure to health-care facilities preceding the development of first symptoms.

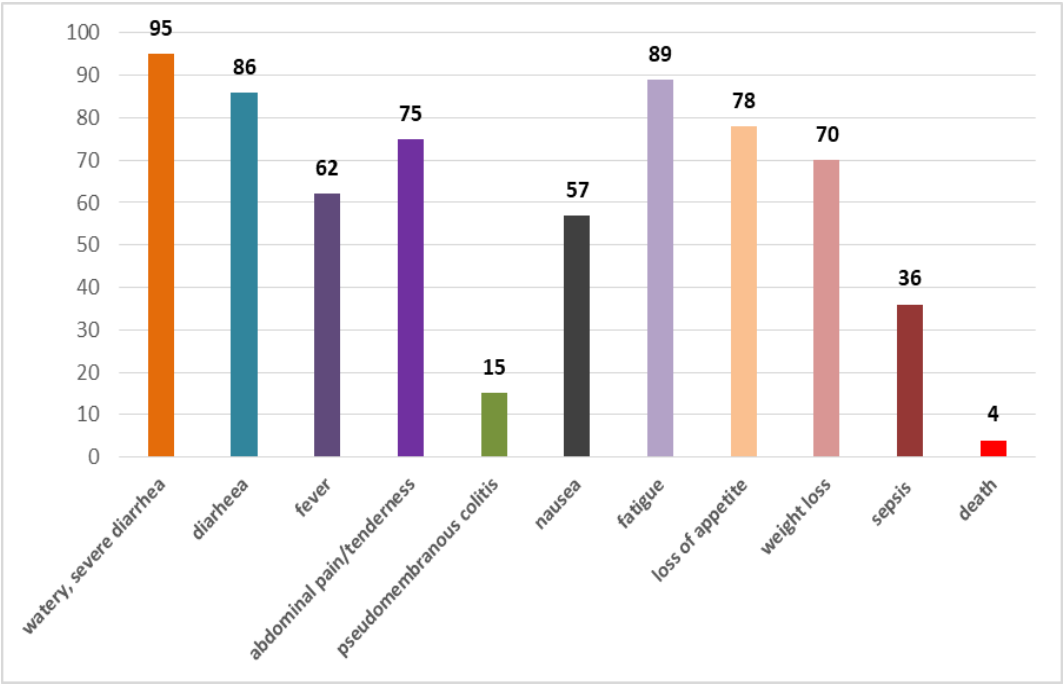

Fig. 5. Symptoms of the patients.

The recurrence rate of CDI was between 27%, 46 patients indicated having CDI more than once, and 20 could not remember. Of the respondents who had CDI more than once, 38 experienced CDI two to four times, while 9 experienced CDI five or more times (fig. 6).

To have therapeutic success for CDI, time to diagnosis is critical. Only 17% of respondents received their diagnosis within the first 48 hrs after the onset of their symptoms, 28% had their diagnosis established between 2 and 5 days, and 30% between 6 and 30 days.

The treatment was established according to the national and international protocols; 35% of the patients indicated that their symptoms resolved within seven days,

29% experienced resolution between 8 to 14 days, 25% were treated for longer than 14 days, and 10% did not experience resolution.

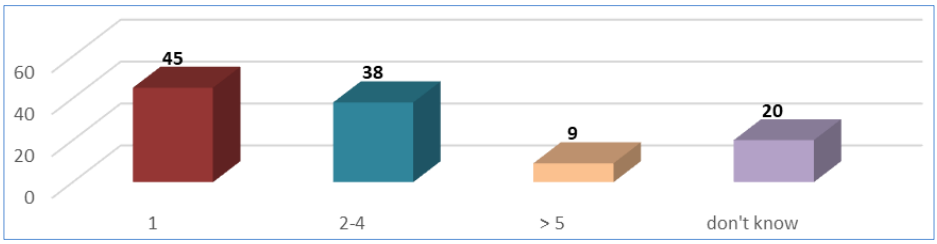

Fig. 6. Number of reinfections.

If antibiotic therapy is inefficient, fecal microbial transplantation (FMT) is reported to be a safe and effective alternative. A total of 3 patients out of 67 who had experienced subsequent CDI indicated treatment by FMT. The three patients who received FMT were treated previously with the complete scheme of antibiotics.

CDI can significantly impact patient's lives, especially on patients with cancer. We were interested in how this infection affects their quality of life (QoL). Our hospital psychologists did a three-question survey with scores ranging from 5 (being able to have a normal activity at home and work with no restrictions) to 1 (unable to take care of themselves and needs institutional or hospital care). Almost 25% of the patients reported that they could not take care of themselves, 35% were unable to work and needed assistance with everyday activities, 37% could have a regular activity at work and home. We observed that someone who experienced a reduced QoL before CDI experienced a more severe impact on their quality of life during active diseases.

Also, we were interested in the long-term impact of CDI on the lives of patients with neoplasia. We asked some participants about their current quality of life. The answers weren't very conclusive because the patients answered in different stages after recovering. Patients at their fourth, fifth reinfection reported QoL at a low level; many suffer from long-term consequences. Some of the patients are afraid of returning in collectivity and to eat regular food without testing every aliment, avoid taking certain medications such as antibiotics, and increase the use of probiotic supplements.

Patients changed preventive behaviors due to having RCDI. More than 40% said that they significantly increased their handwashing, 45% increased their house cleaning, use of bleach, antibacterial wipes, and gels compared to their behavior before being ill. Some avoided public places such as public bathrooms and going to restaurants.

DISCUSSION

Patients who contract CDI often experience painful, embarrassing, and debilitating symptoms, confirmed by our survey of patients with prior RCDI. Diarrhea, abdominal pain, and exhaustion were most likely to be rated as severe, whereas the presence and severity of other symptoms such as fever and headache were more

variable. Almost 39% of the respondents estimated that they could not perform routine daily activities because CDI varied considerably.

Increased hand washing and the use of soap and water for cleaning are evidence-based strategies demonstrated to reduce the acquiring and transmitting infections significantly (23).

Respondents who said they eat out less or avoid public places may impact their quality of life without changing their risk of future illness. Physicians should discuss these behaviors with their patients and assure them that they can still do their everyday activities with appropriate preventive behaviors, such as handwashing (24, 25).

In our cohort, almost 52% of our patients with RCDI were not concerned about avoiding certain medications. Medical literature has shown that proton pump inhibitors (PPIs) increase CDI risk, incident cases, and recurrences (26). Antibiotics also increase CDI risk. The risk is even greater when they are combined with a PPI (27, 28).

Physicians should consider the indications for antibiotic use and PPIs in patients with RCDI. They should empower patients to discuss the risks of RCDI and the benefits of antibiotic treatment; they should encourage their patients to ask about future antibiotic use. Healthcare providers should remind patients to consult with a specialist before stopping a medication because the risks outweigh the benefits (29).

More than 31% of respondents reported increasing their use of probiotics following CDI. Probiotics are microorganism-based products that have been proposed as a therapy to reconstitute disturbed gut microflora due to antibiotic use. The use of probiotics to prevent CDI is still controversial (29, 30).

Individuals currently treated for CDI or with a history but no current treatment for CDI have significantly worse QoL, more significant impairment in their daily activities, and reduced work productivity than those with no history of CDI (31).

The study demonstrates a significant impact of CDI on patients' self-assessed quality of life and the lasting impact that CDI has on patients with cancer, highlighting the importance of early diagnosis and immediate initiation of the treatment.

Nowadays, palliative care is a growing specialization that meets with dignity the needs of the patient with serious illness; this specialty aimed to provide end of life care for patients with advanced cancer and their families, focusing on relieving the pain, controlling the symptoms, and improving the quality of care for patients and their families (32). In our study, the patients with a low quality of life need palliative care assistance.

CONCLUSIONS

This study revealed that cancer patients with CDI experience many overwhelming symptoms which affect their QoL. Different therapeutic options are now available, and new treatments are currently in development to define the best strategies for managing this infection at the first episode and in case of further recurrences. All health professionals must ensure that patients receive appropriate prevention guidance and care. It's compulsory to develop measures for effective management of symptoms in order to improve the QoL.

Reducing infection rate and the spread of CD could benefit the patient's life and reduce the cost of CDI treatment and hospi-

talization. The multidisciplinary approach represents the key to success in the clinical management of CDI neoplastic patients.

AUTHOR CONTRIBUTIONS

All authors equally contributed to collecting of the data, analysis, drafting or revising the article, final approval of the version to be published, and agree to be accountable for all aspects of the work.

CONFLICTS OF INTEREST AND FUNDING

The authors declare no conflict of interest. This research received no external funding.

ETHICAL APPROVAL

The study was approved by the Ethics Committee of "Sf. Parascheva" Hospital of Infectious Diseases Iasi, Romania.

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NEWS

THE USE OF IVERMECTIN FOR COVID-19 IN PREGNANCY

Ivermectin, an antiparasitic agent used to treat parasitic infestations, inhibits the replication of viruses in vitro. The molecular hypothesis of ivermectin's antiviral mode of action suggests an inhibitory effect on severe acute respiratory syndrome coronavirus. Besides the lack of adequate clinical safety of ivermectin in human pregnancies, an additional risk factor to these women would be the coadministration of a P-glycoprotein inhibitor (e.g., clarithromycin, cyclosporin, diltiazem, erythromycin, felodipine, omeprazole, tamoxifen, and some statins to name a few). Studies conducted on mice have shown increased systemic exposure, organ concentrations, or toxicity of ivermectin, and that this combination will likely cause an increased level of ivermectin in the developing embryo or fetus, potentially inducing birth defects (Wise LD, Scialli AR. Ivermectin for COVID-19: Concerns during pregnancy. *Reprod Toxicol*. 2022; 107: 43 / doi: 10.1016/j.reprotox.2021.11.005).

DIETARY PATTERNS IN ROMANIAN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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DIETARY PATTERNS IN ROMANIAN PATIENTS WITH TYPE 2 DIABETES MELLITUS (Abstract): Adherence to healthy dietary patterns leads to improvements in metabolic health and prevents the risk of diabetes and obesity. Current guidelines underscore the importance of focusing on individualized dietary patterns in nutritional therapy of diabetics. The objectives of this study were to identify the dietary patterns in Romanian patients with Type 2 Diabetes Mellitus and to investigate the impact of these dietary patterns on associated anthropometric and metabolic parameters. **Material and methods**: A total of 118 adults were included in this cross-sectional, observational study. Demographic and lifestyle data, information on current pathologies and associated co-morbidities, anthropometric measurements and biological markers were obtained. Dietary intake was assessed via the EPIC food frequency questionnaire. **Results:** We identified three dietary patterns by principal component analysis, defined as *Prudent*, *Western* and *Traditional*. We found that patients belonging to the Western pattern had higher waist-to-hip ratio, higher glycated hemoglobin A1c and were significantly younger compared to those in the Prudent pattern. Alcohol intake was significantly higher in the *Western* compared to the *Prudent* pattern. **Conclusions:** This study demonstrates that the *Western* pattern, characterized by high intake of meat and meat products, eggs and soft drinks, has negative metabolic consequences in Romanian adult patients with T2DM, compared to the *Prudent* pattern. The *Traditional* pattern was closer to the *Prudent* one. **Keywords:** TYPE 2 DIABETES MELLITUS, DIET, DIETARY PATTERN, FOOD FREQUENCY QUESTIONNAIRE, NUTRIENTS.

Type 2 Diabetes Mellitus (T2DM) is a major and complex chronic disease and a worldwide public health issue. The epidemic of T2DM parallels the epidemic of obesity and the adoption of an unhealthy sedentary lifestyle. Epidemiological evidence demonstrates an unprecedented increase in the prevalence of diabetes around the world, from 125 million individuals with diabetes in 1990, to 537 million in 2021 and to an estimated 643 million by 2030. Of particular concern is that the increase incidence of diabetes disproportionately affects populations with a low socioeconomic status (1). In Romania, a recent, large epidemiological study has shown that

11.6% of the adult population has diabetes and 16.5% has prediabetes (2). The high prevalence of excess weight in the Romanian population may partly explain the high number of diabetic individuals since 31.1% of the Romanian adult population is overweight and 21.3% is obese (3).Thus, nutritional assessment (anthropometric, biochemical, clinical, dietary) is important to highlight the relationship with chronic diseases. Anthropometric parameters are predictive factors for cardio-metabolic diseases (4), obesity can be associated with certain infections (5) and malnutrition is frequent in surgical patients (6).

Numerous global changes in the modern lifestyle led to unhealthy eating behaviors (7). The most important changes are lack of physical activity and an increased preference for readily available calorie-rich foods with low nutritional value, leading to a shift toward a Western-like food pattern (1). Recent evidence showed that metabolic health is less influenced by each individual nutrient and is more driven by these global dietary patterns and the consumption of specific foods that have synergistic effects (8). For example, a typical Western diet is generally associated with insulin resistance, hyperlipidemia and pro-inflammatory status, thus increasing the risk of T2DM and cardiovascular diseases. On the other hand, a prudent or healthy diet is associated with a low risk of all-cause mortality (9), diabetes and obesity (10). In particular, there is compelling evidence demonstrating that, in individuals with type 2 diabetes, a healthy dietary pattern improves glycemic control and cardiovascular risk factors (11). Thus, current guidelines emphasize the need of medical nutrition therapy in diabetes to focus on dietary patterns and not on the individual components of the diet and should be individualized to the needs of the targeted population (12).

MATERIAL AND METHODS

Study design

A one-year (2017), cross-sectional observational study was conducted on a group of patients with T2DM who were admitted-for the annual medical evaluation at the Center for Diabetes, Nutrition and Metabolic Diseases at "Sf. Spiridon" County-Clinical Emergency Hospital from Iasi, Romania. The study was approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy Iasi (Registration no 2327/02.02.2017). All patients received information about the study and signed informed consent before any investigation commenced.

Study population

Adult patients (n=118) of which 55 males and 63 females, with an average age of 60.8±10.05 y, with diagnosed T2DM, and on diabetes nutrition therapy with or without oral anti glycemic medication, who provided informed consent were enrolled in this study. Therefore, children, patients with T2DM treated with insulin or GLP-1 receptor agonists, patients with type 1 diabetes mellitus, malnutrition, psychiatric diseases, inflammatory diseases, individuals who were institutionalized or intellectually unfit, or those unwilling to sign the informed consent were excluded from the study.

Data collection

For each patient, socio-demographic and lifestyle data (age, sex, area of residence, smoking status, alcohol intake) and personal medical history including associated co-morbidities were recorded. In addition, anthropometric parameters such as weight, height and waist circumference were taken, using standard methods with a

calibrated scale and stadiometer. Waist circumference (WC) was measured with a tape measure midway between the rib case and the upper edge of the iliac crest, around the abdomen, in a horizontal plane, parallel to the floor, at the end of a normal breath. Normal values were considered <80 cm in women and <94 cm in men, according to the International Diabetes Federation criteria for abdominal obesity (13). Body mass index (BMI) was calculated [Weight (kg) / Height^2 (m^2)] and patients were classified according to the World Health Organization criteria. We also calculated the waist-to-hip ratio (WHR), as the ratio between waist circumference and hip circumference, where normal values are less than 0.85 in women and less than 0.95 in men (14). Blood pressure was measured twice in all patients, using a mercury sphygmomanometer. Fasting blood samples were collected in all patients and the following laboratory tests were performed: plasma glucose, (measured by spectrophotometric method - colorimetric enzyme), glycated hemoglobin (HbA1c) (measured by HPLC assay), total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides (all measured by spectrophotometry, enzymatic method), C-reactive protein (measured by latex immunoturbidimetric assay).

Dietary assessment

Food intake (frequency and quantity) was estimated using the translated, adapted and validated version of the EPIC-Norfolk -FFQ (15). Participants were asked to specify the appropriate frequency of intake for each of the listed foods (133 items), choosing from the nine proposed frequency categories, from never before or less than once a month to more than 6 times a day. We used FETA program (16) for food analysis, which generated the daily average energy and nutrients intake and the daily average intake (in grams/day) of the 13 food groups generated in FETA: cereals and derivatives (which includes bread, pasta, polenta), potatoes, meat and derivatives, fish and derivatives, eggs, fruits, vegetables, milk and derivatives, nuts/seeds, sugar/snacks, fats/oil, soups/sauces, soft drinks (which includes all non-alcoholic beverages, sweetened coffee and tea) and, in addition, alcoholic beverages.

Statistical analysis

The applicability of the principal component analysis for the variables used was verified using the Keyser-Meyer Olkin test (KMO) and the Bartlett test. A KMO value of >0.5 was acceptable and a p value of <0.05 in the Bartlett test was considered significant. Only factors extracted based on the Kaiser criteria (Eigenvalue above 1.2) were considered. These factors were referred to as dietary patterns. To obtain an easy-to-interpret model, the correlation matrix was rotated (orthogonal, varimax). The factor loading over 0.3 was considered significant. If a food group had a loading factor of more than 0.3 in several components, then the food pattern was identified based on the highest value. After rotation, those food groups responsible for the highest load of factors for each factor were identified. Scores that defined each pattern were generated. These scores were the extent to which each individual differed from the average for each score (in standard deviations), therefore higher scores indicated a higher "adherence" to that pattern (17). Dietary patterns were named according to those food groups responsible for the highest load of factors corresponding to each factor. Chi-square test was used for categorical variables and ANOVA for

continuous variables to compare the differences between subjects identified with different food patterns. For non-homogeneous variables t-test was used. Bivariate correlation (Pearson r) was used to identify associations between continuous variables. All data were analyzed using *SPSS Statistics version 20.0, for Windows.*

RESULTS

Subjects general characteristics

We analyzed a group of 118 patients. The general characteristics of the study population are presented in first table.

TABLE I

General characteristics of the study population

Characteristics	Total (n=118)	Men (n=55, 46.6%)	Women (n=63, 53.4%)	*p*
Age (years); mean±SD	60.85±10.05	59.09±10.78	62.38±9.18	NS
Residence (urban); n, %	83, 70.3	41, 49.4	42, 50.6	NS
Duration of DM (years); mean, 95% CI	5.49 (4.5, 6.4)	5.45 (3.8, 7.01)	5.53 (4.3, 6.6)	NS #
Smoking; n, %	11, 11.1	9, 9.1	2, 2	**0.021**
Education; n, %	Higher education 24, 23.8%	Highschool 9, 8.9%	Primary education 8, 7.9%	NS
Occupation; n, %	Retired 30, 25.4%	Active workers 10, 8.5%	Others (unemployed, household) 12,10.2%	**0.022**
Alcoholic beverages intake (g/day); mean, 95% CI	86.67 (62.91, 110.43)	125.8 (86.2, 165.39)	52.51 (26.31, 78.72)	**0.003#**
BMI (kg/m^2); mean±SD	31.96±5.76	31.18±5.94	32.65±5.55	NS
WC (cm); mean±SD	105.72±13.05	106.36±13.18	105.16±13.02	NS
WHR; mean±SD	0.97±0.07	0.99±0.05	0.95±0.08	**0.004#**
Normal weight; n, %	6, 5.1	3, 5.5	3, 4.8	NS
Overweight; n, %	42, 35.6	24, 43.6	18, 28.6	
Obesity; n, %	70, 59.3	28, 50.9	42, 66.7	
Systolic BP (mmHg); mean±SD	141.07±19.96	143.45±19.79	138.98±20.03	NS
Diastolic BP (mmHg); mean±SD	84.24±9.9	85.04±10.45	83.54±9.43	NS
Diagnostic of hypertension; n, %	86, 74.1	37, 43	49, 57	NS
Fasting plasma glucose (mg/dl); mean±SD	143.69±36.23	139.84±33.91	147.06±38.09	NS
HbA1c (%); mean±SD	6.94±1.22	6.97±1.35	6.92±1.1	NS
Total cholesterol (mg/dl); mean±SD	200.69±49.37	196.53±45.66	204.39±52.54	NS
HDL-cholesterol (mg/dl); mean±SD	47.75±13	43.49±9.68	51.58±14.43	**0.001#**
LDL-cholesterol (mg/dl); mean±SD	128.27±44.73	125.24±42.85	130.95±46.52	NS
Triglycerides (mg/dl); mean±SD	170.87±115.98	189.33±151.19	154.5±69.03	NS #
CRP (mg/dl); mean, 95% CI	0.43 (0.34, 0.52)	0.4 (0.26, 0.55)	0.45 (0.33, 0.58)	NS

Equal variances not assumed; *p*, significant difference between sexes; NS, non-significant; BMI, body mass index; WC, abdominal circumference; WHR, waist-to-hip ratio; HbA1c, glycated hemoglobin; CRP, C-reactive protein; SD, standard deviation; CI, confidence interval; SD, standard deviation

Daily intake of energy, macronutrients and food groups

Tab. II shows the average intake of energy and macronutrients in the study population, as derived from FETA analysis of the EPIC questionnaire, for the entire study population and with comparison between sexes. We found significant differences between sexes for consumption of total lipids (32.67% in women, *vs.* 30.59% in men, p=0.029) and polyunsaturated fatty acids (PUFA) (6.7% in women *vs.* 5.96% in men, p=0.006). The analysis also generated the daily average intake of the 13 food categories defined by FETA. Although there were some differences in the consumption of food groups between sexes, these were not statistically significant.

TABLE II

Average daily intake of calories, macronutrients and food groups

Energy, macronutrients, food group intake	Total (n=118)	Men (n=55, 46.6%)	Women (n=63, 53.4%)	*p*
Energy and macronutrients intake mean±SD				
Energy (kcal/day)	1672.60±572.4	1754.99±613.9	1600.67±527.9	NS
Carbohydrates (%)	48.21±5.9	47.76±5.8	48.60±5.9	NS
Lipids (%)	31.66±5.3	30.51±4.8	32.67±5.6	**0.029**
Proteins (%)	19.25±3.2	19.15±3.6	19.33±2.8	NS
SFA (%)	11.03±2.7	10.71±2.4	11.30±2.8	NS
MUFA (%)	11.17±2.5	10.73±2.0	11.56±2.8	NS
PUFA (%)	6.36±1.4	5.96±1.2	6.70±1.6	**0.006**
Fiber (g/day)	18.03±7.2	17.63±7.5	18.38±7.0	NS
Cholesterol (mg/day)	336.79±123.0	366.55±132.2	317.70±123.9	NS
Food group intake (g/day) mean±SD				
Cereals and derivatives	225.52±120.2	222.60±121.4	228.08±120.2	NS
Eggs	23.21±14.9	25.67±15.2	21.07±14.4	NS
Fats/oil	12.69±7.4	11.78±7.2	13.49±7.5	NS
Fish and derivatives	31.6±21.4	33.86±24.0	29.63±18.9	NS
Fruits	270.16±138.5	259.10±144.3	279.83±133.6	NS
Meat and derivatives	124.21±69.0	137.21±74.3	112.87±62.5	NS
Milk and derivatives	235.85±145.3	219.27±146.0	250.34±144.4	NS
Soft drinks	382.51±274.9	372.15±271.8	391.55±279.4	NS
Nuts, seeds	6.23±11.5	4.24±6.9	7.97±14.2	NS
Potatoes	64.77±36.6	62.33±36.6	66.90±36.8	NS
Soups and sauces	215.82±135.1	239.99±163.3	194.72±101.3	NS
Sugar/snacks	13.44±15.7	16.32±19.79	10.93±10.52	NS
Vegetables	288.14±149.32	295.87±162.92	281.39±137.34	NS

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SD, standard deviation; *p*, difference between sexes; NS, non-significant

When correlation analyses were conducted between daily intake of food groups and clinical and metabolic parameters, there was a small, but statistically significant inverse correlations between total cholesterol and consumption of nuts and

seeds (r=-.199, *p*=.032). WHR was negatively correlated with consumption of nuts and seeds (r=-.26, *p*=.007) and positively correlated with intake of soups and sauces (r=.262, *p*=0.006). Fasting plasma glucose correlated negatively with meat and derivatives intake (r=-.208, *p*=.024). CRP was positively correlated with the amount of sugar/snacks (r=.189, *p*=.041) and diastolic blood pressure was positively correlated with fish and derivatives intake (r=.198, *p*=.003) (tab. III).

TABLE III

Correlations between daily average intake of different food groups and clinical and metabolic parameters

Food groups	Pearson's correlation coefficient										
	BMI	WC	WHR	SBP	DBP	FBG	HbA 1c	Total chol	LDL chol	Tg	CRP
Cereals and derivatives	-.050	-.077	-.138	.094	.101	-.030	.062	- 0.018	.024	-.009	-.043
Eggs	-.044	-.067	.020	-.076	-.067	-.061	-.003	- 0.012	.053	-.043	-.089
Fats / oil	-.130	-.166	-.140	.049	.135	-.160	-.033	0.072	.119	-.019	-.066
Fish and derivatives	-.140	-.135	-.119	.110	**.198***	-.030	-.015	- 0.020	.025	-.034	-.128
Fruits	-.066	-.138	-.034	.003	.046	-.083	-.007	- 0.114	-.062	-.029	-.127
Meat and derivatives	-.074	-.084	.020	-.033	-.016	-.208*	-.036	- 0.072	-.008	-.002	-.104
Milk and derivatives	.041	.064	-.059	.060	.045	-.056	-.011	0.038	.079	-.027	-.175
Soft drinks	.177	.079	.049	-.034	-.062	.023	.000	- 0.020	.023	.030	.093
Nuts, seeds	-.078	-.120	**-.260****	.167	.024	-.009	-.138	**- 0.199***	**-.193***	-.078	-.059
Potatoes	-.029	-.074	-.113	.040	-.044	-.148	-.045	- 0.045	.018	.012	-.147
Soups and sauces	.002	.014	**.262****	-.105	-.123	-.036	-.008	- 0.143	-.057	-.048	-.052
Sugar / snacks	-.150	-.055	.006	.024	.062	-.180	-.074	0.061	.140	-.141	**.189***
Vegetables	-.050	-.030	.179	.102	.049	.022	.077	- 0.147	-.080	-.015	.020

*p<0.05; **p<0.01; BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; Tg, triglycerides; CRP, C-reactive protein.

Dietary patterns characterization

There was a high correlation between food groups (KMO = 0.72). Using the principal component analysis, and selecting the varimax rotation, based on the Kaiser criteria and the scree plot (Fig. 1), three food patterns were generated. In the initial solution, these patterns had an Eigenvalue value > 1.2 and accounted for 53.6% of the variance.

The first factor identified that accounted for 29.24% of the variance, was characterized by an increased intake of fat, oil, cereals, potatoes, vegetables, fish, nuts, seeds and fruits. This was named the *Prudent* pattern. The second factor that represented 13.85% of variance and was characterized by increased intake of meat and meat products, eggs and soft drinks, was named the *Western* pattern. Although the sugar/snacks

load did not exceed 0.3, we elected to include it in this pattern since it had the highest loading value, among all three patterns. The third factor that accounted for 10.5% of the variance and was characterized by high intake of milk and derivatives, soups/sauces and vegetables, was named the *Traditional* pattern (tab. IV, fig. 2).

There was a significant difference between patterns, with 21.8% men and 42.9% women included in the *Prudent* pattern, whereas 40% men and 15.9% women belonged to the *Western* pattern (p=0.002). Also, there were significantly more women than men in the *Traditional* compared to the *Western* pattern (p=0.004). Likewise, patients included in the *Prudent* pattern were significantly older than those included in the *Western* pattern (p=0.02). Alcoholic beverages intake was significantly higher in the *Western* compared to the *Prudent* pattern (p=0.005) and patients included in the *Western* pattern had a significantly higher WHR compared to those included in the *Prudent* pattern (p=0.013). HbA1c value was higher in the *Western* pattern (p=0.031) (tab. V).

When correlations analyses between pattern scores and characteristics of the study population were run, there was a significant direct relationship between the *Prudent* pattern and age. WHR was negatively associated with *Prudent* pattern and positively with *Western* pattern (tab. VI).

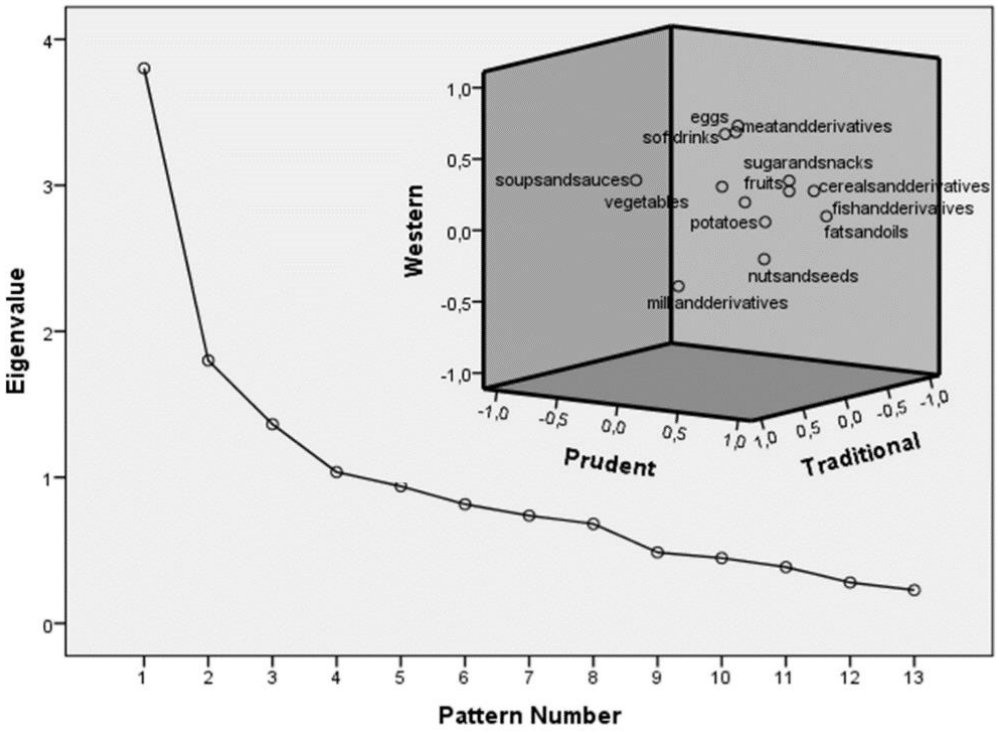

Fig. 1. Number of dietary patterns identified based on Eigenvalue criteria and analyzed by rotated space matrix

TABLE IV

Factor loadings and dietary patterns derived by PCA

	Factor 1 *Prudent* pattern	Factor 2 *Western* pattern	Factor 3 *Traditional* pattern
Fats/oil	**.870**	.121	-.120
Cereals/derivatives	**.761**	.286	-.128
Fish/derivatives	**.639**	.289	-.009
Potatoes	**.607**	.104	.230
Fruits	**.566**	.265	.411
Nuts/seeds	**.376**	-.225	-.090
Meet/derivatives	.278	**.726**	.084
Soft drinks	.133	**.644**	.027
Eggs	.091	**.626**	-.160
Sugar/snacks	.238	**.240**	-.581
Soups/sauces	-.122	.394	**.718**
Milk/derivatives	.192	-.324	**.664**
Vegetables	.457	.380	**.526**

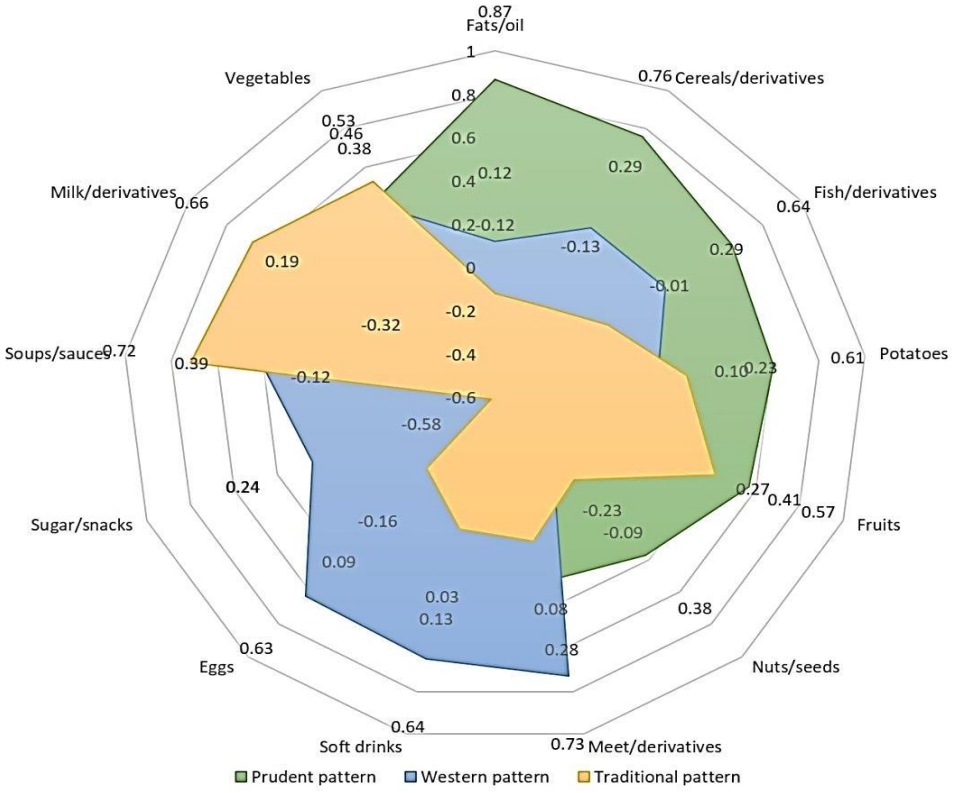

Fig. 2. The three dietary patterns identified

TABLE V

Comparison of the characteristics of the study population included in dietary patterns

Characteristics	*Prudent* pattern (n=39, 33.1%)	*Western* pattern (n=32, 27.1%)	*Traditional* pattern (n=47, 39.8%)	*p*
Men; n, %	12, 21.8	22, 40	21, 38.2	**0.002#**
Women; n, %	27, 42.9	10, 15.9	26, 41.3	**0.004***
Age (years); mean±SD	63.9±9.23	57.44±9.64	60.64±10.38	**0.02#**
Residence (urban); n, %	28, 33.7	23, 27.7	32, 38.6	NS
Duration of DM (years); mean, 95% CI	5.38 (3.82, 6.94)	4.51 (2.79, 6.23)	6.25 (4.61, 7.89)	NS
Smoking; n, %	4, 36.4	5, 45.5	2, 18.2	NS
Alcoholic beverages intake (g/day);mean, 95% CI	44.46 (23.16, 65.76)	140.96 (86.42, 195.49)	84.73 (42.55, 126.92)	**0.005#**
Education; n, %	Higher education 24, 23.8%	Highschool 9, 8.9%	Primary education 8, 7.9%	NS
Occupation; n, %	Retired 30, 25.4%	Active workers 10, 8.5%	Others 12, 10.2%	**0.022**
BMI (kg/m^2); mean±SD	31.04±5.87	32.65±5.23	32.26±6.02	NS
WC (cm); mean±SD	103.18±13.46	107.91±11.38	106.34±13.68	NS
WHR; mean±SD	0.95±0.07	0.99±0.05	0.98±0.07	**0.013#**
Normal weight; n, %	3, 50	0, 0	3, 50	NS
Overweight; n, %	16, 38.1	11, 26.2	15, 35.7	
Obesity; n, %	20, 28.6	21, 30	29, 41.4	
Systolic BP (mmHg); mean±SD	145.13±24.38	136.78±16.29	140.62±17.79	NS
Diastolic BP (mmHg); mean±SD	86.97±10.2	82.53±9.4	83.13±9.72	NS
Diagnostic of hypertension;n, %	33, 38.4	20, 23.3	33, 38.4	NS
Fasting plasma glucose (mg/dl); mean±SD	139.9±33.4	147.38±33.4	144.34±40.53	NS
HbA1c (%); mean±SD	6.65±0.92	7.28±1.37	6.96±1.28	**0.031#**
Total cholesterol (mg/dl); mean±SD	210.64±55.09	198.81±48.27	193.68±44.53	NS
HDL-cholesterol (mg/dl); mean±SD	49.26±13.64	45.76±13.1	47.73±12.48	NS
LDL-cholesterol (mg/dl); mean±SD	135.8±51.9	126.38±44.35	123.38±38.29	NS
Triglycerides (mg/dl); mean, 95% CI	170.95 (145.13, 196.77)	188.06 (119.51, 256.62)	159.47(137.56, 181.38)	NS
CRP (mg/dl); mean, 95% CI	0.38 (0.21, 0.55)	0.48 (0.32, 0.64)	0.43 (0.27, 0.59)	NS

#, sig. between *Prudent* and *Western* pattern; *, sig. between *Western* and *Traditional* pattern BMI, body mass index; WC, abdominal circumference; WHR, waist-to-hip ratio, HbA1c, glycated hemoglobin; CRP, C-reactive protein; SD, standard deviation; CI, confidence interval; *p*, difference between sexes; NS, non- significant

TABLE VI

Correlations between dietary pattern scores and clinical/biological variables

Variables		Pearson's coefficient		
		***Prudent* pattern**	***Western* pattern**	***Traditional* pattern**
Clinical parameters	Age (years)	**.199***	-.163	-.022
	BMI (kg/m^2)	-.138	.014	.069
	WC (cm)	-.166	-.008	.049
	WHR	**-.232***	**.190***	.142
	SBP (mm Hg)	.154	-.116	-.017
	DBP (mm Hg)	.162	-.087	-.061
Biological parameters	FPG (mg/dl)	-.116	-.073	.027
	HbA1c (%)	-.035	.023	.037
	Total cholesterol (mg/dl)	-.029	-.057	-.118
	LDL cholesterol (mg/dl)	.021	.023	-.081
	HDL cholesterol (mg/dl)	-.068	-.063	-.098
	Triglycerides (mg/dl)	-.039	-.011	.024
	CRP (mg/dl)	-.118	.041	-.152

*p<0.05; BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; CRP, C-reactive protein.

DISCUSSION

Eating habits are difficult to change even when patients diagnosed with T2DM are motivated to pursue such changes (18). Our study population presents typical characteristics of individuals with T2DM: mature adults (60.85±10.05 years old), with excess body weight (94.9%), hypertension (74.1%) and borderline glycemic control (HbA1c=6.9±1.2). The average duration of the disease in our patients was 5.5 years and they were provided with nutritional therapeutic education, according to current guidelines for the management of diabetes. Indeed, when we assessed the energy and macronutrient intake of our study population, we observed a balance between macronutrients as percentage of total caloric intake, consistent with the recommendations from food guidelines (12). They reported on average daily intake of 48.2% carbohydrates, 31.6% total lipids and 19.2% protein. However average daily energy intake seen in our study was lower than the recommendations for the general population.,explained either by the typical underestimation seen with self-reported methods of investigation (19) or by the fact that patients with excess weight were reducing caloric intake. A close inspection of the data from the analysis of EPIC questionnaire in FETA revealed several nutritional issues in our patients: low intake of fiber (18 g/day), high consumption of SFA

(11% of total caloric intake) and increased dietary cholesterol (336.8 mg/day), although these observations were consistent with FAO reports (20).

Usually, the terms used in the literature to describe dietary patterns are: "healthy or prudent" that are represented by foods or food groups such as fruits, fish, poultry, whole grains and vegetables) (21); "Western", represented by foods or food groups such as red meat, processed meat, sweets, sugar, snacks, high-fat dairy products and French fries) (22), and "traditional" that is represented by foods or food groups that are country specific, such as white rice, beans, beer, processed and fresh meats, for a common Brazilian meal (23), or such as vegetables, seaweeds, soybean products, fish, fruits, miso soup and green tea, for traditional Japanese pattern (24). Out of the three commonly used dietary patterns, *Western* is the one of concern given that its representative food groups have been associated with high prevalence of chronic diseases (25).

The first pattern we identified is the *Prudent* pattern, characterized by an increased intake of fats, oil, cereals, potatoes, vegetables, fish, nuts, seeds, and fruits. The second is the *Western* pattern, characterized by high intake of meat and meat products, eggs and soft drinks. The third is the *Traditional* pattern, characterized by a high intake of milk and derivatives, soups/ sauces and vegetables. A characteristic of our *Western* pattern was high intake of soft drinks. Fructose-sweetened beverages have many adverse effects such as deposition of visceral fat, increased lipid parameters and increased blood pressure and decreased insulin sensitivity in overweight and obese people (26). We found that patients included in the Western pattern had higher WHR and HbA1c values compared to those in the *Prudent* pattern. These improved health outcomes in patients included in the Prudent pattern could be explained by fish consumption, known for its high contents in omega 3 PUFA, nuts consumption which are high in PUFA and MUFA. Despite the high fat and energy content, regular nut consumption was not associated with increased obesity, but instead it offered benefits in weight control (27). These data confirm our results showing a negative correlation between nut intake and WHR. Furthermore, the *Prudent* pattern is characterized by high intake of fruit, vegetables, cereals that may exert hypoglycemic effects and decrease insulin response via increase in fiber, specific vitamins and minerals, flavonoids, carotenoids (28).

Our results did not show significant associations between dietary patterns and blood pressure, which is consistent with other studies (29).

Dietary analyses and metabolic parameters indicate that *Traditional* pattern is much closer to the *Prudent* rather than *Western* pattern in terms of health outcomes. Moreover, soups and sauces that are included in this pattern are part of the traditional Romanian cooking with "borș", a liquid resulting from wheat bran fermentation, and significant amounts of vegetables and greens. Patients included in the Western pattern, were significantly younger than those who adhered to the *Prudent* pattern. It might be that younger individuals are more exposed to the Western influence in their dietary choices or prefer fast foods. This is important and worrisome since adherence to *Western* pattern from an early age may result in increased risk for T2DM. On average, prudent diets were more prevalent in older adults compared to younger adults (30). In

general, food pattern studies have found that older people, and women in particular, consume less red meat, whole milk and other fatty foods and consume more fruits and vegetables than younger people (31). Higher standard of living, higher education, nutritional knowledge has been associated with a healthy/"prudent" dietary pattern (32). It has been shown that people with lower education have more financial restraints and limited access to healthy food (33). In our study, individuals with higher education degrees and those currently retired, have adhered to the Prudent pattern, whereas those with lower education and employed tended to adopt the Western pattern.

The Romanian Dietary Guidelines promotes a healthy nutrition based on consumption of cereals, fruits, vegetables, foods with low fat content and reduction of processed and high sugar foods (34). Dietary patterns recently studied in the general Romanian population showed a Prudent pattern characterized by large intake of fish, vegetables, legumes, fruits and soymilk, adopted by elderly women and those with a higher education level (35).

Although we have included a large number of T2DM patients, our study has several limitations such as the observational design and lack of a control group consisting of individuals without diabetes. Also, since most subjects were from the urban areas, we cannot make a clear distinction between urban and rural dietary patterns. These patterns are from the Iasi County, situated in the north-eastern region of the country thus data from other regions of the country will be useful in order to get a broader picture of dietary patterns in this population.In addition, our study cannot identify the effects of different oral hypoglycemic treatments or other pathologies that may have influenced patients' food selections.

Also, our subjects did not complete a physical activity questionnaire, thus the extent to which physical exercise might have influenced food selection is difficult to ascertain. Notwithstanding these limitations, our findings demonstrate the value of dietary patterns in nutritional therapy in T2DM in Romanian population.

CONCLUSIONS

This is the first clinical observational study to identify dietary patterns in Romanian patients with type 2 diabetes. Using principal component analysis on 13 food groups derived from the EPIC questionnaire has allowed us to identify three food patterns: *Prudent*, *Western* and *Traditional*. The *Western* pattern was associated with detrimental consequences on abdominal adiposity and glycemic control compared to the *Prudent* pattern. The *Traditional* pattern seems closer to the *Prudent*, than the *Western* pattern. Studies on food patterns, food selections and dietary preferences in patients with metabolic disorders including diabetes in Romania are scarce. Given the differences in country specific food patterns and their impact in disease management, identifying food patterns in patients with Type 2 Diabetes Mellitus could help tailor educational programs in order to target diet and overall lifestyle optimization meant to slow down disease progression and its complications.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding the scientific research.

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THE EFFECTS OF CARDIAC REHABILITATION ON RESTING HEART RATE AND BLOOD PRESSURE IN HEART FAILURE PATIENTS

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THE EFFECTS OF CARDIAC REHABILITATION ON RESTING HEART RATE AND BLOOD PRESSURE IN HEART FAILURE PATIENTS (Abstract): Our study analyzed the efficiency of cardiac rehabilitation programs (CRP) in decreasing resting blood pressure (RBP) and heart rate (RHR), two important prognosis tools for heart failure (HF) patients. **Materials and methods:** A retrospective cohort study was conducted in a regional hospital over a 3-year period. A total of 137 HF patients, who benefited from a physical based CRP with a mean duration of 10 days, were divided into 2 groups according to the severity of their symptoms. All the patients underwent physical and paraclinical evaluation, including cardiopulmonary testing before starting the rehabilitation. RHR and RBP were measured before and at the end of the program. **Results:** Most of the patients in this study were men (73.7%) and hypertensive (55.5%), while the mean age of study's participants was 65.24 ± 9.76 years. A significant decrease was observed in the RHR after rehabilitation (81.37 ± 8.65 bpm *vs*. 77.79 ± 6.77 bpm, p = 0.004). Significant improvements were also observed in resting systolic blood pressure (BP), the mean values decreasing with more than 4% after rehabilitation (122.06 ± 16.2 mmHg *vs*. 116.91 ± 12.14 mmHg, p = 0.001). Mean diastolic BP also decreased at the end of the program (73.81 ± 12.05 mmHg *vs*. 72.8 ± 10.36 mmHg, p = 0.28). Though non-significant, age and baseline HR and BP appeared to be important therapy response predictors. Younger patients and those who had higher HR and BP at the beginning of the program benefited most from CRP. **Conclusions:** A short term CRP proved to be efficient in reducing RHR and RBP of HF patients, while age, baseline HR and baseline BP may be potential therapy response predictors. **Keywords:** HEART FAILURE, CARDIAC REHABILITATION, RESTING HEART RATE, RESTING BLOODPRESSURE.

Heart failure (HF) is the predicted outcome of most cardiovascular diseases (CVD), with an incidence and prevalence which are getting closer to epidemical proportions (1, 2). Consequently, the economic pressure generated by HF upon systems

worldwide is constantly rising, the associated treatment costs being estimated to increase up to 108 billion dollars per year (3, 4).

Effort capacity diminution is one of the main health problems for people suffering from HF. It is also a cause of quality-of-life (QoL) impairment and an important risk factor and prognosis tool for hospitalization and mortality (5, 6). The physiopathology behind this is complex, systolic and diastolic dysfunction having central roles. To maintain the cardiac output, despite a reduced stroke volume, the cardiovascular system activates certain compensatory mechanisms during rest, so that they become insufficient during effort. One of these mechanisms is tachycardia, effort capacity decreasing proportionally with the resting heart rate (RHR). Therefore, its control is an important target when treating these patients, helping increase their quality of life (QoL) and reduce their mortality. Furthermore, blood pressure (BP), is an independent predictor of effort capacity for HF patients and an important risk factor for cardiovascular events, as well, its control being equally important (7, 8).

Apart from pharmacological therapies, which proved their efficiency on reducing the mortality and morbidity rates of HF patients, cardiovascular rehabilitation programs (CRP) are a treatment option increasingly frequently used. Their efficiency is widely recognized especially for patients with moderate-severe symptoms (NYHA II and III), regardless the ejection fraction subtype, the latest guidelines recommending them for all the patients (7, 9). Considering its proved ability to reduce sympathetic stimulation, to down-regulate the funny channels, HCN4 and its effects on neuroendocrinological and vascular level, physical training may produce an important decrease in BP and RHR and consequently an increase of effort capacity and QoL (7, 10).

Our study aimed to assess the clinical and paraclinical profile of HF patients, who underwent a CRP at a regional hospital and to determine its efficiency in reducing resting BP and HR.

MATERIAL AND METHODS

We performed a retrospective cohort study, which included patients diagnosed with NYHA I-III heart failure, who underwent a CRP with a mean duration of 10 days, at a local hospital in Iasi, during a 3-year period. HF diagnosis was defined according to the European Guidelines for Heart Failure, using clinical, echocardiographic and paraclinical (NT-proBNP) criteria. The exercise program was adapted to each patient, using the cardiopulmonary testing and consisted of daily aerobic exercises.

Our cohort included 137 patients with HF, who were divided into two groups according to the severity of their symptoms: group A included patients with NYHA I and II heart failure and group B with more severe symptoms (NYHA III). We excluded from the study those patients for whom we did not have access to all the information needed, especially the resting BP (RBP) and HR before and after the rehabilitation and those patients with NYHA IV HF, who were not able to undergo an exercise-based CRP. All the information was collected anonymously using the hospital's and the rehabilitation department's database. The study protocol received the approval of the ethics committee and the hospital board.

All the patients included into the study underwent complete clinical examination and basic paraclinical testing, consisting of medical history and clinical evaluation, echo-cardiography study, biochemical testing, hemodynamical assessment at the be-

ginning and at the end of the rehabilitation period. Laboratory testing included kidney and hepatic function, fasting glycemia, complete blood count and lipidic profile.

Statistical analysis

The statistical analysis was performed using *SPSS 11.0 software.* For the continuous variables, we calculated the maximum, minimum and the mean values and we also determined the standard deviation. The categorial values are represented as absolute values and percentage. The statistical significance was defined by a $p < 0.05$ and was evaluated using the *t-student test* and the *chi square test.*

RESULTS

The main characteristics of our study group are presented in table I. Most of the included patients were men (73.7%) and hypertensive (55.5%). Apart from hypertension, dyslipidemia was the most common cardiovascular risk factor among these patients (38.7%), followed by diabetes (34.4%) and obesity (23.35%). We did not observe any statistical correlation between the severity of HF and the presence of anemia ($p = 0.507$), patients' gender ($p = 0.194$) or cardiovascular surgical history. Concerning the effects of CRP on the RHR and BP, significative impact was observed in both groups.

TABLE I.
Clinical and paraclinical characteristics of the two-study group

Parameter	Total patients – 137	Group A – 81 (59%) patients	Group B – 56 (41%) patients	p
Mean age (y. o.)	65.24 ± 9.76	64.04 ± 9.48	66.96 ± 9.99	0.04
Gender report				
○ Men	101 (73.7%)	63 (77.7%)	38 (67.85%)	0.19
○ Women	36 (26.3%)	18 (22.3%)	18 (32.15%)	
Surgical history				
○ Revascularization	58 (42.33%)	36 (44.4%)	22 (39.28%)	
○ Valvuloplasty	54 (39.41%)	31 (38.27%)	23 (41.07%)	0.07
○ Both	14 (10.21%)	8 (9.87%)	6 (10.71%)	
○ None	11 (8.05%)	6 (7.4%)	5 (8.92%)	
GFR (mL/min/1.73m^2)	66.83 ± 16.55	68.86 ± 13.73	63.87 ± 19.72	0.06
Anemia				
○ Hb<12mg/dL (women)	91 (66.42%)	52 (64%)	39 (69.6%)	0.53
○ Hb<13mg/dL (men)				
Lipid profile				
○ Total cholesterol (mg/dL)	158.59 ± 43.44	159.02 ± 46.04	157.96 ± 39.67	0.43
○ LDL-C (mg/dl)	87.45 ± 33.77	87.65 ± 36.06	87.15 ± 30.48	0.45
○ HDL-C (mg/dl)	44.35 ± 15.004	43.14 ± 16.69	46.11 ± 12.07	0.17
○ Triglycerides (mg/dl)	133.91 ± 65.5	141.15 ± 69.01	123.46 ± 59.11	0.05
Diabetes	47 (34.3%)	30 (37.03%)	17 (30.35%)	0.41
Mean RHR (bmp)	81.37 ± 8.65	82.04 ± 9.37	80.41 ± 7.46	0.07
Mean resting BP (mmHg)				
○ Systolic	122.06 ± 16.2	123.71 ± 17.36	119.67 ± 14.17	0.06
○ Diastolic	74.27 ± 11.29	73.81 ± 12.05	74.92 ± 10.17	0.28

BP = blood pressure, bpm = beats per minute, GFR = glomerular filtration rate, Hb = hemoglobin, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, RHR =resting heart rate, y. o. = years old

Resting heart rate

At the end of the CRP, 93 (67.8%) of the patients presented lower HR values during rest, the mean values decreasing by 4.4% (3.58/min) for entire group, compared to the beginning of the study ($p < 0.05$). In group A, we observed better results, 60 patients (74.07%) presenting lower RHR at the end of the program, compared to 33 (58.92%) in group B ($p = 0.061$). Furthermore, we observed a higher difference between the mean values of RHR before and after the rehabilitation, in the first group (5.56%, $p < 0.05$ *vs.* 2.68%, $p < 0.05$), the difference being statistically significant ($p < 0.05$) (tab. II).

TABLE II.

RHR response to rehabilitation

	Total	Group A	Group B
Baseline (bmp)	81.37 ± 8.65	82.04 ± 9.37	80.41 ± 7.46
After rehabilitation (bpm)	77.79 ± 6.77	77.48 ± 7.05	78.25 ± 6.37
Difference (bpm)	3.58 (4.4%)	4.56 (5.56%)	2.16 (2.68%)
P value	**0.00463**	**0.00302**	**0.00511**

Bpm=beats per minute

Systolic blood pressure (SBP)

At the end of the study, SBP varied among the included patients between 92 and 156 mmHg, the mean value decreasing from 122.06 ± 16.2 mmHg to 116.91 ± 12.14 mmHg, the 5.15 mmHg (4.22%) the difference being statistically significant ($p = 0.001$). The statistical analysis for each group, revealed a decrease of the mean values from 123.71 ± 17.36 mmHg to 117.28 ± 11.93 mmHg, for group A, respectively from 119.67 ± 14.17 mmHg to 116.37 ± 12.53 mmHg for group B, the differences of 6.43 mmHg (5.19%), respectively 3.3 mmHg (2.76%), being statistically significant ($p = 0.003$, respectively $p = 0.009$) (tab. III). Moreover, a decrease in resting SBP was observed among 92 patients (62.5%), 52 from group A and 35 from group B, which represented 70.37%, respectively 62.5% from each group, the difference not having statistical significance ($p = 0.334$).

TABLE III.

SBP response to rehabilitation

	Total	Group A	Group B
Baseline (mmHg)	122.06 ± 16.2	123.71 ± 17.36	119.67 ± 14.17
After rehabilitation (mmHg)	116.91 ± 12.14	117.28 ± 11.93	116.37 ± 12.53
Difference (mmHg)	5.15 (4.22%)	6.43 (5.19%)	3.3 (2.76%)
P value	**0.00158**	**0.00339**	**0.0097**

Diastolic blood pressure (DBP)

The CRP was not as efficient on the DBP as it was on the systolic one. The statistical analysis revealed a decrease of the mean values of DBP for the entire study group of only 0.7 mmHg which was not significant ($p = 0.28$). Analyzing the data for each group, we obtained the same

results. The mean values decreased with 1.01 mmHg in group A, respectively with 0.26 mmHg in group B (p = 0.28, respectively p = 0.88) (tab. IV). Furthermore, lower DBP values were observed among 69 patients (50.36%), 42 (51.85%) from group A and 27 (48.21%) from group B (p = 0.625).

TABLE IV.
DBP response to rehabilitation

	Total	Group A	Group B
Baseline (mmHg)	73.81 ± 12.05	74.92 ± 10.17	74.27 ± 11.29
After rehabilitation (mmHg)	72.8 ± 10.36	74.66 ± 8.7	73.56 ± 9.73
Difference (mmHg)	1.01 (1.36%)	0.26 (0.34%)	0.7 (0.94%)
P value	0.28	0.88	0.28

DISCUSSION

CVD remain the first cause of mortality worldwide, HF being the main outcome, with its incidence and prevalence increasing, especially in low or middle-income regions (11, 12). The efficiency of CRP in improving the QoL, reducing the morality and the hospitalization rate of HF patients, has already been tested and confirmed by numerous studies so that this type of treatment represents a class IA indication in the current guidelines (9, 13). Even if numerous studies have already validated the efficiency of physical activity in reducing RHR and RBP, very few tested the effects of a CRP of such a short duration.

On epidemiological level, in our study, both the mean age and the gender report of the patients diagnosed with HF matched the date already existed in literature. Consequently, we can reaffirm that HF is a pathology mainly affecting elderly patients and men. This affirmation is valid in most parts of the world, except the low-income regions, such as Sub-Saharan Africa, where HF has a predominantly infectious etiology (14, 15).

67.8% of the patients included in the study presented a decrease in the RHR. The CRP was more efficient for those with less severe symptoms, among whom we observed a better response, with lower RHR at the end, compared to the baseline values (4.56/min *vs.* 2.16/min). Considering the baseline RHR values and the mean age in each group, our results match those obtained in a meta-analysis, which included 181 studies that evaluated the effects of physical training on RHR. The study concluded that the effects of physical training on HR are proportional to the initial values of HR and inversely proportional to the age, without finding a correlation between these two variables. Consequently, we can affirm that CRP may be more efficient, at least when it comes to decreasing RHR, among younger patients (16).

Significant decrease of resting SBP, but not of DBP was observed for all the patients in our study. Similar to RHR, those patients who presented higher baseline SBP values responded significantly better. Our results align to those observed in a meta-analysis which evaluated the impact of physical training on the BP and included 44 randomized controlled studies with physical training programs lasting from 4 to 52 weeks. The meta-analysis concluded that

the baseline BP influenced the BP response and hypertensive patients had a better response than normotensives, with a higher decrease in resting BP values (-2.6/-1.8 mmHg *vs.* -7.4/-5.8 mmHg) (17). A smaller meta-analysis conducted over 23 studies observed the same relationship between physical training induced BP reduction and the baseline BP. The particularity of this study was that BP was evaluated using ambulatory blood pressure monitoring (ABPM), giving a more detailed result (18).

An explanation to our findings could be Wilder's law of initial value, stating that „the higher the baseline level of a physiologic function is, the grater is the response to function-depressing agents". After being published, Wilder's law has been investigated and reconfirmed by numerous researchers worldwide and from all branches of medicine, especially cardiology and despite being published in 1931 it still addresses nowadays problems (19, 20).

The most important limitations of our study are represented by the small sample size and the absence of a control group, which would have let us evaluate more accurately the impact of CRP on HF patients. In addition to this, the lack of ejection fraction-based classification of HF made it impossible to evaluate whether the left ventricle systolic dysfunction influences the response to rehabilitation or not, thus further studies are required to address these limitations.

CONCLUSIONS

Our study reconfirmed that CRP, even on a shorter period, remain an important treatment tool for HF patients, especially for controlling the resting HR and BP. These baseline parameters and the age of the patients represent important therapy response predictors.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they did not receive any specific funding regarding the scientific research.

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NEWS

AVACOPAN – A NEW TREATMENT FOR ANCA-ASSOCIATED VASCULITIS

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis can lead to decrease in kidney function, organ damage and side effects related to the medication used for treatment, such as glucocorticoids. The C5a receptor inhibitor avacopan is being studied in a randomized, controlled study for the treatment of ANCA-antibody associated vasculitis. Patients were divided in two groups which received either avacopan or prednisone on a tapering schedule. Both groups received rituximab or cyclophosphamide followed by azathioprine. Avacopan was superior to prednisone in sustaining remission at the end of the trial (David R.W. Jayne, M.D., Peter A. Merkel, M.D., M.P.H., Thomas J. Schall, Ph.D., and Pirow Bekker, M.D, Ph.D. for the ADVOCATE Study Group. Avacopan for the Treatment of ANCA-Associated Vasculitis. *N Engl J Med* 2021; 384:599-609 / doi: 10.1056/NEJMoa2023386).

INTERDISCIPLINARY IN MANAGING HIV/AIDS INFECTED PATIENTS – THE IMPORTANCE OF TEAMWORK

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INTERDISCIPLINARY IN MANAGING HIV/AIDS INFECTED PATIENTS - THE IMPORTANCE OF TEAMWORK (Abstract): The role of an interdisciplinary approach is useful in maintaining a good quality of life and low mortality rates among HIV-infected people. A better understanding of the evolution of comorbidities may help to improve the clinical management of HIV/AIDS patients. **Material and methods:** We performed a retrospective study between 1st of January and the 1st of December 2022 including 1157 patients who have been admitted to Iasi HIV/AIDS Regional Center, from "Sf. Parascheva" Clinical Hospital of Infectious Diseases, in order to identify the main factors that lead to major imbalances in the status of patients with HIV/AIDS infection, highlighting the most common comorbidities. **Results:** In the study period, 1,157 (70.46%) patients were admitted, and 89 cases (5.48%) were newly diagnosed. The study patients were virologically and immunologically evaluated. It was observed that 12.62% of cases had a CD4 level between 1-200 cells/mm^3, in 58.95% of cases the CD4 value was between 201-500 cells/mm^3, and 28.44% of patients had CD4 over 501 cells/mm^3, with an average CD4 level of 375.28 cells/mm^3. The average of HIV viral load was 107.46 copies/mL. Out of the total of 1157 cases, 146 (12.62%) patients had a CD4 level under 200 cells/mm^3, considered to have a viro-immunological failure, and they were recommended other antiretroviral regimens, most of them being prescribed a single tablet regimen. Depending on the related comorbidities, 62.32% of the 1157 cases admitted in our clinic required interdisciplinary examinations. In descending order, the following conditions were the most common comorbidities that the patients presented: metabolic (45.20%), dermatological (31.20%), ENT (12.79%), gastrointestinal (10.29%), infectious diseases (9.16%), cardiovascular issues (5.10%), psychiatric disorders (4.06%), pulmonary (3.54%), neurological (2.68%), ophthalmic (1.21%), and hematological conditions (0.78%). Out of the newly diagnosed cases, 85.39% were recommended single tablet regimen and in 14,61% cases were given a regimen that took into account their comorbidities. A large percentage of patients (82.20%) were evaluated through psychological consultation to ensure compliance and adherence to antiretroviral treatment, thus helping a better understanding of their disease for effective management. **Conclusions:** Multidisciplinary strategies for HIV have become more prevalent as various scientific environments have evolved. Assessing risk behaviors, clinical manifestations, health status, psycho-behavioral and cultural factors, as well as HIV assistance have emerged. **Keywords**: HIV/AIDS, COMORBIDITIES, ANTIRETROVIRAL THERAPY, INTERDISCIPLINARY.

Over the past four decades, researchers highlighted many challenges in addressing the pandemic with human immunodeficiency virus (HIV). The challenges include geographical, cultural and economic characteristics of affected regions, which could exert influences on how the epidemic presents itself, including its prevalence, severity, accessibility, treatment adherence and associated factors. Stigma, discrimination and barriers to treatment due to poverty are social impacts that can hinder treatment effectiveness and prevention efforts. Furthermore, we must adopt a multi-disciplinary approach to address this disease. HIV and AIDS (acquired immunodeficiency syndrome) are and remains complex diseases that requires extensive, coordinated care including clinical, psychological and social factors to ensure positive outcomes in HIV/AIDS infected patients (1, 2). Specialists have an important contribution to the health care system, specifically in increasing patient awareness by importance of medication adherence, encouraging behavioral changes and providing additional access to care for patients with HIV infection (3). Despite the remarkable progress in medical management, HIV infection continue to be an extremely stigmatized and socially intricate disease. Therefore, it is important to provide psychological support to newly diagnosed patients, by an experienced HIV counselor, as they adjust to HIV diagnosis and get ready to start antiretroviral therapy (4).

People with HIV who are effectively treated with antiretroviral treatment (ART) and with effective sustained viral suppression have a near-normal life expectancy. Even in the era of ART, the identification, mitigation and treatment of HIV-associated comorbidities continue to be a challenge that must be addressed (5).

Obesity, diabetes mellitus, cardiovascular disease, liver disease and neurocognitive illness comorbidities, all contribute significantly to the morbidity and mortality of people with HIV (6).

Dysbiosis, which includes a decline in butyrate-producing bacteria in the general population, is linked to the development of diabetes. A reduced relative abundance of butyrate-producing bacteria and its link with signs of microbial translocation and immunological activation have been observed in people with HIV, despite the lack of a correlation with diabetes (6).

Roomaney *et al*. found that the most common comorbidity among HIV-positive individuals was estimated to be hypertension. Also, tuberculosis (infection with *Mycobacterium tuberculosis)*, TB was the next most prevalent disease, followed by diabetes, heart disease and cancer (7).

Results revealed patterns that show stable hospitalization rates for people with HIV overall, with a drop in AIDS-defining illnesses and an increase in other comorbidities disorders (respiratory, cardiovascular and gastrointestinal causes), (8, 9).

The role of a multidisciplinary approach is useful in maintaining a good quality of life and low mortality rates among HIV-infected persons. A better understanding the evolution of comorbidities may help to improve the clinical management of HIV/AIDS patients including screening for risk factors and optimal selection of ART to balance HIV outcomes. Providing optimal care and treatment to people living with HIV/AIDS is an ongoing challenge (8, 9).

MATERIAL AND METHODS

We performed a retrospective study between January and December 2022 includ-

ing 1157 patients who have been admitted to Iasi HIV/AIDS Regional Center from "St. Parascheva" Clinical Hospital of Infectious Diseases, in order to assess the importance of a multidisciplinary approach in managing patients diagnosed with HIV/AIDS.

RESULTS

Iasi HIV/AIDS Regional Center cares for a total of 1642 patients from 6 counties - Bacău, Botoșani, Iași, Neamt, Suceava, and Vaslui. The patients benefit of periodic clinical, biological, and viral-immunological evaluations from a multidisciplinary team of infectious disease doctors, gastroenterologists, ophthalmologist, ENT doctor, dermatologist, radiologists, psychologist, nurses, and social workers.

Between the 1st of January and the 1st of December 2022, 1,157 (70.46%) patients were admitted, and 89 cases (5.48%) were newly diagnosed. They were all evaluated in our clinic.

Regarding the patients distribution by county, it was observed the following: Iași, 22.73% of patients; Bacău, 18.67%, Botoșani 15.99%, Neamt, 19.53%, Suceava, 16.68%, Vaslui, 6.4% from patients (fig. 1).

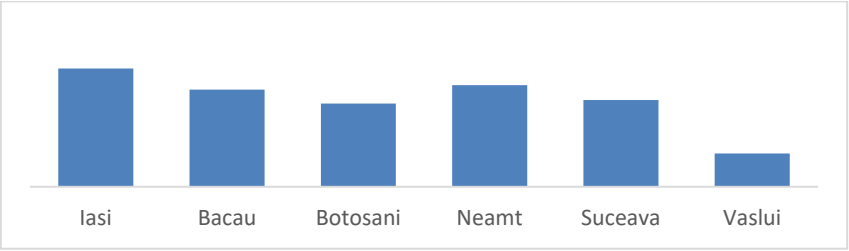

Fig. 1. Distribution of HIV/AIDS cases by county

From total number of hospitalized patients, predominantly 664 (57.39%) were male and 493 (42.61%) were female (fig. 2). The majority of the cases were young adults with ages between 21- 40 years old - 774 (66.9%) patients, followed by the age group 41-60 - 304 (26.27%) patients, 61-80 - 48 (4.15%) patients, and 0-20 - 31 (2.68%) patients (fig. 3). The average age of the study group is 37.78 years old, demonstrating the impairment of young adults.

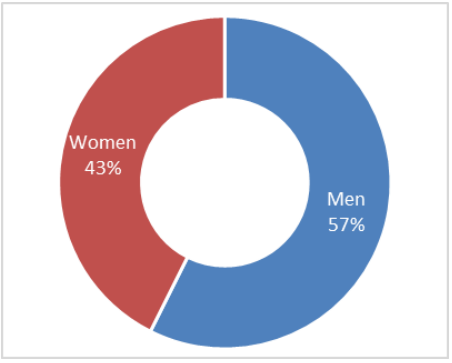

Fig. 2. Distribution of studied cases by gender

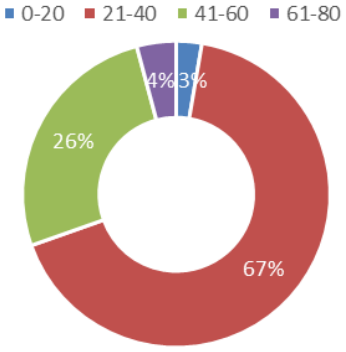

Fig. 3. Distribution of studied cases by age

Regarding the hospitalization way, 577 patients (49.87%) were admitted as outpatients and 580 patients (50.13%) were hospitalized, with an average of 5.72 days of hospital care. The patients were periodically evaluated. Taking into consideration the number of hospitalizations, 690 (59.64%) were admitted just once, 151 (13.05%) between 2 and 4 times, 54 (4.67%) between 5-7 times, 109 (9.42%) between 8-10 times and 153 (13,22%) over 11 times (fig. 4). It was observed that more than half of the study group required only one hospitalization while patients with more than one were most often associated with comorbidities that demanded multidisciplinary evaluations.

All of the patients from the Iasi HIV/AIDS Regional Center were virologically and immunologically evaluated. It was observed that 12.62% of cases had a CD4 level between 1-200 cells/mm^3, in 58.95% of cases the CD4 value was between 201-500 cells/mm^3, and in 28.44% of patients had CD4 over 501 cells/mm^3, with an average CD4 level of 375.28 cells/mm^3 (fig. 5). The average of HIV viral load was 107.46 copies/mL.

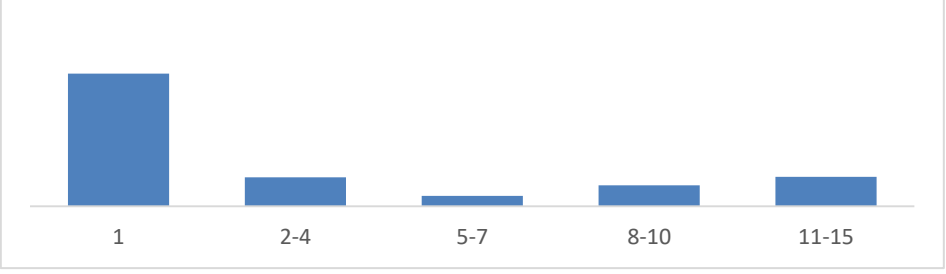

Fig. 4. Distribution of studied cases by number of hospitalizations

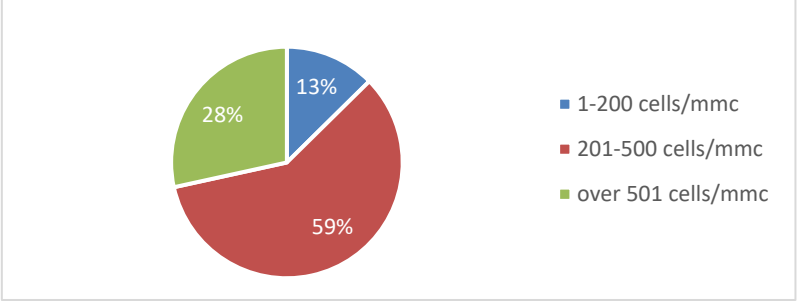

Fig. 5. Distribution of study cases by CD4 levels

Out of the total of 1157 cases, 146 (12.62%) patients had a CD4 level of under 200 cells/mm^3, were considered to have a viro-immunological failure, and they were recommended other antiretroviral regimens, most of them being prescribed a single tablet regimen.

Throughout the studied period, 164 (14.17%) patients needed an antiretroviral treatment (ART) switch. The most common reason was regimen simplification (66%), followed by viro-immunological failure (19%) and comorbidities (11%), (fig. 6).

Between January and December 2022, in Iași HIV/AIDS Regional Center, there were 89 newly diagnosed patients. They

benefited from clinical and biological investigations, and interdisciplinary consultations in order to better understand the infection they are dealing with, and to start antiretroviral treatment tailored to their needs. Out of the newly diagnosed cases, 85.39% were recommended a single tablet regimen and in 14.61% cases, was chosen a regimen that took into account their comorbidities.

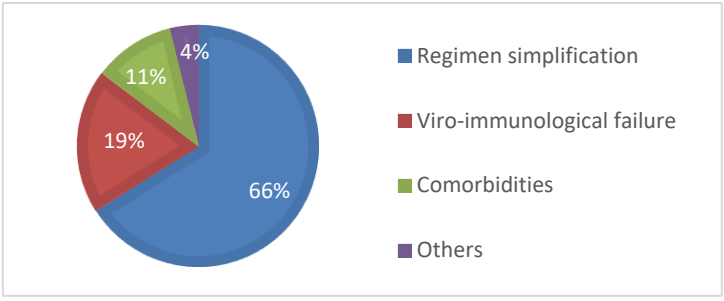

Fig. 6. Reasons for ART switch in the study patients

TABLE I.
Distribution of comorbidities by gender in the study group

Comorbidities		Gender		Total
		M	F	No./%
Infectious diseases	n	56	50	106
	%	4.84	4.32	9.16
Metabolic diseases	n	231	292	523
	%	19.97	25.24	45.20
Dermatological diseases	n	169	192	361
	%	14.61	16.59	31.20
Gastrointestinal diseases	n	82	37	119
	%	7.09	3.20	10.29
Psychiatric disorders	n	27	20	47
	%	2.33	1.73	4.06
ENT diseases	n	73	75	148
	%	6.31	6.48	12.79
Neurological diseases	n	14	17	31
	%	1.21	1.47	2.68
Cardiovascular diseases	n	24	35	59
	%	2.07	3.03	5.10
Ophthalmic diseases	n	8	6	14
	%	0.69	0.52	1.21
Hematological diseases	n	4	5	9
	%	0.35	0.43	0.78
Pulmonary diseases	n	28	13	41
	%	2.42	1.12	3.54

From a total of 1,157 cases hospitalized in our clinic in the mentioned study period, 62.32% required multidisciplinary evaluations, depending on the associated comorbidities. The most common comorbidities presented in the study group were metabolic, dermatological, ENT (otorhinolaryngological), gastrointestinal, infectious diseases, cardiovascular ailments, psychiatric disorders, pulmonary, neurological, ophthalmic and hematological (fig.7, tab. I).

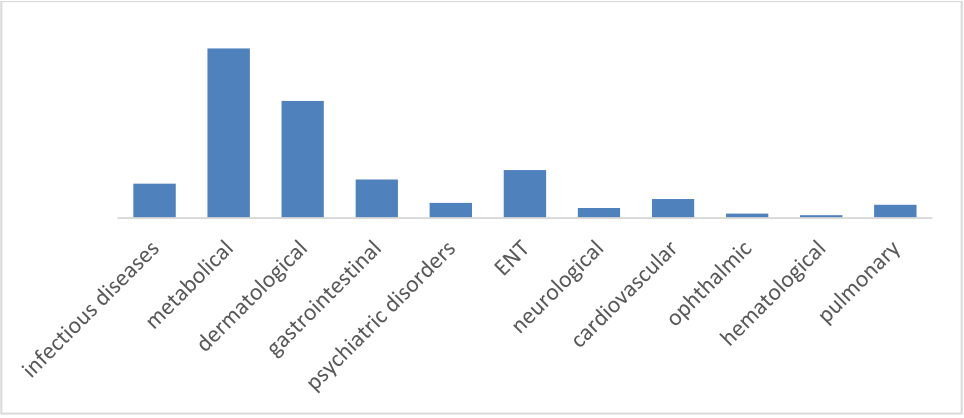

Fig. 7. Distribution of study cases by comorbidities

The metabolically disorders consisted in signs of lipodystrophy, central adiposity, dyslipidemia, increased risk of cardiovascular disease and atherosclerosis. This was observed to be caused by HIV infection and some antiretrovirals. The patients with lipid dysfunction were recommended changes in diet and lifestyle, and assessment in the internal medicine clinic, and in some cases, they benefited from ART switch.

Dermatological disorders were oral candidiasis, herpetic lesions, syphilis, anogenital condylomas, and Kaposi's sarcoma.

The most frequent ENT diseases were represented by sinusitis and otitis, which were evaluated in our clinic.

Gastrointestinal diseases were evaluated biologically, by ultrasound and computer tomograph, the patients received hygienic-dietary recommendations and specific treatment.

Opportunistic infections of HIV/AIDS were evaluated at each periodic visit of the patients in our clinic.

The neurological, cardiovascular, pulmonary, hematological, and psychiatric conditions were assessed, and later the patients received recommendations for consultation in specialized clinics depending on the disease.

Ophthalmic disorders

Were found in 14 cases, 2 patients had *Molluscum contagiosum* on eyelids, 3 cases with *Candida* keratitis, 2 patients had *Cytomegalovirus* retinitis, in 2 cases, *Herpes* keratitis, and in 5 cases was established the HIV-retinopathy diagnosis (fig. 8).

Out of the total of 14 patients with ophthalmic disorders, 3 (21.43%) cases had CD4 levels over 200 cells/mm^3, and 11 (78.57%) cases, under 200 cells/mm^3, with an average of 132.71 cells/mm^3. The average of HIV viral load was 1.025.000 copies/mL (fig. 9).

They were recommended ART switch to a single tablet regimen and specialized

ophthalmological treatment. In 5 cases were associated other conditions, such as oral candidiasis, liver disease and *Cytomegalovirus* infection.

At the one-month evaluation, 9 patients had CD4 level over 200 cells/mm^3 and 5 patients had a CD4 level under 200 cells/mm^3.

Fig. 8. Distribution of ophthalmic diseases in the HIV/AIDS study group

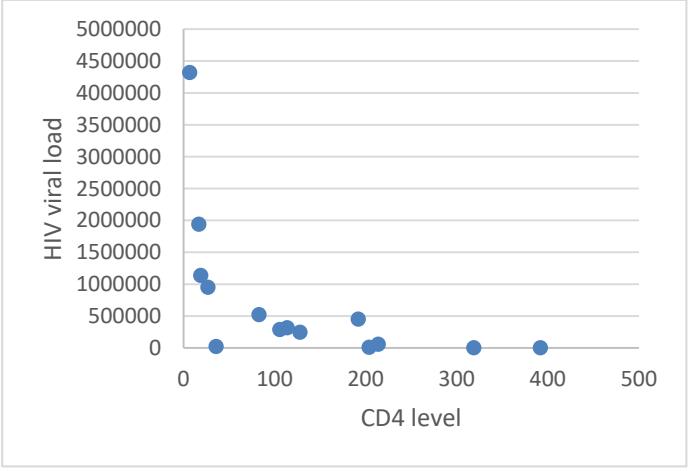

Fig. 9. Distribution of HIV cases associating ophthalmic diseases by CD4 and HIV viral load

A large percentage of patients (82.20%) were evaluated through psychological consultation to ensure compliance and adherence to antiretroviral treatment, thus helping a better understanding of their disease for effective management.

DISCUSSION

The several elements of the HIV multidisciplinary team on ART adherence are examined in this study. Increased attention has been paid to the needs of people with HIV, the chronic diseases treatment. To address the varied demands of people with HIV, interdisciplinary care has evolved as well.

Depending on the related comorbidities, 62.32% of the 1157 cases admitted in our clinic required interdisciplinary examinations. In descending order, the following comorbidities were found metabolic (45.20%), dermatological (31.20%), ENT (12.79%), gastrointestinal (10.29%), and infectious diseases (9.16%), cardiovascular

(5.10%), psychiatric disorders (4.06%), pulmonary (3.54%), neurological (2.68%), ophthalmic (1.21%), and hematological conditions (0.78%).

Roomaney *et al*. found that cardiovascular diseases were more frequent in people with HIV (especially hypertension: 13.3%), next prevalent comorbidities were pulmonary diseases (Tuberculosis was the main cause: 3.5%), followed by metabolic diseases, such as diabetes (3.0%) and cancer (0.4%). Elderly people were more likely to get any of the diseases. The prevalence of diseases like cancer, diabetes, heart disease, and hypertension were higher in women (7).

Katano *et al*. showed that in a cohort of a total of 225 cases opportunistic infectious disease were most frequent (94.4% cases of *Cytomegalovirus* infection and *pneumocystis* pneumonia). The prevalence of non-Hodgkin lymphoma and Kaposi's sarcoma was 37.9% and 15.2%, respectively for people with HIV who had received ART from 1985 to 2012 (10).

Pourcher *et al*. found that people with HIV had significantly higher rates of alcohol misuse (5.8%), chronic renal disease (1.2%), cardiovascular disease (7.4%), dyslipidemia (22%), and hepatitis B (3.8%). Other comorbidities such anemia, malnutrition, mental conditions, and tumors were also more common (11).

Funke *et al*. observed that the most common comorbidities were vitamin D deficiency (29.1%), depressive episodes (27.8%), arterial hypertension (16.3%), and hypercholesterolemia (10.8%) in patients with HIV in Germany (12).

Our results may vary compared to other studies. It is important that physicians should provide patients with varied needs extra care and attention if they want to develop the most effective healthcare plans available.

The global trend in HIV research has been increasing in both quantity and interdisciplinary approaches (13).

CONCLUSIONS

Multidisciplinary strategies for HIV have become more prevalent as various scientific environments have evolved. Assessing risk behaviors, clinical manifestations, health status, psycho-behavioral and cultural factors, as well as HIV assistance have emerged. To provide culturally significant and contextualized evidence of effective therapies to efficiently prevent and manage the condition, more research is needed.

The multidisciplinary approach in the HIV/AIDS research is not surprising. It is absolutely necessary for the scientific fields to intertwine in order to consolidate interdisciplinary.

It is important to identify the main factors that lead to major imbalances in the status of patients with HIV/AIDS infection, whether we are talking about factors related to the disease, community, social factors or factors related to adherence to antiretroviral therapy.

The interdisciplinary approach in HIV/AIDS infection is essential to reach these targets.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no funding regarding this scientific research.

ETHICAL APPROVAL

The current study was approved by the Ethics Committee of "Sf. Parascheva" Clinical Hospital of Infectious Diseases, Iasi, Romania.

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THE HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL PROFILE OF MALIGNANT BREAST TUMORS IN NORTH-EAST ROMANIA

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THE HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL PROFILE OF MALIGNANT BREAST TUMORS IN NORTH-EAST ROMANIA (Abstract): Studies using gene expression profiling have identified five major subtypes of breast cancer, apart from the traditional hormone receptor positive or negative ones: luminal A, luminal B, the HER2 group, the basaloid carcinoma group and the "normal breast-like" group. **Material and methods:** In this retrospective study, 281 patients admitted to the Regional Institute of Oncology Iasi and the Clinical Hospital of Obstetrics and Gynecology Cuza Voda Iasi were included, for whom the oncological records, the operative protocols of the operating room, the unique register of the service were studied; the pathological anatomy and observation sheets were considered for a period of 8 years: 2015-2022. For all 281 invasive mammary carcinomas diagnosed on hematoxylin-eosin staining, they were classified into one of the corresponding histopathological types and subtypes according to WHO criteria. **Results:** The histopathological study allowed: 1. invasive ductal carcinoma NOS (not otherwise specified - non-specific type of invasive ductal carcinoma)-193 cases (68.68%),2. invasive lobular carcinoma-42 cases (14.94%). Immuno-labeling analysis Estrogen receptors (ER) were positive (Allred score ≥ 3) in 61% of cases (169 patients), and progesterone receptors (PR) in 64% of cases (180 patients). Ki 67 immunolabeling analysis highlighted: the presence of positivity in all cases studied, overexpression of the p53 protein in 99 cases and HER 2 immunoassays allowed the highlighting of 42 cases. Identifying cases with p53 protein mutations can select a group of patients with a higher risk of recurrence and death, and testing p53 expression in HER2 positive patients identifies the subgroup with more aggressive tumors that will benefit from a more aggressive treatment. **Conclusions:** The most common histological types were *invasive ductal* carcinoma in 90% of patients. TNM stages was as follows: stage I in 9 cases (3.2%), stage II in 88 cases (31.49%), stage III in 138 cases (48.93%), stage IV in 31 cases (11.03%) ER+/PR- phenotype were more frequent over 50years and tumors larger than 2 cm. Patients under 50 years presented twice as often HER2 positive tumors. Ki67 immunostaining seems to be associated with an unfavorable prognosis (2% of cases in the present study). p53 overexpression occurs most frequently in patients under 50 years, with tumors larger than 2 cm. **Keywords**: BREAST CANCER, DUCTAL CARCINOMA, LOBULAR CARCINOMA, ER, PR, KI67, P53, HER2.

Recently, a variety of molecular techniques, especially the expression profile of certain genes, have redefined the classification of breast cancer from the point of view of prognosis, evolution and expected response to treatment (1).

Studies using gene expression profiling have identified five major subtypes of breast cancer, apart from the traditional hormone receptor positive or negative ones: luminal A, luminal B, the HER2 group, the basaloid carcinoma group and the "normal breast-like" group (2, 3) (tab. I)

Three immunohistochemical markers are used in clinical practice for the molecular classification of breast cancer: estrogen receptors (RE), progesterone receptors (RP) and HER2 expression (4). The best differentiated breast tumors are RE+, RP+ and HER2-, while poorly differentiated tumors are RE-, RP- and HER2+. Exceptions are medullary carcinomas (triple negative), basaloid carcinomas (triple negative) and micropapillary carcinoma (triple positive) (5, 6, 7). Luminal A category carcinomas have a better prognosis, while the basaloid subtype is the most aggressive type of cancer.

TABLE I

The immunophenotype of breast carcinoma

MOLECULAR SUBTYPE	IMMUNOHISTOCHEMICAL PROFILE
Luminal A	RE+ and/or RP+, HER2- and ki67<14% (negative)
Luminal B	RE+ and/or RP+, HER2+ (Luminal group B HER2+)
	RE+ and/or RP+, HER2- high expression ki67 (>14%)
HER 2	RE-, RE+, HER+
Basaloid carcinoma	RE-, RP-, HER2-, CK5/6+ and EGFR+

MATERIAL AND METHODS

In this retrospective study, 281 patients admitted to the Regional Institute of Oncology Iasi and the Clinical Hospital of Obstetrics and Gynecology Cuza Voda Iasi were included, for whom the oncological records, the operative protocols of the operating room, the unique register of the service were studied; the pathological anatomy and observation sheets were considered for a period of 8 years: 2015-2022. The breast tumors was monitored according to age, place of residence, tumor size, tumor location, histopathological type, tumor grading, complications of the disease and material investigated in this study was represented by human material, respectively by breast tissue from patients. For all 281 invasive mammary carcinomas diagnosed on hematoxylin-eosin staining, they were classified into one of the corresponding histopathological types and subtypes

according to WHO criteria.

The aim of immunohistochemical study was to highlight some correlations between the cell markers Ki67, p53, HER2, hormone receptors and the histological grade, the prognosis in breast carcinomas. These pieces of surgical exeresis were fixed in formalin and initially processed by the usual paraffin embedding technique, being brought up to the paraffin block stage. The method used in the *immunohistochemical study* was one of the methods based on soluble immunoenzymatically complexes, called LSAB/HRP (*labelled streptavidin biotin*).

RESULTS

Clinical-statistical study. *The urban/rural ratio* was 1.48, urban residence being found in 167 patients. *Age* The maximum incidence of breast cancer was in the 51-60 age group and included 108 patients, representing 38.43%. The *location* of cancer in the left breast was present in 151 cases(53.73%), in the right breast it was present in 127 cases (45.19%), and bilateral breast cancer was present in 3 cases. *Signs and symptoms* The most common signs and symptoms were breast tumor in 260 patients (92.52%), homolateral axillary adenopathy in 185 patients (65.83%), carcinomatous mastitis in 19 patients (6.67%), arm edema in 11 patients (3.91%), nipple discharge in 5 patients (1.77%), bone metastases in 9 patients (3.2%), liver metastases in 8 patients (2.84 %). *The breakdown by TNM stages* was as follows: stage I was established in 9 cases (3.2%), stage II in 88 cases (31.49%), stage III in 138 cases (48.93%), stage IV was diagnosed in 31 cases (11.03%), the stage was unknown in 5.93% of patients.

Histopathological study. The histopathological study allowed, in a first stage, the classification of the 281 invasive breast carcinomas, according to the WHO classification, into one of the following types and subtypes **: 1.** invasive ductal carcinoma NOS (not otherwise specified - non-specific type of invasive ductal carcinoma) - 193 cases (68.68%)**, 2.** invasive lobular carcinoma - 42 cases (14.94%), **3.** mixed ductal-lobular carcinoma - 14 cases (5.16%), 4. Other types- 26 cases.

Immunohistochemical study. All these tumors were processed immunohistochemically and were smaller or equal to 2 cm in 98 cases and larger than 2 cm in 183 cases.

Immunolabeling analysis for hormone receptors (ER and PR). Estrogen receptors (ER) were positive (Allred score $\geq$ 3) in 61% of cases (169 patients), and progesterone receptors (PR) in 64% of cases (180 patients. Compared to the **histological type,** invasive ductal carcinomas expressed estrogen receptors in 114 cases (58.88%) and progesterone receptors in 122 cases (63.3%), while invasive lobular carcinomas expressed estrogen receptors in 34 cases (80%) and progesterone receptors in 29 cases (70%). Depending on the immunoexpression of hormone receptors, breast carcinomas are classified into four subtypes or phenotypes: **1**. 57% of cases presented both types of receptors with an ER positive/PR positive phenotype.**2**. 32% of cases were completely devoid of hormone receptors having an ER negative/PR negative phenotype. **3-4.** 11% had a heterogeneous phenotype: 7% of cases were ER negative/PR positive, 4% of cases were ER positive/PR negative.

Ki 67 immunolabeling analysis highlighted: the specific marking of Ki 67 is

nuclear and was found in 281 cases. Patients under the *age* of 50 more frequently had a high Ki67 index (over 15% of tumor cells being Ki 67 positive) compared to patients over 50 (60% of cases vs 55.55%); patients with *tumors larger* than 2 cm more frequently had a high Ki 67 index compared to those with *tumors below* 2 cm (71.42% vs 51.43%). Regarding the histological type of the analyzed carcinomas, it was found: the *lobular type* had a low Ki 67 index in all cases; the *ductal invasive type* had a low Ki 67 index in 43.87% of cases and a high index in 56.12% of cases. Related to the histological grade: high-grade tumors (G3) always had a high Ki67 index; low-grade (G1/G2) that had an increased proliferation index in only 8.62% cases.

The analysis of p53 immunolabeling identified the presence of overexpression of the p53 protein (more than 10% of tumor cells labeled with this antibody) in 99 cases (42% of the studied cases, 193 ductal carcinomas and 42 lobular carcinomas). Tumors with p53 overexpression were more frequently encountered in patients under 50 years of age (54 cases, respectively 54.55%) compared to those over 50 (45 cases, respectively 45.45%). p53 positive tumors were more frequently tumors larger than 2 cm. So, 66 tumors among the p53-positive tumors (representing 66.64% of the p53-overexpressing group) were larger than 2 cm in size, while only 33 of the p53-positive tumors (representing 33.33% of the p53-overexpressing group) were larger than 2 cm in size. smaller than 2 cm. Invasive ductal type carcinomas were p53 positive in 85 cases, representing 44.44% of all invasive ductal type cases, and invasive lobular type carcinomas in only 13 cases, representing (30%) of all type breast carcinoma cases invasive lobular Regarding the histological grade, most cases with p53 protein overexpression (73 cases, respectively 73.3%) were of high grade (G3) and only 51 cases (28.33%) had low histological grade (G1 and G2). The immunohistochemical overexpression of p53 was thus found in 46.66% of the 35 HER2 positive cases (score 2+ and 3+) and only in 96 cases (40.85%) of the 200 HER2 negative cases (score 0 and 1+). p53 overexpression (corresponding to an increased amount of p53 protein determined by IHC) was more frequent in HER2-positive breast carcinomas compared to HER2-negative ones (46.66% vs 41.17%).

The analysis of the HER 2 immunoassay allowed the highlighting of 42 cases (representing 14.94% of the studied cases) of invasive breast carcinoma positive for HER 2. Of these, 28 cases (representing 66.66%) were weakly positive (score 2+), and 14 cases (representing 33.34%) were interpreted with a score of 3+ (positive) according to the criteria (Group Biology HER2/CEP 17) Correlating the HER2 immunolabeling of tumors with their histological type, it was observed that 4 cases of lobular carcinoma (10% of lobular carcinomas were HER2 positive) were moderately HER2 positive (score 2+). All other 38 HER 2 positive cases were of ductal type (24 cases with score 2+ and 14 cases with score 3+). Thus, 15.55% of invasive ductal carcinomas were HER2 positive. Following the correlation of HER2 immunomarking with morpho-clinical parameters, it was observed that HER2 positive tumors belonged slightly more frequently to patients under 50 years of age (22 cases, respectively 52.38%) compared to those

over 50 (20 cases, respectively 47, 62%). HER2 positive tumors were more frequently tumors larger than 2 cm. Thus, 22 tumors larger than 2 cm (representing 52.38%) were HER2 positive, while 20 tumors smaller than 2 cm (representing 47.62%) were HER2 negative. Regarding the histological grade, the majority of HER2 positive cases (34 cases, respectively 80.9%) were of high grade (G3) and only 8 cases (representing 19%) had low histological grade (G1 and G2).

DISCUSSION

The clinical study was conducted over a period of 8 years, between 2015-2022 and included 281 patients with breast cancer.

Age. The maximum incidence of breast cancer was in the 50-59 age group and included 79 patients (28.11%). The older the age, the greater the risk of breast cancer. The reason for this reality seems to be related to the existence of multiple somatic mutations (multiple event theory) for breast cancer genes as the population ages (12) (tab. II).

TABLE II

Breast cancer incidence according to age (13, 14, 15, 16)

Author	Patients group	Age (years)	Incidence of breast cancer
Andrew McGuire	26 countries	50-59	71-73%
Allen S. Lichter	247	50-60	51.0%
Edward Obedian	1,029	> 50	67.9%
Coral A. Quiet	826	> 55	50.0%
Richard McQuellon	115	> 57	>50.%

Histological grading The distribution of tumors according to histological grading was as follows: histological grading G1 in 22% of cases; histological grading G2 in 50% of cases; histological grading G3 in 23% of cases. Histological grading is usually based on the criteria established by Bloom. These include tubule formation, mitoses, and nuclear characteristics (size, shape, and hyperchromatism).Black *et al Schuh* (17) divided the nuclear grades into three groups and counted them in reverse order of the histological grading: • nuclear grade1- anaplasia, • nuclear grade2- intermediate differentiation • nuclear grade3- well differentiated.

TNM staging was possible in 94.66% of cases, and in 5.93% of patients the stage was unknown. The breakdown by TNM stage was as follows: • stage I was established in 9 cases (3.2%),• stage II in 89 cases (31.49%),• stage III in 137 cases (48.93%),• stage IV was diagnosed in 31 cases (11.03%),• the stage was unknown in 5.93% of patients.

Histological study. The classification of these invasive carcinomas into the various types according to the WHO criteria demonstrated that an overwhelming number of cases (193 cases, respectively 68.51%) were represented by NOS-type invasive ductal carcinoma, these cases being followed by invasive lobular carcinoma, which was diagnosed in 42 cases, representing 14.94% of all infiltrative breast carcinoma cases studied. *Invasive*

ductal breast carcinoma NOS. It is the most common invasive breast carcinoma, representing between 40% and 75% of invasive breast carcinomas. By definition, the diagnosis of ductal carcinoma NOS is a diagnosis of exclusion, this carcinoma being a malignant epithelial tumor that cannot be classified into any of the special types of invasive breast carcinoma (20). According to the Nottingham score, NOS ductal carcinomas were classified into: NOS G1 carcinomas (48 cases - 17.08%), NOS G2 carcinomas (132 cases - 46.97%) and NOS G3 carcinomas (101 cases - 35.94 %). The separation of breast carcinomas into the 3 histological grades is arbitrary and artificial, because these tumors actually present a continuous scale of malignancy (20).

Lobular carcinoma. It was first introduced by Foote and Stewart in 1941. Invasive lobular carcinoma is the most common type of invasive breast carcinoma after invasive ductal carcinoma, accounting for 5-15% of all breast carcinomas in Europe and the United States, while in Japan this type of carcinoma represents only 1-4% of all breast cancers (21).

The classic subtype of infiltrative lobular carcinoma accounts for approximately 40% of all infiltrative lobular carcinomas (*22*). According to the Nottingham score, invasive lobular carcinomas were classified into carcinomas: G1 16 cases - 19.04%, G2 51 cases - 60.71%, G3 17 cases - 20.23%. Similar results were obtained by specialists and published in recent studies. Thus, using the criteria of the Nottingham score, classified invasive lobular carcinomas in grade 1 - 20% of cases, grade 2 - 66% of cases and grade 3 - 14% of cases and showed that the histological grade of invasive lobular carcinomas (24).

Immunohistochemical study. Hormone receptors. In this study, 61% of invasive breast carcinomas showed estrogen receptors, and progesterone receptors were detected in 64% of cases, this being in accordance with recent data from the literature that records the presence of ER in 63% of patients with invasive breast carcinoma and a PR in 65% of them (25). The majority of breast carcinomas expressed both types of hormone receptors with an ER+/PR+ phenotype (57% of cases) and were followed in frequency by tumors with absent hormone receptors and an ER-/PR- phenotype (32% of cases). Specialist studies cite that approximately 50% of invasive breast carcinomas express both types of hormone receptors, and approximately 25% lack estrogen and progesterone receptors (26). In the present study, the ER+/PR- phenotype was detected in 4% of tumors. Thus, the average level of estrogen receptors in ER+/PR- tumors is only about half that of ER+/PR+ tumors (27). The Ki-67 antigen is a non-histone protein useful for identifying proliferative cells that, not having phase specificity, is expressed in all active phases of the cell cycle (Ki-67 is not expressed in the G0 phase) (28).

Although there are currently several antibodies that can be used on paraffin sections (MM1, NCL-ki-67p, Rah Ki-67), studies show that, nevertheless, the MIB-1 antibody has the highest sensitivity, offering the best visual staining, the reproducibility of the staining index being equally good for any of these antibodies (29). Its distribution by immunohistochemical methods is limited to its appearance in the late G1 phase. Therefore, Ki-67 is recognized as an indicator of cellular mitotic

activity. An increase in Ki-67 expression indicates an increase in cellular mitotic activity and proliferation*(*30). Immunohistochemical expression of Ki 67 correlates well with growth fraction in various models and does not appear to be expressed during the DNA repair process .(31) In breast cancers, Ki67 is used to categorize patients into categories with favorable and unfavorable prognosis correlating with the clinical response to chemotherapy (32). Relative to the histological grade, high-grade tumors (G3) always had a high Ki 67 index, compared to low-grade tumors (G1/G2) which had an increased proliferation index in only 8.62% of cases. All studies that reported correlations of the KI 67 index with the histological grade of the tumors showed that tumors with a high histological grade have an increased proliferative activity compared to those with a low grade (33, 34).

The oncoprotein p53 is a phosphoprotein that has the role of "guardian of the genome", i.e., monitoring the integrity of DNA during cell division. The protein product of normal (wild-type) alleles of the p53 gene negatively regulates cell growth and proliferation, blocking cells in the G1 phase of the cell cycle. In the present study, the overexpression of the p53 protein was found in 42% of the breast carcinoma cases studied, a result well correlated with the data published in the specialized literature that vary between 16% and 48% of p53 positive cases in invasive breast carcinomas (36). Analyzing the overexpression of the p53 protein in the cases included in this study, we found that it was more frequent in patients under 50 compared to those over 50 (54.76% *vs.* 45.24%), with tumors larger than 2 cm (66.64% *vs.* 33.33). Regarding the type and histological grade of the analyzed invasive breast carcinomas, p53 overexpression was more frequently associated with the invasive ductal type compared to the lobular one (95.24% *vs.* 4.76%) and with poorly differentiated tumors (G3) compared to the well/moderately differentiated ones (28.57% *vs.* 71.43%). These results are consistent with recent studies that demonstrated that breast tumors with a high amount of p53 (measured by IHC) are significantly correlated with high histological and nuclear grade (*36*) and they are much less often lobular (37). p53 immunopositivity correlated with the absence of expression of estrogen and progesterone receptors (61.9% of cases were devoid of estrogen receptor expression *vs.* 38.1% of cases showing estrogen receptor immunoexpression) also 54.76% of cases with p53 overexpression had been negative for PR *vs.* 45.24% of positive PR cases.

Co-expression of p53 and HER 2 was found in 7% of invasive breast carcinoma cases included in this study. The rate of breast cancers that coexpress p53 and HER 2 was reported, in previous studies, as varying between 7% and 19.5% of all breast cancers examined (38).

HER2 protein. The product encoded by the HER2 gene is a transmembrane protein of approximately 185 kD that functions as a tyrosine kinase receptor, called the HER2 protein, p185 or c-erbB-2. HER2 is involved in cell differentiation, proliferation, adhesion and motility (39), occupying a key position at the level of the mitogenic signal transduction cascade both in the case of normal and tumor cells. Thus, in addition to hormone receptors, HER2 represents the most accepted prognostic and predictive factor of response or resistance

to treatment and the potential of using new treatments (Herceptin) (40).

In this study, 15% of the analyzed group's tumors were positive for HER2, of which only 5% of the cases overexpressed the HER2 protein with maximum intensity (score 3+). The overexpression rate with maximum intensity of the HER 2 protein was lower in the studied group probably due to the fact that the poorly differentiated tumors (grade G3) were much less (40%) compared to the well and moderately differentiated ones (60%). This fact supports the hypothesis that less aggressive breast tumors have a lower rate of HER2 overexpression and suggests the association between HER2 expression in the tumor and the degree of its malignancy. HER2 positive tumors were more frequently tumors larger than 2 cm. Thus, 8 tumors larger than 2 cm (representing 53.33%) were HER2 positive, while 7 tumors smaller than 2 cm (representing 46.67%) were HER2 negative. Various studies did not find an association between HER 2 overexpression and the sizes of breast tumors analyzed (41,42). Correlating the HER2 immunolabeling of tumors with their histological type found that 10% of lobular carcinomas were HER2 positive. All other HER 2 positive cases were of ductal type (with score 2+ and 3+). Thus, 15.55% of invasive ductal breast carcinomas studied were HER2 positives. Recent data suggest that HER2 overexpression is significantly more frequent in ductal than in lobular carcinomas (43).

Co-expression of HER 2 with that of ER was found in only 10.52% of breast tumors with ER+/PR+ phenotype and in 25% of breast tumors with ER+/PR- phenotype). It should be mentioned here that the presence of hormone receptors in HER positive patients does not affect the benefits of trastuzumab therapy (44).

CONCLUSIONS

The maximum incidence of breast cancer was in the 50-59 age group and included 158 patients (28.11%). The most common histological types were *invasive ductal* carcinoma in 90% of patients, *invasive lobular* carcinoma in 4.6%, carcinoma in situ in 0.8% .

The distribution by TNM stages was as follows: stage I in 9 cases (3.2%), stage II in 88 cases (31.49%), stage III in 138 cases (48.93%), stage IV in 31 cases (11.03%), the stage was unknown in 5.93% of patients. *Invasive lobular carcinomas represented, after invasive ductal carcinoma* type NOS, the most frequent (14.94% of cases) type of breast carcinoma and were presented under a very wide range of variants (classic, solid, alveolar, tubulolobular, pleiomorphic, histiocytoid and with signet ring cells). *The histological grading*. A large number of patients (101 patients representing 35.76%) were identified who presented a high histological grade (G3) correlated with a poor prognosis, compared to patients whose tumors were well differentiated (G1, 48 cases, representing 17.08%).

The subgroup of invasive breast carcinomas is that with the ER+/PR- phenotype, more frequent in patients over 50 who had tumors larger than 2 cm. The group of patients under the age of 50 presented twice as often HER2 positive tumors. Overexpression with maximum intensity of the HER2 protein is more frequently associated with poorly differentiated tumors and those without hormonal receptors. Ki67 immunostaining seems to be associated with

an unfavorable prognosis (2% of cases in the present study). Cell proliferative activity and hormone receptor status were frequent in an inverse relationship. p53 overexpression occurs most frequently in patients under 50 years of age, with tumors larger than 2 cm, of invasive ductal type and with high histological grade. The co-expression of the p53 protein with HER2 has always been associated with an extremely high proliferative activity of the respective tumors. Cases with p53 protein mutations can select a group of patients with a higher risk of recurrence and death.

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PREVALENCE OF LIVER FIBROSIS AND STEATOSIS USING VIBRATION-CONTROLLED TRANSIENT ELASTOGRAPHY IN INDIVIDUALS EMPLOYED IN A NORTH-EASTERN ROMANIAN INDUSTRIAL COMPANY

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PREVALENCE OF LIVER FIBROSIS AND STEATOSIS USING VIBRATION-CONTROLLED TRANSIENT ELASTOGRAPHY IN INDIVIDUALS EMPLOYED IN A NORTH-EASTERN ROMANIAN INDUSTRIAL COMPANY (Abstract): Liver cirrhosis is considered the final stage of liver fibrosis progression and the leading cause of worldwide mortality due to liver diseases. Certain liver infections, such as viral hepatitis B (VHB) and C (VHC), alcohol-related liver disease (ALD), and nonalcoholic fatty liver disease (NAFLD) were found to be significant contributors to liver cirrhosis development. The **aim** of our study was to assess the prevalence of liver steatosis and fibrosis in individuals from the industrial field in the Northeastern region of Romania. **Material and methods:** Five hundred and sixty adult asymptomatic participants, all employees of a large bakery enterprise, were selected for this study. After informed consent was obtained, the demographical, clinical, and physiological description was made for each participant. AUDIT-C questionnaire was applied to each participant. VCTE (Fibroscan) was used to measure liver fibrosis, and hepatitis virus B and C presence was screened using rapid blood tests. **Results:** We found that 56.25% of the screened participants were from rural areas, and 65.7% were male. In our study, 54.64% declared that they had a smoking history, and only 1.7% received blood transfusions in the past. Moreover, 61% of the patients had a body mass index above the normal limit, 1.7% of the participants were positive for the presence of HBs antigen, while 2.85%

exhibited HCV antibody-positive titer. During VCTE examinations, for the majority of the patients M probe was used (75.2%). Liver fibrosis was found in 33.2% of the participants, and liver cirrhosis in 8.2% of the cohort. Increased severity of liver steatosis was observed in 33.4% of the patients. **Conclusions:** By screening a cohort of apparently clinically healthy individuals residing in the Northeastern part of Romania and having different socioeconomic profiles, we observed that the frequency of advanced fibrosis is more increased, as compared to available data. **Keywords:** CHRONIC LIVER DISEASE, FIBROSIS, STEATOSIS, VIRAL HEPATITIS, ALCOHOL-RELATED LIVER DISEASE, NONALCOHOLIC FATTY LIVER DISEASE, VIBRATION-CONTROLLED TRANSIENT ELASTOGRAPHY.

Being the main cause of liver-related deaths, cirrhosis is leading to significant global morbidity and mortality and is setting a major financial burden on the healthcare systems (1). Liver cirrhosis is the final stage of liver fibrosis progression, although the prevalence of compensated cirrhosis is underreported since it presents asymptomatically in the early stages (2). Consequently, a significant proportion of people with compensated cirrhosis remains misdiagnosed until the first sign of decompensation, such as ascites, elevated bilirubin levels, variceal hemorrhage, or encephalopathy. The mortality and morbidity rates are much higher than in compensated cirrhosis, with a 1-year case-fatality rate that can surpass 80% in some situations. Nevertheless, this group of patients is typically rapidly linked to medical care (3).

Currently, chronic hepatitis B (HBV) and C (HCV) infections, non-alcoholic fatty liver disease (NAFLD), and alcohol-related liver disease (ALD) are the main causes of liver cirrhosis (4). NAFLD has emerged as the most prevalent cause of chronic liver disease worldwide, affecting about 25% of the adult population worldwide and contributing to increased global morbidity and mortality (5). This is due to the rapidly shifting landscape of the liver cirrhosis etiology as a result of effective antiviral treatments and the successful implementation of vaccination programs for viral hepatitis (6). Moreover, NAFLD is also a prominent cause of liver transplantation and a rising risk factor for hepatocellular carcinoma (HCC). Simple steatosis is regarded as the "benign form" of NAFLD, while nonalcoholic steatohepatitis (NASH), the "progressive form" with various histological features, is linked to the development of liver fibrosis, including cirrhosis and eventually HCC (5). On the other hand, alcoholism continues to be a serious health issue, with 75 million people globally having an alcohol use disorder and a high risk of ALD. ALD is a catch-all phrase that includes the alcoholic fatty liver, alcoholic hepatitis (AH), and liver cirrhosis; nevertheless, data on prevalence rates and clinical and pathological characteristics of patients with early illness are hard to come by (7). By lowering the prevalence of liver cirrhosis and associated complications, such as liver failure, HCC, and death, early diagnosis of chronic liver illnesses may improve patient outcomes while also lessening the burden of the condition. This strategy must employ simple, readily available, and economically feasible noninvasive techniques for the measurement of liver fibrosis in vast groups of asymptomatic people in order to be effective. The aspartate aminotransferase [AST]-to-platelet ratio index [APRI], the fibrosis-4 index

[FIB-4], the body mass index-AST/ALT ratio-diabetes score (BARD), the Forns score, and the NAFLD score (NAS) are a few serological markers that potentially predict future development of cirrhosis and advanced liver disease in the general population (8).

Over the years, guidelines for fibrosis evaluation have consistently supported vibration-controlled transient elastography (VCTE) as the best non-invasive approach, particularly in the case of chronic viral hepatitis. In summary, this method uses pulse-echo ultrasonic recordings to quantify the speed of a mechanically generated shear wave in a significantly larger hepatic parenchyma, with the propagation velocity being closely correlated with hepatic stiffness. This technique is frequently used in clinical settings around the world since it is quick, simple, painless, and has significant repeatability and reproducibility (9). Additionally, in recent years, the inclusion of CAP in FibroScan® (Echosens, Paris, France) equipment, which reflects the liver's fat impedance, has enabled the simultaneous evaluation of hepatic fibrosis and steatosis. Since CAP measures are a quantitative method with significant sensitivity and specificity, they enable us to detect less severe degrees of steatosis than US (10). Herein, we aimed to assess the prevalence of liver steatosis and fibrosis in individuals from the industrial field in Northeastern region of Romania.

MATERIAL AND METHODS

This study population consisted of apparently healthy individuals residing in two neighboring counties from North-Eastern Romania, all employees in the bakery industry. This geographic area is known at risk for a number of reasons, including increased exposure to risk factors like tobacco use, consumption of calorie-dense and high-fat foods, heavy alcohol intake, physical inactivity, high body mass index (BMI $\geq$ 25 kg/m^2), and restricted access to healthcare services. All participants in this study were above the age of 18, had no prior history of chronic liver disease, and gave their informed consent by signing the study's consent form. Moreover, the individuals included in our study were workers at S.C. MOPAN SUCEAVA S.A., a large bread and bakery factory, and were evaluated due to an association between Institute of Gastroenterology and Hepatology, and this company through Platform for Interdisciplinary Research, "Grigore T. Popa" University of Medicine and Pharmacy. All patients who had a history of chronic viral hepatitis HBV/HCV/ hepatitis D virus (HDV) infection or other causes of chronic liver diseases, pregnant women, individuals with cardiac pacemakers, cancer or end-stage renal diseases, or had unreliable or failed VCTE measurements were excluded. Subjects examined by VCTE were further assessed through rapid diagnostic tests to detect HBs antigen (HBsAg) and anti-HCV antibodies (HCVA): HBV (Wama Immuno-Rapid HBV®) and HCV (Wama Immuno-Rapid HCV®) and completed the AUDIT-C questionnaire to establish the alcohol consumption. The threshold that rules in subjects with excessive alcohol intake usually is 20 g per day in women, and 30 g per day in men according to recent scientific recommendations (11). Using a height meter and a weight scale, measurements of height and weight were recorded. The midpoint between the lower border of the rib cage and the iliac crest was used to calculate waist circumference using a height meter. As proxies for adiposity, the body mass

index (BMI) and waist circumference were determined. The World Health Organization established cut-off values for overweight (25 kg/m^2) and obesity (>30 kg/m^2). Abdominal obesity was defined as having a waist circumference of 80 cm or more for women and 94 cm or more for males. This measurement is frequently used as a proxy for measuring visceral adiposity (12). The Ethics Committee of our Institute approved the study activities, which were conducted in conformity with the Declaration of Helsinki's guiding principles. Before the examination, all subjects gave their free and informed consent.

The FibroScan 520 Compact device (Echosens, Paris, France) was used for the VCTE and CAP procedures. It is outfitted with the M (regular probe - transducer frequency 3.5 MHz) and XL (transducer frequency 2.5 MHz) probes. An experienced doctor who has performed more than 300 examinations conducted each one in accordance with the procedure's instructions (13). The liver fibrosis stages were identified by the following cut-off values: mild fibrosis 5.6 kPa, considerable fibrosis 8.0 kPa, advanced fibrosis 9.6 kPa, and cirrhosis 13 kPa; LSM results were expressed in kilopascals (kPa) ranging from 1.5 to 75 kPa; The degree of steatosis was separated by the following cut-offs: mild steatosis 274 dB/m, (moderate steatosis) 290 dB/m, and severe steatosis 302 dB/m. CAP was expressed in decibels/m, with a range of 100 to 400 dB/m (9).

The *SPSS* software (version 22.0, IBM SPP Inc., Chicago, IL, USA) was used to perform the statistical analyses. Quantitative variables were expressed as means ± standard deviation (SD), whilst qualitative data were expressed as numbers and/or percentages (%). For distribution analysis, the Kolmogorov-Smirnov test was applied. The student's t-test, Mann-Whitney U test, or chi-square test were deemed suitable for comparing group variables. The Pearson correlation coefficient (r) was used to determine the relationship between two variables. Statistical significance was defined as two-tailed p-values 0.05.

RESULTS

In total, 560 individuals had been screened when the call for participants came to an end. Their median age was 48.14 ± 7.9 years, and 65.7% of them were male. Most of them were from rural area (56.25%). Professional school was the most common achieved educational level (43.9%), followed by high school and junior high school levels (30% and 16.8%, respectively). Only 9.3% of the population had the elementary school level in this regard. 306 (54.64%) of respondents said they had ever smoked cigarettes. 7.7% of the population said they have ever received blood transfusions. Also, among them 43.58% were positive for AgHBs and anti-HCV antibodies. On the other hand, 159 (28.4%) of patients reported consuming alcohol. In this cohort the prevalence rate of type 2 diabetes mellitus was 20.4%. 293 patients (52.3%) had high blood pressure, and 47.1% of individuals had dyslipidemia. Regarding body weight, our participant population was distributed as follows: six individuals (1.1%) were underweighted, 212 participants (37.9%) were normal weight, 207 participants (36.9%) were overweight, and 135 participants (24.1%) were obese. The mean BMI value for our cohort was 27.09 ± 3.12 kg/m^2. Also, 23 (4.1%) patients were AgHbs positive, and 16 (2.85%) detectable HCV antibody titer (tab. I).

TABLE I.
Baseline characteristics of studied population

	Overall Cohort N = 560
Age (years)	48.14 ± 7.9
Males, n (%)	368 (65.7)
Weight	79.26 ± 16.11
Height	169.48 ± 9.83
Body mass index (kg/m^2)	27.09 ± 3.12
Underweight, n (%)	6 (1.1)
Lean subjects, n (%)	212 (37.9)
Overweight, n (%)	207 (36.9)
Obesity, n (%)	135 (24.1)
Hypertension, n (%)	293 (52.3)
Dyslipidemia, n (%)	264 (47.1)
Alcohol intake, n (%)	159 (28.4)
HBV, n (%)	23 (4.1%)
HCV, n (%)	16 (2.85%)
History of type 2 diabetes mellitus, n (%)	114 (20.4)
Smoking history, n (%)	306 (54.64)
Blood transfusion, n (%)	43 (7.7)
Education, n (%)	
Elementary school	52 (9.3)
Junior high school	94 (16.8)
High school	168 (30)
Professional school	246 (43.9)

Regarding VCTE examinations, M-probe was utilized in 421 (75.2%) participants whereas a XL probe was employed in 139 (24.8%) subjects. Eight people failed the measurement, or 1.42% of the population under the study. The cut-offs used for liver fibrosis were as follows: F0, when LSM < 5.6 kPa, F1, when LSM = 5.6 – 7.9 kPa, F2, when LSM = 8 – 9.5 kPa, F3, when LSM = 9.7 – 12.9 kPa, and F4, when LSM ≥ 13 kPa. According to fibrosis stage, most of the patients had F2 level of liver fibrosis (33.2%). 161 (28.8%) individuals have no fibrosis, and 46 (8.2%) of patients have cirrhosis. The mean value of LSM was 7.2 ± 5.03 kPa. The following were the hepatic steatosis cut-off values: S0 - < 274 dB/m, S1 – 274 – 289 dB/m, S2 – 290 – 301 dB/m, S3 - ≥ 302 dB/m. Regarding steatosis degree, 33.4% of subjects have severe steatosis, with mean CAP value of 278.35 ± 67.82 dB/m (tab. II).

TABLE II.
Characteristics of VCTE examinations

Characteristics	**Mean ± SD or number (%)**
Probe size	
M	421 (75.2)
XL	139 (24.8)
Steatosis degree (dB/m)	
CAP < 274 (dB/m)	122 (21.8)
CAP ≥ 274 (dB/m)	148 (26.4)
CAP ≥ 290 (dB/m)	103 (18.4)
CAP ≥ 302 (dB/m)	187 (33.4)
CAP (mean ± SD) (dB/m)	278.35 ± 67.82
Fibrosis stage (kPa)	
LSM < 5.6 kPa	161 (28.8)
LSM ≥ 5.6 kPa	102 (18.2)
LSM ≥ 8 kPa	186 (33.2)
LSM ≥ 9.6 kPa	65 (11.6)
LSM ≥ 13 kPa	46 (8.2)
LSM (mean ± SD) (kPa)	7.2 ± 5.03

History of alcohol consumption, and presence of chronic viral hepatitis (HBV or HCV) were linked with risk for developing severe liver fibrosis (≥ F3) (r = 0.27, p = 0.04) and (r = 0.36, p < 0.001) (fig. 1). High values of BMI and the presence of type 2 diabetes mellitus were correlated with increased CAP values among our cohort (r = 0.17, p = 0.021) and (r = 0.27, p = 0.03) (fig. 2).

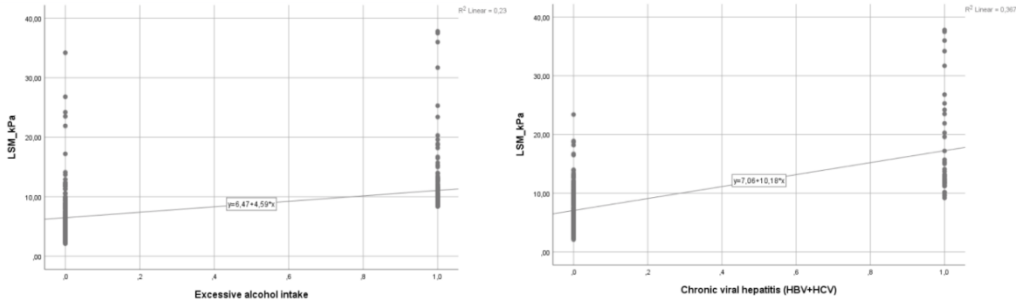

Fig. 1. Correlation between LSM and (A) excessive alcohol intake, (B) chronic viral hepatitis

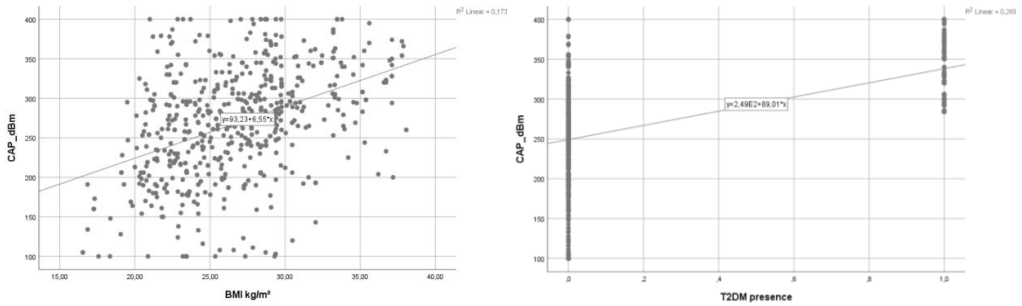

Fig. 2. Correlation between CAP and (A) BMI values, (B) presence of type 2 diabetes mellitus

DISCUSSION

There is a significant financial burden on healthcare systems as a result of liver cirrhosis, which ranks as the 7th most common cause of high disability-adjusted life years and the 11th most common cause of mortality worldwide. The most recent epidemiological research indicate that Europe appears to have the highest global burden of liver disease, and alarming predictions regarding an increase in cases over the next several years are being made (14). The World Health Organization most recent statistics from 2018 show that 8,763 people died in Romania from liver disease, or 3.75% of all deaths. Romania is ranked 47th in the world by age-adjusted death rate, which is 26.90 per 100,000 people. According to data from the Romanian National Institute for Public Health, liver cirrhosis accounted for 34.6 deaths per 100.000 people in 2019 and was the primary cause of mortality in digestive illnesses. With more than 20 deaths per 100,000 people, Lithuania and Hungary both had high mortality rates, at half in comparison with Romania (15).

The analyses of data subsets deriving

from the National Health and Nutrition Examination Survey (NHANES) 2017-2018 revealed that NAFLD had a prevalence of 58.5% and that US fatty liver index (US-FLI) is a reliable scoring system dedicated to diagnosing patients with fatty liver diseases. However, a drawback is that it requires a complementary calculation of insulin levels, for which the odd ratio (OR) was 4.97 in contrast with the indexes for hepatic steatosis (HSI), ORs = 21.2, and fatty liver (FLI) ORs = 18.6 (16).

Another study brought forward both positive and non-significant correlations following CAP and LSM through VCTE assessment of serum uric acid (UA) and marijuana use, whilst no relationships were identified in cases of urinary metabolite concentrations of di-2-ethylhexyl phthalate (DEHP) and vitamin D deficiency. Precisely, Duan *et al.* (17) further emphasize an overall OR of 2.097 and 2.983 and consistence in a sex-matched subgroup, whereas Du *et al.* (18) was able to highlight a low prevalence of steatosis is past ($p = 0.184$) and current users ($p = 0.048$). Recently, Shen *et al.* (19) successfully advanced weight-adjusted-waist index (WWI) to predict hepatic steatosis, WWI suggesting a strong association with hepatic steatosis ($p < 0.0001$) and not liver fibrosis ($p = 0.84$), but extrapolating in relation to excessive alcohol consumption ($p = 0.031$) and hypertension ($p = 0.014$) as multivariate linear regression analysis indicate.

On the other hand, Chen *et al.* (20) failed to observe a significant relationship between liver fibrosis and phthalates for LSM ≥ 8 kPa, but rather in the fourth quartile for mono-(2-ethyl-5-carboxypentyl) phthalate and mono (2-ethyl-5-hydroxyhexyl) phthalate of which the ORs were 2.719 ($p = 0.016$) and 2.073 ($p = 0.037$). This argument of a lack of association extends to the research conducted by Ji *et al.* (21) because the serum vitamin D had a linear trend regardless of participants' group allocation (74.26 *vs.* 72.24 nmol/L for which $p = 0.21$), nor discovered through multivariate logistic regression analysis, instead being uncovered a lower liver fibrosis risk for an OR of 0.89 for a deficiency of vitamin D in NAFLD patients.

Complementary investigations in the field advanced the possibility of enhancing the diagnostic accuracy and thus two teams found out that CAP and LSM identified with an area under the receiver operating characteristic curves (AUROCs) of 0.87 and 0.83 of patients with steatosis, fibrosis and 0.93 those with cirrhosis for the following intervals: 0.77 for S ≥ S1, and S2, and 0.70 for S = S3 at Youden cutoff values of 302, 331, and 337 dB/m, and 0.77 for F ≥ F2, 0.80 for F ≥ F3, and 0.89 for F = F4, respectively, with also Youden cutoff values of 8.2, 9.7, and 13.6 kPa compared with 6.5/12.1 kPa to exclude advanced fibrosis (AF) and cirrhosis at negative predictive values of 0.91 and 0.99. Moreover, a positive predictive value (PPV) of 0.71 and 041, LSM may identify patients with AF and cirrhosis (22, 23). Previous studies from Romania, which were published recently found that in a group of medical students, the prevalence of steatosis and advanced fibrosis was 17.4% and 0.7% respectively, in apparently healthy individuals. Instead, among individuals with type 2 diabetes mellitus the prevalence of advanced fibrosis and cirrhosis was 11.7% and 13.6% respectively (24, 25).

Poverty and chronic liver diseases are linked, due to a variety of factors, such as a higher risk of using tobacco products, eating calorie-dense, high-fat foods, being physically inactive, being overweight or obese, and having less access to healthcare. People who lived in poverty have a higher

mortality rate after being diagnosed with a chronic illness in every country. It's been thought of as rather paradoxical because the poor in middle- and high-income countries tend to be more obese than the wealthy. The same risk factors like in some Romania counties were present in Latin America or Asia, particularly in low- and middle-income nations like Mexico, where the average BMI is approximately 27 kg/m^2, 65% of the population is overweight or obese (BMI $\geq$ 25 kg/m^2), and 26% of the population is obese (BMI $\geq$ 30.27 kg/m^2). Cirrhosis will be one of the most preventable diseases causing death worldwide since it is predicted that the number of deaths worldwide from non-communicable diseases in lower-middle income nations would rise from 13 million in 2005 to approximately 19 million in 2030.

Our study had some limitations. First off, as we included apparently healthy individuals and, no histology data on people with advanced liver fibrosis or cirrhosis were taken due to the lack of liver biopsy. Another drawback is that there was no follow-up to assess if the therapies had changed the CAP score or the LSM, and to maintain monitoring of long-term impacts.

CONCLUSIONS

The assessment of advanced fibrosis by VCTE in a population with particular socioeconomic profile indicates a higher frequency of advanced fibrosis than is apparent in the data available from North-Eastern Romania. In this demographic, obesity is regarded as a separate risk factor for advanced fibrosis. To establish preventive interventions in this demographic area, more research is required.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest.

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PREDICTIVE SCORES FOR HEPATOCELLULAR CARCINOMA OCCURRENCE AFTER HEPATITIS C VIRUS CURE WITH DIRECT ANTIVIRALS

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PREDICTIVE SCORES FOR HEPATOCELLULAR CARCINOMA OCCURRENCE AFTER HEPATITIS C VIRUS CURE WITH DIRECT ANTIVIRALS (Abstract): Hepatitis C virus (HCV) chronic infection has an important tumorigenic propensity and represents a major cause of hepatocellular carcinoma (HCC). Although the therapy of chronic HCV infection has been revolutionized with the introduction of direct acting antivirals (DAAs) resulting in sustained virological response (SVR) in up to 98% of patients, viral clearance does not totally alleviate the risk of liver-related complications, including HCC. The **aim** of our study was to assess the performance of predictive scores for HCC occurrence in patients with chronic HCV infection who obtained SVR with DAAs. **Material and methods:** We conducted a prospective study in which we included 992 patients diagnosed with chronic HCV infection and treated with DAAs, between 1st November 2015 and 31st December 2020. HCC risk scoring systems were applied to each patient enrolled in the study before starting DAA treatment. HCC was diagnosed by imaging methods such as computed tomography and magnetic resonance imaging. **Results:** Of the 992 patients included in the study, 59 (5.9%) were diagnosed with HCC during the follow-up period, mostly women (55.9%) with a mean age of 60-69 years. The mean time to HCC onset was 19.53 ± 11.553 months, with a median of 17 months, with a cumulative incidence at 1, 3, and 5 years of 1.1%, 2.1%, and 2.9%, respectively. The General Evaluation Score (GES), aMAP and ADRESS score had statistically significant higher values in patients with HCC than in those without HCC. GES demonstrated a good discriminating power with an AUC coefficient of 0.804 at a cut-off value of 6.25 which has good sensitivity and specificity (0.746 and 0.799, respectively). **Conclusions:** GES score has a very good predictive power for the risk of HCC after obtaining SVR and could be recommended in clinical practice. Future development and validation of other individualized predictive scores score is needed for a correct and cost-efficient selection of patients with high risk of HCC. **Keywords:** CHRONIC HEPATITIS C INFECTION, DIRECT-ACTING ANTIVIRALS THERAPY, HEPATO-CELLULAR CARCINOMA, PREDICTIVE SCORES.

Hepatitis C virus (HCV) chronic infection has an important tumorigenic propensity and represents a major cause of hepatocellular carcinoma (HCC). Although the therapy of chronic HCV infection management has been revolutionized with the introduction of direct acting antivirals (DAAs), resulting in sustained virological response (SVR) in up to 98% of patients, viral clearance does not totally alleviate the risk of liver-related complications, including HCC (1). Still, there is strong evidence that SVR significantly improves the prognosis in patients with chronic HCV infection by lowering the risk of liver cirrhosis, decompensation and HCC, irrespective of whether it was achieved by using interferon-based regimens or DAAs (2). The high efficacy and availability of DAAs worldwide determined a rapidly growing of the SVR-patients pool, and thus it seems reasonable to consider strategies for a correct stratification of HCC risk.

The main tools for early diagnosis of HCC are screening strategies, which should accomplish the main goal of cancer surveillance that is the decrease of cancer specific mortality at cost-effective terms (3). In line with this, the current guidelines have different recommendations for HCC surveillance in those with SVR. The European Association for the Study of the Liver (EASL) considers the presence of F3 fibrosis and cirrhosis before start of treatment as an indication for HCC surveillance (4), whereas the American Association for the Study of Liver Disease (AASLD) takes into account only those with cirrhosis (5). Interestingly, the recommendations from the Asian Pacific Association for the Study of the Liver does not rely on fibrosis stages and recommends HCC surveillance in all patients with SVR (6). However, it is important to take into consideration the cost-effectiveness of HCC surveillance which is mainly determined by an accurate estimation of the incidence and thus by a correct stratification of HCC risk after SVR. There are numerous studies that evaluated several stratification tools regarding HCC risk in patients with HCV clearance, but data is still scarce (7,8).

In this study, we aimed to assess the performance of several predictive scores for HCC occurrence in patients with chronic HCV infection who obtained SVR with DAAs.

MATERIAL AND METHODS

Study design and study population

We conducted a prospective study in which we included 992 patients diagnosed with chronic HCV infection and treated at the Institute of Gastroenterology and Hepatology in Iasi, between 1st November 2015 and 31st December 2020. All patients met the following inclusion criteria: (1) age over 18 years; (2) diagnosis of HCV infection with detectable HCV-RNA; (3) no contraindication of DAAs therapy; (3) without previous diagnosis of HCC. All patients from the study cohort were followed up until February 1st , 2022.

Clinical and laboratory data

Patients enrolled in the study were followed-up prospectively by clinical-biological and imaging monitoring from study inclusion until 1st February 2022. Demographic variables for the enrolled study population included age and sex. Cirrhosis was defined as the presence of any of the following findings: coarse liver echotexture and nodular liver surface by ultrasonography, clinical features of portal hypertension (e.g., ascites, splenomegaly, or varices), or thrombocytopenia (<150,000/mm3).

Laboratory data included anti-HCV, aspartate and alanine aminotransferases, bilirubin, alkaline phosphatase, Gama glutamyl transpeptidase, albumin, international normalized ratio, prothrombin time, serum creatinine, hemoglobin, platelet count, and alpha-fetoprotein levels. Serum HCV RNA levels were measured in all patients before and at 3 months after the end of treatment (COBAS TaqMan HCV quantitative test (Roche Molecular Systems, Inc. Branchburg, NJ). Transient elastography (FibroScan; Echosens, Paris, France) was used to perform the liver stiffness measurement (LSM) with 13 kPa as the cutoff point for cirrhosis.

Diagnosis of HCC

The diagnosis of HCC was established based on current guideline recommendations using abdominal US as the initial method (4). In case of ultrasound detection of a nodule larger than 1 cm, the diagnosis was confirmed by a more sensitive method such as CT or MRI, and if the result was inconclusive, the following imaging method was used, liver biopsy being reserved for cases uncertain at 2 successive investigations.

Predictive scores

HCC risk scoring systems were applied to each patient enrolled in the study before starting DAA treatment.

The General Evaluation Score (GES) comprises 5 variables scored each as follows: (1) gender: women 0 points, men 3.5 points; (2) age: ≤54 years 0 points, >54 years 1 point; (3) degree of fibrosis: F3 1.5 points, F4 3 points; (4) albumin: ≥ 3.8g/dL 0 points, <3.8g/dL 2 points; (5) AFP: ≤20 ng/ml 0 points, > 20 ng/ml 3 points. The total score can reach a maximum value of 12.5 points and allows placing the patient in one of 3 risk groups: low ≤6, intermediate risk >6 - 7.5, and high risk >7.5.

The aMAP score was calculated using the formula ({0.06 x age + 0.89 x sex (male:1, female: 0) + 0.48 x [(log10 bilirubin x 0.66) + (albumin x −0.085)] - 0.01 x platelet count} + 7.4) / 14.77 x 100. Based on the score, the patients were divided into 3 groups: low risk < 50 points, intermediate between 50 and 60 points, high > 60 points.

The ADRESS-HCC score was calculated based age, presence of type 2 diabetes, race, etiology of cirrhosis, sex and severity of liver disease. Depending on the score obtained, patients were divided into 3 groups: low risk (corresponding to quartile 1), intermediate (quartile 2-3) and high (quartile 4).

Statistical processing was carried out using *Microsoft Excel* and *SPSS version 28* (Inc., Chicago, IL) software. Survival curves were estimated by the Kaplan-Meier method and compared, for univariate analysis, by the log rank test. To evaluate the discriminatory ability for the prediction of HCC, we evaluated the accuracy of prediction for each scoring system. This point was evaluated calculating the area under the receiver operating characteristic (ROC) curve for each staging system.

The study was carried out in accordance with the ethical principles of the Declaration of Helsinki, the protocol being explained in detail to all patients. Informed consent for participation in the study was obtained from all patients and/or their relatives.

RESULTS

Of the 992 patients included in the study, 59 (5.9%) were diagnosed with HCC during the follow-up period, mostly women (55.9%) with a mean age of 60-69 years. The mean time to HCC onset was 19.53 ±

11.553 months, with a median of 17 months; the shortest period of time was 3 months and the longest 51 months. Of the total of 59 patients with HCC, only 6 of them (10.2%) were diagnosed within an interval shorter than 6 months, in the remaining 53 (89.8%) the diagnosis was established after more than six months. Thus, the cumulative incidence at 1, 3, and 5 years was 1.1%, 2.1%, and 2.9%, respectively.

According to the GES score, the vast majority of patients were in the low-risk class (76.6%), followed by the intermediate-risk class (14.5%), the fewest being in the high-risk class (8, 9%) (fig. 1). On the other hand, according to the aMAP interpretation, 60.7% of the patients were in the high-risk category, 33.5% in the intermediate risk category, and only 5.7% of the patients belonged to the low-risk category (fig. 2). Regarding the ADRESS score, 25% of patients had values less than 5.0565 (Q1), 50% had < 5.5557 (Q2) and 25% had > 5.9608 (Q3) (fig. 3).

The comparative analysis of GES, aMAP and ADRESS risk scores in patients with HCC versus those without HCC showed that the score values were significantly higher in patients with HCC (fig. 4).

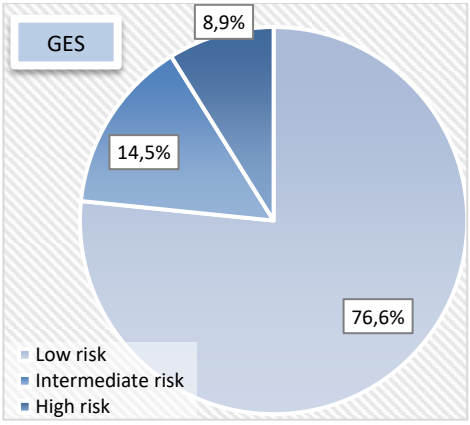

Fig. 1. Risk classes according to GES

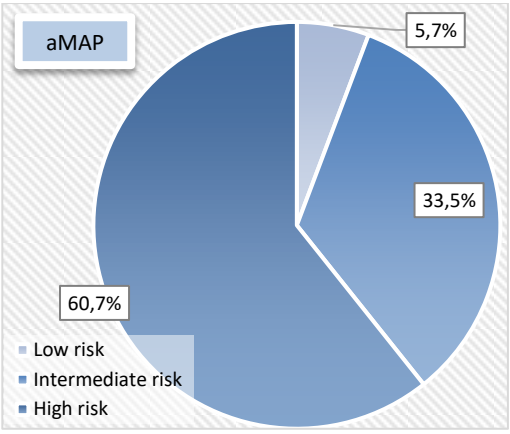

Fig. 2. Risk classes according to aMAP

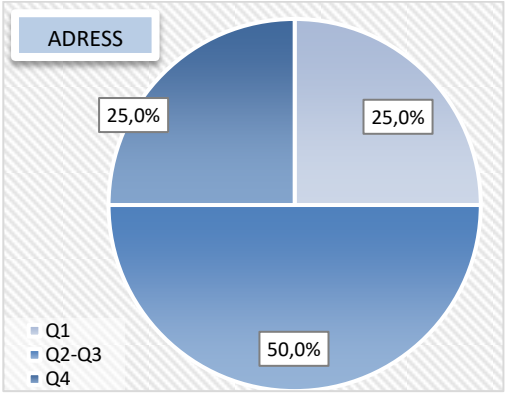

Fig. 3. Risk classes according to ADRESS

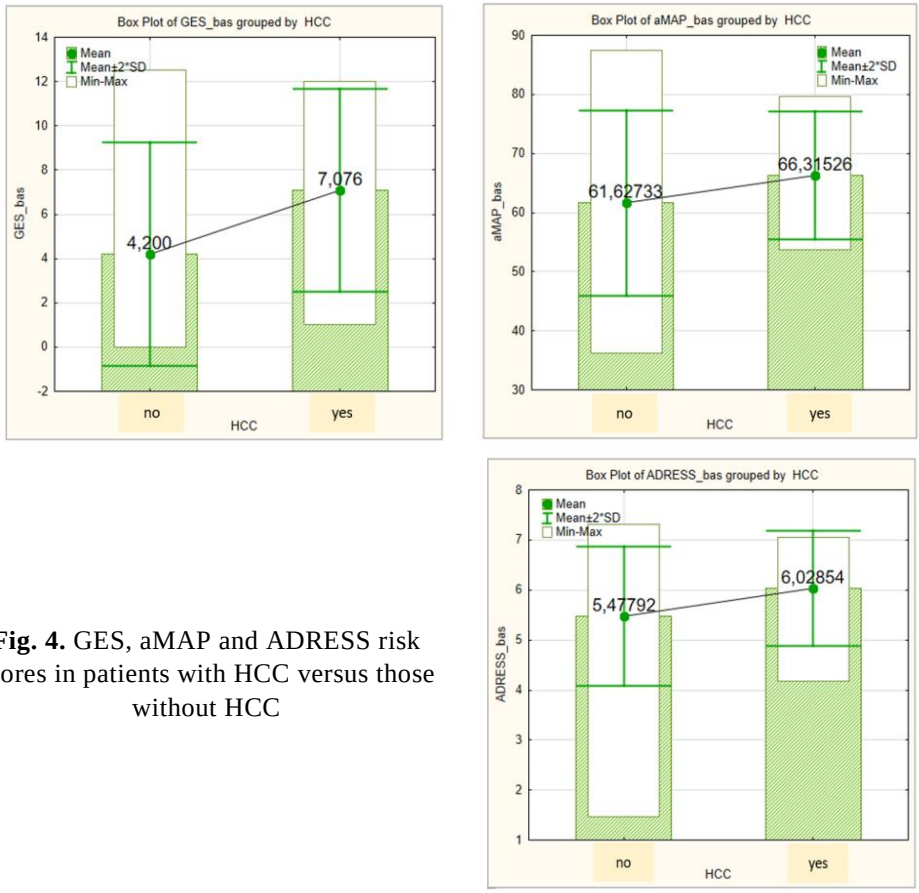

Fig. 4. GES, aMAP and ADRESS risk scores in patients with HCC versus those without HCC

Next, we performed the ROC analysis of the prediction scores, with the aim of identifying their discrimination potential (by evaluating the AUC surface) and the optimal cut-off values (with the best sensitivity-specificity combination) (tab. I).

TABLE I.

ROC analysis of the prediction scores for HCC

	AUC (Area under Curve)	p	Confidence interval 95%		Cut-off value	Sensitivity	Specificity
			Lower limit	Upper limit			
GES	0.804	0.000	0.748	0.860	6.25	0.746	0.799
aMAP	0.700	0.000	0.638	0.761	64.57854	0.737	0.650
ADRESS	0.743	0.000	0.681	0.805	5.6571	0.797	0.599

Only GES had the AUC coefficient greater than or equal to 0.8 - which corresponds to a good or excellent discriminating power for the investigated situation, namely the risk of HCC. The identified cut-off value of 6.25 has both reasonably good sensitivity and specificity. The corresponding ROC curve is illustrated in figure 5.

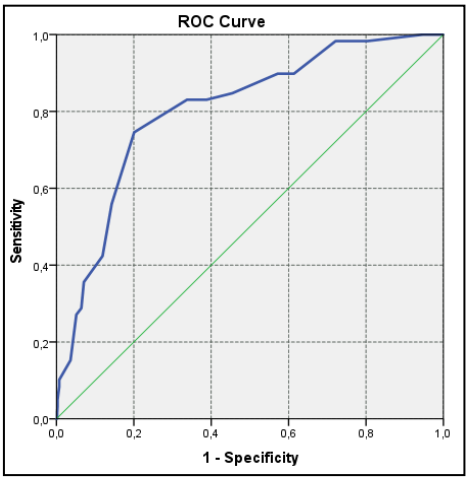

Fig. 5. ROC curve for GES

DISCUSSION

HCC risk scoring systems in patients with chronic HCV hepatitis are derived from prediction models used for chronic HBV hepatitis. Interest in these predictive systems has increased with the introduction of the new DAAs into the therapeutic management of patients with chronic HCV infection. In the pre-DAA era, when limited therapeutic options were reflected in the proportion of viremic patients, scoring systems were developed to expand or narrow the surveillance indications for HCC.

Research in recent years that identified the major risk factors involved in the occurrence of HCC, led to the development of numerous predictive scores, some externally validated and with a very good predictive value, such as the GES score, aMAP and ADRESS. Consequently, one of the aims of this study was to determine the effectiveness of these scores in predicting the risk of HCC after achieving SVR with AAD. Of the 3 scores, only the GES score had excellent discriminatory power for HCC risk at a cut-off value of 6.25 (AUC coefficient: 0.804). Indeed, the GES score was the only one of the 3 whose derivation cohort consisted of patients with chronic HCV infection treated with DAAs (9). However, in contrast to our results, the performance of the GES score was suboptimal in another recent study including 577 patients with HCV infection and liver cirrhosis who achieved SVR with the new DAAs (10). The authors' conclusions were that the GES score failed to correctly discriminate HCC risk, one of the reasons being that the demographic characteristics of the Caucasian patients included in this cohort are different from those of the Egyptian patients in the derivation cohort. However, it should be kept in mind that the results of this study are limited by the small number of patients included.

The aMAP and ADRESS scores, on the other hand, were derived from cohorts of patients with hepatopathies of various etiologies (11,12). The results in the case of the ADRESS score were more satisfactory compared to the aMAP score, but it did not have a good enough discrimination ability to accurately predict the risk of HCC ac-

cording to the 3 risk groups (the AUC coefficient in the case of the ADRESS was 0.743 versus 0.700 in the case of aMAP). Similarly, in a recent study conducted by Shiha *et al.*, the aMAP score failed to predict the risk of HCC in 2085 patients with chronic HCV genotype 4 infection and fibrosis grade F3 and F4 after achieving SVR with AAD (13).

In contrast, other studies evaluated composite HCC risk scores, which were based on combinations of multiple variables, to stratify patients into different HCC risk classes. Four studies focused only on patients with advanced fibrosis or cirrhosis, by proposing a combination of patient-related (age, sex, presence of DM) and biochemical (albumin, GGT, platelet count, AFP) variables, together with data related to by the severity of the liver disease (14,15). Abe *et al.* recently reported that the risk factors for HCC identified in multivariate analysis were pre-SVR serum albumin level for non-cirrhotic chronic HCV infection patients and ALBI score, platelet count and DM for CH patients (14).

CONCLUSIONS

Our study showed that GES score has a very good predictive power regarding the risk of HCC after obtaining SVR and could be recommended in clinical practice for the identification of high-risk patients suitable for surveillance. Future development and validation of other individualized predictive scores score is needed for a correct and cost-efficient selection of patients with high risk of HCC.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest.

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THE USEFULNESS OF NEUTROPHIL-TO-LYMPHOCYTE RATIO FROM THE BRONCHOALVEOLAR LAVAGE (BNLR) IN THE ASSESSMENT OF PATIENTS WITH INTERSTITIAL LUNG DISEASE

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THE USEFULNESS OF NEUTROPHIL-TO-LYMPHOCYTE RATIO FROM THE BRONCHO-ALVEOLAR LAVAGE (BNLR) IN THE ASSESSMENT OF PATIENTS WITH INTERSTITIAL LUNG DISEASE (Abstract): The present study **aims** to evaluate the usefulness of neutrophil-to-lymphocyte ratio from the bronchoalveolar lavage (bNLR) in the assessment of local inflammatory status in patients with the stable interstitial lung disease by comparing a group of patients with IPF *versus* non-IPF patients. A secondary outcome was to see if we can identify values that differentiate idiopathic pulmonary fibrosis from another interstitial lung disease. **Materials and methods:** 42 patients with newly diagnosed ILD (24 with IPF and 18 non-IPF) were included; physical examination, high resolution CT, bronchoscopy with lavage, spirometry, DLCO and blood tests were performed in all patients. **Results:** We did not find any statistically significant difference between bNLR in the two groups IPF *versus* non IPF. bNLR were similar 2.73 + 1.14 in the non-IPF group and 2.98 ±1.72 in IPF patients, p=0.59. bNLR in patients with chronic stable newly diagnosed interstitial lung disease does not seem to bring any additional information. **Conclusions:** Assessment of local lung inflammation in BAL by the bNLR variable can provide minimal differential diagnostic information with higher values in IPF and HP. **Keywords:** NEUTROPHIL-TO-LYMPHOCITE RATIO, BRONHCOALEVOLAR LAVAGE, INTER-STITIAL LUNG DISEASE.

The diagnosis of interstitial lung disease (ILD) is a challenging task and very frequent, despite all efforts, a decisive conclusion cannot be made. The bronchoalveolar lavage (BAL) is part of the assessment of ILD and according to ATS/ERS guidelines, it is recommended when there is no typical UIP appearance on HRCT or if there is a

high degree of uncertainty regarding diffuse interstitial pathology (1). The decision to perform BAL, however, must be analyzed from patient to patient as performing bronchoscopy with BAL can trigger an exacerbation with worsening of the general condition and increased mortality. Serum neutrophil-to-lymphocyte ratio (sNLR) is already a validated marker of systemic inflammation and has been studied in numerous respiratory, infectious, neoplastic, cardiovascular, and autoimmune diseases (2-6). In interstitial lung disease sNLR can be used to assess inflammatory status, disease severity, and short-term prognosis (7,8,9). It has been shown that an sNLR value of 2.8 in patients with non-exacerbated interstitial lung disease is suggestive of systemic inflammation (3). Most studies evaluated NLR from the blood and there have been few studies that have assessed NLR values and its implication in BAL. Data in the literature appraising BAL in patients with ILD follow cell populations rather than NLR value itself, focusing on the identification of exacerbations. Thus, in ILD exacerbations, a high neutrophil count (>25%) and low lymphocyte count (<20%) appear to be associated with a negative prognosis (10). Given the paucity of existing data regarding NLR from bronchoalveolar lavage and the complexity and difficulty of diagnosis in ILD, we attempt to assess the applicability of NRL in current diagnostic practice. The present study aims to evaluate the usefulness of neutrophil-to-lymphocyte ratio from the bronchoalveolar lavage (bNLR) in the assessment of local inflammatory status in patients with stable newly diagnosed interstitial lung disease. Our hypothesis was that if NLR can be used in evaluating inflammation in the blood, it would help evaluate local inflammation and eventually be used to discriminate between various ILDs considering its values, for example IPF versus non-IPF group.

MATERIALS AND METHODS

Study design: it is a prospective cohort study conducted between 2018- 2019 in the "Leon Daniello" Clinic Hospital of Pulmonology, from Cluj-Napoca. **Study population**: 42 patients, over 18 years old with newly diagnosed interstitial lung disease. Exclusion criteria: history of neoplasia, regardless of location; association of known chronic obstructive pulmonary diseases; known genetic disease, diagnosis of pneumoconiosis, or a history of pulmonary tuberculosis, acute infectious disease; patients who received antibiotic treatment 14 days prior to admission, regardless of cause, severe respiratory failure requiring NIV; severe cardiovascular disease; documented psychiatric disease or non-compliant patients. All the patients signed the informed consent. ILD diagnosis was made according to current guidelines after clinical evaluation, imaging, BAL, and multidisciplinary meeting. Bronchoscopy was carried out by the same physician during hospitalization. The analysis of the lavage was performed by the same laboratory physician according to the currently existing good practice guidelines immediately after it was collected, within the first 30 minutes. From the BAL, total cell count and percentage of recovered cells were assessed according to the standard analyzer. By simple rule of 3, from the total cellularity, the absolute value for lymphocytes and neutrophils was calculated to perform the neutrophil/lymphocyte ratio (bNRL). Patients were further divided into two groups those with idiopathic pulmonary fibrosis (IPF) and those with other ILDs

(non-IPF). The non-IPF group included patients with hypersensitivity pneumonitis, sarcoidosis, and undifferentiated ILDs. Spirometry and TLCO were performed at the time of admission according to the ERS/ATS recommendations (11,12). FEV1%, FVC%, TLCO% were noted. The value of DLCO was divided, into 4 groups: 1 > 80%, 2: 50 - 80%, 3: 30 - 50%, and 4 < 30%. The study was approved by the Ethics Commission of the Faculty of Medicine and Pharmacy number 345/1.10.2019 and was reapproved by the Ethics Commission of the Clinical Hospital of Pneumphtysiology "Leon Daniello", Cluj-Napoca 4/2020. Statistical analysis was performed with IBM SPSS v20.0 (Armonk, NY, USA) for Windows. TI-Nspire CX CAS Graphing software is used to perform all statistical analyses and to create all graphical displays.

RESULTS

Forty-two patients with confirmed interstitial lung disease were included in the study: 24 in the IPF group and 18 in the non-IPF group. The bNLR value found was 2.73 + 1.14 in the non-IPF group and 2.98 ±1.72 in IPF patients, p=0.59. No statistically significant differences were found between respiratory function and simple cellularity found in BAL (neutrophils, lymphocytes) in the two groups analyzed (tab. I).

There is no statistically significant difference in bNLR between the two IPF versus non-IPF groups on 0.237 (fig. 1). There is a moderate/weak linear negative correlation r= -0.503 between bNLR value and lung function in IPF patients. The higher the NLR, the lower the lung function (fig. 2). When referring to DLCO there is a weak correlation r=0.302 (fig. 3).

If smokers were removed, the correlation coefficient r increases to -0.575 for FVC and 0.399 for TLCO, suggesting a correlation between them separately from smoking status (figs. 5 and 6). In the non-IPF group no correlation was found between NLR from lavage and lung function assessed by FVC or DLCO.

TABLE I.

Patients functional and blood parameters

	Groups	Number	Median	Standard	Standard	p-value
Neutrophils	non-IPF	18	4.26	2.18	0.51	0.29
	IPF	24	5.01	2.32	0.47	
Lymphocytes	non-IPF	18	1.73	0.77	0.18	0.65
	IPF	24	1.82	0.63	0.13	
NLR	non-IPF	18	**2.73**	**1.14**	0.27	**0.59**
	IPF	24	**2.98**	**1.72**	0.35	
FVC (%)	non-IPF	18	77.78	13.90	3.28	0.18
	IPF	23	71.32	16.08	3.35	
FEV1/FVC	non-IPF	18	77.03	13.24	3.12	0.87
	IPF	23	77.64	11.80	2.46	
FEV1 (%)	non-IPF	18	76.27	20.04	4.72	0.73
	IPF	23	74.23	18.05	3.76	

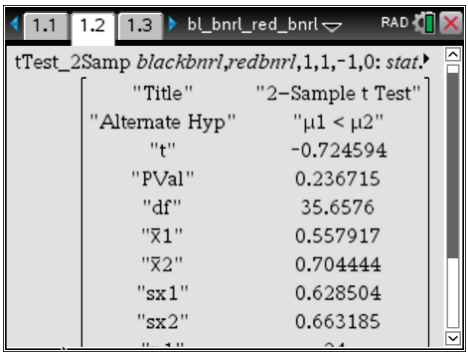

Fig. 1. bNLR values in IPF group versus non IPF group (for data protection, this statistical analysis has been carried out in color. The IPF group was statistically represented as the "black bNRL" blue color and the non-IPF group "red bNRL" the orange color).

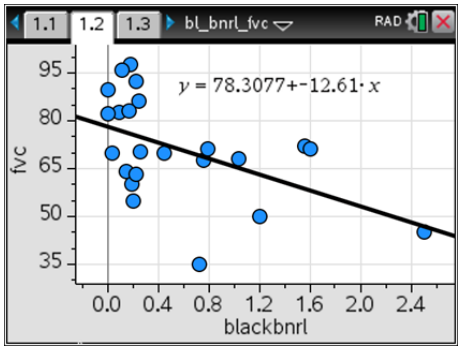

Fig. 2. bNLR correlation with FVC

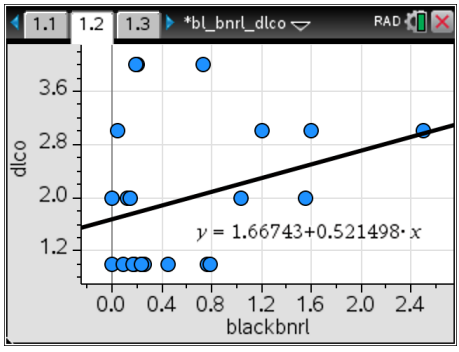

Fig. 3. bNLR correlation with DLCO

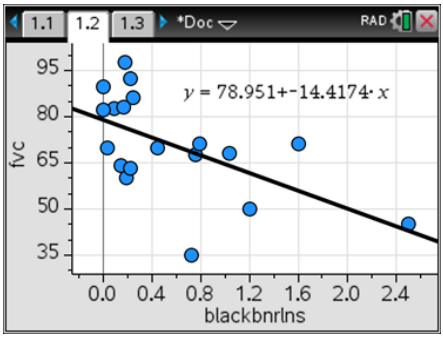

Fig. 4. Correlation between bNLR and FVC in non-smokers

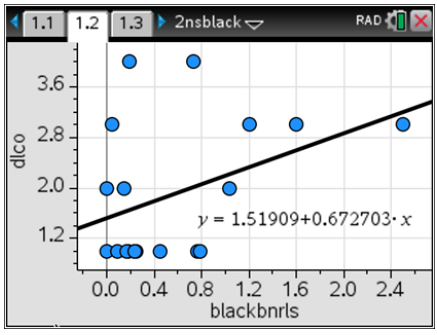

Fig. 5. correlation between bNLR and DLCO in non-smokers

DISCUSSION

The present study assessed the utility of NLR from bronchoalveolar lavage (bNLR) in the evaluation of inflammatory status of patients with stable chronic ILDs (IPF versus non-IPF). No difference was found

between the two groups, bNLR was 2.73 ±1.14 in the non-IPF group and 2.98 ±1.72 in the IPF group, p=0.65. According to our study it is not possible to use bNLR to differentiate between the two groups, as it's the case with serum NLR (3). Studying bNLR in patients with IPF and other fibrosing interstitial lung diseases (PH and sarcoidosis) D'Alessandro and colleagues have identified a statistically significant higher bNLR values in patients with IPF compared to non-IPF group, 2.1±3.8 (IPF) *versus* 0.7±1.9 (sarcoidosis) and 1.6±3.1 (PH) (13). Increased bNRL values were found before severe exacerbation of ILD, frequently associated with an unfavorable outcome (13). A higher bNRL value has also been proposed as an independent risk for lung cancer based on the involvement of inflammation in oncogenesis (13). And this is an important aspect as patients with ILDs are at risk of developing lung cancer that might be undiagnosed in the begging due to extensive parenchymal involvement in some interstitial lung disease (14). Animal studies have shown an increase bNLR in relation with the progression of pulmonary lesions suggesting that bNLR might be an indicator of the severity of lung involvement (15). Concerning threshold values in differentiating different ILD, bNLR showed a sensitivity of 56% and a specificity of 96% for confirmation of PH, when bNRL > 0.48. In the present study the values for both groups were the same, probably as a consequence small sample size and inhomogeneity of the non IPF groups. In healthy nonsmoking individuals BAL contains 85% macrophage, 10-15% lymphocytes and less than 3% neutrophils. Increases or decreases in these percentages have been correlated with different pathologies, but integrated analysis of cellular ratios could increase diagnostic accuracy (16). In patients with ILD at the time of diagnosis and during exacerbation there was an increased percentage of lymphocytes and/or neutrophils in the lavage regardless of the type of ILD (10). The low lymphocyte percentage and high neutrophil percentage (increased bNLR) were independent negative prognostic factors for 90-day survival (10). The above-mentioned study only emphasizes the probability that there is an as-yet poorly understood interconnection between bNRL and lung lesion extension. Similar results were also obtained by Salonen in a study of 20 patients in whom BAL was performed both at baseline and during an AE-ILD. BAL cell count < 20% lymphocytes or > 20% neutrophils during AE-ILD was associated with shorter survival (17). Since bNLR is the neutrophil/lymphocyte ratio in absolute value, we can extrapolate the information and say that bNLR was increased more during exacerbations or even before exacerbations. Concerning bNLR as a predictor of mortality in patients with fibrotic PH and IPF phenotype Barrat *et al.* (18) conclude that increased NLR values are associated with poorer survival in patients with IPF. In ILD patients, a negative prognostic marker remains the reduction in lung function. bNLR appears to correlate weakly/moderately with lung function only in patients with IPF and not in the non-IPF group. So, we can suspect a relationship between lung inflammation as assessed by bNRL and lung volume reduction with a possible prognostic role. Limitations of the study: the small sample of patients, the lack of control group (healthy individuals) and the inhomogeneity of the non-IPF group

comprising various pathologies with different cell profiles. However, it is one of the few studies evaluating bNLR from bronchoalveolar lavage in patients with stable diffuse interstitial lung disease at first diagnosis (*so naïve patients*). But ultimately the question remains whether it is worth the risks of a post-FB exacerbation to assess NLR. Unfortunately, our study, given the small number of patients, cannot transcribe this answer now.

CONCLUSIONS

Assessment of local lung inflammation in BAL by the bNLR variable can provide minimal differential diagnostic information with higher values in IPF and HP. Interestingly enough bNRL shows higher values in the idiopathic disease group, which further supports the inflammatory theory in the pathogenesis and maintenance of IPF. The inflammatory marker bNRL negatively correlates with lung function suggesting the implications of inflammation in lung volume reduction. These findings integrated into broader future research may optimize the management and follow-up of patients with ILD.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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All authors had equal contribution.

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NON-INVASIVE TOOLS TO PREDICT FIBROSIS IN METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE (MASLD). THE RESULTS OF THE PROSPECTIVE STUDY FROM THE HEPATOLOGY TERTIARY CENTER FROM IASI

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NON-INVASIVE TOOLS TO PREDICT FIBROSIS IN METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE (MASLD). THE RESULTS OF THE PROSPECTIVE STUDY FROM THE HEPATOLOGY TERTIARY CENTER FROM IASI **(**Abstract): Metabolic dysfunction-associated steatotic liver disease (MASLD) previously known as non-alcoholic fatty liver disease (NAFLD), represents nowadays the most common cause of chronic liver disease. The gold-standard method for assessing the degree of steatosis and fibrosis is liver biopsy (invasive method). The aim of the study was to establish the role of non-invasive testing (serological and imagistic) in identifying MASLD patients at higher risk to develop advanced fibrosis. **Materials and methods**: a total of 111 patients with MASLD was tested for fibrosis using non-invasive tests. Serological test that were used consisted in the NAFLD fibrosis score (NFS), Fibrosis Index for liver fibrosis (FIB-4) and BARD. Imagistic method consisted in 2D shear wave elastography (2D-SWE.GE elastography). **Results:** The majority of patients (54.9%) had significant fibrosis (F2-F4). The degree of liver fibrosis assessed by 2D-SWE.GE elastography could have been correlated with a range of serological parameters. Liver transaminase values were, in the majority of subjects, the only changes detected. The increase was moderate, and the ASAT/ALAT ratio was subunit. We found statistically significant differences between subgroups of patients with and without advanced fibrosis in the following parameters: BMI, platelets, albumin, blood glucose. All three types of testing identified the cases with significant fibrosis. NFS testing correlated best with advanced fibrosis estimated from 2D-SWE.GE elastometry. **Conclusions:** Serological and imagistic tests are validated tools to predict advanced fibrosis in MASLD patients. The limitation of the study is the relatively small number of patients and the lack of reporting on liver biopsy. **Keywords**: METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE (MASLD), SEROLOGICAL MARKERS, 2D SHEAR WAVE ELASTOGRAPHY.

Metabolic dysfunction-associated steatotic liver disease (MASLD) previously known as non-alcoholic fatty liver disease (NAFLD), represents nowadays the most common cause of chronic liver disease (1, 2). With a global incidence ranging be-

tween 6% and 35%, MASLD is more prevalent in the more developed Western countries (2, 3). The term MASLD encompasses a broad spectrum of different stages of liver damage, ranging from steatosis and metabolic steatohepatitis (MeSH) to MASLD-related cirrhosis and even hepatocellular carcinoma (2, 4). The risk factors for MASLD include waist circumference, blood pressure, plasma triglycerides, plasma high-density lipoprotein-cholesterol, prediabetes, homeostasis model assessment of insulin resistance score, and plasma high sensitivity C-reactive protein. The combination of hepatic steatosis with one of these three metabolic risk stratifications results in the diagnosis of MASLD (5). When assessing the degree of steatosis and fibrosis, the gold standard method is represented by liver biopsy. A number of non-invasive tests (serological markers and imagistic methods) have been developed for replacing the need of a liver biopsy. The aim of the study is to establish the role of the non-invasive testing (serological and imagistic) in identifying MASLD patients at higher risk to develop advanced fibrosis.

MATERIALS AND METHODS

Prospective study, with a group of 111 patients with MASLD (82 male, 29 female patients), aged between 26 and 77 years old (mean age 54.92 years old; p=0.104). The group of patients were registered at the Gastroenterology and Hepatology Institute, of the "Sf. Spiridon" County Clinical Emergency Hospital Iasi. The diagnostic was established from clinical, biological and ultrasound data. Evaluation of each patient consisted in its medical history, clinical exam, laboratory blood testing and 2D-SWE.GE elastography. Including criteria were age over 18 years old, established diagnosis of chronic liver disease (for a minimum of 6 months), MASLD classified. Patients also had to agree to take part in the study and perform all necessary testing. Excluding criteria consisted in pregnancy, lack of discernment (hepatic encephalopathy), chronic liver disease from other causes (B hepatic virus, C hepatic virus, autoimmune disease, alcoholic disease, toxic, drug induced, hemochromatosis, Wilson disease), patients with heart failure, inflammatory bowel disease, intestinal resection, thyroid disease, vascular malformation, focal hepatic lesion on ultrasound, medication that influences portal flow (beta blockers for example). Non-invasive serological tests were the NAFLD fibrosis score (NFS), Fibrosis Index for liver fibrosis (FIB-4) and BARD. The included imagistic method was the 2D shear wave elastography (2D-SWE.GE elastography). NAFLD fibrosis score (NFS) uses 6 variables (age, BMI, blood glucose, platelet count, serum albumin, ASAT/ALAT ratio), and was calculated online. A value <-1.455 has a 90% sensitivity and a 60% specificity in excluding advanced fibrosis, while a score >0.676 has a 67% sensitivity and a 97% specificity for identifying advanced fibrosis (6). Between these values, a "gray area" occurs, in which the diagnosis is uncertain. Fibrosis Index for liver fibrosis (FIB-4) uses parameters like age, ASAT, ALAT and platelet count. Advanced fibrosis is excluded at a score value of <1.30, whilst a score >2.67 confirms the diagnosis. A limitation of this method consists in the existence of indeterminate area between the values. BARD is a test based on ASAT/ALAT ratio, BMI and the presence of diabetes. A value of 0 or 1 has a high negative predictive value (96%) for advanced liver fibrosis (7), while a score ≥2 has a high sensibility for detecting advanced fibrosis, but with a modest specificity (8). 2D-SWE.GE elastography was performed using

a C1-6-D convex probe in Elasto 2D-SWE.GE mode. Liver rigidity (LS) expressed with Young Module (kPa) was saved for each image and automatically calculated. For test validation, the IQR (Inter Quartile Range)/median value ratio must not exceed 30%. The used cut-off values were <6.6 KPa (F0-F1), 6.6-7.9 KPa (F2), 8-10 kPa (F3), >10.1kPa (F4), as proposed by Kim *et al.* (9). Statistical data analysis was made using descriptive statistics and inferential analysis (analytical). Were applied Shapiro-Wilk normality test, t-Student, Chi Squared Test and Spearman's rank correlation coefficient for comparative analysis between samples.

RESULTS

We evaluated the correlation between non-invasive tests (NFS, FIB-4, BARD) with the fibrosis degree evaluated by 2D-SWE.GE elastography. Elastometry value distribution is presented in tab I. Mean values of NFS, FIB-4 and BARD testing are presented in tab. II. In tab. III and tab. IV we present the results of the comparative study of non-invasive diagnosis testing, respectively of post-hoc testing in relation with fibrosis degree. Statistically significant differences were shown between 2 patient groups with different fibrosis degree, F0-F1 versus F3-F4 and F2 versus F3-F4 (p=.000*).

TABLE I.

Distribution of liver stiffness (kPa) in the group of patients with MASLD

Liver stiffness (kPa)	N	Mean value	Standard Deviation	Standard Error	Min	Max
<6,6 kPa	50	5.8378	.58397	.08259	4.48	6.50
6,6-7,9 kPa	30	7.2680	.40771	.07444	6.60	7.98
>7,9 kPa	31	9.6016	1.52407	.27373	8.20	14.70
Total	111	7.2755	1.81484	.17226	4.48	14.70

TABLE II.

General characterization of the parameters analyzed

	Mean value	Standard Deviation	Standard Error	Min	Max	Shapiro-Wilk Test	
						W	p
NFS	1.12163	1.526041	.144845	-1.839	5.652	.98255	.156
FIB-4	1.12480	.629520	.059751	.288	4.811	.84178	.000*
BARD	2.15	1.237	.117	0	4	.90839	.000*

TABLE III.

Comparative study of parameters analyzed by grade of fibrosis

	M ± SD	Odds Ratio	M ± SD	Odds Ratio	M ± SD	Odds Ratio	p
NFS	.59790± 1.458375	-1.839 ÷ 4.059	.82913 ± 1.223576		F0-F1	F2	F3-F4
FIB-4	1.02486 ± .518926	.288 ÷ 2.525	1.03120 ± .507287	.296 ÷ 2.214	1.37658 ± .820716	.507 ÷ 4.811	.047*
BARD	2.02 ± 1.204	0 ÷ 4	1.93 ± 1.311	0 ÷ 4	2.58 ± 1.148	0 ÷ 4	.077

*Statistically significant value ($p < 0.05$).

TABLE IV

Post-hoc testing in relation with fibrosis degree

	F0-F1 *vs.* F2	F0-F1 *vs.* F3-F4	F2 *vs.* F3-F4
	p	p	p
NFS	.464	.000*	.000*

*Statistically significant value ($p < 0.05$).

Figures 1, 2 and 3 shows the mean values obtained from the 3 non-invasive tests (NFS, FIB-4, BARD), for each fibrosis degree. We evaluated the correlation degree between the 3 non-invasive tests. We looked especially for the correlation between risk values and fibrosis degree F3-F4 (advanced fibrosis). The results are shown in tables V, VI and VII.

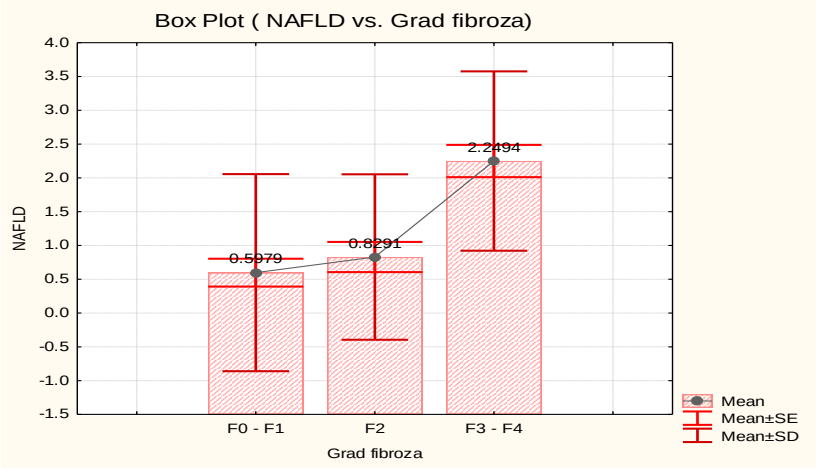

Fig.1. Mean values of NFS *vs.* fibrosis degree

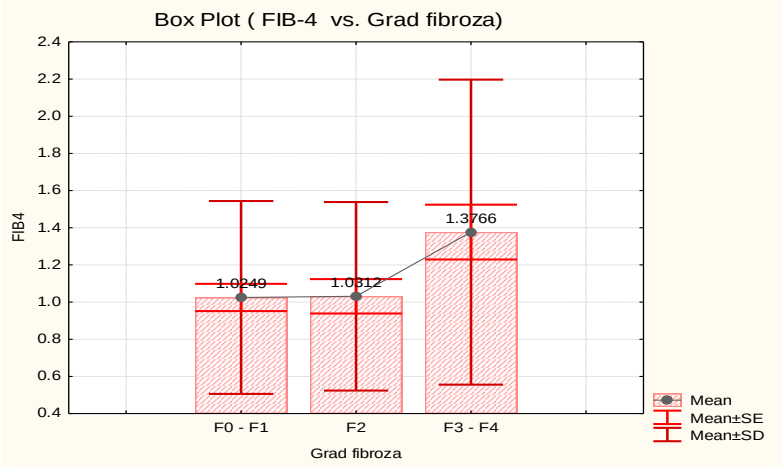

Fig. 2. Mean values of FIB-4 *vs.* fibrosis degree

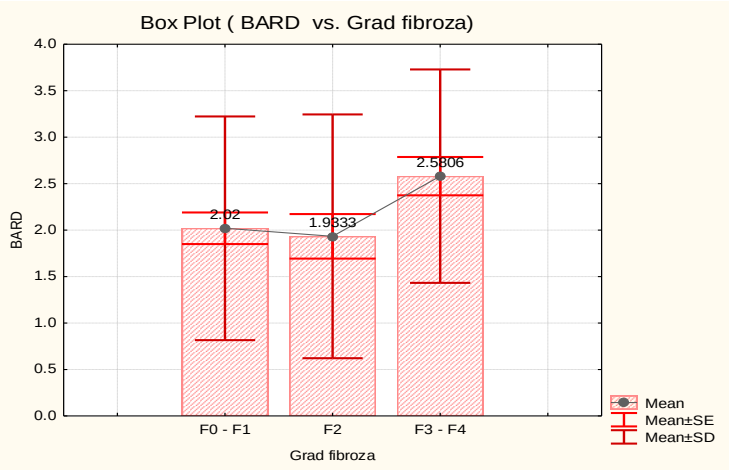

Fig. 3. Mean value of BARD *vs.* fibrosis degree

TABLE V.

Correlation between NFS test and advanced fibrosis

Chi- Squared Test= 13.089, p = .001* **Spearman's rank correlation coefficient = -.251, p = .008***			**F3-F4**		**Total**
			YES	**NO**	
NFS	equivocal	n	3	33	36
		%	9.7%	41.3%	32.4%
	positive	n	28	43	71
		%	90.3%	53.8%	64.0%
	negative	n	0	4	4
		%	0.0%	5.0%	3.6%
Total		n	31	80	111
		%	100.0%	100.0%	100.0%

TABLE VI.

Correlation between the FIB-4 test and advanced fibrosis

Chi- Squared Test = 2.587, p = .108 **Spearman's rank correlation coefficient= .153, p = .110**			**F3-F4**		**Total**
			YES	**NO**	
FIB-4	positive	n	13	21	34
		%	41.9%	26.3%	30.6%
	negative	n	18	59	77
		%	58.1%	73.8%	69.4%
Total		n	31	80	111
		%	100.0%	100.0%	100.0%

TABLE VII.

Correlation between BARD test and advanced fibrosis

Chi- Squared Test = 8.079, p = .004* Spearman's rank correlation coefficient = .270, p = .004*			F3-F4		Total
			YES	NO	
BARD	positive	n	27	47	74
		%	87.1%	58.8%	66.7%
	negative	n	4	33	37
		%	12.9%	41.3%	33.3%
Total		n	31	80	111
		%	100.0%	100.0%	100.0%

In the study group, the NFS test made the best correlation with the patient's advanced fibrosis, with a high sensitivity (90.3%) for the diagnostic of advanced fibrosis. However, we must notice the low specificity (46.2%) of the test. According to our results, the second-best test for diagnostic accuracy is the BARD test, which has an 87.1% sensitivity and a modest 41.3% specificity. The FIB-4 test registered the lowest diagnostic sensibility (41.9%), but with a 73.8% specificity, better than NFS and BARD tests.

DISCUSSION

Most of the patients from the study group (54.9%) had important fibrosis (moderate and severe F2-F4). One explanation for these results is that these patients came to a tertiary hepatology center. The patients with mild steatosis or a small degree of fibrosis are observed in outpatient regime, at the level of primary medicine. Aim of our study was to obtain and study the risk value distribution of clinic and biologic parameters from patients with MASLD. Our results are consistent with literature data. When referring to noninvasive serological tests, the only modified values in most patients in our study group were hepatic transaminases (ASAT, ALAT). The values were moderately elevated (up to 1 to 4-fold the normal value), and the ASAT/ALAT ratio was subunit. The same serological profile is indicated also in other studies (10, 11). However, some studies show that a normal transaminase value does not guarantee the absence of steatohepatitis or advanced fibrosis (11). We evaluated the relationship between fibrosis degree and the values of the used parameters. There were statistically significant differences between the subgroups with and without important fibrosis when referring to the following parameters: BMI, platelet count, albumin, blood glucose levels. These observations are in accordance with literature data (12-15). We consider that the statistic results regarding the correlation between investigated parameters (noninvasive serological and elastometry) and fibrosis degree are relevant because are made from a high enough number of patients in our study group. The relationship between serological parameters and elastometry value shows statistical relevance. Correlation regarding fibrosis, separated for each fibrosis degree, were

affected by the small patient group. For the platelet count parameter, we obtained a single relevant statistic correlation for each fibrosis degree. One possible explanation for the fact that other parameters do not correlate is the small number of patients in one subgroup. The results are in accordance with literature data, which validate the use of elastometry as a non-invasive diagnostic method of advanced liver fibrosis (16). Monitoring the patients with MASLD using non-invasive testing represents a topic of interest because of the risk to develop advanced fibrosis. Non-invasive serological and imagistic testing are useful tools for a quantitative estimation of the fibrosis degree, as shown in numerous studies (6, 16-21). In our study, we put our effort in verifying the specificity and sensibility of three noninvasive serological tests (NFS, FIB-4, BARD), in relation with results from imagistic noninvasive testing (2D-SWE.GE elastography). Numerous studies show that NFS testing and FIB-4 are valid for evaluation of fibrosis degree in patients with non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Two studies from 2015 and 2018 evaluated the severity of steatosis, inflammatory status and degree of fibrosis in a group of 36 patients with non-alcoholic fatty liver disease (diagnostic on ultrasound) using non-invasive testing SteatoTest, ActiTest and FibroTest (22, 23). The authors concluded that non-invasive testing is useful for detection of advanced fibrosis in patients with steatosis on ultrasound, for the selection of patients for liver biopsy and for monitoring disease evolution and treatment efficiency. One meta-analysis regarding the prediction ability for severe fibrosis, included 4 studies, with a total number of 1038 of adult patients and compared data from the studies that investigated the three non-invasive tests that we also used in our study (NFS, FIB-4, BARD) (19). The research investigated sensibility and specificity using Spearman's rank correlation coefficient. Prevalence of severe liver fibrosis among patients in all 4 studies was 13%. For the FIB-4 test, sensibility value was 0.844 and a specificity of 0.685, with a threshold value of 1.30. When raising the threshold value to 3.25, sensibility value decreased to 0.38 and specificity value raised to 0.96. Regarding BARD score, sensitivity value was 0.74, specificity 0.66, significantly lower compared to the FIB-4 testing. The conclusion of the meta analysis was that the FIB-4 test with a threshold value of 1.30 present the highest diagnostic accuracy, better than with a threshold of 3.25 and then NFS, BARD testing (19,20). Similar results were also reported by McPherson *et al.* (21), who compared the diagnostic performance of some non-invasive tests (also including NFS, FIB-4 and BARD) in a study group of 145 patients with NAFLD. In this study, FIB-4 score presented the highest diagnostic accuracy for severe fibrosis (0.86), followed by ASAT/ALAT ratio (AUROC 0.83), NFS (AUROC 0.81), BARD (AUROC 0.77) and ASAT values (AUROC 0.67). A common and important aspect of the mentioned studies is represented by the fact that liver biopsy can be avoided in more than a half of the cases with NAFLD by using non-invasive testing (ASAT/ALAT ratio, FIB-4, BARD testing). The results obtained from our study regarding the diagnostic accuracy of non-invasive tests are different from literature data. All three tests identified the cases with significant fibrosis. Separated analysis showed that NFS test correlated the best with advanced fibrosis

estimated by 2D-SWE.GE elastometry. For our study group, NFS test correlated best with advanced fibrosis, with a sensitivity of 90.3%, but with a rather low specificity of 46.2%. The following test in order of diagnostic accuracy was BARD testing, with a sensitivity of 87.1% and a modest specificity of 41.3%. FIB-4 testing presented the lowest sensitivity (41.9%), but with the highest specificity (73.8%), better than the other tests. We also mention that the FIB-4 test recorded the lowest share of false positive results (26%), lower than the other 2 tests. In contrast with the mentioned studies, we haven't used liver biopsy for identifying advanced fibrosis, instead we used elastometry. We correlated the results of non-invasive serological testing with imagistic testing. We consider that as a possible explanation for the discordant results. The prevalence and incidence of patients with MASLD in daily practice shows the need to develop new, non-invasive, cost-efficient, reproducible methods for monitoring and management of those patients. Considering the raised number of patients with MASLD there is a higher need for developing a diagnostic algorithm and a higher demand for using prediction markers with good replicability.

CONCLUSIONS

In our study group we managed to correlate the presence of fibrosis degree (evaluated with 2D-SWE.GE elastometry) with a series of biological parameters (serological tests NFS, FIB-4, BARD). For our group, all three tests identified the cases with advanced fibrosis. Separate analysis showed that NFS test correlated best with advanced fibrosis estimated with 2D-SWE.GE elastometry. Serological and imagistic tests are validated tools to predict advanced fibrosis in MASLD patients. The limitation of the study is the relatively small number of patients and the lack of reporting on liver biopsy.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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DIAGNOSTIC APPROACHES AND MANAGEMENT OF DEEP NECK SPACE INFECTIONS

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DIAGNOSTIC APPROACHES AND MANAGEMENT OF DEEP NECK SPACE INFECTIONS (Abstract): Deep neck infections (DNI) represent critical medical conditions involving the deep cervical spaces and demand urgent and precise diagnostic measures to prevent severe complications such as airway obstruction, mediastinitis, and sepsis. Accurate and early diagnosis is paramount for effective management and improved patient outcomes. The study has in mind an analysis that provides a component for the efficiency of the medical act and resources, in order to optimize diagnostic strategies and to prevent the occurrence of sepsis. **Materials and methods**: An observational analytical retrospective study was conducted on a group of 110 patients selected from January 1, 2016 to December 31, 2022. **Results**: The most frequent clinical presentation was the infection of the lateral cervical space, the frequency of cases being 61 (55.45%), followed by the parapharyngeal and prevertebral locations 24 (21.82%). Considering the laboratory investigations, the white blood cell count was above the normal value with a mean of 15410.1 (SD: 5455). Positive culture was obtained from 61 patients (55.45%). Isolated aerobic bacteria were isolated in 29 (48.3%), gram positive aerobic bacteria were identified in 38 (34.55%), and Gram-negative aerobic bacteria in 8 (7.27%). Nonbacterial culture was observed in 47 patients (42.73%). All 110 patients received intravenous broad-spectrum antibiotics, which were subsequently upgraded based on culture and sensitivity report. Complications were encountered in 46 patients (41.82%). Airway Obstruction was presented by 24 patients (21.82%), and Mediastinitis was found in 31 patients (28.18%) (tab. III). The average number of days of hospitalization in the hospital was 17.3 days (SD: 10.49), and it varied between 2 days and 51 days. **Conclusions**: Success in the therapy of deep neck infections lies in early and accurate diagnosis, appropriate use of antibiotics, timely surgical intervention when necessary and a multidisciplinary approach to care. This ensures effective infection control and minimizes the risk of complications, ultimately improving patient outcomes. **Keywords**: DEEP NECK SPACE INFECTIONS.

INTRODUCTION

Deep neck infections (DNI) are infections in the potential spaces and fascial planes of the neck with abscess formation or cellulites (1). These involve the deep spaces of the neck and are bacterial infections originating in the upper aero digestive tract. DNI represents pathology with multiple implications, being a major surgical emergency with rapid evolution towards complications that, if not treated timely, can lead to death (2).

The incidence of this disease was relatively high before the advent of antibiotics, requiring prompt recognition and early interventions (3). Odontogenic infection is one of the most common causes, especially in developing countries (4). Clinical manifestations of DNI depend on the spaces involved and include pain, fever, malaise, fatigue, swelling, odynophagia, dysphagia, trismus, dysphonia, otalgia, and dyspnea (5, 6).

Descending necrotizing mediastinitis is the most feared complication and results from retropharyngeal extension of infection into the posterior mediastinum. Septic shock is associated with a mortality rate of 40-50% (7, 8). In addition, suppurative thrombophlebitis of the internal jugular vein associated with pulmonary septic embolism, cavernous sinus thrombosis, and carotid artery erosion have been reported (9).

Management of deep cervical infections includes prompt diagnosis, antibiotic therapy, airway management, and surgery. The therapeutic approach is traditionally based on prompt surgical drainage of the abscess followed by antibiotics or non-surgical treatment using appropriate antibiotics in the case of cellulites (10). Proper diagnosis and prompt management can effectively overcome the disease and provide a cure without complications. However, for this to be possible, otolaryngologists must have a good insight into the presentation, etiology, investigations, and access to appropriate medical and surgical interventions (11, 12).

The aggressiveness of this pathology with a mortality of 20-25%, which can reach 40% of cases through mediastinal extension, requires multidisciplinary, Otolaryngology, oral and maxillofacial treatment, thoracic surgery, anesthesia and intensive therapy (13, 14). These infections are frequently polymicrobial, with a symbiotic relationship between aerobic and anaerobic bacteria (15). Streptococci, *Peptostreptococcus species*, *Staphylococcus aureus* and anaerobes are the most commonly cultured organisms in DNI (16). Many authors recommend urgent drainage, as the infection from cervical abscesses can easily spread and have life-threatening complications such as acute obstruction of the upper respiratory tract, mediastinitis, sepsis, even death (16, 17).

The aim of our study was to present the experience of the Otolaryngology and Oromaxillofacial Departments of the "Sf. Spiridon" County Clinical Emergency Hospital in Iasi, Romania regarding clinical aspects, involved locations, bacteriology, management and complications in deep neck infections. The study focuses on an analysis that provides a component for the efficiency of the medical act and resources, in order to optimize diagnostic strategies and to prevent the occurrence of sepsis.

MATERIALS AND METHODS

An observational analytical retrospective study was conducted on a group of 110 patients selected from January 1st, 2016 to December 31st, 2022. Patients included in

the study were hospitalized for deep throat infection, surgical and medical treatment. The medical data recorded in the patients' electronic records were processed, alongside appropriate imaging (computed tomography and intraoperative photo images). The study was approved by the Clinical Research Ethics Committee at the "Sf. Spiridon" County Clinical Emergency Hospital in Iasi, Romania 15/03/2021.

Inclusion criteria comprised of hospitalized patients who were diagnosed with deep throat infection; with bacteriological examination performed; CT examination and who required surgery.

Exclusion criteria included patients with fistulized or incised peritonsillar phlegmon without affecting the deep cervical spaces, patients with infections in the cervical region that did not require surgical interventions, as well as patients with fistulized intra-oral abscesses.

The identification and documentation of the cases considered the existing changes at the cervico-facial level, the associated lesions and associated pathology, etiology, imaging investigations, treatment method, time elapsed from the diagnosis to the surgical intervention, follow-up in the dynamics of postoperative healing, the complications, surgical reinterventions to avoid the extension of the infection to mediastinum.

The following parameters were analyzed: patient age, sex, antibiotic therapy before admission, imaging and radiological investigations, symptoms, comorbidities, location of infections, treatment, intraoperative findings, bacteriological culture results, complications, length of hospitalization. Abscess locations were divided into 6 regions: submandibular and retrostylian, parapharyngeal and prevertebral. lateral cervical, anterior cervical, retropharyngeal and cervical mediastinitis. All patients were operated under general anesthesia. All 101 patients received IV antibiotic therapy. The choice of antibiotic drugs was decided according to the patient's age, comorbidities and whether he had previously received antibiotic treatment. Antibiotic treatment was reviewed based on culture results and patient response. The selected antibiotics were: cefort and gentamicin or with amoxicillin and clavulanic acid; amoxicillin and clavulanic acid with clindamycin; meropenem, clindamycin and vancomycin +/- colistin; ceffort and clindamycin; cefort and metronidazole.

Microbiology cultures were obtained for all 110 patients for both aerobic and anaerobic agents. The identification of pathogens in the first 24-48 hours was carried out by bacteriological examination of secretions from the abscess, taken by puncture or from the surgical wound. To identify the germs, the MALDITOF device was used, the antibiograms were performed on cards on Team Freedom Evo. Results were checked after the institution of the treatment according to the antibiogram to see if negative.

Statistical Analysis. The statistical analysis of data was performed using a *SPSS version 29* statistical package program. Descriptive statistics of the analyzed variables were performed. Continuous variable was reported as mean values and standard deviation (SD) or median with 25th–75th percentiles (range), depending on the normality and homogeneity of the data series. The Kolmogorov–Smirnov test was applied to verify the normal distribution of the variables. The qualitative variables were presented as absolute (n) and relative (%) frequencies, and the comparison among the groups was based on the results of the

Pearson chi-square test. The significance level calculated in utilized tests (p-value) was considered for the values of $p < 0.05$.

RESULTS

In the "Sf. Spiridon" County Clinical Emergency Hospital in Iași, Romania between January 1st, 2016 and December 31st, 2022, 1,921 patients were treated in the departments of Otolaryngology and Oro-maxillo-facial. According to the inclusion and exclusion criteria mentioned in the material and method section, 110 patients with deep neck infections (DNI) were included in the study. The analysis of patient characteristics indicated a large predominance of male patients (74.55%). Their average age in the analyzed group was 53.77 (standard deviation: 15.3).

Considering the laboratory investigations, the white blood cell count was above the normal value with a mean of 15410.1 (SD: 5455). The mean values and standard deviations of the results of laboratory investigations are presented in table I. The mean C reactive protein (CRP) level was 25.58 mg/L, and the mean blood sugar level was 125.8 mg/dL. There were 34 patients with diabetes (30.91%) (tab. I).

The most frequent clinical presentation was the infection of the lateral cervical space, the frequency of cases being 61 (55.45%), followed by the parapharyngeal and prevertebral locations 24 (21.82%) (tab. I). The most common etiology in our study proved to be the pharyngo-tonsillar localization of the infection, constituting 52.7% of cases. Sore throat 81 (73.64%) and dysphagia were the most common symptoms 84 (76.36) (tab. I).

Comorbidities were found in 52.73% of patients (58 cases). Very frequently they were associated, 82.3% of patients presented two or more associated comorbidities (tab. I). Diabetes mellitus was found in 30.91% of cases (34 patients), and cardiovascular diseases were reported in 35.45% of cases (39 patients). Cardiovascular disease included coronary heart disease or other prior myocardial disease, congestive heart failure, prior stroke, or transient ischemic attack. Immunocompromised status was also found in 20.19% of cases (23 patients) and malignant tumors in 3.64% of cases (4 patients). In the group of immunocompromised patients, we included patients receiving drugs for autoimmune diseases or other known immunodeficiencies. Alcohol excess was mentioned in the patient's history in 5.45% of cases (6 patients) (tab. I).

Positive cultures were obtained from 61 patients (55.45%). Isolated aerobic bacteria were isolated in 29 (48.3%), gram positive aerobic bacteria were identified in 38 (34.55%), and Gram-negative aerobic bacteria in 8 (7.27%). Nonbacterial culture was observed in 47 patients (42.73%) (tab. II).

All 110 patients received intravenous broad-spectrum antibiotics, which were subsequently upgraded based on culture and sensitivity report. Complications were encountered in 46 patients (41.82%). Airway obstruction was presented by 24 patients (21.82%), and Mediastinitis was found in 31 patients (28.18%) (tab. III). The average number of days of hospitalization in the hospital was 17.3 days (SD: 10.49), and it varied between 2 days and 51 days (fig.1).

There was no significant relationship between length of hospitalization and patients' comorbidities ($p = 0.0874$). Also, the statistical analysis did not reveal a statistically significant relationship between the hospitalization time and the location of deep neck infections. ($p = 0.0791$).

TABLE I.

Baseline characteristics of the patients with deep neck infections

Characteristics	Group study,n=110 cases
Gender, male / female, n (%)	82 / 28 (74.55 / 25.45)
Age, years, mean (SD)	53.77 (15.13)
Blood test	
Leukocytes, mm^3, mean (SD)	15410.1 (5455)
C-reactive protein, mg/L, mean(SD)	25.28 (9.04)
ESR (erythrocyte sedimentation rate), mean (SD)	49.2 (16.75)
Fibrinogen, mg/dL, mean (SD)	595.6 (98.8)
Presepsin, pg/m, mean (SD)	527.2 (114.4)
Blood sugar, mg/dL, mean (SD)	125.8 (57.7)
Neutrophil ratio (%)	81.2 (13.5)
Creatinine (µmol/L)	76.9 (11.6)
Location	
submandibular and retrostylian	15 (13.64)
parapharyngeal and prevertebral	24 (21.82)
lateral cervical	61 (55.45)
anterior cervical	17 (15.45)
retropharyngeal	14 (12.73)
cervical mediastinitis	21 (19.09)
Etiology factors	
odontogenic infections	9 (8.18)
pharyngo-tonsillar infections	58 (52.73)
epiglottis	16 (14.55)
foreign body	3 (2.73)
congenital cyst or trauma	14 (12.73)
laryngo-tracheal infections	12 (10.91)
lymphadenopathy	15 (13.64)
Symptoms	
pain	39 (35.45)
sore throat	81 (73.64)
dysphagia	84 (76.36)
dyspnea	19 (17.27)
dysphonia	7 (6.36)
otalgia	6 (5.45)
chest pain	9 (8.18)
fever	57 (51.82)
Comorbidities	58 (52.73)
Diabetes mellitus	34 (30.91)
Cardiovascular diseases	39 (35.45)
Alcohol abuse	6 (5.45)
Immunocompromised status	23 (20.91)
Malignant tumors	4 (3.64)

Continuous variables were expressed as mean (standard deviation),
the variables did have a normal distribution. Categorical variables: number (%).

TABLE II.

Pathogen spectrum in deep neck infections

Pathogens	Group study, n=110 cases
Species	
Monomicrobial	46 (41.82)
Gram-positive aerobe	38 (34.55)
Gram-negative aerobe	8 (7.27)
Polymicrobial	15 (13.64)
2 pathogens	11 (10)
3 pathogens	4 (3.64)
Gram-positive aerobes	
Streptococcus viridans	7 (6.36)
Staphylococcus aureus	14 (12.73)
Group C Streptococci	5 (4.55)
Streptococcus B hemolyticus	4 (3.64)
Streptococcus constellatus	12 (10.91)
Streptococcus anginosus	3 (2.73)
Pseudomonas aeruginosa	8 (7.27)
Klebsiella pneumoniae	7 (6.36)
Other gram-positive cocci	4 (3.64)
Enterococcus species	2 (1.82)
Peptostreptococcus	2 (1.82)
Gram-negative aerobes	
Escherichia coli	4 (3.64)
Haemophilus influenzae	2 (1.82)
Acinetobacter	5 (4.55)
Enterobacter cloacae	3 (2.73)
Fusobacterium species	1 (0.91)

Categorical variables: number (%).

TABLE III.

Morbidity and mortality of patients with deep neck infections

	Group study, n=110 cases
Complications	46 (41.82)
Without complications	64 (58.18)
Airway Obstruction	24 (21.82)
Mediastinitis	31 (28.18)
Pneumonia	9 (8.18)
Necrotizing fasciitis	5 (4.55)
Spontaneous fistulization	15 (13.64)
Internal Jugular Vein Thrombosis	3 (2.73)
Renal insufficiency	15 (13.64
Mortality	10 (9.09)
Number of days of hospitalization, mean (SD)	17.3 (10.49)

Categorical variables: number (%).

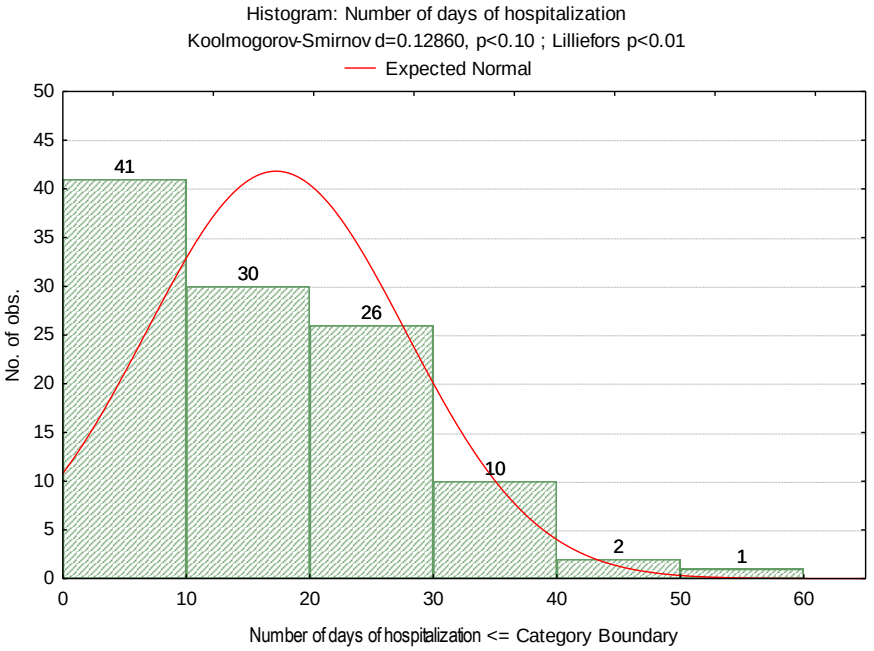

Fig. 1. Histogram of the hospitalization length (days)

DISCUSSION

Recent studies indicate that the incidence of DNI has not significantly declined despite advances in antibiotic therapy, likely due to increased rates of antibiotic resistance and the prevalence of comorbid conditions such as diabetes mellitus and immunosuppression (18). Common pathogens include *Streptococcus species*, *Staphylococcus aureus*, MRSA, and anaerobic bacteria. The rise in odontogenic infections as a primary source of DNI has also been noted, suggesting the need for improved dental care and early intervention for dental abscesses (19).

Computed tomography (CT) with contrast remains the gold standard for diagnosing DNI, offering detailed information on the extent of the infection and the presence of abscesses. Magnetic resonance imaging (MRI) has shown promise in specific scenarios, such as when evaluating potential complications like vascular involvement or when CT results are inconclusive (20). Ultrasound has gained traction for initial evaluations and guiding needle aspirations, particularly in pediatric populations (21).

Empirical broad-spectrum antibiotic therapy remains a cornerstone in managing DNI, with regimens typically including coverage for both aerobic and anaerobic bacteria. Studies highlight the increasing importance of tailoring antibiotic therapy based on culture results to combat antibiotic resistance effectively (22).

There is ongoing debate regarding the necessity of surgical intervention in all cases of DNI. Recent data suggests that in selected cases, particularly with smaller abscesses (<3 cm), conservative management with antibiotics alone may be sufficient (23). However, surgical drainage

remains essential for larger abscesses and when there is imminent risk of airway compromise or progression to mediastinitis. Minimally invasive procedures, such as CT-guided needle aspiration and endoscopic drainage, have been increasingly utilized and show promise in reducing the morbidity associated with traditional open surgical procedures. These techniques are particularly useful for patients with significant comorbidities who may not tolerate more invasive surgery (24).

Recent literature highlights the high risk of complications associated with DNI, including airway obstruction, mediastinitis, and septic shock (25). Early intervention remains critical in preventing these severe outcomes. The role of corticosteroids in reducing inflammation and preventing airway compromise is being explored, with some studies indicating potential benefits in selected patients (26).

Management of DNI in pediatric populations requires special consideration due to anatomical and immunological differences compared to adults. Studies emphasize the importance of conservative approaches and the judicious use of imaging and surgical interventions to minimize long-term sequelae in children (27).

Deep neck infections are serious and potentially life-threatening conditions that require prompt diagnosis and effective management. They involve the potential spread of infection in the deep spaces of the neck, which can lead to severe complications such as airway obstruction, sepsis, and mediastinitis (28).

Identifying the profile of the patient with deep throat infection allows doctors to provide appropriate and timely treatment to manage the infection. Prompt airway protection for patients with deep neck infections and mediastinitis is extremely important. Upper airway obstruction and subsequent respiratory failure require emergency tracheostomy. Effective management of the deep neck infections requires a multidisciplinary approach involving otolaryngologists, infectious disease specialists, radiologists, and critical care teams to address both the infection and its potential complications (29).

Deep neck infections occur in the potential spaces and fascial planes of the neck. The management of such infections typically involves the use of diagnostic imaging, such as CT scans or MRI, to accurately locate and determine the extent of the infection. Treatment generally includes a multidisciplinary approach engaging various specialists like otolaryngologists, infectious disease experts, head and neck surgeons, radiologists, microbiologists, and intensive care specialists (30).

The primary aims of treatment are to control the infection and prevent complications. This often involves surgical intervention to drain any abscesses and debride necrotic tissue. Antibiotic therapy is essential and typically broad-spectrum to cover a variety of bacteria until specific pathogens can be identified. In advanced cases, reconstructive surgery or tissue grafts may be necessary to repair or replace tissues damaged by the infection (31). Continuous research and advancements in head and neck reconstructive techniques are crucial for improving long-term outcomes and restoring both function and appearance for patients following a deep neck infection (32).

In order to reduce the duration and costs of hospitalization, we propose dynamic evaluation of bacteriological examinations, of local evolution through serial computer

tomography examinations, as well as establishing the moment of surgical intervention (tracking the number of days that elapse from hospitalization to the surgical procedure (33). Information regarding the sequencing in the dynamics of biological and imaging parameters will contribute to the safety of the therapeutic act. The retrospective, but especially prospective, follow-up study will bring information on bacteriological variability, which will contribute to preventive therapeutic strategies (targeted antibiotic therapy) for severe complications (IJV thrombophlebitis, sepsis).

Deep abscesses of the cervical region are of major importance due to their frequency and severe complications. Similarly, with Singhal *et al*., 2022, our studies observed a higher incidence in the young male population (34).

However, Huang *et al*. and other studies also show an upward trend in the incidence of infections in elderly patients and patients with systemic diseases. In this group, defense mechanisms would be less effective, recovery rates would be slower and complications would be more frequent (35).

In the pre-antibiotic era, pharyngeal, tonsillar infections were responsible for 70% of deep throat infections (36).

Currently, many studies show a significant decrease in incidence, with odontogenic infections being the most common cause. In our study, bacterial tonsillitis was the most common cause (31.68%), followed by odontogenic infection (23.7%), totaling 55.3% of our samples (37).

In a study by Kusumoto *et al*., the dental focus was the origin of the most frequent abscesses in 37% of patients, while pharyngeal tonsils and disorders were present in 20% of cases, not being possible to identify the source of infection in 33% of patients (38). For Sennes *et al*. odontogenic infection was the cause in 42.1% of patients, tonsillitis in 17.5%, post infections of the upper airways in 15.8% and unknown cause in 8.8% lymphadenitis (39).

Other authors have also reported a significant proportion of deep cervical space infections of unknown primary origin, reaching up to 50% of cases (40).

In 14.8% of our patients, the etiology infection could not be determined, probably because the initial infection was not promptly diagnosed, although it preceded the abscess. Thus, the average time from symptom onset to diagnosis in our study was 8 days, but it was up to 20 days and the main symptoms were fever and sore throat (41).

Knowledge of the anatomical relationships between the spaces of the neck is important for therapeutic management, because the fascia limiting these spaces represent important anatomical barriers to the spread of infection, but also serves to direct infection once its natural resistance is overcome (42).

Other indicators of health care use, such as medications used before and frequency, were not possible to obtain because most patients received treatment for their acute episodes in remote locations, either by their primary care physicians or by their local ENT specialist. However, this may probably be caused by the history of antibiotic abuse and antibiotic resistance explained by the indiscriminate use of antibiotics, especially in colds and other viral infections, which are more prevalent in children than in adults. Prior antibiotic use is correlated with greater recovery of resistant organisms and increased incidence of lactamase-producing bacteria (43).

Adults have infections of the cervical

spaces with multiple interest, this being due to the increased incidence of comorbidities in adults compared to children. Patients with comorbidities tend to have weaker defenses against infections and thus result in higher rates of severe infections that also lead to death.

The microbiology of deep cervical suppurations is characterized by being generally polymicrobial infections, including aerobic and anaerobic, gram positive in particular. Among the pathogens commonly found were: *Streptococcus viridans*, *Streptococcus milleri*, *Prevotella spp.*, *Pepto streptococcus spp.* and *Klebsiella pneumoniae*, the latter being more common in diabetic patients (26). Sennes found *Streptococcus viridans* in 41.5% of cases, *Staphylococcus aureus* in 20.7% of them and 3.8% with *Haemophilus influenzae* (44).

Many studies have shown the association of diabetes mellitus with deep suppurations (9, 11). In patients with diabetes, hyperglycemia can affect several humoral defense mechanisms of the host, such as the modification of neutrophil values, function: adhesion, chemotaxis and phagocytosis, leading to increased predisposition to infection and complications (21). Huang *et al*. found high rates of *Klebsiella pneumoniae* infection in diabetic patients (35).

The management of deep neck infections has evolved significantly over the years, due to ongoing input from by clinical studies, retrospective analyses, and expert consensus.

The literature consistently prioritizes airway management due to the potential for rapid deterioration in patients with DNI. Early recognition of airway compromise and proactive measures, such as intubation or tracheostomy, are critical to prevent life-threatening complications (17).

There is strong consensus on starting broad-spectrum antibiotics immediately upon diagnosis to cover the common pathogens (e.g., *Streptococcus*, *Staphylococcus*, and anaerobes). Empiric regimens often include combinations like ampicillin-sulbactam or clindamycin with a third-generation cephalosporin (25).

Adjusting antibiotic therapy based on culture results and sensitivity patterns is emphasized to avoid resistance and ensure efficacy. This tailored approach is supported by numerous studies highlighting improved outcomes with targeted therapy.

CONCLUSIONS

The management of DNI requires a nuanced approach that balances prompt, aggressive treatment with careful consideration of individual patient factors. Advances in diagnostic imaging and minimally invasive techniques offer new avenues for improving patient outcomes while reducing the risks associated with traditional surgical interventions. Ongoing research and clinical trials are essential to refine these approaches further and address the challenges posed by antibiotic resistance and evolving pathogen profiles. The key to successful management lies in early and accurate diagnosis, appropriate use of antibiotics, timely surgical intervention when necessary, and a multidisciplinary approach to care. This ensures effective control of the infection and minimizes the risk of complications, ultimately improving patient outcomes.

CONFLICT OF INTEREST AND FUNDING

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PREDICTIVE FACTORS FOR HEALTH-RELATED QUALITY OF LIFE IN TYPE 2 DIABETES PATIENTS

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PREDICTIVE FACTORS FOR HEALTH-RELATED QUALITY OF LIFE IN TYPE 2 DIABETES PATIENTS (Abstract) **Aims:** Diabetes-related characteristics and associated chronic conditions are said to influence the health-related quality of life (QoL) of patients with type 2 diabetes mellitus (T2DM). This study assessed the relationships between QoL, demographic, and disease-related variables in T2DM patients without established atherosclerotic disease. **Materials and methods**: This post hoc analysis included outpatient data from a cross-sectional study. Descriptive and inferential statistics were used to examine patient characteristics in relation to QoL scores and identify predictive factors for physical and mental health perceptions. QoL was evaluated with the 36-item Short Form Health Survey (SF-36) questionnaire. **Results**: The 138 T2DM patients enrolled were 49.3% men, 57.86 ± 8.82 years old, with T2DM for 1 to 21 years, without optimal glycemic control under oral glucose-lowering drugs (median HbA_{1c}7.8%). Mean physical and mental component summary scores (PCS, MCS) were 50.44 ± 16.22 and 47.78 ± 12.10, respectively. The lowest SF-36 scores were reported for physical functioning and vitality, and the highest for bodily pain. Women had significantly lower PCS scores compared to men (46.85 *vs.* 54.03, $p = 0.01$). The diastolic index E/e', CHA_2DS_2-VASc score and diabetes duration predicted lower PCS ($p \leq 0.01$), while the impact of age, glycemic control, neuropathy, diastolic dysfunction, and comorbidities on QoL was non-significant. The diastolic index E/e', CHA_2DS_2-VASc score and diabetes duration predicted lower PCS ($p \leq 0.01$). **Conclusions:** These results provide a nuanced view of what is clearly a complex set of relationships. Further longitudinal studies are necessary to ascertain the effects of these variables on the quality of life of T2DM patients. **Keywords:** QUALITY OF LIFE, SF-36V2, TYPE 2 DIABETES.

Health-related quality of life (HRQoL) is a multidimensional concept first introduced half a century ago that provides an accurate, comprehensive, and concise view of patients' perceived impact of chronic disease (with or without comorbidities) on their

lives. It considers physical, mental, cognitive, and psychosocial aspects. Diabetes mellitus has been independently associated with lower HRQoL scores, similar to pulmonary, cardiovascular, and oncologic conditions. The quality of life of patients with diabetes is a psychosocial concern with implications for their long-term adherence to treatment (1, 2, 3). Current recommendations highlight diabetes-related psychosocial screening and care protocols as an important component that should be integrated into annual routine diabetes management practices to prevent or delay complications and optimize quality of life (4, 5).

Self-reported data on general health, physical status, social functionality, emotional well-being, and self-care capacity provide a meaningful context for achieving the clinical and biological goals of medical, nutritional and pharmacologic interventions. HRQoL has been studied as a primary or secondary outcome in both observational and interventional studies, often using the Short Form 36 (SF-36), a standardized, freely available, easy-to-administer questionnaire (6, 7). SF-36 assesses perceived health status across multiple scales - general, physical, mental, and social functioning. Although SF-36 was not developed specifically for people with diabetes, it helps explore relevant issues in the lives of these patients (8).

This study aimed to analyze the relationships between QoL data surveyed with SF-36v2 and clinical-biological parameters in type 2 diabetes (T2DM) patients with incidental echocardiographic findings of diastolic dysfunction (DD), but without established atherosclerotic disease.

MATERIALS AND METHODS

This is a post hoc analysis of outpatient data collected from T2DM patients presenting at the Clinical Centre of Diabetes, Nutrition, and Metabolic Diseases Iasi between June 2016 and February 2018. It is part of a larger prospective observational study approved by the Ethics Committees of "Grigore T. Popa" University of Medicine and Pharmacy Iași and of "Sf. Spiridon" Emergency Clinical Hospital Iași.

The study enrolled 138 consenting T2DM patients treated with metformin and/or sulfonylurea or acarbose, and who were eligible for additional incretin-based medication due to inadequate glycemic control (HbA_{1c} > 7%). The exclusion criteria were: HbA_{1c} < 7%, type 1 or secondary pancreatic diabetes, elevated triglycerides >400 mg/dL, uncontrolled blood pressure (>140/90 mmHg), cardiovascular disease (atherosclerotic manifestations, valvular heart disease, moderate/severe mitral annular calcification, dysrhythmias, cardiac pacemakers), history of inflammatory acute/chronic conditions (pancreatitis, liver failure, gastrointestinal and kidney diseases, malignancies), psychiatric disorders, pregnancy or intention to become pregnant, and smoking or recent history of smoking (< 1-year withdrawal). The enrollment process was described in more detail in Grigorescu *et al*, 2021, 2023 (9, 10).

The patients underwent physical examination, blood and urine sample tests (lipids, glycated hemoglobin HbA_{1c}, glycemia, insulin, C-peptide, high sensitivity C-reactive protein, interleukin (IL)-6, Tumour Necrosis Factor (TNF)-alpha, uric acid, etc.), and resting electrocardiography (11). Values of the ratio between mitral E wave velocity and mitral A wave velocity (E/A), mitral annular velocity with tissue Doppler imaging (e'), left ventricular ejection fraction (LVEF), fractional shortening (FS), E-

wave deceleration time (EDT), isovolumic relaxation time (IVRT), interventricular septum (IVS), left ventricular posterior wall (LVPW), left atrium volume (LAV) were measured by transthoracic echocardiography. The estimated glomerular filtration rate (eGFR) was computed using the CKD-EPI creatinine equation. The methods used for measuring HbA_{1c} and immunological parameters were DCCT-aligned ion-exchange high-performance liquid chromatography (Bio-Rad D-10™) and chemiluminescence (IMMULITE 1000). The thromboembolic risk score CHA_2DS_2-VASc was calculated based on age > 75 years, female sex, the presence of diabetes, congestive heart failure, hypertension, vascular disease, and past stroke events (12).

The patients were also asked to complete the Romanian version of the SF-36v2 questionnaire, consisting of 36 items grouped into 8 domains: Physical Functioning Scale (10 items), Physical Impairment Problems Scale (4 items), Social Functioning Scale (2 items), Physical Pain Scale (2 items), Mental Health Scale (5 items), Problems Caused by Emotional States Scale (3 items), Vitality Scale (4 items). All responses were re-coded to a linear 0-100 scale, with higher scores indicating better HRQoL. The two summary scores - the *physical component summary* (PCS) and the *mental component summary* (MCS) - were calculated according to the user's manual: the PCS was comprised of the scales PF, RP, BP, and GH, while MCS was computed from the remaining RE, SF, MH, and VT scales (6).

The statistical analysis was performed using IBM SPSS Statistics for Windows (version 20, SPSS Inc, Chicago, IL, USA). P-values < 0.05 were considered statistically significant. Continuous variables were assessed for conformity to normal distribution using the Kolmogorov-Smirnov test. Parametric and non-parametric tests (ANOVA, Kruskal-Wallis, Mann-Whitney U) were applied to compare mean/median questionnaire scores or mean ranks between specific groups (i.e., stratified by gender, presence of neuropathy). Receiver-operating characteristic (ROC) curves and areas under the curves (AUCs) were also considered (13).

RESULTS

Data collected from 138 patients aged 57.86±8.82, of whom 49.3% were men, are summarized in tables I and II. The patients had a diabetes history of 1 to 21 years (median value 5) and HbA_{1c} levels >7% on enrollment. The SF-36 scores averaged 50.44 ± 16.22 for self-reported physical health (PCS) and 47.78 ± 12.10 for the mental component (MCS). More details are shown in tables II and IV.

PCS had a weak association with eGFR ($r = 0.217$, $p = 0.011$), CHA_2DS_2-VASc ($r = -0.219$, $p = 0.01$), and E/e' ($r = -0.213$, $p = 0.013$), while MCS was associated with E/e' ($r = -0.202$, $p = 0.019$) and TNF-alpha ($r = -0.169$, $p = 0.048$) values. The following significant correlations were also noted between SF-36 components and patient characteristics:

- General health (GH) correlated with weight ($r = -0.169$, $p = 0.048$), abdominal circumference (WC, $r = -0.186$, $p = 0.029$), and LDL-cholesterol ($r = 0.194$, $p = 0.023$);
- Vitality (VT) correlated with left atrium length ($r = -0.185$, $p = 0.043$);
- Bodily pain (BP) correlated with CHA_2DS_2-VASc ($r = -0.250$, $p = 0.003$), HOMA-IR ($r = -0.190$, $p = 0.025$), index C-peptide ($r = 0.169$, $p = 0.047$), E/e' ($r = -$

0.173, p = 0.044), E/A (r = 0.170, p = 0.047), and EDT (r = 0.204, p = 0.017);

- Physical functioning (PF) correlated with CHA_2DS_2-VASc (r = -0.194, p = 0.022), C peptide (r = 0.184, p = 0.031), and HOMA-IR (r = 0.169, p = 0.048);

- Role physical (RP) correlated with age (r = -0.174, p = 0.041), CHA_2DS_2-VASc (r = -0.201, p = 0.018), and TNF-alpha (r = -0.208, p = 0.014).

Subgroup analyses based on age, sex, DD, degree of inadequate glycemic control, neuropathy, and other comorbidities (hypertension, dyslipidemia, obesity, metabolic dysfunction-associated fatty liver disease) revealed significant SF-36 score differences only for self-assessed physical health between men and women (54.03 vs. 46.85, p = 0.01) (table III). The presence of comorbidities did not seem to affect the PCS and MCS scores (16.7% of patients had three comorbidities, 42% had two, and 32.6% had just one). Patients with mitral annular calcifications and/or aortic atheromatosis had higher MCS scores (50.43 ± 12.06 vs 45.49 ± 11.74, p = 0.016), similar to patients with dyslipidemia.

Further analyses based on the qualitative variables revealed significant differences for the following scales:

- patients with obesity had significantly lower scores (51.63 ± 17.89 vs 44.90 ± 16.88, p = 0.032) for **general health** compared to those with BMI < 30 kg/m^2;

- men reported higher scores for **bodily pain** compared to women (mean ranks 82.49 vs 56.89, p<0.01), and patients with neuropathy had lower scores for this scale (62.30 vs 75.30, p = 0.05);

- **social functioning** scores were lower in patients with ≥ 3 comorbidities (p = 0.041);

- **emotional role** scores were lower in patients with ≥ 4 criteria for metabolic syndrome (41.38 *vs.* 78.82, p = 0.05), which described almost all patients.

TABLE I.

Patient characteristics overall and based on QoL scores

Studied Variable	Overall (N = 138)	PCS		*p-value*	MCS		*p-value*
		< median (N = 69)	> median (N = 69)		< median N = 68	> median N = 70	
Age (years)	57.86±8.82	58.87 ± 8.43	56.84 ± 9.14	0.178	58.21 ± 8.99	57.51 ± 8.69	0.647
Sex male (n)	68 (49.30%)	26 (18.84%)	42 (30.43%)	**0.006**	31 (22.47%)	37 (26.81%)	0.39
Sex female (n)	70 (51.70%)	43 (31.16%)	27 (19.57%)		37 (26.81%)	33 (23.91%)	
Weight (kg)	91.11 ± 14.71	91.72 ± 13.52	90.51±15.84	0.631	91.95 ± 15.31	90.30±14.17	0.513
BMI (kg/m^2)	32.65 ± 5.50	33.43 ± 5.10	31.86 ± 5.81	0.095	33.21 ± 5.89	32.10 ± 5.08	0.241
WC (cm)	109.13±10.74	110.46±11.01	108.08±10.43	0.194	110.04±11.40	108.52±10.11	0.408
DM duration * (y)	5 (8)	7 (8)	4 (8)	**0.046**	6 (9)	5.5 (7)	0.904
Neuropathy + (n)	61 (44.2%)	34 (24.64%)	27 (19.57%)	0.23	32 (23.19%)	29 (21.01%)	0.31
Neuropathy - (n)	77 (55.8%)	35 (25.36%)	42 (30.43%)		36 (26.09%)	41 (29.71%)	
HbA_{1c} * (%)	7.8 (1.11)	7.85 (1.08)	7.88 (1.4)	0.443	7.85 (1.33)	7.8 (1.2)	0.917
Fasting glycemia * (mg/dL)	162 (46)	158.5 (58)	166.5 (45)	0.592	160.5 (56)	162 (49)	0.371
Insulin * (µIU/mL)	11.2 (9.39)	12 (9.51)	12.3 (9.44)	0.676	13.3 (12.29)	10.25 (7.98)	0.274
C-peptide * (ng/mL)	3.26 (2.22)	3.35 (2.09)	2.89 (1.53)	0.371	3.38 (2)	2.83 (1.49)	0.273
HOMA-IR	5.74 ± 3.87	5.79 ± 3.75	5.68 ± 4.02	0.874	6.07 ± 3.70	5.41 ± 4.03	0.322

Studied Variable	Overall (N = 138)	PCS		*p*-value	MCS		*p*-value
		< median (N = 69)	> median (N = 69)		< median N = 68	> median N = 70	
HOMA C-peptide	4.02 ± 2.10	4.16 ± 2.07	3.89 ± 2.14	0.447	4.30 ± 2.07	3.76 ± 2.13	0.138
Index C-peptide *	0.24 (0.19)	0.22 (0.28)	0.27 (0.18)	0.371	0.21 (0.17)	0.27 (0.21)	0.085
Total cholesterol (mg/dL)	195.33 ± 46.11	189.90 ± 46.25	201.77 ± 45.14	0.101	195.07 ± 46.77	195.59 ± 45.80	0.948
LDL-cholesterol (mg/dL)	103.12 ± 38.96	97.63 ± 36.78	108.62 ± 40.54	0.098	103.34 ± 40.43	102.92 ± 37.76	0.949
HDL-cholesterol (mg/dL)	56.79 ± 15.27	56.62 ± 16.13	56.97 ± 14.65	0.894	56.61 ± 16.39	56.97 ± 14.20	0.892
Triglycerides (mg/dL)	202.57 ± 90.46	197.62 ± 90.12	207.51 ± 91.19	0.523	208.87 ± 88.57	196.44 ± 92.49	0.422
eGFR (mL/min/1.73 m^2)	82 ± 16.37	79.07 ± 17.77	87.12 ± 17.43	**0.008**	84.09 ± 18.49	82.13 ± 17.58	0.525
ACR (mg/g)	27.14 ± 48.64	28.19 ± 50.80	26.02 ± 46.15	0.826	28.37 ± 49.78	26.02 ± 48.04	0.811
Uric acid (mg/dL)	5.48 ± 1.43	5.53 ± 1.37	5.53 ± 1.37	0.694	5.37 ± 1.41	5.59 ± 1.45	0.380
hsCRP * (mg/L)	5.35 (9.18)	5.87 (9.17)	5.33 (8.6)	0.332	5.38 (9.51)	4.91 (9.15)	0.914
IL-6 * (pg/mL)	3.52 (4.66)	2.48 (1.86)	2.05 (0.95)	0.123	2.39 (1.64)	2.00 (1.12)	0.099
TNF-α * (pg/mL)	8.73 (7.66)	7.94 (3.65)	7.35 (3.91)	0.079	7.89 (3.26)	6.72 (3.95)	**0.025**

*Data are expressed as medians and IQR (non-normal distribution); PCS: physical component summary; MCS: mental component summary; BMI: body mass index; WC: waist circumference; DM: diabetes mellitus; HbA_{1c}: glycated hemoglobin; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; HOMA C-peptide: Homeostatic Assessment Model of C-peptide; eGFR: estimated glomerular filtration rate; UACR: albumin-to-creatinine ratio in urine; hsCRP: high-sensitive C-reactive protein; IL-6: interleukin 6; TNF-α: tumor necrosis factor-alpha.

TABLE II.

The evaluation of chronic conditions overall and based on QoL scores

Studied Variable	Overall (N = 138)	PCS		*p*-value	MCS		*p*-value
		< median (N = 69)	> median (N = 69)		< median N = 68	> median N = 70	
Comorbidities							
Dyslipidemia + (%)	99 (71.7%)	43 (31.16%)	56 (40.58%)	0.128	43 (31.16%)	56 (40.58%)	**0.023**
Dyslipidemia - (%)	39 (29.3%)	23 (16.66%)	16 (11.60%)		25 (18.12%)	14 (10.14%)	
Hypertension + (%)	93 (67.4%)	48 (34.78%)	45 (32.60%)	0.358	47 (34.06%)	46 (33.33%)	0.404
Hypertension - (%)	45 (32.6%)	21 (15.22%)	24 (17.40%)		21 (15.21%)	24 (17.40%)	
MAFLD+ (%)	106 (76.81%)	55 (39.86%)	51 (36.96%)	0.420	55 (39.86%)	51 (36.96%)	0.180
MAFLD - (%)	32 (23.19%)	14 (10.14%)	18 (13.04%)		13 (9.42%)	19 (13.76%)	
Obesity + (%)	92 (66.67%)	51 (36.96%)	41 (29.71%)	**0.052**	48 (34.78%)	44 (31.88%)	0.217
Obesity - (%)	46 (33.33%)	18 (13.04%)	28 (73.68%)		20 (14.50%)	26 (18.84%)	
Cardiac status							
With DD (%)	98 (71%)	49 (35.50%)	49 (35.50%)	0.574	50 (36.23%)	48 (34.78%)	0.325
Without DD (%)	40 (29%)	20 (14.50%)	20 (14.50%)		18 (13.04%)	22 (15.94%)	
LVEF (%)	67.14 ± 9.35	67.68 ± 9.78	66.59 ± 8.93	0.497	67.09 ± 9.55	67.19 ± 9.26	0.951
FS (%)	38.16 ± 7.89	38.27 ± 8.20	38.05 ± 7.59	0.875	37.95 ± 8.16	38.37 ± 7.64	0.766
E/A *	0.93 (0.68)	1.06 (0.83)	0.89 (0.63)	0.818	0.87 (0.72)	0.95 (0.63)	0.620
E/e'	6.54 ± 1.84	6.97 ± 1.96	6.10 ± 1.10	**0.006**	6.89 ± 1.93	6.21 ± 1.69	**0.031**
EDT *	190 (58)	177.5 (44)	203 (65)	**0.010**	185 (48)	195 (65)	0.545
IVRT *	104 (25)	100 (20)	100 (30)	0.509	100 (70)	100 (20)	0.752

Studied Variable	Overall (N = 138)	PCS		*p*-value	MCS		*p*-value
		< median (N = 69)	> median (N = 69)		< median N = 68	> median N = 70	
IVS *	11 (2)	12 (2.8)	11 (2)	0.505	12 (3)	11 (2)	0.178
LVPW *	11 (2)	11 (2)	11 (2)	0.260	11 (2)	11 (2)	0.446
LAVi *	42 (18)	42 (20)	39 (18)	0.725	39 (14)	43 (19)	0.280
CHA_2DS_2-VASc *	3 (1)	3 (2)	2 (1)	**0.009**	3 (1)	3 (4)	0.542
NT-proBNP * (pg/mL)	63 (77.1)	65 (88.6)	59 (71.24)	0.615	74 (90.24)	54.5 (66.14)	0.276

* Data are expressed as medians and IQR; PCS: physical component summary; MCS: mental component summary; MAFLD: Metabolic dysfunction-associated fatty liver disease; DD: diastolic dysfunction; LVEF: left ventricular ejection fraction; FS: fractional shortening; E: mitral E wave velocity (rapid filling) with pulsed Doppler; A: mitral A wave velocity (atrial contraction) with pulsed Doppler; e': mitral annular velocity with tissue Doppler imaging; EDT: E-wave deceleration time; IVRT: isovolumic relaxation time; IVS: interventricular septum; LVPW: left ventricular posterior wall; LAVi: indexed left atrium volume; CHA_2DS_2VASc: thromboembolic risk score; NT-proBNP: N-terminal pro-brain natriuretic peptide.

TABLE III.

Associations between QoL components and diabetes-related factors

Studied Variable		PCS	*p*-value	MCS	*p*-value
Age (years)	< 50	51.50 ± 13.72	0.713	47.45 ± 13.45	0.934
	50 - 64	50.86 ± 16.82		48.12 ± 12.00	
	> 64	48.37 ± 17.25		47.27 ± 11.34	
Sex	Male	54.03 ± 16.59	**0.010**	48.63 ± 12.39	0.418
	Female	46.95 ± 15.18		46.96 ± 11.74	
Diabetes duration (years)	< 5	52.17 ± 14.92	0.331	46.19 ± 12.05	**0.031**
	5 - 10	50.70 ± 17.30		52.09 ± 12.01	
	> 10	47.12 ± 17.15		45.90 ± 11.43	
BMI (kg/m^2)	< 25	52.22 ± 16.31	0.870	51.27 ± 11.94	0.754
	25 - 29.9	51.32 ± 16.95		47.98 ± 9.97	
	> 30	49.93 ± 16.04		47.46 ± 13.04	
HbA_{1c} (%)	< 7.5	49.22 ± 17.18	0.824	46.49 ± 12.72	0.658
	7.5 - 8	50.91 ± 16.64		48.77 ± 12.28	
	> 8	51.16 ± 15.20		48.14 ± 11.49	
Neuropathy	with	49.46 ± 15.67	0.531	47.17 ± 11.30	0.597
	without	51.21 ± 16.44		48.27 ± 12.75	
Hypertension	with	50.09 ± 16.45	0.721	47.80 ± 12.26	0.983
	without	51.15 ± 15.91		47.75 ± 11.90	
Dyslipidemia	with	51.76 ± 16.00	0.128	49.00 ± 12.14	**0.023**
	without	47.09 ± 16.52		44.68 ± 11.58	
MAFLD	with	49.13 ± 15.79	0.567	47.44 ± 12.03	0.841
	without	50.92 ± 16.43		47.91 ± 12.19	
Obesity	with	49.69 ± 16.13	0.441	47.35 ± 13.01	0.518
	without	51.95 ± 16.49		48.65 ± 10.12	
Met. syndrome criteria	≤ 3	51.16 ± 16.33	0.678	47.82 ± 10.92	0.979
	≥ 4	49.98 ± 16.24		47.76 ± 12.87	

Studied Variable		PCS	*p*-value	MCS	*p*-value
Diastolic dysfunction	with	51.24 ± 15.72	0.336	47.98 ± 11.26	0.786
	without	48.47 ± 17.44		47.30 ± 14.64	
CHA_2DS_2-VASc	< 3	53.46 ± 16.21	**0.048**	47.84 ± 12.46	0.961
	≥ 3	47.97 ± 15.92		47.74 ± 11.89	

PCS: physical component summary; MCS: mental component summary; BMI: body mass index; MAFLD: Metabolic dysfunction-associated fatty liver disease; CHA_2DS_2VASc: thromboembolic risk score.

TABLE IV.

SF-36 scales - descriptive and associative statistics

SF-36	GH	BP*	MH	PF*	RE*	RP*	VT	SF*	PCS	MCS
Mean/median*	47.13	67.50	41.65	35	66.66	50	36.81	50	50.44	47.78
Std. Dev. / IQR	17.45	35	9.12	40	100	100	13.64	15.63	16.22	12.10
r - PCS	0.206	0.699	0.036	-0.23	0.433	0.841	0.036	0.103	1	0.448
p-value	0.016	<0.001	NS	0.007	<0.001	<0.001	NS	NS	-	<0.001
r - MCS	0.208	0.316	0.130	-0.378	0.866	0.547	0.211	0.382	0.448	1
p-value	0.014	<0.001	NS	<0.001	<0.001	<0.001	0.013	<0.001	<0.001	-

GH, general health; BP, bodily pain; MH, mental health; PF, physical functioning; RE, role emotional; RP, role-physical; VT, vitality; SF, social functioning; PCS, physical component summary; MCS, mental component summary; NS statistically non-significant: p > 0.05, r: coefficients of correlations with PCS and MCS.

ROC curves predictive of lower PCS were found for E/e' (AUC = 0.643, CI 0.549-0.736, p = 0.005), CHA_2DS_2-VASc (AUC = 0.623, CI 0.530-0.716, p = 0.013), and diabetes duration (AUC = 0.613, CI 0.549-0.736, p = 0.005), as illustrated in first figure.

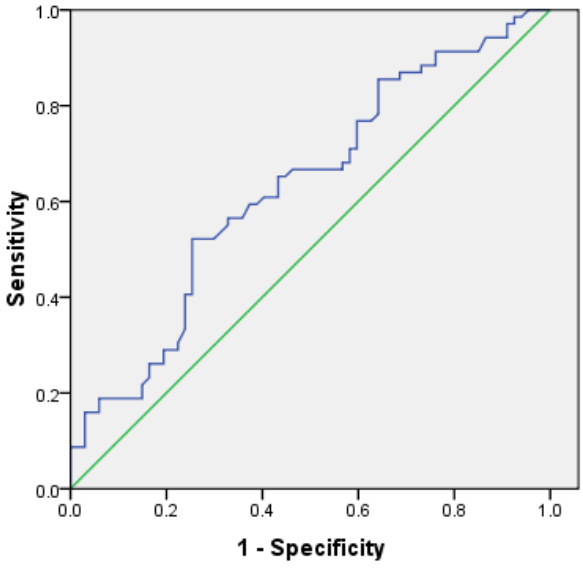

Fig. 1. Receiver-operating characteristics (ROC) curve for E/e'

DISCUSSION

There is growing interest in understanding and addressing the factors associated with a decline in health-related quality of life for people with type 2 diabetes as a way to improve healthcare services and patient adherence. This post hoc analysis of 138 T2DM Romanian patients without overt manifestations of atherosclerotic disease focused on the associations between patient-reported outcomes and characteristics with proported effects on QoL (metabolic profile, pre-existing chronic conditions).

QoL was assessed using the PCS and MCS scores of the SF-36v2 questionnaire, similar to other published and ongoing observational and interventional research from Europe and elsewhere (14, 15, 16). A minimum SF-36 score change of 3-5 points has been proposed as clinically relevant, with due caution when interpreting it as a significant improvement or decline. Furthermore, comparisons across studies should be made with circumspection, acknowledging the substantial heterogeneity of the studied populations in terms of their general, metabolic, and pathology-related characteristics (17, 18, 19).

In our study, the lowest SF-36 scores were reported for physical functioning, followed by vitality, and the highest were for bodily pain. Compared to previous research on age-standardized Romanian general population, our patients' PCS and MCS scores were substantially lower: 74.52 ± 19.44 vs. 50.44 ± 16.22 and 71.70 ± 19.02 vs 47.78 ± 12.10, respectively (20). At the same time, the mean PCS and MCS values were within the range of scores reported for patients with diabetes from other geographical regions/countries: Greece (39.96 and 38.24), Spain (44.9 and 51.2), Netherlands (49.4 and 53.9), India (52 and 46), Ethiopia (40.15 and 48.11), and United States (52 and 46) (21, 22, 19, 23, 24, 25). Moreover, the mean values of our patients' PCS and MCS scores were comparable to those reported in other clinical research investigating the effects of new drugs or weight loss programs (26, 27, 28, 16). For example, in an interventional clinical trial on 563 uncontrolled T2DM patients of similar mean age (55 years), slightly higher HbA_{1c} (8.32%), and higher BMI (37.1 kg/m^2), an intensive weight loss program yielded no significant differences in SF-36 scores assessed after 12 months, compared to a control group: PCS 45.7 vs 45.2, and MCS 51.5 vs 51.6 (16).

While some researchers have demonstrated associations between glycemic control and HRQoL domains, in other studies and our analysis, HbA_{1c} was not associated with PCS and MCS (29). In a multivariate analysis of 5-year data from 1,876 patients with T2DM from Denmark, the Netherlands, and the UK, no significant associations were identified between HbA_{1c} and SF-36 scores after adjustment for age, sex, BMI, type of antihyperglycemic drugs, and education status. Even so, 60% of the patients with HbA_{1c} below 7% and who were treated with oral medication had higher SF-36 scores (PCS 46.2 and MCS 54.6) (30). In another study where 1,352 patients with T2DM filled out the SF-36 questionnaire, a multivariate analysis adjusting for disease duration and other risk factors found significantly higher PCS and MCS scores in patients with an HbA_{1c} below 6.9% versus above 8.6% (31). These differing results across studies suggest that we have yet to learn about the complexities of the proport-

ed relationships.

At the same time, systematic reviews and meta-analyses confirm that patients with a longer duration of diabetes, chronic complications and hypertension have lower QoL scores. In our data, patients' quality of life decreased as the duration of the disease increased, which is consistent with the results of other studies (14). However, the difference was significant only for MCS.

Comorbidities were highly prevalent in our study, especially hypertension (67.4%), dyslipidemia (71.74%), and MAFLD (75.46%) only twelve patients had none. Almost all patients had abdominal obesity and metabolic syndrome, of which 60.9% met at least 4 criteria. In addition, 83 patients had diastolic dysfunction diagnosed according to the 2016 guideline, of whom 71 with grade 1 severity (and all the patients had normal systolic function). By comparison, the prevalence of these common chronic conditions in other studies varied from 27.71% to 78.2% for hypertension, from 43.5% to 98.3% for dyslipidemia, and was only 32.8% for the metabolic syndrome (32, 24, 33, 34). Our data set was, therefore, suitable for exploring the relationship between comorbidities and QoL.

Available research into the impact of comorbidities on the quality of life in type 2 diabetes patients has been reviewed recently in a meta-analysis, with the key finding that each additional chronic condition decreased the PCS score by approximately 3% (36). Nonetheless, in our study, the number of comorbidities did not appear to undermine the health-related quality of life, nor did the presence of subclinical diastolic dysfunction, which is consistent with other studies that evaluated such relationships (19, 37). This raises interesting questions for further research, e.g. could a satisfying relationship with one's physician, good adherence to medical treatment, or some other aspects of healthcare give patients a certain level of psychological comfort and reassurance that counterbalances the otherwise negative impact of the disease severity and complexity?

In addition, DD did not correlate with QoL either, similar to the findings of Edelman *et al.*, 2011 (38*).* However, we noted that the diastolic index E/e' was a significant predictor for lower scores attributed to physical health. E/e' was also significantly higher in patients with PCS and MCS scores below median values. This could be investigated further, considering the well-known role of echocardiographic parameters in one's objective vitality, as well as recent research supporting the inclusion of the self-reported SF-36 vitality item in the cardiovascular risk stratification algorithms for T2DM patients (39).

In our previous research, DD was predicted by the CHA2DS2-VASc score (40). Typically, CHA2DS2-VASc serves as a decision-making tool when establishing the need for anticoagulant therapy in patients with atrial fibrillation. Recently, more potential applications have been explored, such as for the prediction of rehospitalization and all-cause mortality or in the risk assessment for cardiac and cerebrovascular events even in patients without atrial fibrillation (41, 42, 43). In this study, CHA2DS2-VASc was predictive of a poorer quality of life in terms of perceived physical health. From a practical perspective, this score is based on readily available data and is easy for diabetes specialists to calculate during routine check-ups, so it is worth knowing and exploiting its full predictive potential.

The relatively small number of participants is a limit of this study, and further multivariate analysis was not appropriate because of insufficient demographic data (marital, educational or employment status), psychological data (stress or anxiety levels), or health literacy levels. The nature of this post hoc analysis could not support assertions of causality explaining the observed associations. Moreover, all patients had HbA_{1c} levels above 7% and no severe complications, so comparisons with data from patients with adequate glycemic control were not possible in our study.

We recognize the complex relationship between HRQoL, diabetes-specific, and other factors. The use of a validated tool to assess patient-reported outcomes, the nuanced analysis, and the identification of predictors for a lower quality of life are the main strengths of this study. The results can contribute to international data collection efforts and allow the experiences of Romanian patients to be included in forthcoming meta-analyses.

CONCLUSIONS

In this study of T2DM patients without established atherosclerotic disease, self-reported quality of life was mostly independent of diabetes-related factors such as glycemic control, chronic complications, and comorbidities. Conversely, diabetes duration, the diastolic index E/e', and CHA_2DS_2-VASc score, were associated with lower physical health perception. This and other forthcoming studies can inform the design of prospective and interventional research, as well as educational programs targeting better quality of life for T2DM patients as a primary or secondary endpoint.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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RETROSPECTIVE STUDY ON THYROID FUNCTION MODIFICATIONS IN PATIENTS DIAGNOSED WITH POLYCYSTIC OVARY SYNDROME

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RETROSPECTIVE STUDY ON THYROID FUNCTION MODIFICATIONS IN PATIENTS DIAGNOSED WITH POLYCYSTIC OVARY SYNDROME (Abstract): Polycystic ovary syndrome (PCOS) is a complex endocrine condition, and its association with thyroid dysfunction is variably reported in the literature. The **aim** of the study was to retrospectively evaluate the degree of association between PCOS and thyroid impairment in a cohort of 269 patients. **Materials and methods:** The study had a retrospective observational design. The data were analyzed descriptively and using a multivariate logistic regression model to analyze the association between thyroid status and the type of thyroid disorder with each PCOS phenotype individually. The results were reported as relative risk ratios (RRR) and 95% confidence intervals (CI). **Results:** The presence of phenotype A in the context of advanced age increased the risk of developing hyperthyroidism by 9% (RRR: 1.06, 95% CI: 1.01-1.12, p=0.019), while in the context of a normal or low BMI, it decreased the risk of developing hyperthyroidism by 93% (RRR: 0.07, 95% CI: 0.08-0.68, p=0.021). Phenotype B in the context of a normal or low BMI increased the risk of developing hyperthyroidism by over 15 times (RRR: 15.86, 95% CI: 1.23-30.3, p=0.03). **Conclusions:** Early identification of thyroid abnormalities in patients with PCOS is warranted and requires an individualized therapeutic approach. **Keywords:** POLYCYSTIC OVARY SYNDROME; THYROID DISORDERS; HYPOTHYROIDISM; HYPERTHYROIDISM.

INTRODUCTION

Thyroid disorders are diagnosed in approximately 14% of adult women, particularly those of reproductive age (1). Female sex is one of the most significant clinical risk factors associated with the onset of thyroid dysfunction, with this risk factor increasing the likelihood of these disorders by 3-5 times in the female population compared to the male population (2, 3).

Additionally, certain ethnic groups or those with specific clinical characteristics are more prone to thyroid dysfunction, with patients from the Asian region or those who are overweight presenting the highest risk (4, 5). On the other hand, there is a documented dual relationship in the literature between thyroid disorders and polycystic ovary syndrome (PCOS), with significant metabolic and reproductive effects in the context of this association (6).

Subclinical hypothyroidism is linked to

the presence of the polycystic ovarian syndrome (PCOS), with these patients at increased risk for dyslipidemia or insulin resistance (7). Patients diagnosed with autoimmune thyroid disorders, such as Graves' disease or Hashimoto's thyroiditis, also present an increased risk of developing infertility and/or premature ovarian insufficiency (8).

The effects of thyroid dysfunction on the female reproductive system are complex and can manifest both directly and indirectly (9). Thyroid hormones control the release of kisspeptin and GnRH at the hypothalamic-pituitary level by directly influencing the secretion of these hormones and also through their response to metabolic signals such as prolactin and leptin (10-12).

Furthermore, thyroid hormones influence the availability of sex hormones by modifying binding proteins (13). An euthyroid status promotes the proper functioning and growth of the female reproductive system and regulates the development of the placenta and fetus during pregnancy (14, 15).

A thorough examination of the complex relationship between the thyroid and the female reproductive system is necessary to fully understand the factors that influence the clinical phenotype of the association between PCOS and thyroid dysfunction (16, 17).

To date, data from the literature support the association of thyroid dysfunctions with a range of reproductive abnormalities such as premature ovarian insufficiency, PCOS, infertility, irregular menstrual cycles, abnormal bleeding, and adverse obstetric outcomes.

However, the direct impact of thyroid hormones on reproduction is complicated by the frequent connection between autoimmune diseases and dysfunctions of both the thyroid system and the reproductive system.

Fluctuations in thyroid hormone levels among patients with PCOS continue to be a subject of debate. A study conducted in Denmark demonstrated that patients diagnosed with PCOS have a two-fold higher risk of developing hyperthyroidism compared to age-matched patients without PCOS (18).

Several studies have shown a higher prevalence of hypothyroidism in patients with PCOS compared to the prevalence of hyperthyroidism (19, 20). However, another study indicated that there is no significant difference in the incidence of thyroid diseases between patients with or without this condition (21).

In this study, we aimed to retrospectively evaluate the degree of association between polycystic ovary syndrome and thyroid hormonal disorders in a cohort of patients who presented for evaluation at a referral center in the Moldova region.

MATERIALS AND METHODS

In this retrospective cohort study, we analyzed all patients diagnosed with PCOS who sought care at the endocrinology clinic of “Sf. Spiridon” County Clinical Emergency Hospital in Iași, during the period from January 1st, 2017, to December 31st, 2022. We confirm that all the procedures were followed in accordance with the relevant guidelines of the Helsinki declaration. Ethical approval for conducting this study was obtained from the Research Ethics Committee of the “Grigore T. Popa” University of Medicine and Pharmacy in Iași (No. 132/14.12.2021).

The database query was made based on discharge diagnoses from the department,

using the ICD code (International Classification of Diseases and Related Health Problems, version 2010) corresponding to PCOS - E28.2 Polycystic Ovary Syndrome. All data extracted from the query were anonymized and statistically processed.

In addition to the diagnosis of PCOS, we used the following inclusion criteria for this cohort study: age of patients between 18 and 80 years, complete medical records, patients with or without thyroid dysfunction, patients with or without thyroid-stimulating medication, definitive diagnosis of PCOS according to Rotterdam criteria (22, 23).

Patients were excluded from this study if they met any of the following criteria: pregnant patients, underaged or patients over 80 years of age, incomplete diagnosis or monitoring, those who did not sign consent for processing personal data upon admission, patients with severe comorbidities (advanced malignant neoplasms, HIV/AIDS infection, severe neurological, cardiovascular, and pulmonary conditions).

Medical data from 269 patients who met the inclusion criteria were subsequently analyzed. We focused on the following types of data: age, body mass index (BMI), living environment, PCOS phenotype, presence of obesity, dyslipidemia, diabetes, chronic arterial hypertension (HBP), hirsutism, or infertility, thyroid status: euthyroidism, hypothyroidism, or hyperthyroidism, type of thyroid impairment: autoimmune thyroiditis, non-toxic multinodular goiter, thyrotoxicosis, Graves' disease.

Patients with PCOS were segregated according to the phenotype of the disease based on the Rotterdam criteria into (22, 23):

- phenotype A, characterized by the association between clinical or biochemical hyperandrogenism, ovulatory dysfunction, and typical PCOS morphology (group 1, n= patients);
- phenotype B, characterized by the association of clinical or biochemical hyperandrogenism with ovulatory dysfunction (group 2, n= patients);
- phenotype C, characterized by the association of clinical or biochemical hyperandrogenism with typical PCOS morphology on ultrasound (group 3, n= patients);
- phenotype D, characterized by the combination of ovulatory dysfunction and ultrasound morphology of PCOS (group 4, n= patients);

The first stage of our statistical analysis was the description of the data used, for which we employed histogram-type graphs to assess the distribution of various characteristic types among the analyzed groups. Nominal characteristics were subsequently compared using chi-square or Fisher's exact tests depending on the type of distribution. Numerical variables were also compared among the main study groups using ANOVA and the Bonferroni post-hoc test. Violin plots and boxplots were used to illustrate the differences between the serum parameters across the evaluated groups and subgroups. We also analyzed the degree of correlation between serum hormone levels and thyroid impairment.

Subsequently, we created a multivariate logistic regression model to analyze the association between thyroid status and the type of thyroid disorder with each PCOS phenotype separately. The model was adjusted for the age and body mass index of the patients, and the results were reported as relative risk ratios (RRR) and 95% confidence intervals (CI). Statistical analyses were conducted using *STATA SE software*

(version 17, Stata Corp LLC, College Station, TX, USA). A nominal p-value less than 0.05 was considered statistically significant.

RESULTS

Table I presents a comparison of the clinical characteristics among the main study groups. We found that the studied groups were homogeneous in terms of patient age and living environment. On the other hand, we noted that patients diagnosed with phenotype A of the disease exhibited significantly higher rates of obesity (A: 45.53% vs. B: 14.44% vs. C: 14.71% vs. D: 13.64%, p<0.001), dyslipidemia (A: 30.89% vs. B: 12.22% vs. C: 20.59% vs. D: 13.64%, p=0.009), and diabetes mellitus (A: 14.63% vs. B: 1.11% vs. C: 2.94% vs. D: 0%) compared to the other phenotypes.

TABLE I.
Clinical characteristics of the study groups

	Group 1 (Phenotype A, 123 patients)	Group 2 (Phenotype B, 90 patients)	Group 3 (Phenotype C, 34 patients)	Group 4 (Phenotype D, 22 patients)	p-value
Age years (median, IQR)	30 (18-71)	27 (18-74)	24 (18-70)	26 (19-58)	0.86
Environment (%)	Rural= 39.02% Urban= 60.98%	Rural= 34.44% Urban= 65.56%	Rural= 38.24% Urban= 61.76%	Rural= 27.27% Urban= 72.73%	0.71
Obesity (%)	45.53%	14.44%	14.71%	13.64%	<0.001
Dyslipidemia (%)	30.89%	12.22%	20.59%	13.64%	0.009
Diabetes Mellitus (%)	14.63%	1.11%	2.94%	0%	0.001
Chronic Arterial Hypertension (%)	9.76%	7.78%	5.88%	4.55%	0.78
Infertility (%)	3.25%	1.11%	0%	0%	0.45

IQR- interquartile range

Figure 2 shows the distribution of types of thyroid impairment according to the disease phenotype. The highest rates of hypothyroidism were found in patients with phenotype D (18.18%) and A (15.45%) compared to those with phenotype B (7.77%) and C (2.94%).

Additionally, the highest rates of hyperthyroidism were found in patients diagnosed with phenotype A (8.13%) and C (5.88%), followed by phenotypes D (4.54%) and B (3.33%) in terms of hyperthyroidism prevalence.

It is noteworthy that there was a relatively high prevalence of euthyroidism for all analyzed phenotypes: phenotype C (91.18%), B (88.89%), D (77.27%), and A (76.42%). The overall distribution of hypothyroidism in the studied population was 11.52%, hyperthyroidism was 5.94%, and euthyroidism was 82.53%.

As shown in figure 3, autoimmune thyroiditis had a high prevalence across all evaluated groups. Thus, the highest prevalence was found in phenotype D (81.82%) and C (67.65%), while the prevalence of hypothyroidism was relatively similar for phenotypes A and B (52.03% and 52.22%).

Non-toxic multinodular goiter was relatively common in the studied population, with rates of this condition being 24.39% for phenotype A, 18.89% for phenotype B, 17.65% for phenotype C, and 13.64% for phenotype D.

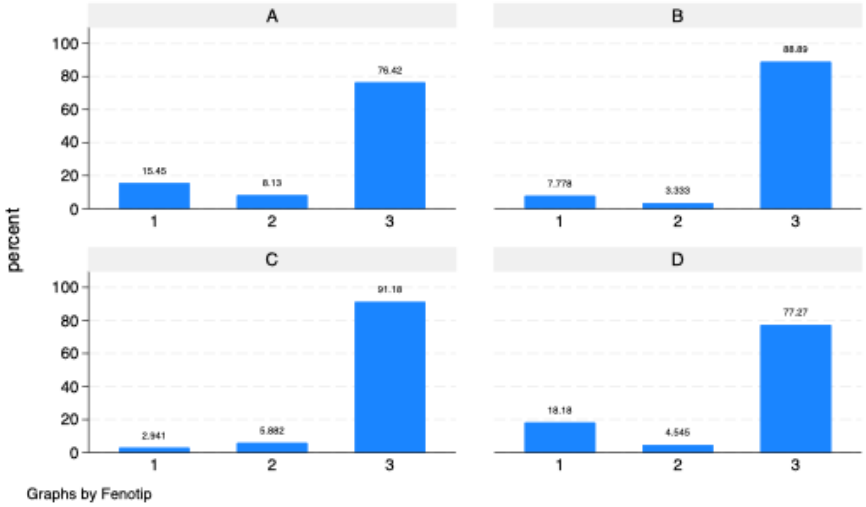

Fig. 1. Distribution of the type of thyroid impairment according to the studied phenotype. Legend: 1 - hypothyroidism, 2 - hyperthyroidism, 3 - euthyroidism.

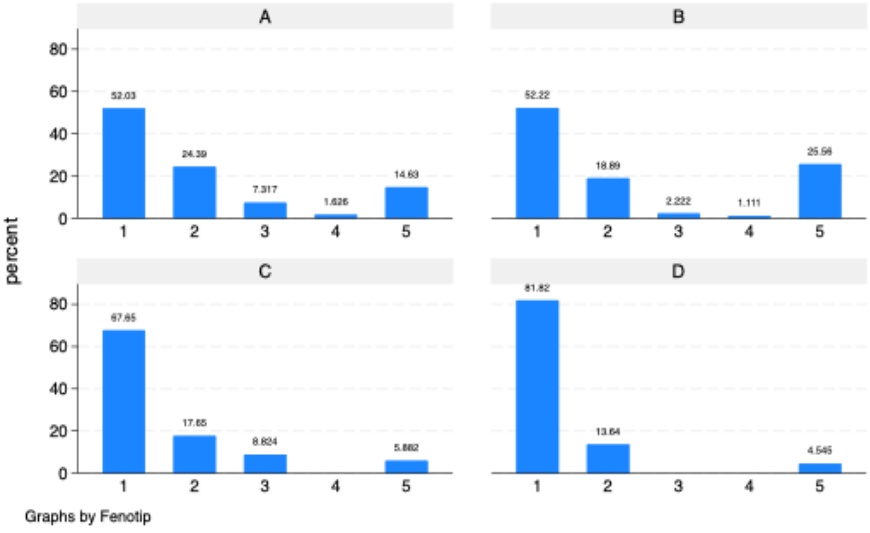

Fig. 2. Distribution of thyroid disorders according to the studied phenotype. Legend: 1 - autoimmune thyroiditis, 2 - non-toxic multinodular goiter, 3 - thyrotoxicosis, 4 - Graves' disease, 5 - without disorders.

Thyrotoxicosis was seen in 8.825% of patients with phenotype C, 7.31% of patients with phenotype A, and 2.22% of patients with phenotype B. Lastly, Graves' disease was observed in 1.62% of patients with phenotype A, 1.11% of patients with phenotype B, and 0% of patients with phenotypes C or D. The rate of patients without thyroid disorders in the examined groups was 25.56% for phenotype B, 14.63% for patients with phenotype A, 5.88% for patients with phenotype C, and 4.54% for patients with phenotype D.

The overall prevalence of thyrotoxicosis in the examined cohort was 56.51%, nontoxic multinodular goiter was 20.82%, thyrotoxicosis was 5.20%, and Graves' disease was 1.11%. A total of 16.36% of patients showed no evidence of thyroid impairment.

We subsequently evaluated a multinomial logistic regression model that aimed to identify the association between thyroid status and each PCOS phenotype individually. The model was adjusted for the age and body mass index of the patients (tab. II).

TABLE II.

The results of the adjusted multinomial logistic regression model that evaluated the association between PCOS phenotypes and thyroid status.

Adjustment	Thyroid Status	Phenotype A	P	Phenotype B	P	Phenotype C	p	Phenotype D	P
		RRR and 95%CI		RRR and 95%CI		RRR and 95%CI		RRR and 95%CI	
Age	Hypo T	0.99 (0.95- 1.04)	0.98	0.99 (0.92-1.05)	0.77	0.95 (0.71-1.26)	0.72	0.98 (0.87- 1.10)	0.80
	Hyper T	1.06 (1.01- 1.12)	0.019	0.99 (0.90-1.09)	0.98	1.08 (0.98-1.20)	0.10	0.79 (0.42-1.47)	0.46
BMI	Hypo T	0.42 (0.14- 1.22)	0.11	3.32 (0.53- 20.45)	0.19	1.35 (0.32-1.90)	0.99	2.89 (0.15- 55.08)	0.48
	Hyper T	0.07 (0.08- 0.68)	0.021	15.86 (1.23- 30.3)	0.03	4.25 (0.16-10.9)	0.38	5.31 (0.48- 15.3)	0.99

RRR- relative risk ratio, CI- confidence interval, BMI- body mass index, T- thyroidism.

Our results showed that the presence of phenotype A in the context of advanced age (over 35 years) increased the risk of developing hyperthyroidism by 9% (RRR: 1.06, 95% CI: 1.01-1.12, p=0.019).

Additionally, the presence of phenotype A in the context of a normal or low BMI (less than 25 kg/m²) decreased the risk of developing hyperthyroidism by 93% (RRR: 0.07, 95% CI: 0.08-0.68, p=0.021).

On the other hand, phenotype B in the context of a normal or low BMI (less than 25 kg/m²) increased the risk of developing hyperthyroidism by over 15 times (RRR: 15.86, 95% CI: 1.23-30.3, p=0.03).

Finally, the presence of any phenotype did not significantly increase the risk of developing hypothyroidism when adjusted for age or body mass index. For example, the decrease in the risk of hypothyroidism ranged between 1 and 5 percent in the context of a young patient age (under 35 years): phenotype A (RRR: 0.99, 95% CI: 0.95-1.04, p=0.98), phenotype B (RRR:

0.99, 95% CI: 0.92-1.05, p=0.77), phenotype C (RRR: 0.95, 95% CI: 0.71-1.26, p=0.72), or phenotype D (RRR: 0.98, 95% CI: 0.87-1.10, p=0.80).

Subsequently, we evaluated a multinomial logistic regression model that aimed to identify the association between thyroid impairment and each PCOS phenotype individually. The model was adjusted for the age and body mass index (BMI) of the patients, and the results are presented in third table.

TABLE III.

The results of the adjusted multinomial logistic regression model that evaluated the association between PCOS phenotypes and thyroid impairment.

Adjustment	Thyroid Status	Phenotype A	P	Phenotype B	P	Phenotype C	p	Phenotype D	P
		RRR and 95%CI		RRR and 95%CI		RRR and 95%CI		RRR and 95%CI	
Age	Auto immune Thyroiditis	1.02 (0.97- 1.08)	0.33	1.00 (0.96- 1.06)	0.69	1.34 (0.88-2.06)	0.16	1.06 (0.76- 1.49)	0.71
	Nodular Goiter	1.08 (1.02- 1.15)	0.006	1.05 (0.99- 1.11)	0.08	1.59 (1.01-2.50)	0.04	1.61 (0.73- 3.55)	0.23
BMI	Auto immune Thyroiditis	0.14 (0.04- 0.49)	0.002	2.8 (0.88- 6.41)	0.98	1.96 (0.42-3.98)	0.99	4.98 (0.76- 16.22)	0.99
	Nodular Goiter	0.29 (0 .07- 1.17)	0.08	5.22 (0.19- 25.48)	0.98	4.27 (0.35-11.8)	0.99	3.16 (0.55- 13.4)	0.99

RRR- relative risk ratio, CI- confidence interval, BMI- body mass index.

Our results indicated that phenotype A significantly increased the risk of developing non-toxic nodular goiter by 8% in the context of advanced age (RRR: 1.08, 95% CI: 1.02-1.15, p=0.006). Additionally, our model indicated a significant 59% increase in the risk of developing non-toxic nodular goiter in the context of advanced age and phenotype C (RRR: 1.59, 95% CI: 1.01-2.50, p=0.04).

Last but not least, the risk of developing autoimmune thyroiditis was significantly increased in the context of being diagnosed with phenotype A and having an increased body mass index (greater than 25 kg/m²) (RRR: 0.14, 95% CI: 0.04-0.49, p=0.002). On the other hand, our results did not indicate an increased risk of developing autoimmune thyroiditis when adjusted for patient age.

Thus, the risk of developing autoimmune thyroiditis in the context of having phenotype A was 2% (RRR: 1.02, 95% CI: 0.97-1.08, p=0.33), with phenotype B it was 0% (RRR: 1.00, 95% CI: 0.96-1.06, p=0.69), with phenotype C it was 34% (RRR: 1.34, 95% CI: 0.88-2.06, p=0.16), and with phenotype D it was 6% (RRR: 1.06, 95% CI: 0.76-1.49, p=0.71).

DISCUSSION

The global prevalence of polycystic ovary syndrome (PCOS) is estimated to be between 6% and 10% for unselected populations (24). The literature identifies a variety of risk factors that increase the likelihood of developing this condition as well as the risk of developing thyroid disorders (25, 26).

This retrospective study provided an ex-

tensive description of PCOS pathology in terms of clinical and paraclinical characteristics, as well as the associations of this pathology with thyroid dysfunction. As such, we demonstrated that the studied groups were homogeneous in terms of patient age and living environment. Additionally, we showed that patients diagnosed with phenotype A of the disease presented significantly higher rates of obesity (A: 45.53% vs. B: 14.44% vs. C: 14.71% vs. D: 13.64%, $p<0.001$), dyslipidemia (A: 30.89% vs. B: 12.22% vs. C: 20.59% vs. D: 13.64%, $p=0.009$), and diabetes mellitus (A: 14.63% vs. B: 1.11% vs. C: 2.94% vs. D: 0%) compared to the other phenotypes.

From this perspective, phenotype A appears to be associated with most of the metabolic dysfunctions present in the studied cohort. Our data agree with findings published in the literature.

The prevalence of PCOS phenotypes is variably reported in the literature. Thus, the prevalence of phenotype A has been reported to range from 44.1% to 64.5%, phenotype B from 7.9% to 32.6%, phenotype C from 2.9% to 28.8%, and phenotype D from 1% to 22.6% (352-355). In our study, the prevalence of phenotype A was 45.72%, phenotype B was 33.45%, phenotype C was 12.63%, and phenotype D was 8.17%, which overlaps with statistics from other countries.

Regarding the association of obesity with PCOS and its phenotypes, data from the literature confirm this link. For example, Moran and colleagues highlighted obesity rates of 48.2% for phenotype A, 22.7% for phenotype B, and 1.7% for phenotype C (27).

Bariatric surgery has proven to be a successful strategy for the treatment of morbid obesity; however, there are limited studies regarding its effect on PCOS in terms of improving clinical aspects, hormones, and comorbidities.

In a prospective observational study involving 1,013 patients diagnosed with PCOS who underwent bariatric surgery at a single center conducted by Bhandari and collaborators, the authors investigated the effectiveness of these procedures on the clinical and hormonal manifestations of PCOS (28).

In the studied cohort, 993 patients exhibited hirsutism before the surgical intervention, and 741 (74.6%; $p < 0.001$) had complete resolution of hirsutism by the end of the 6-month follow-up period. A total of 202 patients (20.3%) experienced moderate reduction in hirsutism at the 1-year follow-up, 5 patients had minimal reduction in hirsutism by the end of the 2-year follow-up, and 45 (4.5%) of patients reported no change in hirsutism at 4 and 5 years of follow-up (28).

Additionally, 93% of patients restored their normal menstrual cycle within 6 months after surgery, while the remaining 7% reported regular menstruation at 2 years postoperatively and continued to have a normal menstrual pattern throughout the entire follow-up period (28).

Similarly, associated comorbidities such as type II diabetes (79.7%), chronic hypertension (78.7%), dyslipidemia (93.2%), and obstructive sleep apnea (98.5%) resolved completely by the end of the follow-up period, highlighting the importance of performing these types of procedures in patients with PCOS and obesity (28).

Our results showed that the presence of phenotype A in the context of advanced age (over 35 years) increased the risk of developing hyperthyroidism by 9% (RRR: 1.06, 95% CI: 1.01-1.12, $p=0.019$). Additionally, the presence of phenotype A in the context of a normal or low BMI (less than 25

kg/m²) decreased the risk of developing hyperthyroidism by 93% (RRR: 0.07, 95% CI: 0.08-0.68, p=0.021).

On the other hand, phenotype B in the context of a normal or low BMI (less than 25 kg/m²) increased the risk of developing hyperthyroidism by over 15 times (RRR: 15.86, 95% CI: 1.23-30.3, p=0.03). Finally, the presence of any phenotype did not significantly increase the risk of developing hypothyroidism when adjusted for age or body mass index.

A study published in 2024 analyzed the association between PCOS and subclinical hypothyroidism and determined the potential impact of subclinical hypothyroidism on the clinical and metabolic characteristics of patients with PCOS (29). The results indicated a statistically significant difference between the two groups regarding obesity rates ($p = 0.014$) (29). Compared to the group of patients with PCOS, those with an association of both conditions exhibited significantly higher levels of TSH ($p < 0.001$), triglycerides ($p = 0.025$), and HOMA-IR ($p < 0.001$), while LH levels were significantly lower ($p = 0.048$).

However, the multivariate linear regression analysis revealed that TSH, triglycerides, LH, and HOMA-IR were not determinants for the occurrence of subclinical hypothyroidism in patients with PCOS (29).

Bonakdaran and colleagues evaluated the incidence of hypothyroidism in patients with PCOS and compared it in a group of healthy patients (30). Furthermore, the impact of hypothyroidism on metabolic parameters, particularly on insulin resistance, was examined in patients with PCOS.

The results of the study indicated that patients with PCOS had a higher incidence of hypothyroidism and a higher level of anti-peroxidase antibodies compared to the control group (30). HDL cholesterol was significantly higher in patients with PCOS and hypothyroidism than in those without hypothyroidism, although no significant differences were observed regarding other metabolic markers (30).

The results of this study should be analyzed considering a number of limitations: the small cohort of patients, the limited number of serum parameters included, and the lack of a control group without thyroid impairment.

On the other hand, our findings managed to describe aspects related to the prevalence of PCOS phenotypes in the evaluated patients, as well as their association with thyroid status and the most commonly encountered thyroid disorders.

CONCLUSIONS

The most frequently encountered PCOS phenotypes in the studied population were A and B. These phenotypes were significantly more associated with the development of thyroid disorders such as autoimmune thyroiditis, non-toxic multinodular goiter, or Graves' disease. Additionally, patients classified as phenotype A exhibited significantly higher rates of comorbidities such as dyslipidemia or chronic hypertension.

COMPETING INTERESTS

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

CONFLICTS OF INTEREST AND FUNDING

All the authors declare no funding received and no conflict of interest.

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EVALUATION OF THE SUICIDAL MENTAL STATE OF PATIENTS HOSPITALIZED AT THE "SOCOLA" INSTITUTE OF PSYCHIATRY IAȘI

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EVALUATION OF THE SUICIDAL MENTAL STATE OF PATIENTS HOSPITALIZED AT THE "SOCOLA" INSTITUTE OF PSYCHIATRY IAȘI (Abstract): Suicidal ideation and attempts are the most important predictive factors for death by suicide. Accordingly, in this study we performed a retrospective analysis of cases admitted to the "Socola" Psychiatric Institute in Iași regarding suicide attempts and/or suicidal ideation throughout 8 years of pre-pandemic period. **Materials and methods:** This retrospective study was based on data collected from the electronic archive of the "Socola" Institute of Psychiatry. To search for primary and secondary diagnoses, we used the following keywords: *suicide attempts, suicidal attempt, suicidal ideation* and *autolytic ruminations*. For statistical analysis, was used *Microsoft Excel* 2007. **Results:** The search yielded 257 cases, more than a quarter of which were young people, mostly women (63%). Of the patients whose brain activity was investigated (43%), 6% presented an altered EEG. In terms of occupation, 31% were professionally active people or students, the majority being retirees and those without occupation (56.4%). Of the 37% of patients whose religious denomination was recorded, 95% were Orthodox Christians. Also, 55% of all patients consumed alcohol, 69.6% suffered from family conflict, 6.6% were in a state of mourning, 18% performed the act for demonstrative purposes, and 42% were subjected to a psych stressful context. It was observed that depression, in various forms, represented around 35% of the total disease codes associated with suicidal attempts and ideation. Hypertension was the most frequent associated organic condition. It was also observed that people in the segment with primary or secondary education had the most suicidal attempts and ideation, and more than half of the patients presented an IQ bordering on normality. **Conclusions:** The analysis found that suicidal ideation and attempts continued to have a significant incidence in hospitalized cases, while depression, alcohol, and family conflicts were the most significant triggers. The study may represent an important landmark in relation to the Covid-19 pandemic and post-pandemic period. **Keywords:** SUICIDAL IDEATION, SUICIDAL ATTEMPTS, SUICIDE METHODS, DEPRESSION, RELIGION.

INTRODUCTION

Suicide is a global phenomenon but geographically unevenly distributed, with 77% of suicides occurring in low- and middle-income countries. Suicide occurs across the lifespan and is the fourth leading cause of death among young people aged 15-29 years worldwide (1). It is estimated that there were 700,000 suicide deaths worldwide in 2021 (2). In addition to deaths by suicide, increased attention should be paid to non-fatal suicide ideation and attempts (3) given

that overall lifetime prevalence rates are approximately 9.2% for suicidal ideation and 2.7% for attempts (Nock *et al*., 2008).

It is generally known that suicidal ideation and attempts are the most important predictors of death by suicide. In addition, suicide attempts are one of the main causes leading to disability, including premature death. Therefore, improved packages of preventive and therapeutic measures are needed in the medium and long term to better understand the phenomenon both globally and at the individual level, so that the insertion and judgment of each case are not absorbed by treatment models considered universally valid, but rather related to a social behavior pattern associated with an individual psychological and religious profile.

In addition, the prevention not only of suicide attempts, but also of suicidal ideation, perhaps also represents an act of moral responsibility of each member of the local community, based not only on the rationale of social responsibility towards a fellow human being in critical suffering, but also on that unconditional empathetic emotion, characteristic of human nature in general. Accordingly, Scientific investigations focused on the current scale of the phenomenon, its characteristics and proposals for improving the act of prevention must be carried out with increased frequency, being critical in relation to new social realities and the stressors associated with them in order to be able to both adjust traditional methods of therapeutic support as quickly as possible and to introduce new ones, if necessary, depending on the patient's requirements.

In this study, an analysis of cases admitted to the "Socola" Institute of Psychiatry in Iași regarding suicide attempts and/or suicidal ideation was carried out throughout 8 years of pre-pandemic period, in order to evaluate the dimension of the phenomenon, highlight one- or two-way relationships between the data collected based on the statistical interpretation of the results, and correlation with data from the specialized literature.

MATERIALS AND METHODS

This retrospective study was based on data collected from the electronic archive of the "Socola" Institute of Psychiatry. To search for primary and secondary diagnoses, we used the following keywords: *suicide attempts, suicidal attempt, suicidal ideation* and *autolytic ruminations.* For statistical analysis, was used *Microsoft Excel* 2007.

RESULTS

Following the analysis, 257 cases were found, the majority of them women (63%), the casuistry being approximately equally divided (48% having rural origin) in terms of the environment of origin. On average, patients were hospitalized 11.3 (±12.3) times during the analyzed period, the number of days per year and per patient being 16.5 (±15.2). The average age of the patients was 43.8 (±14.8) years, 26.5% being young patients aged ≤ 35 years. Of the patients whose brain activity was investigated (43%), 6% presented an altered EEG. In addition, 27% of the patients were brought to the hospital by ambulance or police, and 11% were referred by the UPU service. In terms of occupation, only 31% were professionally active people or students, the majority being pensioners and those without employment (56.4%).

Age of patients. Regarding the age of patients, more than a quarter of them were

young people. It is known that the incidence of suicidal ideation increases during adolescence (5). An explanation offered by some theoretical models (6), would be that some interpersonal conflicts as well as socialization difficulties would be predictors (7) and ultimately factors of suicide in adolescents. The death of a loved one has also been linked to suicidality in adolescents (5, 7). In addition, experiencing the suicide of a family member may constitute a behavioral model for already vulnerable young people (7, 8) or it could simply be viewed by a young person as a solution to a personal problem (8).

It is also interesting in this study that almost all the people in this study, with two exceptions, are younger than 65 years old. Basically, even though the study shows that almost 40% of the patients analyzed in this study are retired, they have been retired for a short time. Therefore, it is possible that the shock of not adapting to the new social condition may constitute a cause for the emergence of a suicidal state.

Risk factors for suicidal ideation and behavior among middle-aged and older adults (50-65+ years) include psychiatric disorders (especially depression), alcohol and drug use disorders, impaired physical health, chronic pain, weak social support/connection, loneliness, family problems, depression, financial problems, perceived burden, and a history of suicide attempts (9). On the other hand, protective factors against suicide include marriage, mental coping strategies, family connection, and religiosity (participation in religious activities, giving importance to religious beliefs, and activation of religious and moral objections to suicide) (9).

Religion. Regarding religion, given that it was only recorded in 37% of cases, the statistical analysis would suffer from precision. However, it is observed that Orthodox Christians had more hospitalizations (95%) than those belonging to other religious denominations, including in relation to their share in the general population. This can be explained not necessarily by a different, more efficient doctrine or set of religious rules, but especially by better psychosocial mechanisms of integration and cohesion between members of minority religious denominations who, conditioned precisely by the fact that they are a minority, in the existential desire to preserve their religious identity in a "sea" of Orthodox Christianity (representing almost 90% at the national level), develop better interpersonal social community relationships, including religious ones, with increased integrative potential, so that they manage to maintain psychological support, and not only, for the entire community, at least in times of crisis for its members.

Regardless of the religious denomination of the person, religion is generally seen as a protective factor against suicide. Some individuals with suicidal behavior have reported that developing a stronger relationship with God (10) has aided their recovery, while others have found that religion has prevented them from committing suicidal acts (11, 12). However, spirituality/religion has not always been discussed as a means of recovery (6).

Alcohol consumption and family conflicts. More than half of the patients consumed alcohol before the suicide attempt or are chronic alcohol consumers. Alcohol consumption before committing the suicide act is a known fact in the case of suicides. In the case of this study, 179 family conflicts were recorded, but it cannot be specified whether these were also based on the

patients' alcohol-related problems or whether alcohol consumption became chronic as a result of family problems.

Considering that in the case of almost 50% of the patients a psycho-stressful context was recorded, including the state of mourning, it is possible that family support was completely precarious and, moreover, the family itself was among the primary stressors that led to the suicide attempt. This can be verified and to some extent validated by the fact that almost 20% of the patients acted for demonstrative purposes, without having the intention of committing a complete suicide, but only to warn the family about their condition.

In particular, 16.3% of them were assessed by CEML or CEMRCM. As a rule, these cases committed a lethal or extremely serious aggression, based on a lethal intent, on a person in the family or outside the family, as a result of which they attempted suicide.

Impulsive temperament can lead to suicide rather than a calm one that leads to depression. From the psychological analyses included in the observation sheets it appears that impulsivity occupies an important place alongside anxiety for potentially suicidal patients. The Hamilton Depression Scale is relevant in the case of patients at risk or even attempted suicide, but the HAMA anxiety scale and the WM (Woodworth-Mathens) personality tests should not be ignored.

Frequency of mental and organic disorders associated with suicidal attempts and ideation. The study also analyzed the frequency of mental disorders associated with suicidal attempts and ideation. These disorders were considered main disorders. 127 such disease codes were found, which were used 803 times. The 20 most frequent codes (representing 15.7% of the 127 codes) had a frequency of use of 66.5%.

It is observed that recurrent depressive disorder, severe current episode with or without psychotic symptoms has the highest frequency (22% - of the total disease codes). Overall, depression, in various forms, represented around 35% of the total disease codes associated with suicidal attempts and ideation. On average, approximately 63 mental illness codes were used for each patient with suicidal attempts and ideation.

The frequency of organic diseases associated with suicidal attempts and ideation was also analyzed. These diseases were considered secondary diseases. 634 such disease codes were found, which were used 2256 times. The 20 most common codes (representing 3.15% of the 634 codes) had a frequency of use of 26.6%.

It is observed that arterial hypertension, in both its forms, presents the highest frequency (4.3% - of the total disease codes). In addition, spondylosis together with osteoporosis form 4.25% of the total disease codes. The three diseases therefore form 8.55% of the total of 634 secondary diseases associated with suicidal attempts and ideation. On average, approximately 8.8 organic disease codes were used for each patient with suicidal attempts and ideation.

Level of education. The analyses performed show an apparently lower incidence of suicidality in uneducated people (2.6%), in those with post-secondary education (1.3%) and in those with higher education (6%). In contrast, apparently, people with primary or secondary education, as well as those with secondary education, were more prone to suicidal attempts.

However, in order to obtain a correct

picture of the relationship between the level of education of the patients and their suicidal state, the data obtained must be compared with the percentages of the educational levels of Romanian citizens at national level. The comparison clearly shows that only in the case of people with primary or secondary education this remains true, the percentage of people in the analyzed population characterized by a suicidal state being double compared to their share at national level. In other words, people in the segment with primary or secondary education had the most suicidal attempts and ideation. At the opposite end, people with higher education are the least likely to commit suicide attempts or have suicidal ideation.

Period of hospitalization. According to the data obtained in this study, the seasons of the year can influence the suicidal state. The graph describing the dependence of the number of days of hospitalization on the months in which the hospitalization took place has an oscillatory profile (Fig. 1), presenting more pronounced maxima in the months of January-February (winter) and October-November (autumn-winter) and a less intense maximum in the period of May-June (spring-summer). The absolute minimum is found in the month of August (summer). This is interesting because in the specialized literature there are studies that show a seasonal distribution of the suicidal phenomenon (13, 14, 15), this being more accentuated in spring and autumn (16), i.e. overlapping only partially with the periods of hospitalization in the hospital of the patients characterized by suicidal states analyzed in this study. Therefore, the suicidal state (suicidal thoughts and/or attempts, more or less demonstrative) is, on average, approximately constant throughout the year, with the exception of summer. During the year, however, there are "explosions" in the suicidal state materialized in the complete commission of suicide. Probably only the desire to die is greater in spring and autumn, but it is present as a background state throughout the year.

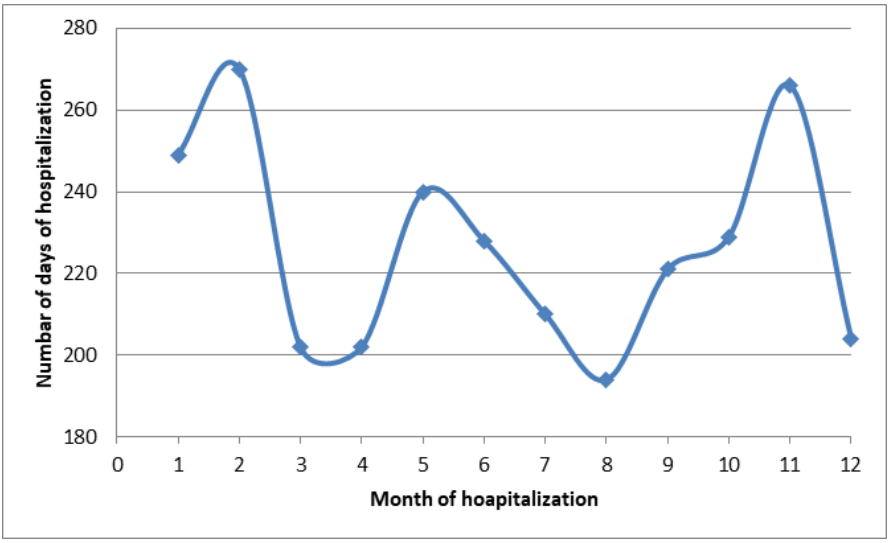

Fig. 1. Dependence of the number of days of hospitalization on the hospitalization period.

Marital status

The study of cases according to marital status is valuable, including for verifying reports that assert the positive influence of the family environment. The family status of the patients was recorded for 101 people out of the 257 investigated. Thus, 48 people were married, 29 unmarried, 15 divorced, 7 widowed, while 2 cases were cohabiting as a consensual union/cohabitation. Apparently, immediately after married people, unmarried people had the highest frequency of hospitalization. However, it is observed that, in relation to the share in the general population, unmarried people had the lowest hospitalization rate among all categories. With the exception of unmarried people and people living in cohabitation, both married people and divorced and widowed people presented higher hospitalization rates.

Methods used in suicide attempts

The main methods used in suicide attempts were intoxication, as the most common method (46.5%), followed by hanging (17.6%), chemical ingestion (8.8%) and self-mutilation (8.1%), with other methods accounting for 19% of all cases.

Drug intoxication correlates with the higher number of female hospitalizations, given that this method is one of the most common methods used by women to commit suicide. Drug intoxication allows for a longer rescue time than other more brutal methods that are usually chosen by men. Hence the higher success rate in saving female individuals, in line with their higher number of hospitalizations. The 257 patients accumulated 499 multiple suicide attempts and 159 single attempts throughout their numerous hospitalizations. Suicidal ideation was also recorded in 667 cases, and autolytic ruminations in 40 cases.

MMSE and GAFS evaluation

The MMSE (Mini Mental State Examination) is a test used to assess cognitive impairment in adults, with a maximum score of 30, with lower scores indicating more or less severe cognitive problems. The MMSE score that defines cognitive function as "normal" is usually set at 24 (17). Of the 170 people who took the MMSE, only 7.6% showed no cognitive impairment (fig. 2). However, the majority, 65.3%, were borderline normal.

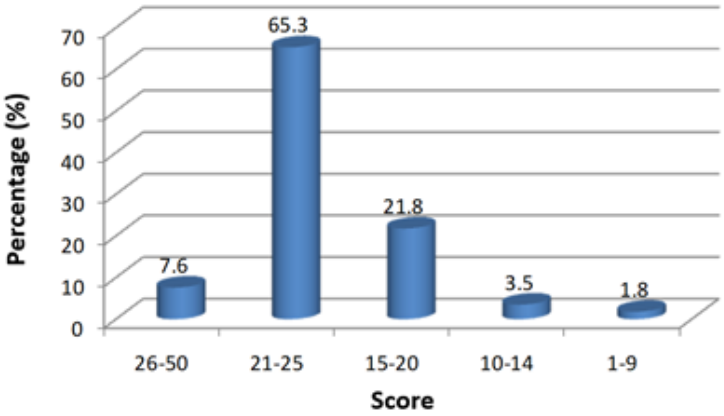

Fig. 2. Score obtained by patients in the MMSE test

The GAFS (Global Assessment of Functioning Scale) test was performed on 251 patients and shows that practically all patients presented serious symptoms ranging from severe, obsessive suicidal thoughts, communication deficits, psychosis, to delusions and hallucinations (fig. 3).

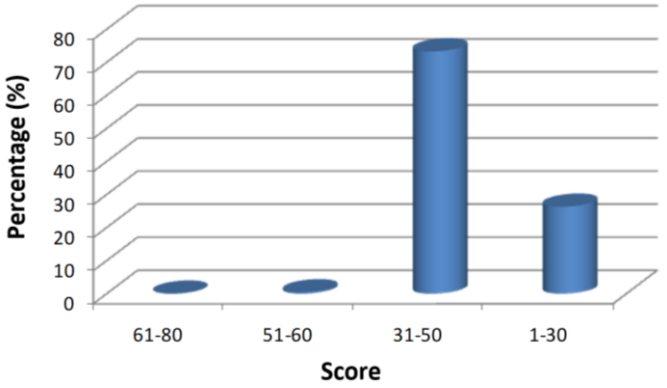

Fig. 3. Score obtained by patients in the GAFS test

Intelligence Quotient (IQ)

Only about 23% of the patients investigated had normal intellectual functioning (fig. 4). However, more than half of the patients had an IQ bordering on normality. The mean IQ was 74.85 (S.D. ± 17.26), placing them in the borderline intellectual functioning range.

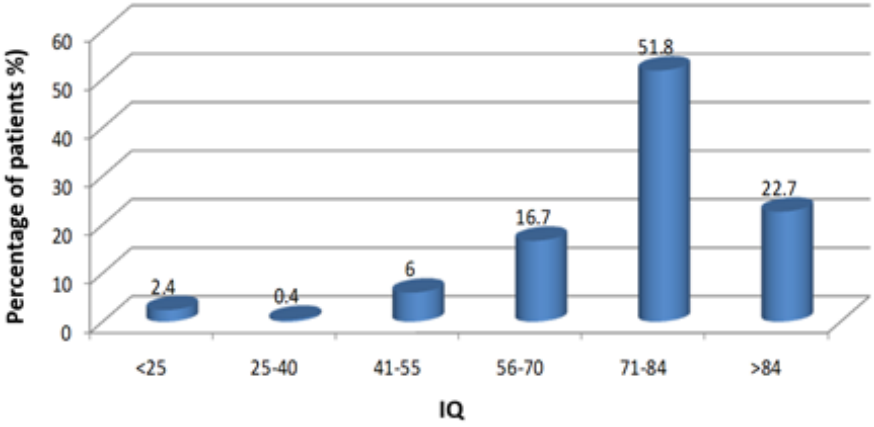

Fig. 4. The score obtained by the patients in the intelligence test

The relationship between IQ and suicidality, including suicide attempts or completed suicide, is controversial. A number of authors argue that the degree of intelligence also plays a role in the suicidal act. According to a 2004 study (18), people with high intelligence may be, overall, less adapted to different general life situations and may become more susceptible to suicide.

Also, another study from 2018 shows that poor academic performance in school, at age 16, was a robust predictor of suicide attempt in adulthood and appeared to account for the association between lower childhood IQ and suicide attempt (19).

On the other hand, another study from 2005 showed that there were no significant differences in IQ types between suicidal and non-suicidal inpatients, with logistic regression showing no evidence of an association between IQ and suicide (20).

Assessment of depression degree. In the study, the state of depression was assessed using the 21-item Hamilton Depression Rating Scale (HAM-D) which was completed and recorded for 209 patients (81.3%). The Hamilton Depression Rating Scale is used to assess the severity of depressive symptoms in patients diagnosed with a depressive disorder. The HAM-D17 contains 17 items that reflect: depressive mood, but also feelings of guilt, suicidal ideation and/or behavior, insomnia, difficulties in professional functioning, psychomotor inhibition, agitation, mental and somatic anxiety, gastrointestinal symptoms, general somatic symptoms, hypochondria, weight loss (21). Compared to the HAM-D17, the HAM-D21 contains 4 additional items: diurnal variation of mood, derealization, paranoid ideas and obsessive ideas. These items do not assess the intensity of depression and are rarely encountered in depressive syndrome, the HAM-D21 being therefore used mainly in research (21). 96.7% of the patients in the current study had severe depression and only 3.3% had moderate depression (fig. 5).

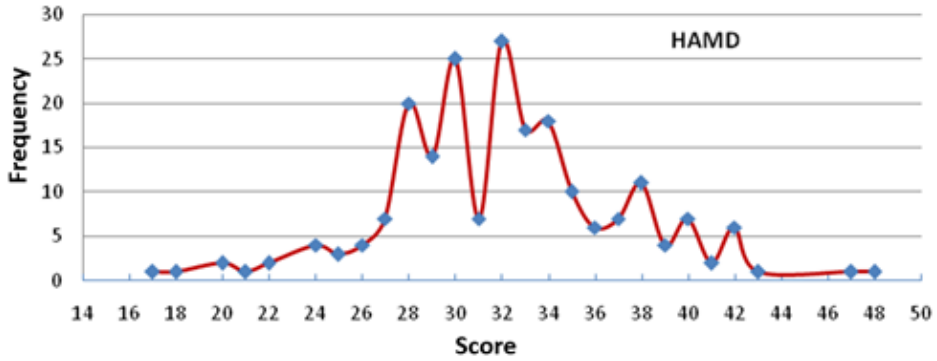

Fig. 5. The score obtained by patients in the HAMD test and its associated frequency.

From the data obtained, an association was observed between severe depression and essential hypertension for patients with suicidal ideation and behavior. Given that primary hypertension is the result of long-term psycho-organic processes, this connection can be explained by the state of stress, of inner tension that has been grinding the minds of patients for a longer period of time, generated by an increasingly accentuated depressive background. Therefore, in the case of the current study, hypertension can be considered a potential non-specific marker associated with suicidal ideation. In other words, an initially unidirectional dependency relationship

could be built between persistent stress - increased primary blood pressure - moderate/severe depression that culminates in suicidal ideation and attempt. Once built, however, the causal relationship becomes non-specific, multidirectional through persistence. Thus, the continuous presence or increased frequency of suicidal ideation can induce a state of considerable stress, leading to depression and even to the maintenance and exacerbation of cardiovascular pathology. The parameters of the relationship become interchangeable, even if their participation, influence or hierarchy present different levels in the etiology of the disease.

Medication. In this study, we also wanted to carry out a brief evaluation of the treatment addressed to both psychiatric and somatic conditions administered to patients with suicidal ideation and state. Thus, during the analyzed period, over 200 commercial names of drugs were used with a more or less high frequency, belonging to different drug classes. The top 5 used drugs were Carbamazepine, Cipralex, Rivotril, Halo-peridol, and Coaxil. Given that each patient with suicidal ideation and attempts was administered on average more than 14 different drugs, in random combinations, the evaluation of drug interaction should be a priority.

CONCLUSIONS

In this study, we performed a retrospective study, including the cases hospitalized over eight years with suicidal ideation and attempts. We have found that the depression, along with alcohol, and family conflicts were the most significant triggers of their suicide-driven ideation. Given the endurance and consistency of the suicidal ideation and attempts, we consider that increasing and diversifying the screening methods for identifying people at risk and risk factors such as depression or substance abuse, could represent one of the most direct and successful alternatives for reducing suicidal acts. The study may represent an important landmark in relation to the COVID-19 pandemic and post-pandemic period.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding.

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HIGH-FREQUENCY ULTRASONOGRAPHIC ANALYSIS IN THE EVALUATION OF THERAPEUTIC RESPONSE IN PATIENTS WITH *PSORIASIS VULGARIS*

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HIGH-FREQUENCY ULTRASONOGRAPHIC ANALYSIS IN THE EVALUATION OF THERAPEUTIC RESPONSE IN PATIENTS WITH *PSORIASIS VULGARIS* (Abstract): Plaque psoriasis is a chronic, immune-mediated disease which represents a global health problem. The main objective of the study was to determine the monitoring performance of non-invasive tests of high frequency ultrasonography (HFUS) in psoriasis vulgaris. **Materials and methods:** In a prospective interventional analytic study, we aimed to assess whether the first chronologically obtained change in psoriasis plaque assessment in monitored patients was a decrease in psoriasis plaque thickness and subepidermal hypoechoic band as compared to baseline values. The study was carried over a period of 8 weeks and included 50 patients diagnosed with psoriasis vulgaris in the Dermatology Clinic of the "Sf. Spiridon" County Clinical Emergency Hospital, Iasi. We assessed the evolution under topical (calcipotriol/betamethasone 50 micrograms/0.5mg/g gel or fluticasone propionate 0.05% cream in combination with lipolotion urea 10%) combined with systemic (Etanercept) in severe forms of disease. Target lesions of psoriasis vulgaris were analyzed by clinical examination and by HFUS. **Results:** The results showed that the first change obtained chronologically was the decrease in psoriasis plaque thickness and subepidermal hypoechoic band in the target plaque as compared to baseline values (i.e. at week 4 compared to week 0). After comparing the mean values of psoriasis plaque tegument thickness and hypoechoic subepidermal band thickness, we found that they decrease significantly both in week 4 compared to week 0 and in week 8 compared to week 4. **Conclusions.** HFUS examination allows an objective and reproducible measurement of skin thickness and is a useful technique for a non-invasive assessment of treatment efficacy in psoriasis. **Keywords:** HIGH FREQUENCY ULTRASONOGRAPHY (HFUS), PSORIASIS VULGARIS, TOPICAL THERAPY, SUBEPIDERMAL LOW ECHOGENIC BAND (SLEB), MONITORING.

INTRODUCTION

Skin ultrasonography is a non-ionizing imaging method useful in the in vivo study of skin lesions using ultrasound as a vector for imaging. Miller and Alexander reported that A-mode (Amplitude) sonography is an

effective method of determining skin thickness (1). Subsequently, in 1987, the introduction of 20-Megahertz (MHz) B-mode (Brightness) ultrasound scanning systems with axial and lateral resolution of 200 μm and 75-80 μm respectively made two-dimensional (2D) imaging possible (2). Psoriasis is a chronic immune-mediated inflammatory disease characterized by an excessive hyperproliferation of keratinocytes with extracutaneous manifestations, that affects about 1-3% of the world's population (3, 4).

The inflammatory pathology represented by psoriasis vulgaris is much more than just a skin condition and is a global health problem, with over 125 million individuals affected by this multisystemic inflammatory condition (5). Worldwide, the prevalence of psoriasis has increased from 758 cases per 100,000 population in 1990 to 812 per 100,000 population in 2017. In Europe and the U.S. 21-55% of patients have a moderate to severe form of the disease (6, 7, 8).

The examination of psoriasis plaque lesions with HFUS may indicate the following: hyperechogenic band representing the epidermis with hyperkeratosis and parakeratosis; echogenic or hypoechogenic band corresponding to the elongation of the dermal papillae as one of the most common features, hyperechogenic band given by the reticular dermis and subcutaneous hypoechogenic layer. The subepidermal hypoechogenic band is due to edema and inflammatory infiltrate in the papillary dermis. Furthermore, the overall thickness of the integument in psoriatic plaques is increased and its reduction during therapy can be objectively demonstrated (9, 10). High frequency ultrasonography (HFUS) is a proper method for psoriasis assessment without causing subjective differences in clinical scoring systems such as PASI (Psoriasis Area Severity Index), TLS (Target Lesions Score) or DLQI (Dermatology Life Quality Index) (11,12).

MATERIALS AND METHODS

The main objective of the present study was to determine the monitoring performance of non-invasive test of HFUS in psoriasis vulgaris. In a prospective interventional analytic study justified by the need to improve monitoring modalities for plaque psoriasis vulgaris, our goal was to determine whether the first chronologically obtained change in psoriasis plaque assessment in monitored patients was a decrease in psoriasis plaque thickness and subepidermal hypoechoic band as compared to baseline values. Thus, the current research aims to evaluate the contribution of imaging techniques such as HFUS in the algorithm of diagnosis and monitoring of psoriasis plaques, starting from the hypothesis that this technique may provide the possibility of establishing prognostic factors in the therapeutic management of psoriasis vulgaris.

The prospective study was carried over a period of 8 weeks (January-February 2020) and included patients diagnosed with psoriasis vulgaris in the Dermatology Clinic of the "Sf. Spiridon" Emergency Hospital, Iasi. The study was conducted on a group of 50 patients with psoriasis vulgaris composed mainly of men (78%) from urban areas (54%). The average age of the patients was 48,12 ± 14,11 years, with variations between 18 and 75 years. The parameters analyzed in the group of patients with psoriasis vulgaris were disease-related data (sex, rural/urban area), disease severity scores (PASI , DLQI) and Target Lesions Score/ TLS in weeks 0, 4, 8. Calculation of PASI scores was performed by the same examiner in weeks 0, 4, 8 with values between 0 and 72 and DLQI scores were

obtained in weeks 0 and 12 by the method of the questionnaire completed by the patient, with values between 0 and 30.

Clinical evaluation of erythematous-squamous lesions was performed by calculating a score called TLS which assesses erythema, scales and the presence of infiltration by assigning the following values: 0 - no, 1 - weak, 2 - moderate, 3 - severe. The TLS score has a maximum value of 8 and a minimum of 0 (when psoriasis plaque resolution is complete) and was clinically determined at weeks 0, 4, 8. Skin lesions with a score of 3 for scales were excluded to avoid interference with hyperkeratosis. The evaluation of the TLS therapeutic response was performed with the distribution of the therapeutic response in full resolution, major improvement, minor improvement or no response/non-responder. Others studied factors were the ultrasonographic diagnosis of the psoriasis target plaque. They were performed at weeks 0, 4 and 8 defined by the following parameters: thickness (mm) of the plaque analyzed in weeks 0, 4, 8; thickness (mm) of the hypoechogenic band in weeks 0, 4, 8; thickness (mm) of normal adjacent integument in weeks 0, 4, 8; thickness (mm) of hypoechogenic band normal integument adjacent to psoriasis plaque analyzed in weeks 0, 4 and 8.

We compared the evolution under topical and/or systemic treatment of patients with plaque psoriasis vulgaris both by classical means (clinical examination) and by non-invasive imaging techniques (HFUS). Inclusion criteria were patients clinically diagnosed and histopathologically confirmed with psoriasis vulgaris; age older than 18 years; presence of accessible lesions with cutaneous localization that can be examined using ultrasonography probe. The criteria considered for imaging selection of erythemato-squamous lesions (target lesion) required that the skin lesion examined had an active character, with onset less than 6 months; the lesion was visible and well defined. Using exclusion criteria we eliminated patients on immunosuppressive therapy other than Etanercept, on systemic corticosteroids or lithium-containing, beta-blocking medicines during the study, or 1 month prior to examination, or on other topical therapies other than those administered during study assessments; patients on current phototherapy or 6 weeks prior to study; patients with neuropsychiatric pathology; patients with the presence of tuberculosis infection, severe comorbidities, diabetes, diseases of autoimmune character, exogenous or endogenous eczema such as atopic dermatitis; patients with marked photoaging (with history of chronic sun exposure) or sun exposure within the last 6 weeks or during the study; patients with contraindications for topical treatment with calcipotriol/betamethasone gel or fluticasone propionate combinations: bacterial, viral or mycotic infections, with skin atrophy or allergic contact dermatitis due to corticosteroids or formulation constituents; pregnant or lactating patients; smoking patients.

For the study we used a high frequency ultrasound scanner (Dermascan C Cortex Technology Denmark®) with a 20 MHz transducer, 12.1 mm wavelength, with a 13 mm focus (maximum 15 mm penetrability), with 1580 m/s velocity, 0.06 mm axial resolution/0.3 mm lateral resolution, mode A of examination. 19 patients enrolled in the study received topical treatment (calcipotriol/betamethasone 50 micrograms/0.5mg/g gel in combination with lipolotion urea 10% 1application/day) or fluticasone propionate 0.05% cream in combination 2 applications/day and emulsion urea 10% 1 application/day combined with systemic treatment (Etancercept) previously initiated in severe forms of disease (31 patients).

We chose this way of dividing the topical therapy, according to the severity of the disease from a clinical point of view, quantified by PASI and DLQI score.

Patients without biological systemic therapy received topical treatment with combination of vitamin D3 analogues and dermatocorticoids (calcipotriol with betamethasone propionate gel) for their synergistic effect and obtaining a better therapeutic effect quantified by severity scores. These topical therapies (topical dermatocorticoids fluticasone propionate, combinations of dermatocorticoids with calcipotriol vitamin D3 analogues with betamethasone dipropionate) and emollient (10% urea emulsion) have been applied as recommended in current psoriasis vulgaris treatment guidelines by the American Dermatology Association as well as that of the Romanian Society of Dermatology. The research was carried out in accordance with the Declaration of Helsinki 1964 and subsequent amendments and was approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy Iasi and by the Ethics Committee of the "Sf. Spiridon" County Clinical Emergency Hospital, Iasi. Volunteers were informed about how the research would be carried out and gave their written consent by signing the informed consent form before the start of the study. The statistical data processing was performed using STATISTICA var. 7.0 software and Microsoft Excel.

RESULTS

In the current study, psoriasis plaque thickness values ranged in week zero between 1.70 mm and 3.77 mm, in week four between 1.34 mm and 3.56 mm, and in week eight between 1.11 mm and 3.31 mm (tab. I). The clinical and ultrasound analysis of target psoriasis plaques showed that thickness decreased continuously from one assessment in week 0 to the next in week 4 and 8.

TABLE I.
Medium skin thickness of psoriasis plaque

Variables	N	Mean	Minimum	Maximum	SD
Skin thickness plaque psoriasis (mm) in Week 0	50	2.71	1.70	3.77	0.56
Skin thickness plaque psoriasis (mm) in Week 4	50	2.50	1.34	3.56	0.57
Skin thickness plaque psoriasis (mm) in Week 8	50	2.21	1.11	3.31	0.55
N, participants number; SD, Standard Deviation					

Comparison of the mean skin thickness of the psoriasis plaque between weeks 0, 4 and 8 using the t test for repeated measurements showed that the mean thickness decreased continuously from one assessment to another:

- compared to week 0, the mean skin thickness of the psoriasis plaque decreased significantly by 0.21 mm on average in week 4, from 2.71 mm to 2.5 mm ($t=9.82$, $p <0.0000001$);
- compared to week 4, the mean skin thickness of the psoriasis plaque decreased significantly by 0.29 mm on average in week 8, from 2.5 mm to 2.21 mm ($t=7.32$, $p <0.0000001$).

The analysis of target psoriasis plaques continued in the present prospective study with the comparison of mean hypoechoic subepidermal band psoriasis plaque thickness at weeks zero, four and eight. Comparison of the mean thickness of the hypoechoic subepidermal band between weeks 0, 4 and 8 using the t test for repeat-

ed measurements showed that the mean thickness decreased continuously from one assessment to another:

- compared to week 0, the average thickness of the hypoechoic subepidermal band decreased significantly by 0.23 mm on average in week 4, from 0.60 mm to 0.37 mm ($t = 10.39$, $p < 0.0000001$);

- compared to week 4, the average thickness of the hypoechoic subepidermal band decreased significantly by 0.24 mm on average in week 8, from 0.37 mm to 0.13 mm ($t = 6.21$, $p < 0.0000001$) (tab. II).

Given that the first change obtained chronologically when assessing the target plaque of cutaneous psoriasis was the decrease in psoriasis plaque thickness and subepidermal hypoechoic band in the target plaque as compared to baseline values (i.e. at week 4 compared to week 0), after comparing the mean values of psoriasis plaque tegument thickness and hypoechoic subepidermal band thickness, we found that they decrease significantly both in week 4 compared to week 0 and in week 8 compared to week 4. It was noted that the mean thickness of the hypoechoic subepidermal band of the adjacent normal integument did not change.

Comparison of mean scores of TLS between week 0, 4, and 8 using the T test for repeated measurements (repeated assessments over time) showed that the mean score decreased continuously from one assessment the high:

- compared to week 0, the average TLS score decreased significantly by 3.42 points on average in week 4, from 6.94 points to 3.52 points ($t = 21.82$, $p < 0.0000001$);

- compared to week 4, the mean TLS score decreased significantly by 2.96 points on average in week 8, from 3.52 points to 0.56 points ($t = 17.04$, $p < 0.0000001$).

TABLE II.

Comparison of the average thickness of the hypoechoic subepidermal band in week 0, 4 and 8

Comparisons between	Mean 1	SD 1	Mean 2	SD2	N	Difference	SD of the difference	t	p
Week 0 with week 4	**0.60**	0.34	***0.37***↓	0.29	50	0.23	0.15	10.39	***<0.0000001***
Week 0 with week 8	**0.60**	0.34	***0.13***↓	0.24	50	0.47	0.35	9.48	***<0.0000001***
Week 4 with week 8	**0.37**	0.29	***0.13***↓	0.24	50	0.24	0.28	6.21	***<0.0000001***
N, number of participants; SD, Standard Deviation									

In order to study the relationship between the clinical and ultrasonographic appearance of the psoriasis target plaque Pearson r correlation coefficients were calculated, being considered significant those coefficients that were at a significance threshold $p < 0.05$. The analysis of the correlations between the clinical and ultrasonographic appearance of the psoriasis target plate showed that:

- in the first week, no significant correlations were found; from the fourth week, some small but statistically significant direct correlations were found, as follows:

- between the skin thickness of the psoriasis plaque from week four and TLS from week four ($r = 0.3970$, $p = 0.004$), between the skin thickness of the psoriasis plaque from week eight and TLS from week eight ($r = 0.3432$, $p = 0.015$); between the thickness of the hypoechoic subepidermal band at week eight and the TLS at week eight (r

= 0.3475, p = 0.013).

The results revealed that the average PASI scores decreased significantly from one assessment to another, as follows:

- an average decrease of 5.66, from 23.62 in week 0, to 17.96 in week 4, (t = 7.44, p <0.0000001);
- an average decrease of 4.96, from 17.96 in week 4, to 13 in week 8, (t = 8.59, p <0.0000001);
- an average decrease of 4.3, from 13 in week 8, to 8.7 in week 12, (t = 8.82, p <0.0000001).

Similarly, the average DLQI scores decreased significantly from one assessment to another, showing that:

- an average decrease of 10.48, from 23.08 in week 0, to 12.6 in week 8, (t = 12.48, p <0.0000001);
- an average decrease of 2.54, from 12.6 in week 8, to 10.06 in week 12, (t = 11.96, p <0.0000001).

Significant direct correlations were not obtained between skin thickness of psoriasis plaque or hypoechoic subepidermal band and PASI score.

DISCUSSION

HFUS of the integument or nail apparatus can be used in the evaluation of response to local and/or systemic treatment (13, 14, 15). In a pilot study of 30 patients with psoriasis vulgaris compared to 10 healthy volunteers who used topical clobetasol propionate 0.05% foam differently (one arm of patients with psoriasis vulgaris applied twice daily for 14 days, the second arm applied once daily for one month, and volunteers once daily for four weeks) Lacarrubba *et al*. reported reduced psoriasis plaque thickness when monitoring treatment response with 20 MHz HFUS (16).

This resulted in equal values of integument thickness to that of adjacent healthy skin in all treated plaques, with no differences between the two psoriasis patient groups, and no sonographic variations in skin thickness were observed in the healthy group at the end of the study (16). In contrast to Lacarruba's study, in the current study we evaluated the therapeutic response not only under topical treatment (Fluticasone Propionate 0.5g/g cream or Calcipotriol/Betamethasone dipropionate 50 micrograms/0.5 mg/g gel and 10% Urea Emulsion) but also systemic, with 31 patients also under biological therapy with Etanercept. Similarly, in the 20 MHz ultrasonographic examination using Dermascan C® the study published by Micali *et al.* showed that at the 15-day examination, psoriasis plaque values were reduced by 33.5% in Etanercept-treated patients, by 63.6% at 30 days, and by 79.3% at 60 days. In total, after 60 days of treatment, 23 of the 42 psoriasis plaques analyzed showed complete normalization (13).

Subepidermal hypoechoic band can also be found in other inflammatory conditions such as atopic dermatitis or contact dermatitis (17). As a result, patients with these pathologies were excluded. In addition to the Subepidermal Low Echogenic Band (SLEB), in psoriasis we may detect thickened entry echo and streaky which is perpendicular to the entry echo shadows caused probably by air bubbles trapped between the scales (18). HFUS of the normal skin shows a clear separation of the skin layers (19). Camerota *et al*. argue that entry eco is due to the difference in acoustic impedance between gel and skin (20). The average epidermal thickness in healthy individuals is 0.6 mm, being between 0.1-0.8 mm (21).

For inflammatory dermatoses like psoriasis vulgaris, eczema, atopic dermatitis as well as in mycosis fungoides, similarly to the skin elastosis SLEB may be easy to

identify (22, 23). Maria C *et al*. (24) compared the efficiency of 20 MHz skin ultrasonography but also 40 MHz conventional ultrasonography in the assessment of 16 plaques psoriasis in three consecutive patients. The measurement was made at baseline and after hydrocortisone acetate 1% ointment six-week application. In contrast with our study parameters (total psoriasis plaque thickness including epidermal and dermal thickness and hypoechogenic band), their parameters were represented by epidermal and dermal thicknesses for gray-scale ultrasonography. Their results indicated that epidermal thickness was significantly reduced with 20 MHz and 40 MHz sonography, while dermal thickness varied insignificantly with 20 MHz and 40 MHz sonography. Gutierrez *et al*. (25) have showed that dermal thickness has been found to be correlated with disease severity measured using PASI score and other scales assessing the severity or extent of disease (11). Similarly, Fernando Alfageme (26) indicated that in a multicenter study HFUS examination showed a reduction in plaque thickness and Doppler signal intensity in the dermis of patients treated with infliximab.

One limitation of the study is the lack of biological and hematological data such as inflammatory syndrome indicated by Erythrocyte sedimentation rate (ESR) or imagistic tests like joint x-rays which can facilitate the diagnosis of severe form of psoriasis accompanied by psoriatic arthritis.

CONCLUSIONS

HFUS examination allows an objective and reproducible measurement of skin thickness and is a useful technique for a non-invasive assessment of treatment efficacy in psoriasis. Psoriasis plaque thickness and subepidermal hypoechoic band thickness can be markers of progression and therapeutic efficacy. The efficacy indicators of therapy objectified by HFUS are decreases in epidermal and dermal thickness and disappearance of the hypoechoic band. In the current study, the first chronological change obtained in the assessment of the target plaque of cutaneous psoriasis was a decrease in the thickness of the psoriasis plaque and the subepidermal hypoechoic band in the target plaque as compared to baseline values (i.e. at week 4 versus week 0), and after comparing the mean values of psoriasis plaque integument thickness and hypoechoic subepidermal band thickness, we found that they decrease significantly both in week 4 as compared to week 0 and in week 8 as compared to week 4.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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CONE BEAM COMPUTED TOMOGRAPHY ASSESSMENT OF CRANIOMANDIBULAR DISORDERS. A PROSPECTIVE STUDY FOR OSSEOUS COMPONENTS AND RISK FACTORS

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CONE BEAM COMPUTED TOMOGRAPHY ASSESSMENT OF CRANIOMANDIBULAR DISORDERS. A PROSPECTIVE STUDY FOR OSSEOUS COMPONENTS AND RISK FACTORS (Abstract): Craniomandibular disorders (CMD) are a series of clinical dysfunctions on the level of masticatory muscles, temporomandibular joint (TMJ) or both. Our aim was to use CBCT imagery to evaluate the morphology of the TMJ's components, to document any variations, and to corelate this data with the anatomy of the developing TMJ and the risk factors of the severity of CMD in patients from NE Romania. **Materials and Methods:** Our prospective study was conducted on 105 patients (70.5% were females and 53.3% were aged 26 to 45 years). All patients were carefully examined, underwent orthopantomogram and cone beam CT scans and were categorized according to the current diagnostic criteria for TMJ disorders. Group 1 (73 patients) were identified with symptomatic CMDs (clicking / crepitation, joint pain, muscle pain, limited mouth opening and non-harmonic movement of the jaw), while the remaining 32 patients represented Group 2 with asymptomatic presentation. **Results:** Linear measurements of TMJ spaces displayed statistically significant differences with age in the intervals of 18-25, 26-35 and 46-55years, while in the age groups of 36-45 and over 55 years, the results were similar on the right *vs.* left side. In Group 1, left-right differences were significantly greater in females (0.2265 ± 0.04029) compared to males (0.2067 ± 0.03152), with the highest amplitude being observed in the 18-25 and 26-35 age subgroups. The left-right differences remained statistically significant between the two groups even in comparative analysis of patients with/without intense weekly physical activity and in those with/without parafunctions (grinding, thumb sucking, or tongue thrusting). **Conclusions:** The severity of CMDs is more common in young age females. Oral status and morphology represent a significant risk factor for TMJ disorders.
Keywords: CRANIOMANDIBULAR DISORDER, CBCT, TEMPOROMANDIBULAR JOINT, CONDYLE, GLENOID FOSSA.

INTRODUCTION

The temporomandibular joint (TMJ) is defined as a ginglymo-arthrodial synovial joint consisting of an articular disk, fibrous capsule, articular eminence, synovial membrane and fluid, and associated ligaments.

The mandibular condyle is the inferior part of the articular surface of the TMJ, whereas in the superior part of the articular surface one finds the articular eminence of the temporal bone and the mandibular fossa (glenoid fossa). This joint is not only pivotal for hinging and gliding movements of the mandible across all spatial planes, but it also plays a vital role in maintaining the dynamic of mandible (1, 2).

The volume and size of the TMJ can be associated with the final dimensions of the mandibular arch and the relationship between the two arches. Also, the evaluation and the assessment of the volume and morphology of condyle are of utmost importance (3). Nowadays, there is no objective criterion for the ideal position of the mandibular condyle within the glenoid fossa. When the posterior joint space width is less than the anterior joint space, the condyle is retracted, and when the anterior joint space is greater, the condyle is protracted (4).

Craniomandibular disorder (CMD) is a general term for a group of diseases affecting temporomandibular joint (TMJ), masticatory muscles, and/or the surrounding tissues (5, 6). The main symptomatology is represented by muscle pain in the head and neck region, noise and pain in the temporomandibular joint, and limitation of the mandibular mobility. Other possible symptoms encountered are headache, posterior neck pain and dental pain (7). CMD is the second most common musculoskeletal disorder which leads to pain and disability (8, 9).

The recent advancements in three-dimensional (3D) technology, especially for osseous components, is represented by cone beam computed tomography (CBCT) that have enabled us to analyze the TMJ in a more comprehensive manner than before (10).

The aim of this research was to use CBCT imaging modality to evaluate the morphology of the mandibular condyle, glenoid fossa, other TMJ components and to document any morphological variations. The data obtained may provide valuable information on the anatomy of the development of the TMJ and joint spaces.

MATERIALS AND METHODS

Study design. A prospective study was conducted on patients 105 patients, of which three-quarters were women (70.5%), and half were aged between 26 and 45 years (53.3%). Among them, 73 patients (69.5%) were identified with symptomatic CMDs (clicking / crepitation, joint pain, muscle pain, limited mouth opening and non-harmonic movement of the jaw), while the remaining 32 patients (30.5%) represent Group 2 of asymptomatic patients.

All patients were carefully examined, underwent orthopantomogram (OPG) and CBCT scans, and were categorized according to the current diagnostic criteria for TMJ disorders. Full-volume CBCT scans using a Green X CBCT (VATECH) machine were taken at a centric relation position with the patient's mouth, closed in the natural head position, from October 2023 to September 2024. These CBCT images were taken at 90 kV and 8 mA with a volume size of 24 x 17 cm^2, a voxel size of 0.4 mm, and an exposure time of 3.7 s.

The inclusion criteria for the study were patients who have: been at least 18 years of age; filled written informed consent; completed the questionnaire PHQ-9 Patient Health Questionnaire-9 (7); and completed the personalized merged version of questionnaire TMD-Pain Screener (2).

The exclusion criteria for the study were patients who have/were: refused to participate in the study; non-cooperative; systemic joint disorders; neurological diseases; psychiatric diseases; cervical spine alterations; facial paralysis; previously diagnosed temporomandibular joint (TMJ) pathology.

Measurements. All CBCT images were oriented and analyzed using a digital software RadiAnt-DICOM Viewer version 2024.1. The axial slice of 0.4 mm was selected for measuring medio-lateral and antero-posterior dimensions. The joint spaces, eminence height and eminence inclinations were measured using sagittal slice of thickness of one mm from medial condyle to orbital.

The eminence inclination was measured using two methods: the best-fit (bf) line method and top-roof (tr) line method. The best-fit line method measures the angle between Ebf (eminence best-fit) and F (Frankfort), top-roof line method measures the angle between Etr (eminence top-roof) and F.

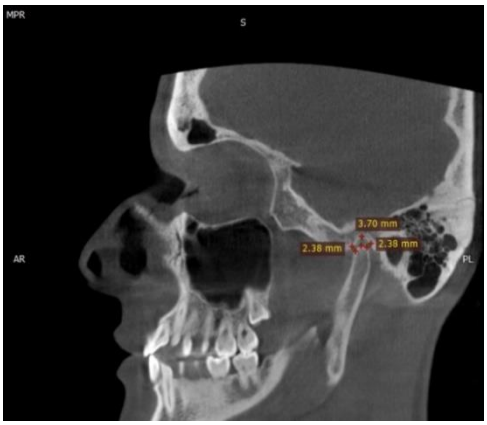

Fig. 2. Measurement of Sjs - Superior Ajs -Anterior joint space, and Pjs – Posterior joint space.

In sagittal plane, the thickness of the glenoid fossa was defined as the distance between the outer cortical outline and the inner cortical outline.

Metric evaluation of morphology of the mandibular condyle and glenoid fossa, including its condylar height, width, length, roof of glenoid fossa (RGF) thickness, anterior, posterior, and superior joint space, and volume of the condyle were recorded.

Fig. 1. Measurement of ML - Medio-Lateral joint space, width of condyle, a nd AP - Antero-Posterior width of condyle.

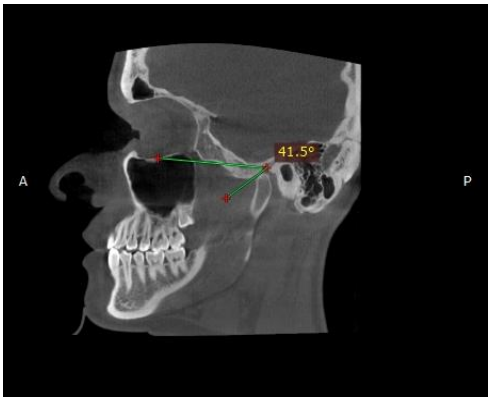

Fig. 3. Measurement of EBFA - Eminence image Best Fit Angle, ETRA - Eminence Top sagittal Roof Angle, F-Frankfort horizontal line.

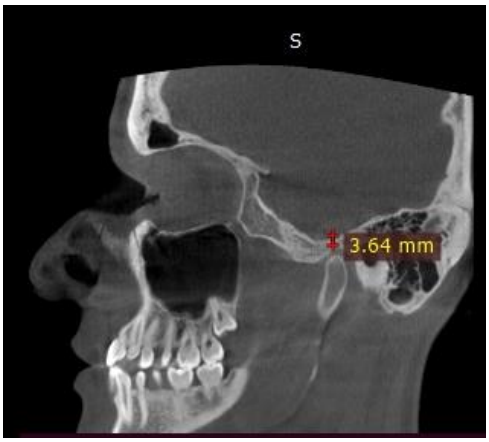

Fig. 4. Cone beam computed tomography image and schematic representation of the sagittal view showing glenoid fossa roof thickness measurement between the outer and inner cortical outline of the bone.

A 2D sagittal slice with a clear appearance of the condyle and glenoid fossa was selected. The superior-most portion of the mandibular condyle was denoted as superior mandibular condyle (SCo). Two points, one posterior and one anterior, on either side of the condyle were selected as posterior-most mandibular condyle point (PCo) and anterior-most condylar point (ACo). From this selected sagittal slice, the condylar length was measured.

The areas of maximum convex curvature on either side of the condyle, in the coronal section, were chosen as the medial condyle (MCo) and lateral condyle (LCo). Condylar width was measured as the linear distance between the mandibular lateral and medial poles.

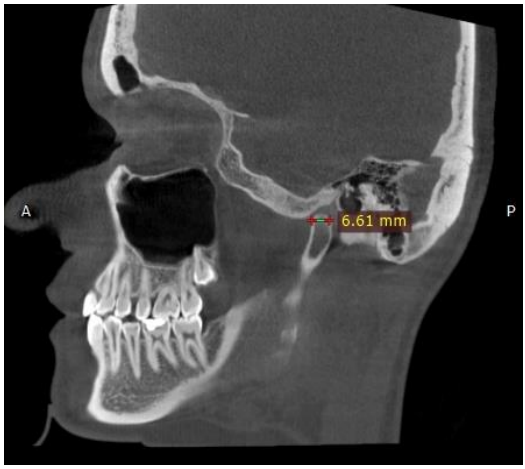

Fig. 5. Cone beam computed tomography image in sagittal view of the condylar length in the anteroposterior direction. (ACo, anterior-most condylar point; PCo, posterior-most mandibular condyle point; SCo, superior mandibular condyle)

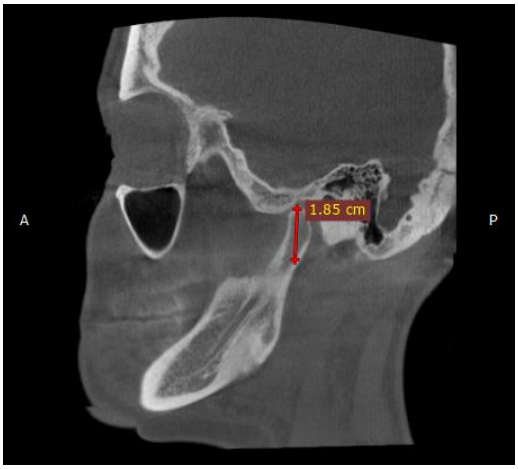

Fig. 6. Cone beam computed tomography image and schematic representation of the sagittal view showing condylar height measurements. (Inf Sig, inferior-most point of the sigmoid notch; SCo, superior mandibular condyle)

The **intercondylar distance** in the context of the temporomandibular joint (TMJ) refers to the space between the medial or lateral femoral condyles.

Fig. 7. Cone beam computed tomography image tomography of intercondylar distance.

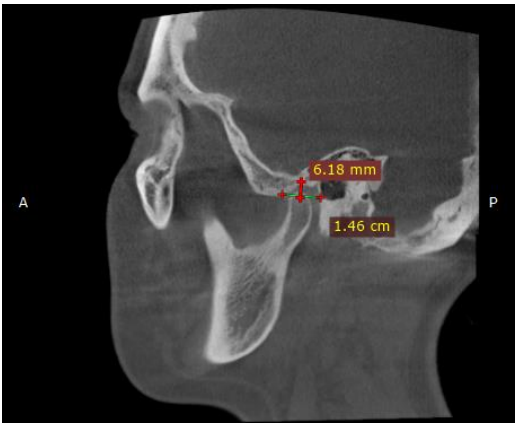

Fig. 8. Cone beam computed tomography image of the glenoid depth.

Data analysis. The data were centralized in *SPSS 24* databases and processed with suitable statistical function. Both descriptive and analytical methods were used. We compared average percentages between various genders and severity levels using the Pearson Chi-squared test, with a significance level of error set at 95%. We also performed the Mann-Whitney test (parametric) to compare two samples or groups, with low numbers of individuals in each group, which are not normally distributed, and where the data are continuous.

RESULTS

Comparative study of the parameters investigated in the sample of patients with CM dysfunctions versus asymptomatic patients

A cohort of 105 patients was investigated, of which three-quarters were women (70.5%), and half were aged between 26 and 45 years (53.3%). Among them, 73 patients (69.5%) were identified with CM dysfunctions (Group 1), while the remaining 32 patients (30.5%) made the Group 2 asymptomatic patients. Specifically, Group 1 displayed a heterogeneous distribution across age groups, covering the entire range from 18 to 75 years, whereas the vast majority of patients in Group 2 - asymptomatic patients (75.0%) were young adults aged between 26 and 45 years. Most patients with CM dysfunctions exhibited only mild dysfunctions (80.8%), with 19.2% of cases identified as having moderate dysfunctions (tab. I).

Additionally, the values of the differences between the left-right symmetric measurements across the entire sample (general statistical characterization) are presented in table II.

The study of the correlations between left-right measurement differences and clinical parameters of the patients

The first parameter considered for potential correlations was the patients' age. The only measurement found to be correlated with age was the articular slope - specifically, the left-right difference in its values - which showed a weak but positive and statistically significant correlation with patient age ($r = 0.222$) (tab. III).

TABLE I.

Demographic and clinical characteristics of the samples investigated

		SAMPLE				Total		Pearson Chi-squared test
		Group 1 -CM Dysfunction		Group 2 - Asymptomatic patients				
		N	%	N	%	N	%	
Gender	male	21	28.8%	10	31.3%	31	29.5%	$Chi^2 = 0.066$
	female	52	71.2%	22	68.8%	74	70.5%	p = 0.797
Age group	18-25 years	16	21.9%	5	15.6%	21	20.0%	$Chi^2 = 12.298$
	26-35 years	14	19.2%	15	46.9%	29	27.6%	p = 0.015*
	36-45 years	18	24.7%	9	28.1%	27	25.7%	
	46-55 years	19	26.0%	3	9.4%	22	21.0%	
	over 55 years	6	8.2%			6	5.7%	
Dysfunction degree	mild	59	80.8%					
	moderate	14	19.2%					
Total		73	100.0%	32	100.0%	105	100.0%	

TABLE II.

Left-Right differences between the cephalometric measurements - statistical characterization across the entire sample

GLOBAL SAMPLE	N	Average (m)	Std. deviation (SD)	Min	Max	Median	IQR	
							Q1	Q3
Condyle - Glenoid Cavity Distance - Anterior: DIFF	105	0.1852	0.06300	0.10	0.31	0.2000	0.1100	0.2100
Condyle - Glenoid Cavity Distance - Superior: DIFF	105	0.1834	0.06389	0.10	0.31	0.2000	0.1100	0.2100
Condyle - Glenoid Cavity Distance - Posterior: DIFF	105	0.1849	0.06338	0.10	0.31	0.2000	0.1100	0.2100
Articular Slope: DIFF	105	1.7114	0.44383	0.90	2.30	2.0000	1.1000	2.0000
Condyle Height: DIFF	105	0.1823	0.06426	0.08	0.33	0.2000	0.1100	0.2100
Condyle Length: DIFF	105	0.1836	0.06363	0.09	0.33	0.2000	0.1100	0.2100
Small Diameter: DIFF	105	0.1815	0.06312	0.09	0.32	0.2000	0.1050	0.2100
Large Diameter: DIFF	105	0.1993	0.18844	0.10	2.00	0.2000	0.1000	0.2100
Glenoid Cavity Thickness: DIFF	105	0.1810	0.06433	0.01	0.31	0.1900	0.1000	0.2100
Glenoid Cavity Depth: DIFF	105	0.1836	0.06256	0.09	0.32	0.2000	0.1100	0.2000
Intercondylar Distance: DIFF	105	33.7039	4.56098	23.50	40.90	32.7000	29.9000	38.8000
DIFF=Differences								

TABLE III.

Study of the correlation between the left-right differences of the investigated cephalometric measurements and the patients' age

AGE vs.	**Spearman rho Correlation Coefficient**	**p**	**95% CI**		**Interpretation**
			Lower limit	**Upper limit**	
Condyle – glenoid cavity anterior distance DIFF	0.087	0.377	-0.112	0.280	Null correlation
Condyle – glenoid cavity superior distance DIFF	-0.034	0.729	-0.230	0.164	Null correlation
Condyle – glenoid cavity posterior distance DIFF	0.035	0.724	-0.164	0.230	Null correlation
Articular slope values DIFF	0.222	0.023*	0.026	0.402	Weak positive correlation, statistically significant
Condyle height values DIFF	0.119	0.228	-0.080	0.309	Negligible correlation
Condyle length values DIFF	0.037	0.710	-0.162	0.232	Null correlation
Small diameter values DIFF	-0.040	0.685	-0.235	0.158	Null correlation
Large diameter values DIFF	-0.039	0.690	-0.235	0.159	Null correlation
Glenoid cavity thickness values DIFF	0.027	0.788	-0.172	0.223	Null correlation
Glenoid cavity depth values DIFF	0.103	0.296	-0.096	0.294	Negligible correlation
Intercondylar distance values DIFF	-0.024	0.811	-0.222	0.176	Null correlation
DIFF=Differences					

DISCUSSION

The accuracy of CBCT for linear measurements was confirmed in various studies by Lascala *et al.* (10) and Soumalainen *et al.* (11). Furthermore, Lascala *et al.*, consider that CBCT images underestimate the actual distances on the base of the skull between different points and are accurate for linear measurements of these structures (10). Soumalainen *et al.*, stated that the error in linear measurement and evaluation using CBCT technique was lower than that of CT (11).

Dalili *et al.* in their study, concluded that males have larger linear measurements of joint spaces in comparation with females, especially the posterior and the superior spaces (12).

In our Group 1 (CM dysfunction), statistically significant differences were observed between right and left measurements, with the values recorded on the right side being statistically significantly higher than those recorded on the left side. Among male patients, although the measurements on the right side were also higher than those on the left, the observed differences did not reach statistical significance; however, among female patients, the differences were greater and statistically significant (tab. II).

The results of our study are aligned with other studies, wherein a significant difference was observed between the two genders when linear dimensions of posterior and superior joint spaces were compared (13). Another explanation could be due to the difference in the overall size of tem-

poral fossa and mandibular condyle in males, because of sexual dimorphism (14).

In our study, linear measurements of the joint spaces displayed significant differences with age. The same statistically significant differences were also found in the age groups 18-25 years, 26-35 years, and 46-55 years; in patients aged 36-45 years and those over 55 years, the measurements on the right and left sides were similar (tab. III). This agrees with the results of a study by Alexiou *et al*. (15), who concluded that linear measurement of the joint spaces decreases with age.

In our study, the volume of the condyle was significantly greater in males (tab. II, tab. III) and was aligned with the results of Tadej *et al*. (16), the latter relating the overall size of the mandibular condyle to sexual dimorphism.

Comparing the measurements of the posterior and superior space among left and right sides, a significant difference was observed in both genders. This asymmetry observed in our study in the condylar position within the left and right sides of glenoid fossa could be explained by the presence of a preferred side for mastication, as also stated by Blaschke and Blaschke (17) and by Tecco *et al*. (18). Moreover, the overall asymmetry in the joint structure, that we also emphasized, must be due to the preferred side of chewing among the subjects with CM disorders, in their effort to mitigate discomfort, but with consequences in altered morphology and volume (19).

The volume of the condyle decreased with age, and this was significant in females. Our findings are also supported by Takayama *et al*, who reported it in both males and females; volumetric and morphologic changes in TMJ being both adaptive and degenerative over a long period of time (20).

In Group 1 these left-right differences are statistically significantly greater in females (0.2265 ± 0.04029) compared +to males (0.2067 ± 0.03152). The largest differences were observed in the 18-25 and 26-35 age groups (tab. III), reconfirming Chaurasia *et al*. who noted that the condylar height and width are maximum between 20 and 30 years of age, followed by a gradual reduction with age (21).

The significant difference among the right and left sides observed in our study suggested that the right side has a more posteriorly placed fossa, a narrow glenoid fossa width, and greater inclination of the anterior wall, while the left condyle has increased condylar width and vertical inclination.

We observed also significant differences between the right and left side measurements when separately analyzing patients with mild and moderate dysfunctions, as well as those with mild or moderate depression. The differences between right and left side measurements remained statistically significant even in separate analyses of patients with or without parafunctions (tab. II). Bruxism is one of the most common parafunctions, consisting in repetitive jaw muscle activity, clenching or grinding of the teeth and/or bracing or thrusting of the mandible. It is generally accepted that extreme bruxing activity may lead to a breakdown in the stomatognathic system, orofacial pain and TMD, thus raising the need for reliable and validated screening tools, and structured clinical examination (22).

Our study highlighted the relationship

between morphology of TMJ in symptomatic and asymptomatic TMDs. We can state that craniomandibular disorders (CMDs) is a significant concern that should be screened and addressed accordingly, especially in the population at greater risk, and CBCT can be a valuable diagnostic aid in the evaluation of various morphometric analysis of the TMJ.

CONCLUSIONS

Our research proved there was a significant variation in the linear condylar measurements of width and volume between the left and right side of the temporomandibular joint (TMJ), which leads us to state that each condyle should be evaluated and assessed as a separate entity prior to any treatment.

According to our results, the severity of the craniomandibular disorder is more common in females, and at a young age. Oral status and joint morphology have been established as a significant risk factor for TMJ disorders, apart from parafunctional habits (grinding, thumb sucking, or tongue thrusting), intense physical activity, and/or pathologic occlusion.

This study advocates an increased awareness in the medical community for the relationship between temporomandibular joint (TMJ) screening based on CBCT and the role of morphological and external factors for an efficient future therapeutic approach.

ETHICS

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Grigore T. Popa University of Medicine and Pharmacy of Iasi (271/14.02.2023) for studies involving humans.

Informed Consent Statement. Informed consent was obtained from all subjects involved in the study.

CONFLICT OF INTERESTS AND FUNDING

The authors declare no conflict of interests.

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DETERMINATION OF HEART RATE VARIABILITY, A POSSIBLE MARKER FOR ASSESSING INTRINSIC CAPACITY

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DETERMINATION OF HEART RATE VARIABILITY, A POSSIBLE MARKER FOR ASSESSING INTRINSIC CAPACITY (Abstract): The assessment of heart rate variability (HRV) parameters is a non-invasive method of multidimensional evaluation of geriatric patients, independent of complex geriatric and clinical-biological assessment, with an impact on the early identification of nutritional disorder, cognitive status, psycho-emotional status, frailty, cardiovascular mortality risk, as well as functional disorders and maintaining quality of life. **Aim:** To determine HRV parameters and their role in assessing intrinsic capacity in old patients. **Materials and methods:** We determined HRV in a group of 83 patients over the age of 65 who were admitted to the Geriatric Clinic of the Dr. C. I. Parhon Hospital in Iasi, and we also performed a complex geriatric assessment by evaluating cognitive status (MMSE - Mini-Mental State Exam), nutritional status (MNA - Mini Nutritional Assessment), psycho-emotional (GDS - Geriatric Depression Scale), risk of falling (Downton Scale), the presence or the absence of the urinary incontinence, as well as determining body composition using bioimpedance. **Results:** The average age was 75.64 ± 6.64 years with a median of 75 years, most patients being women (63.9%). The distribution by age group shows that most patients were between 65 and 74 years old (48.2%), falling into the young old category; of these, 60% were female. Low scores on the MNA, as well as low muscle strength, body mass index, bone mass, visceral fat values or urinary incontinence are correlated with decreased heart rate variability (HRV) parameters. The presence of anemia or depressive symptoms is associated with reduced levels of HRV parameters. **Conclusions:** Data obtained support the existence of a causal relationship between heart rate variability (HRV) and a number of factors, both dependent on the patient's clinical-biological status and geriatric profile, and independent of it. Early identification of these factors in the old population is essential for preventing adverse changes in body composition, appearance in psycho-emotional disorder, urinary incontinence, as well as for mitigating their impact on autonomy and quality of life. **Keywords**: HEART RATE VARIABILITY, INTRINSIC CAPACITY, BODY COMPOSITION, NUTRITIONAL DISORDER, OLD PATIENTS.

INTRODUCTION

Assessing intrinsic capacity in all five dimensions (functional status, cognition, vitality, sensory functioning, and psycho-emotional assessment) is a central concept in modern geriatrics (1, 2, 3). This is achieved either through clinical- biological assessment or by using complex geriatric assessment scales: Mini-Mental State Examination (MMSE), Mini Nutritional As-

sessment (MNA), Geriatric Depression Scale (GDS), risk of falling - Downton Scale (3). These diagnostic methods are time-consuming and energy-intensive, limiting the development of a personalized treatment plan. Therefore, in the context of contemporary medical practice, the integration of advanced technological innovations contributes to the optimization of diagnosis and monitoring of therapeutic interventions (2).

Determining heart rate variability is a modern, non-invasive method that consists of identifying normal fluctuations in the interval between two successive heartbeats-RR intervals - a mechanism controlled by the autonomic nervous system (ANS) (1, 4, 5). Heart rate variability (HRV) parameters are classified into several main domains, namely the time domain (e.g. SDNN standard deviation of intervals, SDANN standard deviation of average intervals for each 5-minute segment, RMSSD mean square of successive interval differences) and the frequency domain (Power - ULF, VLF, LF, HF, Absolute power of ultra-low, very low, low, and high frequency bands, Peak - ULF, VLF, LF, HF, Peak frequency of ultra-low, very low, low, and high frequency bands, LF/HF Ratio between low and high frequency power, each providing complementary information on autonomic heart rate regulation) (1).

The assessment of these parameters provides information about the sympathetic-vagal balance, estimation of cardiovascular and overall mortality risk, as well as identification of neurocognitive function decline and the nutritional disorders, systems that are part of the assessment of intrinsic capacity (6, 7, 8, 9, 10). A high level of HRV is considered a sign of autonomic balance, physiological resilience, and adaptability, while low HRV indicates autonomic dysfunction, chronic stress, inflammation, or increased cardiovascular risk (11, 12, 13, 14, 15).

In old patients, age-related changes and chronic comorbidities frequently lead to a reduction in parasympathetic activity and, implicitly, to a decrease in HRV, which is associated with an increased risk of developing, and increased mortality. Therefore, the need to integrate heart rate variability assessment into geriatric clinical practice can lead to the collection of fundamental data on multidimensional health status, as well as medical outlook, prognosis, and quality of life (16, 17, 18, 19).

MATERIALS AND METHODS

We present a prospective study that started in January 2024, from which we present the preliminary data collected in January - October 2024, on a group of 83 patients, aged over 65, hospitalized in the "Dr. C.I. Parhon" Clinical Hospital Iasi.

The exclusion criteria were as follows: age under 65 years, absence of informed consent, unavailability of relevant clinical or biochemical data, severely compromised functional status (patients bedridden or unable to maintain orthostasis which prevented the assessment of body composition by bioelectrical impedance analysis), presence of an active implantable device (e.g. pacemaker).

All patients enrolled in the study underwent intrinsic capacity assessment using the following scales: MMSE, MNA, GDS, fall risk - Downton scale, the presence or the absence of the urinary incontinence, as well as cardiovascular assessment (EKG, Holter EKG, echocardiography) (20, 21, 22). We also determined muscle strength in both upper limbs using a dynamometer and extracted data on biochemical levels to assess nutritional status (total protein level, hemoglobin level). Furthermore, all patients underwent body composition assess-

ment using the Tanita BC-545N weighing scale bioimpedance device.

Muscle strength was assessed with a dynamometer, and values were interpreted according to sex and age, with specific thresholds for men and women, defining normal, low, or high values (23). For men aged 65 to 69, normal muscle strength ranged from 28.2 kg to 44.0 kg, and for those aged 70 to 99, from 21.3 kg to 35.1 kg. For women aged 65 to 69, normal muscle strength ranged from 15.4 kg to 27.2 kg, and for those aged 70 to 99, from 14.7 kg to 24.5 kg. Values below the lower limit were considered low, and those above the upper limit were considered high (23).

Cognitive status was determined using the MMSE scale (0-30 points), with normal cognitive function between 25-30, mild impairment between 20-24, moderate impairment between 10-19, and severe impairment below 10 points (24).

Nutritional assessment was based on the MNA questionnaire (24-30 indicates normal nutrition, 17-23.5 risk of malnutrition, <17 malnutrition) (24), as well as on body mass index, with standard categories of underweight (18.5 kg/m²), normal weight (18.5 and 24.9 kg/m²), overweight (25 and 29.9 kg/m²), between 30 and 34.9 kg/m² obesity grade I, between 35 and 39.9 kg/m² obesity grade II, and values above 40 kg/m² morbid obesity (25).

Biochemical parameters were taken from recent data (≤ 7 days), with normal ranges for total protein (66-87 g/L) and hemoglobin (11.5-15.7 g/dL), values below and above the limits being considered abnormal.

Visceral fat was measured by bioelectrical impedance using Tanita BC-545N, classifying values as normal (1-12), high (13-19), or very high (>19), adjusted for age, sex, height, and patient profile.

Emotional state was assessed using the 15-item Geriatric Depression Scale (GDS), considering scores below 5 as normal, 5-8 as mild depression, 9-11 as moderate depression, and above 11 as severe depression (26).

Data on urinary incontinence were taken from the patient's medical history or observation sheet and were noted as present or absent.

The completing of the questionnaires was done after obtaining the informed consent of the patient, being made by the same trained medical team.

The data were collected by a geriatric team and were analyzed using SPSS 18.0 software. ANOVA Test, Chi-square Test and Paired T-test, Kruskall-Wallis and Pearson correlation for quantitative analyze where performed. The statistical significance was defined in 95% interval of confidence ($p < 0.05$).

RESULTS

The patients included in the study had a mean age of 75.64 ± 6.64 years, with a median age of 75 years. Most of the subjects were female (63.9%). The distribution by age group showed that 48.2% of participants fell into the "young old" category (65-74 years), 39.8% into the "adult old" category (75-84 years), and 12% were over 85 years old. Most patients included in the study had relatively balanced cognitive characteristics of their psychoemotional status (tab. I).

Approximately 30% of subjects had reduced muscle strength in one of their upper limbs, either dominant or non-dominant. In terms of body composition, most patients had an increased body mass index (BMI), thus falling into the overweight category, and about 70% had a high percentage of total body fat.

TABLE I.
Characteristics of the analyzed patients.

Parameter (Mean ± SD, Median (IQR) / No, %)	Value
MNA	23.5 ± 3.69, 24 (21-26)
MMSE	26.47 ± 3.62, 27 (25-29)
GDS	5.8 ± 3.34, 5 (3-9)
Muscle force - right arm	21.7 ± 9.41, 20.8 (14.1-27.8)
Muscle force - right arm (Category)	
Low	24 (28.9%)
Normal	52 (62.7%)
High	7 (8.4%)
Muscle force - left arm	20.87 ± 8.85, 19.8 (14.4-26.9)
Muscle force - left arm (Category)	
Low	26 (31.3%)
Normal	51 (61.4%)
High	6 (7.2%)
Total fat	34.72 ± 8.68, 34.3 (29.1-40.9)
Total fat (Category)	
Low	2 (2.4%)
Normal	23 (27.7%)
High	58 (69.9%)
BMI	28.97 ± 5.72, 28.3 (25.9-31.9)

Nutritional Status Assessment

Based on the geriatric assessment and MNA scores, Spearman's correlation analysis (ρ) revealed positive associations between the nutritional score and certain parameters of heart rate variability: Variability ($p = 0.046$; $\rho = 0.220$), SDNN ($p = 0.047$; $\rho = 0.219$), SDANN ($p = 0.015$; $\rho = 0.267$), and ULF ($p = 0.013$; $\rho = 0.271$) (fig. 1).

Total protein values were positively correlated with SDANN ($p=0.046$; $\rho = 0.221$), and hemoglobin showed positive correlations with Variance and SDNN ($p \approx 0.046$-0.047; $\rho \approx 0.218$-0.220), suggesting an association between nutritional status and heart rate variability parameters (fig. 2).

Body composition assessment, as part of nutritional status analysis, revealed significant positive correlations between bone mass and LF ($p = 0.025$; $\rho = 0.245$) and HF ($p = 0.016$; $\rho = 0.263$), indicating an association of higher bone mass with increased sympathetic and parasympathetic autonomic activity. Body mass index (BMI) was positively correlated with HF ($p = 0.044$; $\rho = 0.222$), suggesting a similar relationship with parasympathetic activity.

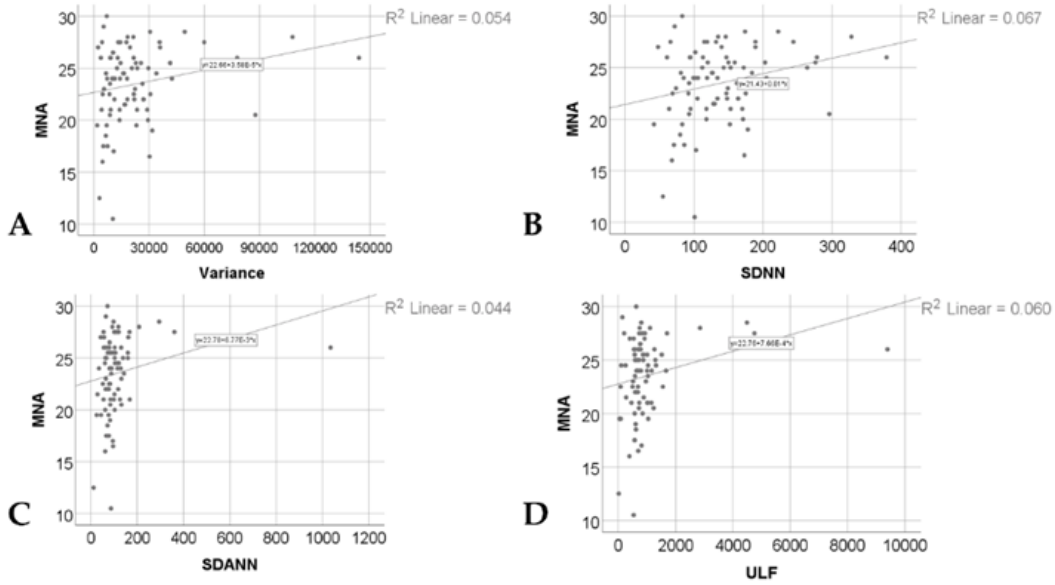

Fig. 1. Correlations between **MNA** and Variance (A), SDNN (B), SDANN (C) and ULF (D).

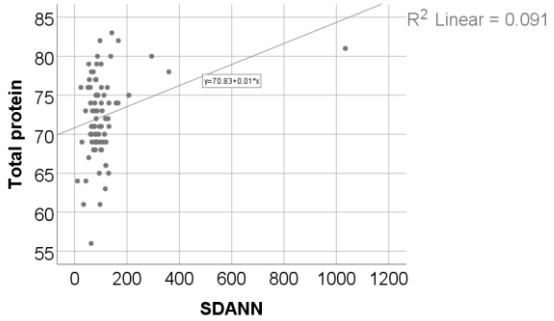

Fig. 2. Correlation between **Total protein value** and **SDANN**

Right arm muscle strength showed significant positive correlations with heart rate variability (Variance, SDNN, SDANN, ULF, VLF, nLF, LF/HF; p between 0.002 and 0.042; ρ between 0.224 and 0.343), reflecting increased cardiovascular autonomic tone. Left arm muscle strength was also significantly correlated with SDANN, ULF, VLF, nLF, and LF/HF (p between 0.005 and 0.041; ρ between 0.225 and 0.304).

Neurocognitive status Assessment

Data analysis showed that 80.7% of patients had no neurocognitive disorder, 12% had mild cognitive impairment, and 7.2% had moderate impairment, according to the MMSE. nLF values were significantly lower in the mild impairment group compared to the unaffected group (p = 0.031),

while differences between the other groups were not significant. GDS scores are associated with lower values of these heart rate variability indicators.

Psycho-Emotional status Assessment

Univariate linear regression models, with the GDS score as the dependent variable, showed significant negative correlations with the Variance parameters (B= 0.000036; 95% CI: -0.000066 - -0.000007; p=0.017), SDNN (B = -0.013657; 95% CI: -0.024875 - -0.002439; p=0.018), ULF (B = -0.000625; 95% CI: -0.001234 - -0.000017; p=0.044) and VLF (B = -0.000202; 95% CI: -0.000403 - -0.000002; p=0.048), indicating that higher (figs. 3, 4).

Urinary incontinence

In this study, the presence of urinary incontinence was significantly associated with lower values of several HRV parameters. Specifically, patients with urinary incontinence showed significantly reduced TINN values (446.12 ± 234.32) compared to those without this condition (575.38 ± 272.12), with a p-value of 0.042. Similarly, Triang-IX values were also lower in the incontinence group (48.64 ± 22.57) *versus* the control group (65.84 ± 29.18), reaching statistical significance (p = 0.010). Frequency domain analysis further indicated that patients with urinary incontinence had significantly lower ULF values (median = 636, IQR = 525-865.5) compared to those without incontinence (median = 771, IQR = 581.7-1030.7), with a p-value of 0.048.

Additionally, normalized high-frequency power (nHF) was also significantly reduced in the incontinence group (median = 43, IQR = 39.2-46) compared to the control group (median = 44.5, IQR = 42.25-48.38), with a p-value of 0.047 (fig. 5).

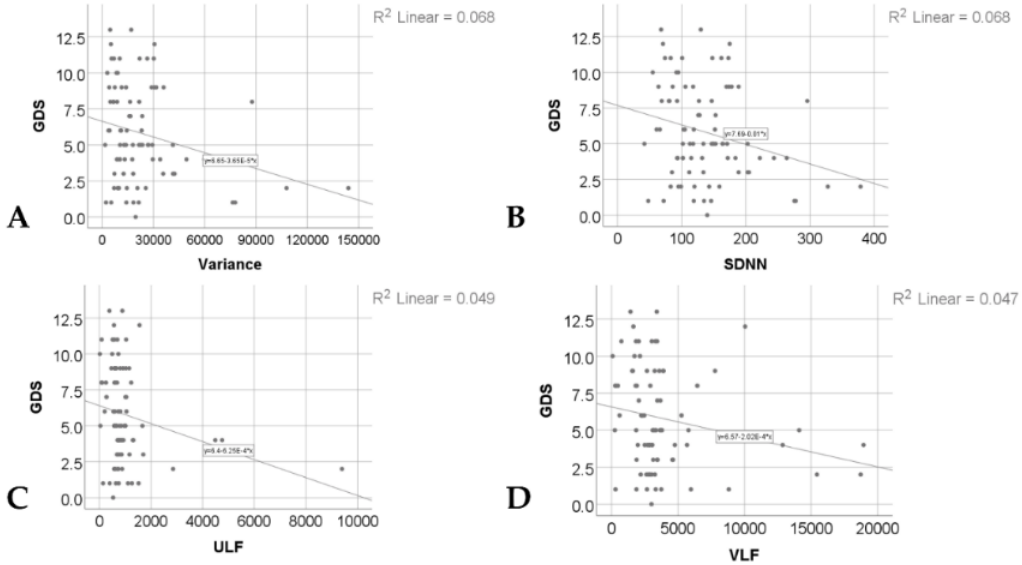

Fig. 3. Correlations between **GDS** and Variance (A), SDNN (B), ULF (C) and VLF (D).

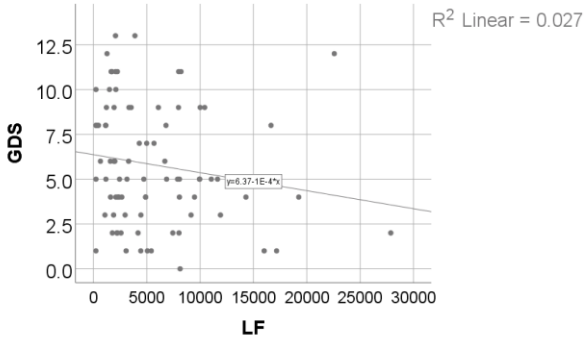

Fig. 4. Correlation between **GDS** and LF.

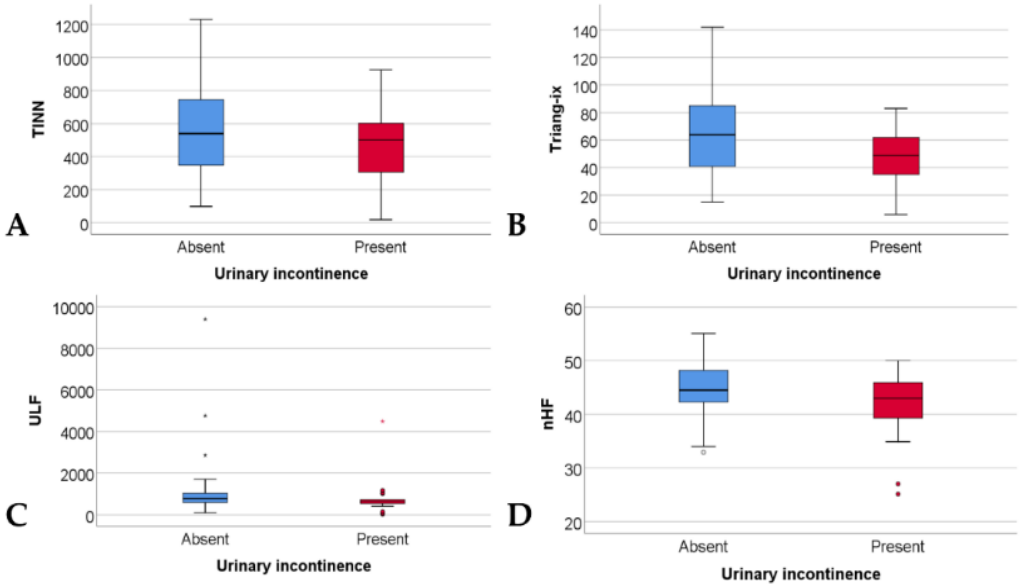

Fig 5. Comparison of TINN (A), Triang-ix (B), ULF (C) and nHF (D) according to the existence of **urinary incontinence**

The results of this study highlight a significant association between the presence of urinary incontinence and decreased HRV, both in the temporal and spectral domains. Patients with urinary incontinence had significantly lower HRV parameters, which may indicate an imbalance in autonomic cardiovascular regulation. These findings suggest that autonomic nervous system dysfunction may play a role in the pathophysiology of urinary incontinence and may represent a potential marker of severity or progression of this condition.

DISCUSSION

The results of our study highlight positive correlations between nutritional status

assessed by the MNA test and heart rate variability parameters (variance, SDNN, SDANN, ULF), which is confirmed by previous studies supporting the hypothesis that good nutritional status is closely linked to better cardiovascular tone (27, 28). The determination of bone mass and body mass index, as an integral part of nutritional status, reflects the link with some HRV parameters as a marker of parasympathetic activity and the autonomic nervous system. Data from the literature support the fact that weight loss in overweight individuals leads to increased parasympathetic activity and increased HRV (11, 13, 14, 15).

Determining muscle strength and the nutritional status (total protein level, hemoglobin, MNA), contributes to a comprehensive assessment in geriatric evaluation and provides information on autonomic function (13, 14, 23).

The results obtained sustain that superior nutritional status is associated with better cardiovascular autonomic activity.

Data on neurocognitive function are partially confirmed by the present study. Patients with mild neurocognitive disorder have lower HRV parameters, concordant with the literature indicating autonomic dysfunction as a biomarker of early cognitive decline. The results obtained are due to the profile of the enrolled patients, who predominantly present with no neurocognitive disorder or mildly impaired neurocognitive function.

Further studies are needed to provide data on involvement in moderate and severe cognitive dysfunction (27).

The lack of significant correlations between HRV parameters and the risk of falling, as assessed by the Downton scale, indicates the need for complementary methods for a more accurate assessment of the risk of falling (28).

Regarding psycho-emotional status, the significant negative correlations between GDS scores and HRV parameters (Variance, SDNN, ULF, VLF) are consistent with multiple studies that have highlighted the link between affective disorders and autonomic nervous system dysfunction (20).

At the same time, this study highlights a significant link between urinary incontinence and decreased heart rate variability, suggesting a possible involvement of autonomic dysfunction in the mechanisms of this condition. The lack of comprehensive studies in the current literature highlights a gap in knowledge, emphasizing the importance of addressing this issue in future clinical practice.

Data on functional status and frailty are currently being analyzed and will be published later as part of intrinsic capacity in old patients.

CONCLUSIONS

This study supports the importance of determining heart rate variability as part of a comprehensive holistic assessment of senior patients.

HRV and its spectral components can be used as biomarkers for early detection and monitoring of the progression of nutritional and psycho-emotional disorders, as well as urinary incontinence in patients over 65 years old.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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SALIVARY OXIDATIVE STRESS BIOMARKERS AND ORAL HEALTH IN PATIENTS WITH ACTIVE INFLAMMATORY BOWEL DISEASE: PRELIMINARY RESULTS

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SALIVARY OXIDATIVE STRESS BIOMARKERS AND ORAL HEALTH IN PATIENTS WITH ACTIVE INFLAMMATORY BOWEL DISEASE: PRELIMINARY RESULTS (Abstract): The oral cavity and the intestine are interconnected through shared microbial and immunological pathways, supporting a bidirectional relationship between oral health and inflammatory bowel disease (IBD). Oral pathological conditions are more prevalent and severe in IBD, while oxidative stress represents a key mechanistic link between oral and intestinal inflammation. However, salivary oxidative stress biomarkers remain insufficiently characterized in IBD, and comparative data with healthy controls are limited. **Materials and methods:** This cross-sectional study evaluated adult participants at a single time point, who underwent standardized oral examinations and unstimulated saliva collection. Salivary oxidative stress biomarkers, including superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), and total protein levels, were assessed using validated biochemical methods. Correlation and linear regression analyses were performed to examine associations between salivary biomarkers and clinical dental indices in patients with clinically active IBD, compared with a healthy control group. **Results**: In patients with active IBD, salivary oxidative stress biomarkers (SOD, GPx, MDA) and total protein levels showed weak, non-significant associations with oral health indices. MDA demonstrated modest positive trends with caries experience (DMFT; $r \approx 0.45$) and periodontal status (PDI; $r \approx 0.54$), while SOD and GPx showed minimal correlations across oral parameters (all $p > 0.05$). Compared with controls, patients with IBD showed lower mean salivary SOD and GPx activities and higher MDA levels; however, these intergroup differences did not reach statistical significance. **Conclusions:** Salivary oxidative stress biomarkers were not significantly correlated with dental indices in patients with active IBD. Nevertheless, the observed trends for MDA suggest that lipid peroxidation may more closely reflect the shared chronic inflammatory burden affecting oral and intestinal tissues, warranting further investigation in larger, adequately powered studies. **Keywords:** ORAL HEALTH, PERIODONTAL DISEASE, INFLAMMATORY BOWEL DISEASE, OXIDATIVE STRESS, SALIVARY BIOMARKERS.

INTRODUCTION

The oral cavity and the intestine are distinct yet interconnected components of the orodigestive tract, linked through shared anatomical, microbial, and immunological pathways that generate bidirectional influences on host health. Increasing evidence indicates that oral diseases and inflammatory bowel diseases (IBD) should not be regarded as isolated conditions, but rather as components of a complex and interdependent pathogenic network (1).

Inflammatory bowel diseases, comprising ulcerative colitis and Crohn's disease, are characterized by chronic inflammation of the gastrointestinal tract. Its etiology is multifactorial, involving genetic predisposition, disruption of the intestinal epithelial barrier, and a dysregulated immune response to luminal contents, alongside influences from lifestyle, diet, microbiota composition, and environmental exposures. Certain factors, such as smoking, increase the risk of Crohn's disease, whereas a Western-style diet rich in animal proteins and fats, the frequent use of antibiotics or nonsteroidal anti-inflammatory drugs, and psychosocial stress can trigger or exacerbate IBD (2) (3). Modern dietary patterns, through their profound influence on both the oral and intestinal microbiome, contribute to the increasing susceptibility to IBD and other chronic metabolic disorders (1).

Periodontitis is a chronic inflammatory disease of the tooth-supporting structures, arising from the complex interplay between microbial dysbiosis and the host immune response. This microbial imbalance is characterized by the proliferation of pathogenic oral bacteria and the reduction of protective commensal species, while disease initiation is linked to the accumulation of dental plaque and the subsequent activation of inflammatory pathways that progressively destroy periodontal tissues. In this context, periodontitis is not merely a localized condition but a disorder with significant systemic implications (4).

The relevance of the oral cavity in IBD extends beyond extraintestinal manifestations and reflects a complex interplay between local and systemic inflammatory processes. Crohn's disease may involve oral mucosa, lips, tongue, teeth, and periodontium, with oral involvement reported in approximately 0.5% to 20% of cases (5). Moreover, an increased burden of periodontitis has been documented in patients with IBD compared with the general population, underscoring the bidirectional relationship between these two conditions (6, 7, 8). Additionally, a higher prevalence and severity of periodontitis has been consistently reported among individuals with IBD compared with healthy cohorts, highlighting the influence of intestinal health on the oral cavity (9).

Through the dissemination of oral pathogens and the promotion of low-grade systemic inflammation, periodontitis may contribute to the development or exacerbation of systemic conditions such as IBD, cardiovascular disease, diabetes, and other metabolic or inflammatory complications (10).

The primary aim of our study was to evaluate the relationship between salivary oxidative stress biomarkers, specifically the salivary antioxidant enzymes superoxide dismutase and glutathione peroxidase, as well as malondialdehyde, as a marker of lipid peroxidation, and clinical oral parameters in patients with active IBD, in order to explore their potential relevance as non-invasive indicators of systemic inflammatory burden. A secondary aim was to assess

the associations between these salivary bio markers and established oral health Periodontal Disease Index (PDI) (11), the Simplified Oral Hygiene Index (OHI-S) (12), and the Decayed, Missing, and Filled Teeth (DMFT) (13) with the objective of determining the extent to which salivary oxidative stress reflects oral health status in the context of IBD.

MATERIALS AND METHODS

Patients and study design. A cross-sectional study was conducted, including 30 patients with IBD and a control group of 5 healthy subjects, who were clinically and biologically evaluated at the time of inclusion. Patients with confirmed IBD were recruited from the Institute of Gastroenterology and Hepatology, "Sf. Spiridon" County Clinical Emergency Hospital, Iași. Nineteen patients were diagnosed with ulcerative colitis (UC) and eleven with Crohn's disease (CD). The diagnosis of IBD was established based on clinical, biological, imaging, and endoscopic criteria.

The inclusion criteria comprised adult patients (≥18 years) with a confirmed diagnosis of active IBD (ulcerative colitis or Crohn's disease), without recent major surgical interventions and with no use of antioxidant supplements during the preceding four weeks. The control group included healthy subjects of comparable age, with no systemic inflammatory or autoimmune diseases and a healthy oral status, defined by periodontal indices PDI, oral hygiene OHI-S, and dental caries DMFT within normal ranges, with no evidence of active periodontitis or active carious lesions. The exclusion criteria included patients with diabetes mellitus, advanced cardiovascular or renal diseases, acute oral conditions, recent use of antibiotics or anti-inflammatory drugs, heavy smoking, chronic alcohol consumption, a history of recent dental interventions (<3 months), as well as pregnant or breastfeeding women.

The study protocol was reviewed and approved by the local Ethics Committee, and all procedures were conducted in accordance with the principles of the Declaration of Helsinki. Prior to participation, all subjects received detailed information regarding the study objectives and procedures and provided written informed consent.

Demographic and clinical characteristics were compared between the control group and patients with active IBD. For each participant, age, sex, smoking status, and body mass index (BMI) were recorded. The distribution of categorical variables (sex and active smoking status) was analyzed using Fisher's exact test, while continuous variables (age and BMI) were compared using independent-samples t-tests, with corresponding p-values reported. The study enrolled patients with clinically active IBD, as defined by disease-specific activity scores (Mayo score for UC, Crohn's Disease Activity Index) and elevated inflammatory markers, treated with conventional therapy (5-aminosalicylates and/or immunomodulators) or advanced biologic therapies.

All study patients and subjects underwent oral clinical evaluation as well as biological evaluation of unstimulated saliva samples. Associations between salivary oxidative stress biomarkers (SOD, GPx, MDA, and total proteins) and dental clinical indices were assessed.

Oral Clinical Evaluation. All participants underwent a comprehensive intraoral examination performed by an experienced

dentist. Oral hygiene status was assessed using the Oral Hygiene Index-Simplified (OHI-S), according to standardized methodology. The index was determined by examining six representative dental surfaces (upper right molar, upper left molar, lower right molar, lower left molar, and two central incisors), each evaluated for dental plaque and calculus accumulation. The individual scores were subsequently averaged to obtain the final OHI-S value.

Periodontal status was evaluated using the Periodontal Disease Index (PDI), which includes the examination of the six Ramfjord teeth. For each tooth, gingival inflammation and periodontal attachment loss were assessed, with the final score representing the mean of the recorded values. Higher scores indicate increased periodontal disease severity.

Gingival inflammation was additionally assessed using the Gingival Index (GI), which classifies the severity of gingival inflammation on a scale from 0 (healthy gingiva) to 3 (severe inflammation with bleeding and edema).

Caries experience was evaluated using the DMFT index (Decayed, Missing, Filled Teeth), determined by examining all permanent teeth and recording the number of decayed teeth, teeth missing due to caries, and filled teeth. The total DMFT score reflects cumulative caries experience and overall oral health status.

Biological Evaluation. Following the selection interview and clinical examination, unstimulated saliva samples were collected from all participants included in the study. Unstimulated whole saliva was collected by passive drooling into sterile tubes and transferred using a pipette. Approximately 0.5-1 mL of saliva was collected into sterile Eppendorf tubes. Samples were immediately frozen at -50°C to prevent biomolecule degradation and to avoid repeated freeze-thaw cycles. For biochemical analyses, samples were gradually thawed from -50°C to 4°C, then diluted in cold phosphate-buffered saline (PBS) and homogenized. Superoxide dismutase (SOD) activity was determined using a commercial colorimetric assay kit (SOD Assay Kit, Sigma-Aldrich), based on the competition between xanthine oxidase and SOD for superoxide radical generation. Quantification was performed by measuring absorbance changes at 450 nm. Enzymatic activity was expressed as percentage inhibition of the reaction, according to the manufacturer's instructions.

Glutathione peroxidase (GPx) activity was measured using a commercial enzymatic assay (Glutathione Peroxidase Cellular Activity Assay Kit, Sigma-Aldrich), which quantifies NADPH consumption during the GPx-catalyzed reaction. The decrease in absorbance at 340 nm was recorded kinetically, and GPx activity was calculated in enzymatic units normalized to sample volume and dilution factor.

Malondialdehyde (MDA) concentrations, as a marker of lipid peroxidation, were determined using the thiobarbituric acid (TBA) reaction method, considered a standard approach for quantifying end products of lipid peroxide degradation. The MDA-TBA complexes formed were measured spectrophotometrically at 532 nm, and concentrations were calculated based on a standard calibration curve generated with MDA bis(diethyl acetal). Results were expressed as nmol MDA/mL supernatant.

Total soluble protein levels were determined using the Bradford method with a commercial kit (Rapid Protein Quantification Kit, Sigma-Aldrich). Absorbance was

measured at 595 nm, and protein concentrations were calculated using a bovine serum albumin standard curve. TSP concentrations were used to normalize enzymatic activities (SOD U/mg TSP, GPx U/mL TSP) and, optionally, MDA levels expressed relative to protein content (nmol MDA/mg TSP). Routine hematological and biochemical analyses were performed in patients with active IBD, including C-reactive protein (CRP), fecal calprotectin, fibrinogen, hemoglobin, ferritin, total proteins, and electrolytes. These parameters were interpreted according to the standardized recommendations of the CALM study and were used to confirm disease activity at the time of inclusion.

Statistical Analysis. Clinical and demographic variables are presented as mean ± standard deviation (SD), whereas salivary biomarker data are expressed as mean ± standard error of the mean (SEM).To assess the associations between salivary oxidative stress biomarkers (SOD, GPx, MDA, and total proteins) and dental clinical indices, correlation and regression analyses were performed. For each biomarker-parameter pair, scatter plots were generated to visualize the distribution of individual values. Linear regression models were applied using ordinary least squares (OLS) fitting to estimate the direction and magnitude of the relationships.

The strength of the associations was quantified using Pearson's correlation coefficient (r), while statistical significance was evaluated using two-tailed Pearson correlation tests, with corresponding p-values displayed on each plot. This combined graphical and analytical approach enabled an integrated assessment of both linear trends and the statistical robustness of the observed associations.

RESULTS

Clinical characteristics of study patients and subjects are presented in first table, with no significant differences between groups.

Regarding the specific activity of SOD, a key first-line antioxidant enzyme involved in the defense against oxidative stress development, patients diagnosed with IBD exhibited lower mean SOD activity (0.0207 U/mg protein) compared with the control group (0.0618 U/mg protein) (fig. 1). This difference did not reach statistical significance ($p > 0.05$). The error bars illustrate the within-group variability, indicating a more homogeneous distribution of values among patients and greater dispersion in the control group.

TABLE I.

Clinical characteristics of the control group and patients with IBD

	Controls (n=5)	IBD patients (n = 30)	p- value
Age (years), Mean ± SD	45 ± 5.7	48.8 ± 16.3	0.35
Sex, n (Male/Female)	1/4	20/10	0.14
Active smokers, n (%)	1 (20%)	4 (13.3%)	0.56
BMI, kg/m^2 Mean ± SD	20.26 ± 5.48	23.84 ± 3.04	0.22
Disease duration (years), Mean ± SD	-	7.07 ± 6.77	

BMI: Body-Mass Index

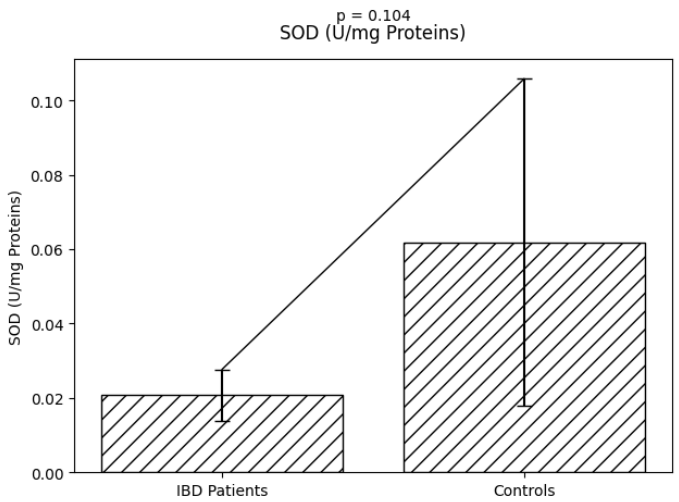

Fig. 1. Specific activity of superoxide dismutase (U/mg protein) in patients with IBD compared with the control group. Values are presented as mean ± SEM.

Regarding the second antioxidant enzyme, the mean GPx activity was 0.2367 ± 0.0044 U/mL in the IBD group, compared with 0.3112 ± 0.0468 U/mL in healthy controls (fig. 2). This difference did not reach statistical significance (p = 0.187). Nevertheless, the observed distribution suggests a tendency toward reduced GPx activity in IBD patients relative to controls, possibly reflecting increased enzymatic consumption under conditions of chronic inflammation and oxidative stress.

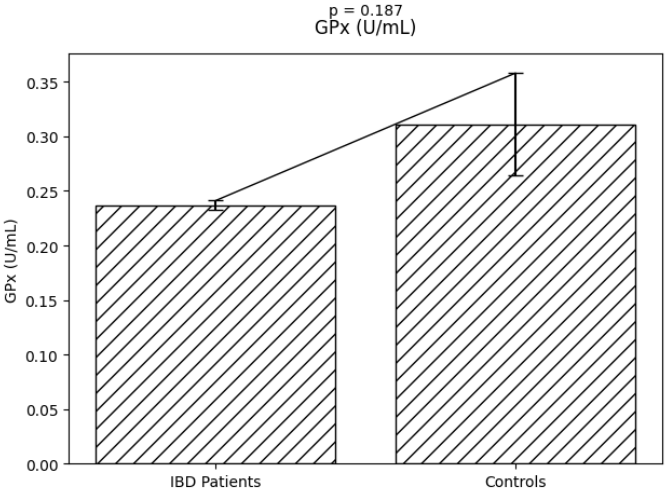

Fig. 2. Glutathione peroxidase (U/mL) activity in patients with IBD compared with the control group. Values are expressed as mean ± SEM. The statistical comparison yielded p = 0.187

Compared to healthy controls, patients with IBD exhibited higher mean salivary MDA concentrations (47.64 ± 3.22 nMol/mL versus 29.43 ± 7.34 nMol/mL) (fig. 3). The error bars reflect within-group variability, showing greater dispersion among controls and a more consistent pattern within the IBD group. A borderline trend toward increased MDA levels in IBD patients relative to controls was observed ($p = 0.066$).

To complement the descriptive analysis of salivary oxidative stress biomarker levels, regression-based graphical evaluations were performed to explore whether these biochemical changes were reflected in oral health status. While the group comparisons illustrated the overall patterns of MDA, SOD, GPx, and total protein concentrations, the scatter plots with linear regression lines provided a detailed view of how these markers aligned with individual dental indices. Together, these two approaches allowed us to assess not only the magnitude of biomarker alterations but also their potential associations with caries experience, periodontal indicators, and cumulative dental disease.

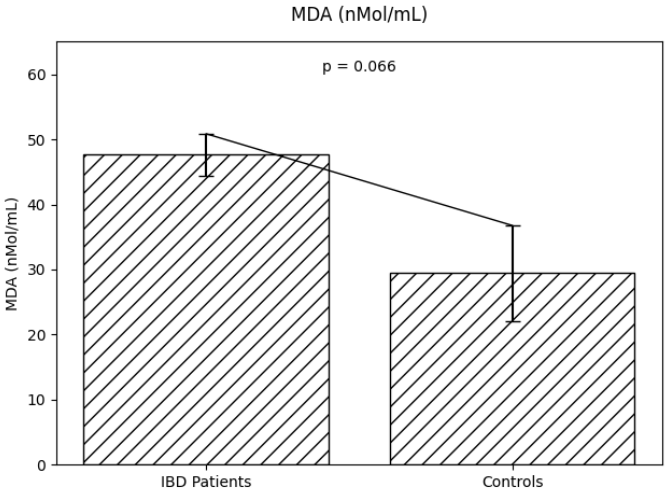

Fig. 3. Mean salivary concentration of MDA (nMol/mL) in patients with IBD compared with the control group. Values are expressed as mean ± SEM. A non-significant trend was observed ($p = 0.066$)

Correlation analysis revealed no statistically significant relationships between SOD activity (fig. 4) and the assessed clinical oral parameters. Only weak positive trends were observed between SOD activity and the filling score ($r = 0.21$; $p = 0.55$) as well as the DMFT index ($r = 0.21$; $p = 0.55$), suggesting that antioxidant activity does not increase proportionally with dental caries severity. The strongest association was identified between SOD activity and the oral hygiene index (OHI-S) ($r = 0.40$; $p = 0.25$); however, this correlation did not reach statistical significance.

Regarding the second antioxidant enzyme, GPx (fig. 5) its activity showed no statistically significant associations with any of the oral clinical parameters evaluat-

ed. Scatterplot analyses revealed only weak and nonsignificant correlations between GPx and the number of cavities, filled teeth, missing teeth, and the overall DMFT index, as well as with PDI and OHI-S. Nevertheless, several upward or downward tendencies could be observed: GPx displayed mild positive associations with cavities (r = 0.26), filled teeth (r = 0.27), DMFT (r = 0.26), and PDI (r = 0.35), suggesting a possible compensatory antioxidant response in individuals with greater caries or periodontal involvement. Conversely, negative trends were noted for missing teeth (r = -0.35) and OHI-S (r = -0.33), indicating that higher tooth loss or poorer oral hygiene may relate to lower GPx activity.

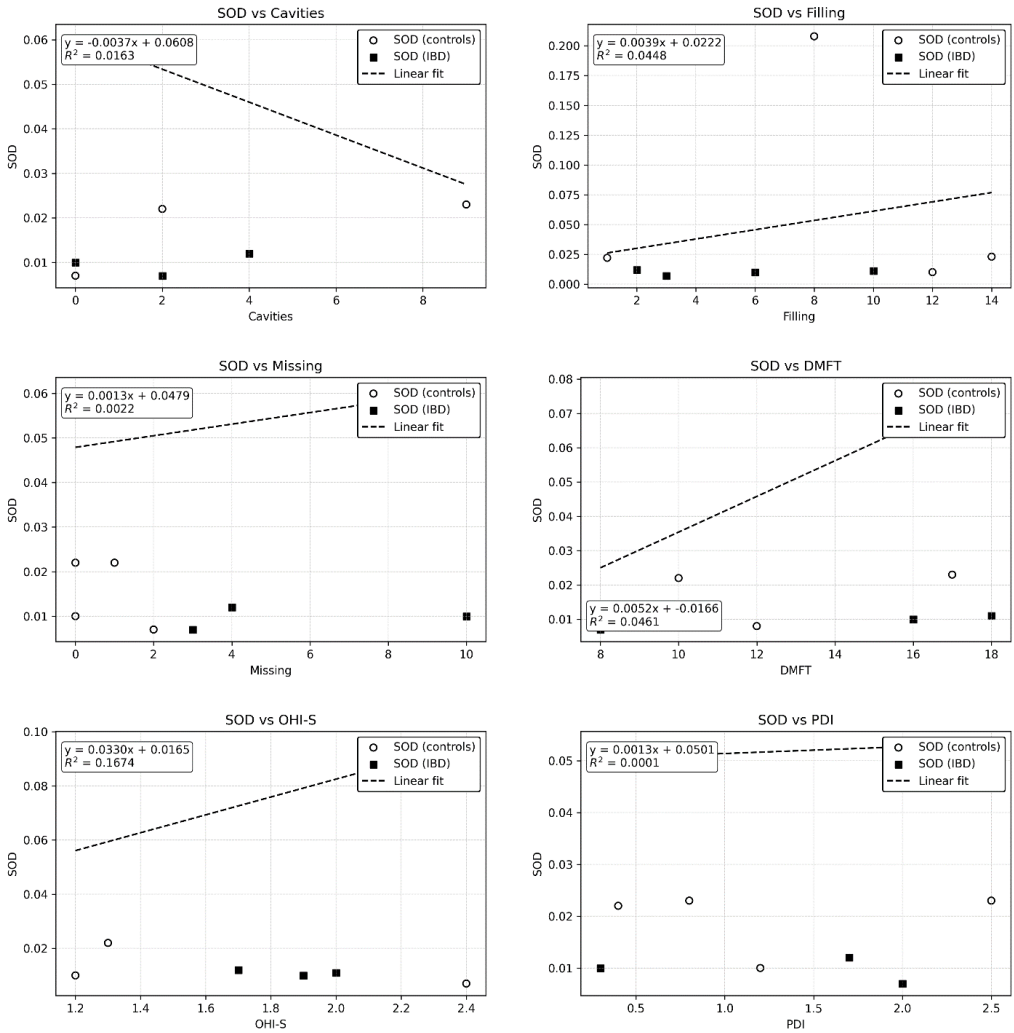

Fig. 4. Scatter plots with linear regression lines illustrating the relationships between salivary SOD and dental clinical indices in controls and patients with IBD.

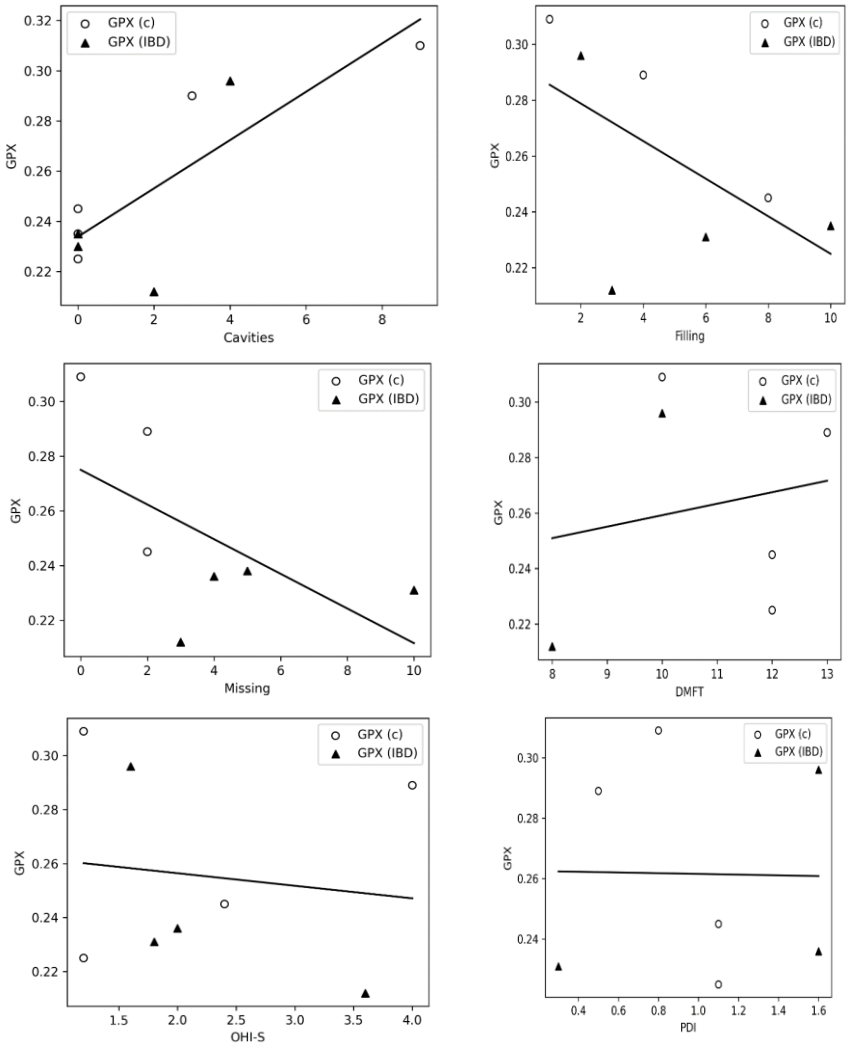

Fig. 5. Scatter plots with regression lines showing the relationship between salivary GPx and dental indices in controls and patients with IBD.

In the case of MDA, an important marker of lipid peroxidation, correlation analyses revealed only weak to moderate, non-significant associations with the evaluated clinical oral parameters (fig. 6). Scatterplot analyses demonstrated a slightly negative trend between MDA levels and the number of carious lesions ($r = -0.37$), suggesting that higher oxidative stress was not accompanied by an increase in active caries within this cohort. In contrast, weak positive correlations were observed with the filling index ($r = 0.38$) and the number of missing teeth ($r = 0.29$), indicating that MDA levels tended to be higher in individuals with a history of dental restorations or tooth loss, although these associations did not reach statistical significance. A moderate positive associa-

tion was also detected with the total DMFT score (r = 0.45) and the periodontal disease index (PDI) (r = 0.54), potentially reflecting a link between cumulative oral disease burden and increased oxidative stress. The weakest relationship was observed with the OHI-S (r = 0.07), suggesting that current oral hygiene status exerted minimal influence on salivary MDA levels. Overall, none of the correlations reached statistical significance; however, several biologically plausible trends point toward a potential cumulative effect of chronic oral pathology on oxidative stress.

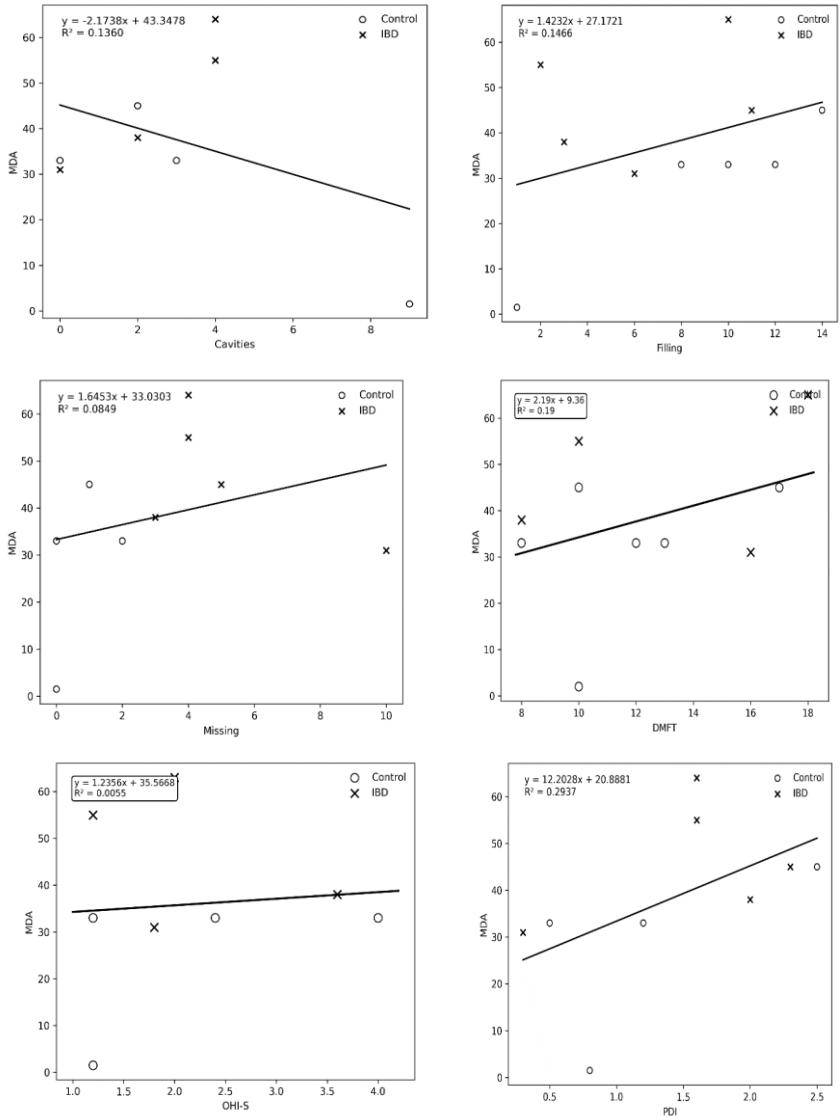

Fig. 6. Scatter plots with linear regression lines illustrating the relationships between salivary MDA and dental clinical indices in controls and patients with IBD

The analysis of total salivary protein levels revealed no statistically significant correlations with the evaluated oral clinical parameters (fig. 7).

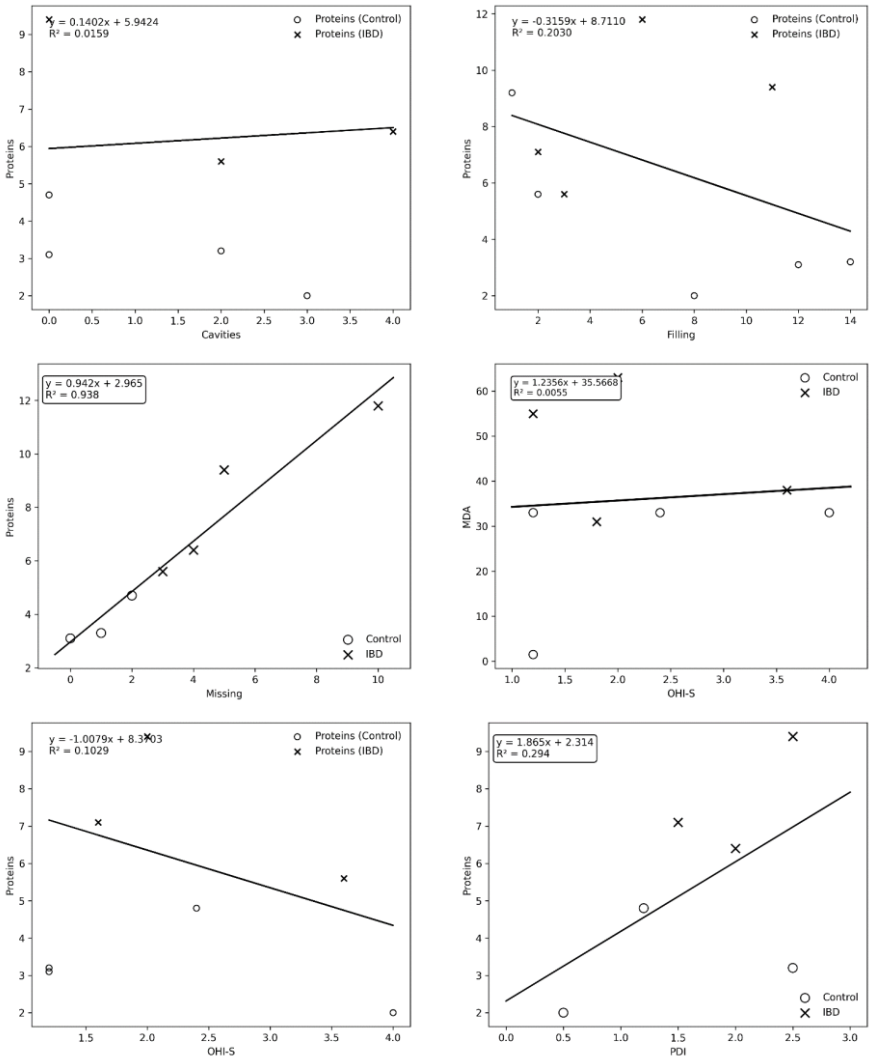

Fig. 7. Scatter plots with linear regression lines illustrating the relationships between salivary protein levels and dental clinical indices in controls and patients with IBD

Scatterplot assessment indicated that protein concentrations exhibited mild positive but nonsignificant trends in relation to the number of filled teeth ($r = 0.18$, $p > 0.05$), missing teeth ($r = 0.22$, $p > 0.05$), and the overall DMFT score ($r = 0.24$, $p > 0.05$), suggesting that individuals with greater cumulative caries experience may present slightly elevated salivary protein levels. In contrast, negative or near-flat associations were observed between protein levels and the number of decayed teeth ($r =$

−0.09, $p > 0.05$), indicating that active carious lesions were not accompanied by increases in salivary protein content. PDI and OHI-S also demonstrated weak and nonsignificant relationships with protein concentrations (PDI: $r = 0.17$, $p > 0.05$; OHI-S: $r = -0.11$, $p > 0.05$), with minimal inter-individual variability. Overall, these findings indicate that salivary protein levels remained relatively stable in both the control and IBD groups, with no significant correlations with caries activity or periodontal status in the present sample.

DISCUSSION

Salivary oxidative stress markers showed biomarker-specific patterns, with MDA levels consistently higher in IBD patients than in controls. Although correlations between MDA and dental indices were weak or borderline significant, their direction suggested an association between greater cumulative dental disease experience and increased lipid peroxidation, with the strongest positive-moderate but nonsignificant-associations observed for DMFT ($r \approx 0.45$) and PDI ($r \approx 0.54$). In contrast, SOD and GPx exhibited low values and minimal variability, resulting in very weak and nonsignificant correlations with clinical oral parameters, indicating limited sensitivity in this small pilot cohort.

In light of our pilot data and previous work from our group, oxidative stress alterations in IBD may reflect not only intestinal inflammation but also associated systemic comorbidities. Our prior studies on chronic inflammatory and hepatobiliary disorders support a broader, multisystem involvement affecting metabolic and oxidative pathways. For example, patients with extrahepatic cholestasis caused by choledocholithiasis were reported to exhibit increased liver stiffness values (6.2-18.4 kPa), which normalized after biliary drainage, with this improvement correlating significantly with reductions in serum bilirubin (14). Furthermore, Trifan *et al*. demonstrated that a 12-week regimen of paritaprevir/ritonavir, ombitasvir and dasabuvir plus ribavirin was safe and achieved nearly 100% sustained virologic response in elderly patients (≥70 years) with HCV genotype 1b compensated cirrhosis (15).

Periodontitis progression has been linked to local oxidative stress, with affected patients showing elevated MDA, hydrogen peroxide, and oxidative DNA damage levels (16). Periodontitis has been associated with reduced SOD and catalase activity and altered oxidative stress biomarkers, including total antioxidant capacity, MDA, and 8-hydroxydeoxyguanosine, compared with healthy controls (17). The pathogenesis of periodontal tissue destruction involves oxidative stress as a central mechanism (16). In a large study, Almerich-Silla *et al*. (17) emphasized that oxidative stress levels in the periodontium are significantly higher in periodontal disease compared with gingivitis or health, displaying a linear trend that parallels disease severity and bleeding on probing (BOP). Patients with periodontitis show increased biomarkers of ROS-mediated tissue damage as well as compensatory increases in antioxidant enzymes within inflamed periodontal tissues and gingival crevicular fluid.

In our study, the MDA-PDI scatterplot showed an upward trend with a steep regression slope, indicating higher MDA levels with increasing periodontal inflammation. Patients with more severe PDI scores exhibited elevated salivary lipid peroxidation, suggesting a relevant association between periodontal severity and oxidative damage. In contrast, GPx and SOD activity showed minimal variation across PDI levels (tab. II), indicating limited antioxidant compensatory responses.

TABLE II.
Summary of the direction and strength of associations between salivary oxidative stress biomarkers and periodontal disease index

Salivary biomarker	Direction of association with PDI	Correlation strength
MDA	Positive (↑)	Moderate
SOD	Very Weak (≈0)	Very Weak
GPx	Weak Positive (↑)	Weak

Regarding dental caries, current evidence indicates that carious lesions are associated with an altered redox response, characterized by increased lipid peroxidation and a reduction in antioxidant capacity during the progression of the disease (18). The direction of these correlations suggests that a greater cumulative dental disease burden may be accompanied by higher salivary lipid peroxidation. Studies on dental caries in children further report that total antioxidant capacity (TAC) decreases in the presence of carious lesions, whereas higher levels of gingival inflammation are paradoxically associated with increased TAC (19). Some studies have reported higher SOD levels in caries-free dentition (18), although these findings remain inconsistent across cohorts. In our study, both SOD and GPx exhibited generally low values and showed very weak correlations with DMFT or the number of carious lesions, suggesting limited sensitivity in detecting oral redox imbalances.

A major limitation of this study is the relatively small sample size, which reduces statistical power and limits the ability to identify robust associations between salivary oxidative stress biomarkers and clinical oral parameters. The substantial biological variability of salivary markers-shaped by disease activity, oral microbiome composition, behavioral factors, and treatment status-may introduce measurement noise that obscures true biological relationships. Particularly relevant is the potential confounding effect of biological therapies used in IBD: anti-TNF agents (infliximab, adalimumab) can suppress systemic inflammation and reactive oxygen species production, potentially lowering MDA levels and increasing antioxidant activity (SOD, GPx). Likewise, IL-12/23 blockade (ustekinumab) and JAK1 inhibition (upadacitinib) may significantly modulate oxidative pathways, while vedolizumab, despite its gut-selective action, may indirectly influence periodontal inflammation through improved intestinal disease control. Consequently, the salivary oxidative stress profile observed in this cohort may reflect both disease-related alterations and treatment-related modulation, making it difficult to attribute changes solely to oral or intestinal pathology. The limited sample size also restricts the generalizability of the findings, underscoring the preliminary nature of these results and the need for larger, longitudinal studies to validate these observations.

CONCLUSIONS

This pilot study suggests that salivary oxidative stress markers, especially MDA, may provide complementary insight into the oral-intestinal inflammatory axis in patients with IBD, although their diagnostic utility appears limited in small, heterogeneous samples. The absence of strong associations for SOD and GPx likely reflects substantial inter-individual variability, disease-related heterogeneity, and the modulatory effects of biological therapies. Larger, longitudinal studies

integrating salivary biomarkers, microbiome profiling, and disease activity indices are required to clarify the role of salivary oxidative stress as a non-invasive marker of systemic and oral inflammation in IBD.

CONFLICT OF INTERESTS AND FUNDING

The authors declare no conflict of interests. No financial support was received for perfecting this article.

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RELATIONSHIP BETWEEN FRACTION OF EXHALED NITRIC OXIDE AND SPIROMETRY PARAMETERS IN ASTHMA

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RELATIONSHIP BETWEEN FRACTION OF EXHALED NITRIC OXIDE AND SPIROMETRY PARAMETERS IN ASTHMA (Abstract): **Aim:** Asthma is a chronic airway inflammatory disease with significant social and economic impacts, being associated with a high annual rate of premature deaths. Asthma severity and response to inhaled corticosteroids therapy can be assessed by spirometry parameters and fractional exhaled nitric oxide (FeNO) evaluation. The aim of this study was to evaluate the relationship between spirometry parameters and FeNO values in a group of asthmatic patients. **Materials and methods:** We performed a retrospective analysis on 124 patients with history of asthma of different degrees of severity. All patients have been classified according to the last Global Initiative for Asthma (GINA) criteria and had a complete spirometry and a FeNO measurement. **Results:** The analyzed cases showed a female predominance (62.9%) and a mean patient age of 50.6±16.0 years. A significant association between Forced Expiratory Volume in one second (FEV1) values ($F = 231.167$, $p < 0.001$) or FEV1/FCV ratio ($F = 52.630$, $p < 0.001$) and the degree of asthma control was observed in all patients, regardless of inhaled corticosteroids administration. An inverse correlation between FEV1 and FeNO values ($r = -0.52$, $p < 0.01$) was registered. **Conclusions:** Spirometry parameters and FeNO represent valuable tools in the management of asthma, with higher FeNO values being associated with impaired lung function. **Keywords:** ASTHMA, FEV1, FCV, FeNO.

INTRODUCTION

Asthma is a chronic respiratory disease characterized by wheezing, chest tightness, shortness of breath and cough, with a variable incidence in the population, between 1% and 29%, in different geographical areas (1). According to the latest Global Asthma Report, asthma affects 9.1% of children and 6.6% of the adult population worldwide (2). Due to its continuous increasing incidence in recent decades, asthma is considered a global health problem with impact on the quality of life and is responsible for over 450,000 deaths each year (3). Epidemiological studies on asthma estimate an increase in its incidence in the population worldwide, affecting approximately 520 cases per 100,000 people by 2050 (4).

Mainly produced by epithelial cells, endothelial cells, and neurons in the lung, NO promotes ciliary motility and surfactant secretion, acting also as an airway bronchodilator factor (5-7). However, NO may also be generated by inducible nitric oxide synthase (iNOS) in lung, supporting Th2 lymphocyte differentiation and inhibiting Th1 and Th17 Ly activity in the bronchial mucosa, reflecting its chronic inflammatory status that lead to bronchial epithelial lesions, goblet cell hyperplasia, increased vascular permeability and eosinophilia (6, 7). Thus, NO plays a

dual role, as it can also act as a potent pro-inflammatory factor that supports the airway hyper-responsiveness in T2 asthma (6, 8).

Although the role of NO is still controversial, the evaluation of the fractional exhaled nitric oxide (FeNO) may be an adjunct to diagnostic tools used to assess the type 2 airway inflammation in asthma. Moreover, the addition of FeNO to blood eosinophil counts has been considered as a valuable marker for the assessment eosinophilic bronchial mucosa inflammation in the asthmatic patients (7). Last but not least, FeNO measurement is a non-invasive test with increased compliance in adult and pediatric population, its increased values being correlated with lower lung function, limited response to inhaled corticosteroids, and higher risk for asthma exacerbations (4).

In this context, our study aims to evaluate the association between FeNO values and spirometry parameters in a group of asthmatic patients.

MATERIALS AND METODS

The study group consisted of 124 asthmatic patients undergoing inhaled corticosteroid therapy, diagnosed in the Specialty Ambulatory of the Pneumology Clinic Hospital, Iasi, Romania. Clinical data was anonymously collected from the medical records without active patient participation, in accordance with the principles of the Declaration of Helsinki. Participants were included in the study group based on informed consent and a set of clearly defined inclusion criteria, including age over 18 years, non-smokers (total non-smoker or former occasional smoker), absence of other chronic or acute lung diseases, and the capacity to adequately and correctly perform a spirometry test. The exclusion criteria in the study group were smokers, inability to adequately and correctly perform spirometry tests, and patients with other chronic or acute lung diseases. The FeNO level assessment was performed using the NIOX® device, in accordance with the manufacturer's recommendations. A FeNO value $\geq$ 25 ppb in patients with a medium dose of inhaled corticosteroid therapy and a FeNO value > 20 ppb in patients with a high dose of inhaled corticosteroid therapy were considered pathological according to current guidelines (3). Spirometry test has been performed after FeNO level assessment, by using Vitalograph Alpha Touch after a minimum six-hour withdrawal from a short-acting inhaled beta-agonist, in agreement with the American Thoracic Society recommendations (9). The test was repeated three times and the best spirometry indices values were recorded (9). Forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and FEV1/FVC ratio were registered for each patient. A gender, age and height-wise comparison of spirometry indices was done, being automatically calculated by the device. Airway obstruction was defined as an FEV_1/FVC ratio below the lower normal limit (LLN; Z-score < -1.64).

Statistical analysis has been performed using *SPSS version 25* (IBM, Armonk, NY, USA) and *Microsoft Excel* 2016. Means and standard deviations were computed for continuous variables. Differences in FeNO levels between patient subgroups were analyzed using ANOVA and Pearson correlation tests. A p-value <0.05 was considered as statistically significant.

RESULTS

The predominant female gender was registered in our study group, with 78 cases (62.9%), compared to 46 male cases (37.1%), with a women-to-men ratio of 1.69:1. The most patients were from an urban area (81 of cases; 65.32%) and only 43 patients (34.68%) from the rural area. The mean patients' age was 50.6±16.0 years, showing relatively similar gender ranges, with 51.5±16.4 years in men and 50.1±15.9 years in women. The youngest patient included in the study was 19

years old, while the oldest was 83 years old, and the highest number of cases was observed in the 50-69 age group (28.2%) followed by 60-70 age group (20.2%) (fig. 1).

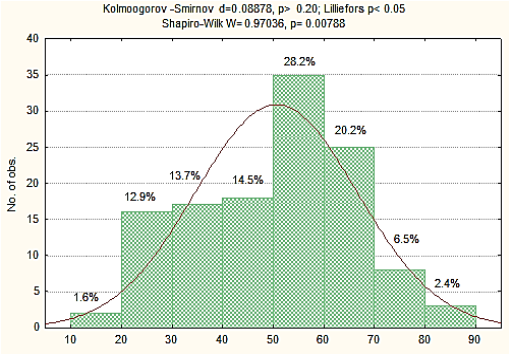

Fig. 1. Age group distribution of the asthmatic patients

Following clinical examination and respiratory exploratory tests, the patients have been included into the following stages: 62 cases (50%) of usual well-controlled (intermittent/mild) asthma, 36 cases (29.03%) of partially controlled (moderate) asthma, and 26 cases (20.97%) of uncontrolled (severe) asthma.

The mean value of FEV1 (% of predicted) for the study group was 78.2%, ranging between 24.1% and 147%, the lowest mean values being registered in the uncontrolled (severe) asthma cases (45.9±9.4), with a minim 24.1% and maximum values of 55.9%. FEV1/FVC ratio had the lowest mean values in uncontrolled asthma patients (55.9±8.0) and the highest values in well-controlled asthma cases (77.6±7.5) (tab. 1). Statistical analysis showed a statistically significant association between asthma severity levels and FEV1 ($F = 231.167$, $p < 0.001$) or between asthma severity and FEV1/FCV ratio ($F = 52.630$, $p < 0.001$).

TABLE I.

Patients' distribution according to FEV1 values (% of predicted) and FEV1/FVC ratio (%) in the study group

Bronchial asthma	FEV1		FEV1/FVC ratio	
	mean ±SD	minimum; maximum	mean ±SD	minimum; maximum
Well-controlled (intermittent/mild)	99.75±10.7	80.3; 147.0	77.6±7.5	59.4; 91.5
Partially controlled (moderate)	68.8±6.3	59.2; 79.8	66.4±7.0	51.2; 78.0
Uncontrolled (severe)	45.9±9.4	24.1; 55.9	55.9±8.0	39.6; 69.2

FEV1 - forced expiratory volume in one second; FVC - forced vital capacity; SD - standard deviation

Asthma duration ranged from 1 to 40 years among the study group patients (Figure 2). The mean asthma duration was 13.1±9.6 years in uncontrolled (severe) asthma, 12.9±11.1 years in partially controlled (moderate) asthma, and 8.25±8.6 years in usual well-controlled (intermittent/mild) asthma.

Most of the asthmatic patients (79 of cases; 63.7%) displayed FeNO values ranging between 20-60 ppb, while 41 patients (33.06%) had FeNO values between 20-40 ppb. A significant FeNO increased value (> 60ppb) has been registered in 31 asthmatic patients (25%) (tab. II).

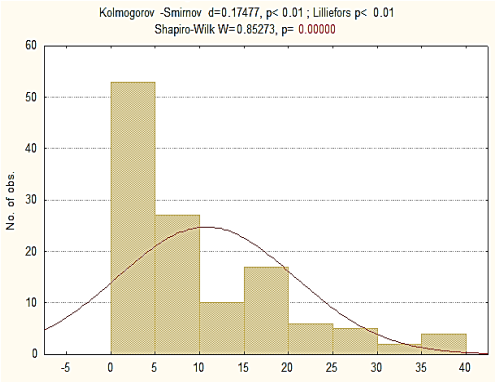

Fig. 2. Distribution of the asthma duration (in years) in the study group

TABLE II.
FeNO values distribution in the study group

	Number of cases	%
0<FENO<=20	14	11.29%
20< FENO <=40	41	33.06%
40< FENO <=60	38	30.65%
60< FENO <=80	11	8.87%
80< FENO <=100	12	9.68%
100< FENO <=120	4	3.23%
120< FENO <=140	4	3.23%
Total	124	

The highest FeNO value in patients with uncontrolled asthma was 126 ppb, while it was only 75 ppb in patients with partially controlled asthma. The lowest FeNO value of 6 ppb was observed in a patient with well-controlled asthma. The mean FeNO value increased progressively with disease severity, being 33.75±17.2 in well-controlled asthma, 43.1±14.9 in partially controlled asthma, and 86.1±22.7 in uncontrolled asthma. Statistical analysis showed a statistically significant association between FeNO levels and asthma severity ($F = 43.905$, $p < 0.001$).

Pearson correlation analysis demonstrated a moderate inverse correlation between FEV1 and FeNO values ($r = -0.52$, $p < 0.01$, 95% CI) (fig. 3).

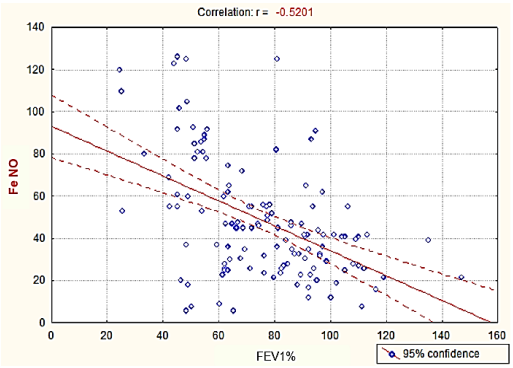

Fig. 3. Scatter plot with regression line showing the correlation between FEV1 and FeNO

DISCUSSION

Although substantial advances have been achieved in the management of bronchial asthma, a significant improvement in patient's quality of life has not been yet demonstrated, despite the implementation of recent standardizations and clinical guideline and the availability of effective therapies (9).

This study was conducted on a group of asthmatic patients diagnosed under background treatment. The study group was predominantly female, a finding that aligns with global epidemiological data, which suggest a higher incidence of asthma in women than in men (9-11). This shift is linked to potentially stronger immune responses in women, smaller airways, and hormonal changes (estrogen and progesterone) affecting airways, leading to more severe symptoms and poorer asthma control in adult women (11). Moreover, estrogens and progesterone were associated with an enhancement of type 2 (T2) airway inflammation by supporting the antigen presentation, Th2 lymphocyte polarization, eosinophils infiltration, and mast cell degranulation (12, 13). Most patients were from urban areas, which may reflect an easier access to medical services and the potential role of the environmental factors (e.g. air pollution), along with unhealthy diet and sedentary life style (11, 14).

GINA emphasizes that asthmatic patients may have a decreased lung ventilation function, expressed by a reduction in FEV1 and FEV1/FVC values. Therefore, spirometry is an important diagnostic tool for asthma management, helping the assessment of the functional status of the patient and guiding the therapy (3). A decrease in FVC and FEV1 values compared to the predicted value was observed in our study, which was significant in patients with partially controlled or uncontrolled asthma. These data are consistent with similar studies that also reported reduced FEV1 and FEV1/FCV values in most asthmatic cases (9, 15). However, low values of these functional parameters could be par-

tially influenced by various factors, such as incorrect inhalation technique, low adherence to treatment, and irregular follow-up visits to medical centers. As a result, additional biomarkers, such as FeNO assessment, may offer supplementary data regarding both diagnosis and disease progression, providing information about the local inflammatory status of the bronchial mucosa (6, 7).

Chronic inflammatory status is a one of the hallmarks of asthma, as in other chronic inflammatory diseases (16-20). According to this criterion, asthma is currently divided into type-2 high, type-2 low, and mixed endotypes (6, 21). Type-2 high asthma is associated with increased eosinophilic airway inflammation and elevated values of inflammatory cytokines (e.g., IL-2, IL-4, IL-5, and IL-13), whereas Type-2 low asthma is characterized by a predominantly neutrophilic inflammation of the bronchial mucosa (6, 22). The mixed asthma includes features of both endotypes (6).

NO secretion in the airway tract may occur *by* enzymatic and non-enzymatic pathways, high FeNO level being correlated with the **T2-high (Type 2) asthma (**6, 22). The NO enzymatic synthesis is controlled by nitric oxide synthases 1-3 (NOS1-3), while NOS-1 and NOS-3 isoforms are constitutively expressed (6). NOS-2 overexpression is induced by pro-inflammatory cytokines (e.g. TNF-α, IL-4, and IL-13), which are intensely produced in **Type 2 asthma** (6). Increased NO levels in asthmatic patients may induce bronchial mucosae hyperemia and edema, bronchial hyper-reactivity, decreased apoptosis of inflammatory cells, and mucus hypersecretion in the bronchial epithelium (7). Therefore, FeNO is considered an adjunct biomarker, reflecting underlying airway inflammation. In this context, an increased concentration of FeNO was detected in asthma patients, including well-controlled ones, compared to normal individuals in different studies (23, 24). These data are consistent with our results, when a mean FeNO value of 33.75±17.2 was identified even in mild forms of asthma, its mean values increasing with asthma severity. Additionally, a meta-analysis performed on a large cohort of asthmatic patients showed that FeNO exhibits higher specificity than sensitivity, which supports its role in the diagnosis of asthma (25). Moreover, the combination of spirometry test results, value of symptom intensity scales, and FeNO measurement increased the asthma diagnostic accuracy with area under curve of 0.76 (95% confidence interval 0.66-0.84) in a group of untreated patients with symptoms suggestive of asthma (26).

However, FeNO evaluation remains an additional tool for asthma diagnosis, high FeNO values increasing the probability of asthma diagnosis, while a negative result does not exclude asthma (3). This is attributable to the fact that FeNO level may be influenced by numerous factors, including time of the day, age, gender or allergen exposure. In this regard, FeNO has a higher value in men than in women, in the afternoon than in the early morning or in patients with other allergic diseases (allergic rhinitis or atopic dermatitis), respectively a lower value in patients who smoke, take inhaled corticosteroids or have a reduced lung function (3, 6). Even so, a FeNO > 20 ppb, added to blood and sputum eosinophils is considered the cut-off for Type 2 inflammation of severe asthma, according to GINA criteria, and it is used to assess this asthma phenotype (3, 6). In addition, a FeNO value > 50 ppb in adults is associated with eosinophilic inflammation in bronchial mucosa, feature that is used to predict the anti-inflammatory therapy response. Furthermore, FeNO value added to lung function parameters is a useful tool to predict the future risk of symptoms exacerbation and loss of asthma control in practice (3, 7, 27). Not least, glucocorticoids are

used to reduce the eosinophilic inflammation in asthmatic patients, therefore FeNO evaluation has a significant role to predict the corticotherapy response of these patients (28). Regarding FeNO and FEV1, a moderate inverse correlation between these two variables has been identified in our study, which is partially consistent with the literature. Thus, a strong correlation between FeNO and FEV1 have been observed in different asthmatic patients (29-32), while other studies have not reported such an association (33, 34). These contradictory results are most likely due to the heterogeneity of the asthma patients' groups studied so far. Despite its limitations, a refractory Type 2 inflammation in asthma is associated with a FeNO > 20 ppb value in patients with high-dose inhaled corticosteroids and itself has been demonstrated to be the strongest predictor of asthma exacerbation in the same patients, compared to periostin and peripheral blood eosinophils count (7, 35). Thus, alongside spirometry, FeNO is a non-invasive method to assess airway inflammation and a significant tool for the asthma therapy management.

CONCLUSIONS

FeNO evaluation is a cost-effective method that is used to detect Type 2 airway inflammation in asthma.

The highest FeNO levels were observed in patients with uncontrolled asthma compared to partially controlled or well-controlled asthma, and these were negatively correlated with FEV1 and FCV values.

Although its value may be influenced by different factors, FeNO may serve as a bio marker for asthma diagnosis, prediction for lung function decline, evaluation of poor asthma control, future symptoms exacerbation risk, and assessment of response to the inhaled corticosteroid therapy in practice.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no funding regarding this research.

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Z-SCORE: AN INDICATOR OF PROTEIN-CALORIE MALNUTRITION

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Z-SCORE: AN INDICATOR OF PROTEIN-CALORIE MALNUTRITION (Abstract). According to the World Health Organization, malnutrition is a chronic disorder of nutritional status, especially among infants and young children, triggered by the imbalance between the nutritional intake and the body's needs to ensure the harmonious growth and development and the fulfillment of the body's specific functions. **Aim:** WHO introduced the Z-score, which has been used for child growth standards; since then, malnutrition and its severity have been determined according to this score. **Material and methods:** We conducted a retrospective study of the cases of malnutrition admitted to the "Pediatric Recovery" department between May and December 2016. The sample considered for the study includes 74 infants showing signs of malnutrition at the moment of hospitalization: significant weight loss, asthenia, fatigue etc. **Results:** Of all the indicators used for the assessment of malnutrition, the Z-score is the most reliable. It expresses the anthropometric value in the number of standard deviations that are inferior or superior to the mean reference value. The graphical processing of the data showed that the Z-score for the height on admission generally ranged from -3.5 to -0.5, being below the reference limit. **Conclusions:** The Z-score is harder to calculate, but it is age-independent and it accurately diagnoses malnutrition. **Keywords:** Z-SCORE, PROTEIN-CALORIE MALNUTRION, DYSTROFIC INFANT.

Half of the nutritional deficiency states occur in the first 6 months of life, overlapping the maximum period of development of the central nervous system, given that the neural multiplication takes place up to 18 months of life. Thus, nutritional deficiency causes severe and often irreversible mental disabilities (1). According to the World Health Organization, malnutrition is a chronic disorder of nutritional status, especially in infants and young children, triggered by the imbalance between the nutritional intake (both caloric and protein intake) and the body's needs to ensure the harmonious growth and development and the fulfillment of the body's specific functions. It is characterized by low birth weight, low weight-for-age and, in chronic forms, by a low weight-for-height (2). In 2006, WHO introduced the Z-score, which has

been used for child growth standards; since then, malnutrition and its severity have been determined according to this score (3). The term protein-calorie malnutrition is used to cover a large number of clinical conditions in adults and children, including: growth failure, marasmus, cachexia, nutritional nanism, kwashiorkor and nutritional edema or hunger edema. Regardless of the clinical differences between these situations, most of the physiological, biochemical and compositional features of the body are common to all forms of severe malnutrition (4).

MATERIAL AND METHODS

We conducted a retrospective study of the cases of malnutrition admitted to the "Pediatric Recovery" department between May and December 2016. The sample considered for the study includes 74 infants showing signs of malnutrition at the moment of hospitalization: significant weight loss, asthenia, fatigue, decreased resistance to cold and infections, dry skin, changes in pigmentation, edema, hypotension and bradycardia, diarrhea and hepatomegaly. The data were taken from the observation sheets of the "Pediatric Recovery" department of the "Sf. Maria" Clinical Emergency Hospital in Iasi, respecting the code of ethics and deontology for scientific research. The study was designed so as to achieve the following objectives: to assess the nutritional status in newborns and infants and to determine the risk factors for malnutrition in newborns and infants in the Iasi area. The observation sheets were used to collect data such as the child's sex, his or her age at the moment of hospitalization, his or her mother's age, the data related to the mother's educational and socioeconomic background, the age of gestation and the child's place of birth as well as the data on his or her condition at birth (Apgar score, weight, height), the way the child was fed before the admission to hospital, the period of hospitalization, the anthropometric indicators on admission and on discharge from hospital and the Z-score calculated for each anthropometric indicator.

RESULTS

Out of the total of 74 infants in the study group (admitted due to malnutrition symptoms), female was found to have a higher incidence (65%) compared to male. The analysis of the statistical data shows that more than half of the mothers were aged less than 25 years old (70%), with a predominance of those under the age of 20, which is correlated with their lack of education. Mothers' marital status was also associated with underweight children for their age. More than half of the mothers in the study are unmarried (66%), and this has a negative influence on the growth and development of children. Most mothers of the children admitted to hospital and considered for this study had more than 4 pregnancies and a low number of abortions, which means they belong to families with many children. This is caused by the mother's lack of education, the low level of living standards and the area where they live – most of them are from rural areas.

The infants included the study group showed symptoms of malnutrition at the moment of admission, although 69% of them were born on term; more than a quarter of them had a birth weight of less than 2500 grams. The birth weight of a child represents an indicator of his / her mother's health and nutritional status and, at the same time, of the population's socioeconomic level. The birth height of the infants included in the study was generally normal, ranging from

47 to 55 cm. This indicator is known to be the most reliable for the infant's age, as it is not influenced by malnutrition in the long term. Most of these infants were hospitalized for one month. The statistical data show that about three quarters of them had an admission weight of less than 5 kg and during the hospitalization they had a weight gain of 500-1,000 grams. The infants' measurement showed that less than half of them had a smaller height than normal and that they gained 1 or 2 cm in height during the month of hospitalization.

Of all the indicators used for the assessment of malnutrition, the Z-score is the most reliable. It expresses the anthropometric value in the number of standard deviations that are inferior or superior to the mean reference value. The graphical processing of the data showed that the Z-score for the height on admission generally ranged from -3.5 to -0.5, being below the reference limit. The normal Z-score must be 0 with ± 1 SD. 4.05% of infants had a Z-score of less than -4.5, 10.81% had a Z-score between -4.5 and -3.5, 16.22% had a Z-score between -3.5 and -2.5, 28.38% had a Z-score between -2.5 and -1.5, 25.68% had Z-score between -1.5 and -0.5, 12.16% had a Z-score between -0.5 and 0.5 and 2.7% had a Z-score above 0.5. The Z-score for the height on discharge was also calculated – its values slightly increased, ranging from -2.5 to -0.5. This showed that the statural deficit decreased; however, the normal values were not reached. The Z-score for the height on discharge indicated that 1.35% of infants had a Z-score below -4.5, 10.81% had a Z-score between -4.5 and 3.5, 10.81% had a Z-score between -3.5 and -2.5, 33.78% had a Z-score between -2.5 and -1.5, 27.03% had a Z-score between -1.5 and -0.5, 14,86% had a Z-score between -0.5 and 0.5 and 1.35% of infants had a Z-score above 0.5.

The indicator weight-for-age was used to calculate the Z-score; along with the processed data, it showed that the nutritional deficiency of the infants included in the study was increased compared to the weight they had on admission. This fact is also confirmed by the literature (7). The analyzed data showed that the Z-score for weight on admission was generally between -5 and -2, which means that the nutritional deficit of these infants was very high. Upon discharge, the Z-score for weight was one standard deviation higher than the normal value – this showed that the care and nutrition during the hospitalization period had the expected effect on the infants in the study group.

Regarding the Z-score for weight on admission, 10.81% of infants had a Z-score of less than -5, 18.92% had a Z-score of -5 to -4, 28.38% had a Z score of -4 and -3, 29.73% had a Z-score between -3 and -2 and 12.16% of infants had a Z-score which above -2. Upon discharge, 4.05% of infants had a Z-score of less than -5, 16.22% of infants had a Z-score of -5 to -4, 25.68% had a Z-score for weight between -4 and -3, 29.73% had a Z-score between -3 and -2, 18.92% had a Z-score between -2 and -1 and 5.41% of infants had a Z-score for weight above -1 (fig. 1). The Z-score for weight-for-height compares the weight of the child under analysis with the weight of a child of the same height in the control population. As for the Z-score for weight-for-height on admission, it appears that 1.35% of infants had a Z-score of less than -5, 14.86% of them had a Z-score ranging from -5 to -4, 24.32% had a Z-score between -4 and -3, 21.62% had a Z-score between -2 and -1, 31.08% had a Z-score

between -1 and 0, 5.41% had a Z-score between 0 and 1 and 1.35% of infants had a Z-score above 1.

On discharge, 1.35% of the infants had a Z-score for weight-for-height which was below -5, 6.76% had a Z-score between -5 and -3, 18.92% had a Z-score between -3 and -2, 24.32% had a Z-score between -2 and -1, 35.14% had a Z-score between -1 and 0, 10.81% had a Z-score between 0 and 1 and 2.70% of infants had a Z-score above 1 (fig. 2).

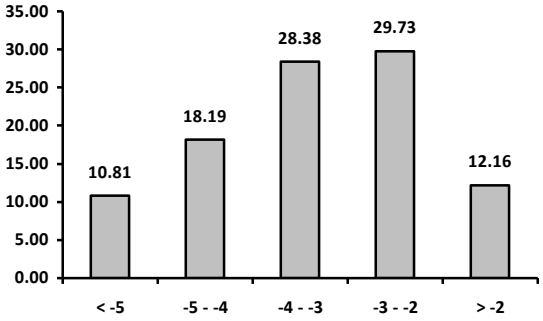

Fig. 1. Z-score for weight on admission

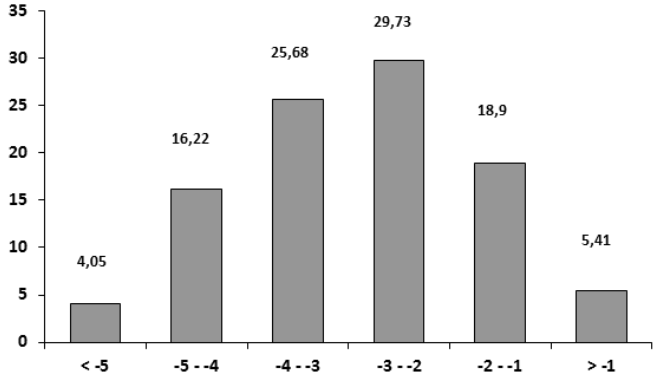

Fig. 2. Z-score for weight on discharge

DISCUSSION

The literature shows that the gender distribution of malnutrition in infants is relatively equal, with a slight predominance of the male gender (7).

In our study, the age distribution shows that more than half of infants were aged less than 3 months old at the moment of their admission to hospital, which corresponds to literature data (5). According to the literature, the ideal age to be a mother is between 25 and 34 years; babies born from mothers under 18 or over 40 years have a higher risk of having health problems (8). The literature shows that the mothers who have a job have higher income and use this income to take care of their children. Therefore, mothers' partici-

pation in generating family income is crucial to improving children's nutritional status (9).

In the current research, mothers gave birth in a maternity unit, most of them in a natural way. The findings of our study are similar with the se from the literature (6).

The scientific literature also shows that, in developing countries, 11% of children have a birth weight below the reference value of 2,500 grams, while in developed countries this percentage is only 2% (10, 11). A study conducted by UNICEF in Romania showed that most cases of low birth weight babies were in Moldavia. Half of the pregnancies were not monitored by medical staff because the access to specialized medical services of the people in the countryside is limited. These data are also confirmed by the literature, which shows that in other parts of the world, more than half of rural mothers do not go to a physician for consultations during pregnancy (3, 4, 6). The literature shows that the prevalence of low weight-for-height is generally below 5%, even in poor countries. In Romania, the percentage is 2.3%, which is the limit between a mild deficit and a moderate deficit; in Moldavia, the prevalence is lower than in the other regions, but this is also due to the smaller size of the children in this region. It has also been found that the environment of origin influences the prevalence of low weight-for-height, which higher in the rural environment (5, 6). Previous studies carried out by UNICEF showed that the low height-for-age in Romania has improved; even if the value of 5% is above the reference value for age groups, it does not exceed the maximum value showing a slight deficit (3, 5, 12).

CONCLUSIONS

In our study, we found that the Z-score is hard to be calculated, but it is age-independent and it accurately diagnoses malnutrition. Malnutrition remains the most common cause of mortality and morbidity among children worldwide. A low socio-economic level means a lack of access to utilities, infrastructure and proper health services, as well as inadequate housing and, most of all, a low-nutrient and vitamin diet. Therefore, the frequency of malnutrition is increased among children from disadvantaged families. Our research shows that with appropriate care and diet children with malnutrition could be recovered.

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NEWS

DOSAGE OF REACTIVE C PROTEIN AND TUBERCULOSIS PROPHYLAXIS IN HIV-INFECTED PATIENTS

Prophylaxis of tuberculosis (TB) using isoniazid, in HIV-infected patients (HIVIP) is a challenge because it requires exclusion of the presence of active TB and may increase the risk of isoniazid mono-resistance. The diagnosis of TB is, however, lengthy and costly, which delay the initiation of isoniazid prophylaxis (IP). According to the World Health Organization, the eligibility of HIVIP for IP can be established based on the presence of any one of four predictive symptoms of active TB (current cough, fever, night sweats, weight loss in the past 30 days). This method of clinical triage allows the initiation of IP in less than one third of eligible HIVIP. In this context, it is necessary to use other triage method with greater sensitivity and specificity. Numerous studies have shown that an increase in C-reactive protein (CRP) greater than 10 mg / L is predictive of active TB, regardless of the patient's immune status. A recent study conducted by C. Yoon and collaborators showed that the CRP test can improve active TB screening and eligibility for IP in HIVIP. The study group included 201 patients, of whom 196 HIVIP and 5 HIV-TB co-infection. The results showed that of the 196 HIVIP, 129 patients were eligible for IP using the CRP as screening test, while only 42 patients were eligible for IP on the basis of clinical triage. The results also established that the CRP test had a sensitivity of 80% and a specificity of 87% for the detection of active TB while the clinical triage sensitivity was 100% but with a specificity of 21%. These results have demonstrated the utility of CRP as a quick and inexpensive triage test for establishing eligibility for IP in HIVIP (Yoon C, Davis JL, Huang L, Muzoora C, Byakwaga H, Scibetta C, Bangsberg DR, Nahid P, Semitala FC, Hunt PW, Martin JN, Cattamanchi A. Point-of-care C-reactive protein testing to facilitate implementation of isoniazid preventive therapy for people living with HIV. *J Acquir Immune Defic Syndr* 2017; 65(5): 551-556).

Cătălina Luncă

ARTIFICIAL NEURAL NETWORKS - A NEW APPROACH IN NON-INVASIVE MONITORING OF INFLAMMATORY BOWEL DISEASES

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ARTIFICIAL NEURAL NETWORKS - A NEW APPROACH IN NON-INVASIVE MONITORING OF INFLAMMATORY BOWEL DISEASES (Abstract): Our aim was to use artificial neural networks (ANN) for assessing inflammatory bowel disease (IBD) activity using various biological and historical data. **Material and methods**: The study group included 100 patients aged 18-80 years, diagnosed with ulcerative colitis (UC) - 59% and Crohn's Disease (CD) - 41% based on clinical, biological, imaging and histopathological criteria. Both pre-diagnosed and newly diagnosed cases were included. Study database contains multiple parameters obtained through anamnesis, physical exam, evaluation of nutritional status (body mass index), laboratory tests, imaging investigations. We built the neural network using MATLAB 7.0. **Results**: The accuracy of the mathematical model was measured in terms of mean square error (MSE) and mean absolute percentage error (MAPE). Calculated MAPE values for Ulcerative Colitis Disease Activity Index (UCDAI) score and Rachmilewitz score were 52,34% and 47,15% respectively. None of the scores were estimated with excellent or high precision. The Rachmilewitz score was estimated with average accuracy because the network included currently few registered patients. All other scores were estimated with low precision. **Conclusions**: The partial results obtained in the first stage of this study are encouraging given the small number of patients enrolled. Thus, for the next phases of the study, with the ongoing development of the database, we expect to achieve results with higher accuracy. **Keywords**: INFLAMMATORY BOWEL DISEASE, NON-INVASIVE METHODS, ARTIFICIAL NEURAL NETWORKS.

Inflammatory bowel diseases (IBD) include Crohn's disease (CD) and ulcerative colitis (UC). IBD are chronic disorders with recurrent evolution. Complex and inaccurate methods of diagnosis and treatment are conditioned by the unknown etiopathogenesis.

Currently, the techniques used to monitor disease progression are invasive and costly. For this reason, efforts of the scientific community are focused on identifying non-invasive and cost-effective methods capable of accurately assessing disease activity.

Biomarkers are noninvasive or micro invasive, objective, fast and at a lower cost

than other techniques. An ideal biomarker for IBD should be straightforward, cheap, easy to obtain, noninvasive or micro invasive, fast and reproducible. Unfortunately, there is not yet a biomarker that satisfies all these conditions (1, 2, 3, 4, 5).

Recent studies have been published that highlight the utility of artificial neural networks and automatic learning methods in the characterization of various diseases (6, 7, 8, 9). Although the methods are promising, their application in the medical field is still on its way. Therefore, this research area is open to further exploration and can add to the originality of the published works.

The link between biological data, disease history and IBD activity is a complex biological system. Most data have multidimensional and nonlinear links with the severity of the disease. Traditional statistical calculus, such as linear regression models, are too simple to achieve reliable results due to poor statistical stability. Therefore, it is difficult to measure the severity of IBD through traditional statistical models (10). Artificial neural networks are a new computer model inspired from the functioning of human brain. They can build non-linear statistical models to deal with complex biological systems. Artificial neural networks have become a common modeling method in engineering (11). In recent years, artificial neural networks have been successfully introduced for various clinical contexts (10-12). The artificial neural network model has proved to be more accurate and with better performances than multiple logistic regression (13).

In this study, we tried to investigate the possibility of establishing a complex neural correlation between the various biological and historical data of the disease and IBD activity.

MATERIAL AND METHODS

The study group included 100 patients aged 18-80 years, diagnosed with UC (59%) and CD (41%) based on clinical, biological, imaging and histopathological criteria. Both pre-diagnosed and newly diagnosed cases are included in the study.

Exclusion criteria: diagnosis of colorectal cancer; ethanol consumption (≥ 50g/day for men and ≥ 30g/day for women); *Clostridium difficile* infection; neurological, renal, pulmonary, infectious or chronic heart disease

All patients included in the study were interviewed on education, occupational status, smoker status, ethanol consumption.

Laboratory tests. Blood tests include the following: complete blood count with red blood cell count, erythrocyte sedimentation rate (ESR), serum fibrinogen, C reactive protein (CRP), total proteins, protein electrophoresis, sodium (Na), potassium (K), chlorine (Cl), alkaline reserve. These were taken by venous puncture and transmitted to the Hematology and Biochemistry Laboratory of "Sf. Spiridon" County Clinical Emergency Hospital in the context of patient's routine revaluation.

Lower gastrointestinal endoscopy was performed with an Olympus EVIS EXERA II. Investigation was carried out in the context of routine reassessment of patients.

Computed tomography (CT) exam was performed and interpreted by a specialist doctor in the Radiology and Medical Imaging Department of "Sf. Spiridon" County Clinical Emergency Hospital from Iasi.

Study database contains multiple parameters obtained through: anamnesis and disease history considering environmental factors and behaviors (smoker/non-smoker, ethanol consumption, medication, alimentary behavior); physical exam and evalua-

tion of nutritional status (body mass index); laboratory tests; imaging investigations (lower gastrointestinal endoscopy, CT exam).

All personal data is confidential. The database contains patient initials, personal information and study data that can only be accessed by the research team.

Before entering the study, each patient signed an informed consent. Only patients capable of giving their consent were included. Research is not conducted on vulnerable groups.

Artificial neural networks

This research proposes a new approach for addressing the problem of biomarkers in inflammatory bowel diseases. Thus, an interdisciplinary method has been implemented: artificial neural networks. These systems are known in literature as "learning" systems. They can be trained with a set of well-known data (patients already evaluated) and then interrogated for new cases.

An artificial neural network can mimic a biological neural system, both structurally and functionally. The network contains an input layer, an output layer and one or more hidden layers. It is made up of a set of complex processing units (neurons) interconnected by weighted links (analogy to synapses in the nervous system). Units (neurons) receive information from other neurons. If a threshold is reached, the neuron sends information to the other connected neurons; otherwise it does not send anything. In the learning phase, data with results already known are introduced into the system and the interneuronal connection weights are updated according to a training algorithm. Training process ends when the total system error is minimal. A trained neural network extracts its calculation rules from the matrix of weights between neurons. This allows to predict the outcome of new cases that have never been introduced into the system (14, 15, 16).

In this study, we built the neural network using Matlab 7.0.

Active parameters for the input layer (i.e. those with the highest estimation power), were determined using statistical methods (t-student and chi² tests). Statistical significance was set to $p = 0.05$.

Therefore, input layer contains 25 neurons that are:

- *clinical:* smoker status; disease duration; number of kilograms lost in the last three months; presence of asthenia; clinical data from the scores used in the output layer (UCDAI, Rachmilewitz etc.)

- *biological:* white blood cells count (WBC); hemoglobin (HGB); hematocrit (HCT); platelet count (PLT); red cell distribution weight (RDW); ESR; fibrinogen; cholesterol; iron; ferritin; CRP; protein electrophoresis.

The output layer consists of the following scores: Ulcerative Colitis Disease Activity Index (UCDAI); Rachmilewitz; Ulcerative Colitis Endoscopic Index of Severity (UCEIS); Geboes (histological score); Crohn's Disease Endoscopic Index of Severity (CDEIS); Simple Endoscopic Score for Crohn's Disease (SES-CD).

Hidden layers were used to allow the development of complex relationships between input and output neurons. Hyperbolic tangent transfer functions were used in the hidden and output layer. Each variable was normalized in the range (0, 1).

80 patients were assigned to the training cohort and 20 patients were assigned to the validation cohort.

The number of hidden layers and the number of neurons within hidden layers

were determined by a trial and error process. After repeated attempts, a four-layer network with ten neurons in each hidden layer was considered the best mathematical model.

RESULTS

A descriptive statistic for a part of the data in the input layer is shown below, describing separately the training cohort and the validation cohort (tab. I).

TABLE I

Descriptive statistics

	Training cohort			Validation cohort		
	Min	Max	Mean + SD	Min	Max	Mean + SD
WBC	2,86	15,12	10,1 +/- 3,1	3,63	13,85	9,88 +/-4,7
PLT	132	550	390,2 +/- 6,5	144	470	363,3 +/- 10,1
RDW	29	62	44,9 +/- 4,7	32	58	40,3 +/- 5,2
ESR 1h	1	35	13,5 +/- 4,2	3	27	12,7 +/- 5,8
CRP	0,1	31,6	8,3 +/- 6,8	0,12	35,8	10,1 +/- 6,5

The accuracy of the mathematical model is measured in terms of mean square error (MSE) and mean absolute percentage error (MAPE). MSE is calculated based on the desired and estimated value and then the average for all data is determined. It is used as a good fit indicator of the model. MAPE indicates the mean deviation from the desired value and is usually expressed as a percentage. The precision of a model is considered excellent if MAPE value is less than 10%.

MAPE value from 10 to 20%, from 20 to 50% and above 50% is high, medium and low accuracy (17).

The formulas of MSE and MAPE are presented below:

$$MSE = \frac{1}{n}\sum_{i=1}^{n}\left(Y_i - Y_{i'}\right)^2$$

$$MAPE = \frac{1}{n}\sum_{i=1}^{n}\frac{\left|Y_i - Y_{i'}\right|}{Y_i} \times 100\%$$

where n represents the number of inputs, Y_i is the desired value for the ith input, $Y_{i'}$ is the calculated value for the ith input.

The calculated MSE and MAPE values for the output layer scores are (tab. II):

TABLE II

Calculated MSE and MAPE values

	MSE	MAPE
UCDAI	0,092	52,34%
Rachmilewitz	0,065	47,15%
UCEIS	0,212	65,37%
GEBOES	0,342	70,23%
CDEIS	0,156	62,48%
SES-CD	0,301	69,52%

None of the scores were estimated with excellent or high precision. The Rachmilewitz score was estimated with average accuracy, although with a value close to the medium range upper limit.

The lack of significant results is because the network included currently few registered patients. Neural networks need hundreds, up to thousands of records to deliver significant results.

In this context, it is encouraging that Rachmilewitz score was estimated with an average accuracy. Thus, the main direction for the next phase of the study is to populate the database with as many patients as possible. This will improve accuracy of the results.

Romania is a country with limited re-

sources and restricted access to expensive medical investigations. Finding an artificial neural network system capable of non-invasively evaluating inflammatory bowel disease activity, will reduce the number and cost of invasive endoscopic and histo-pathological investigations.

CONCLUSIONS

The partial results obtained in the first stage of this study are encouraging, given the small number of patients enrolled. Thus, for the next phases of the study, with the ongoing development of the database, we expect to achieve results with higher accuracy.

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NEWS

EVALUATION OF AGREEMENT BETWEEN FUNGAL CULTURE AND PCR FOR DIAGNOSIS OF ONYCHOMYCOSIS

Fungal culture for diagnosis of onychomycosis has the disadvantage of being time consuming and less sensitive. PCR techniques are faster and may be more suited for diagnosing nondermatophyte mold infections. In a study by Gupta *et al*, PCR methods were compare to fungal culture to measure their agreement for detection of microorganisms causing onychomycosis. For this purpose, single samples from 167 patients with suspected onychomycosis and repeated serial samples from 43 patients were analyzed using both methods to detect 16 dermatophyte species and 5 nondermatophyte molds. Kappa statistic (κ) was used to measure the agreement between methods. Regarding the identification of all infecting organisms, the two methods showed a good agreement ($\kappa = 0.32$ for single samples and $\kappa = 0.38$ for repeated samples respectively). In the case of dermatophytes, the agreement was moderate ($\kappa = 0.44$ for single samples and $\kappa = 0.42$ for repeated samples respectively). In the case of nondermatophyte molds there was a poor agreement for single samples ($\kappa = 0.16$) but a good agreement for repeated samples ($\kappa = 0.25$). The study concluded that there was a three to four times higher risk of a false-negative result when using fungal culture compared with PCR. The authors also recommend the use of serial samples, since repeated sampling increases the agreement of nondermatophyte mold identification. (Gupta AK, Nakrieko KA.Onychomycosis Infections. Do Polymerase Chain Reaction and Culture Reports Agree? *J Am Podiatr Med Assoc* 2017; 107(4): 280-286)

Teodora Vremera

PSYCHOLOGICAL ASPECTS IN PEDIATRIC PATIENTS AFTER ONE YEAR OF TREATMENT FOR HEPATITIS C

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PSYCHOLOGICAL PROBLEMS RELATED TO HEPATITIS C IN PEDIATRIC PATIENTS AFTER ONE YEAR OF TREATMENT (Abstract). The treatment of hepatitis C in pediatric patients determines physical and psychological effects. **Aim**: to identify psychological problems in children infected with HCV. **Material and methods**: a number of 32 children (20 boys and 12 girls) with no psychiatric problems were evaluated after one year of treatment for hepatitis C. Variables like age, gender and the presence of asthenia, irritability, agitation, sadness, somnolence, fatigability, insomnia and behavioral problems were considered. Data were recorded in the medical paper, as results of self-evaluation and aspects declared by the parents. Statistical data were processed using SPSS 17.00. **Results:** A number of 6 patients (18.75%) presented insomnia. Fatigability was recorded in 96.88% of questioned patients and somnolence was identified in 25% of them. A number of 30 patients (93.75%) claimed that they felt sad at the time of the evaluation. Significant statistical correlations were identified between some variables. **Conclusions**: Results are important to prove the necessity of psychological care during the treatment for hepatitis C, for both patients and parents. **Keywords**: HEPATITIS C, CHILDREN, FATIGABILITY, SADNESS, BEHAVIORAL PROBLEMS.

Hepatitis C is a liver disease caused by the hepatitis C virus: the virus can cause both acute and chronic hepatitis, ranging in severity from a mild illness lasting a few weeks to a serious, lifelong illness. Globally, *World Health Organization* (WHO) statistics estimate that 71 million people have chronic hepatitis C infection. Hepatitis C virus (HCV) causes both acute and chronic infection. Acute HCV infection is usually asymptomatic, and is only very rarely (if ever) associated with a life-threatening disease. About 15-45% of infected persons spontaneously clear the virus within 6 months of infection without any treatment (1).

Eastern Mediterranean and European regions are the most affected, with a prevalence of 2.3% and 1.5%, respectively. According to WHO statistics, the prevalence of HCV infection in other WHO regions varies from 0.5% to 1.0% and can be concentrated in certain populations (for example, among people who inject drugs) and/or in general populations. There are multiple strains (or genotypes) of the HCV virus and their distribution varies by region (1). According to the *Centers for Disease Control*

and Prevention, a prevalence of 2% or greater is defined as regions/countries with high risks of HBV infections. Regions from sub-Saharan Africa, from Central and Southeast Asia, and China are considered very high-risk regions (2).

Apart from geographical area criteria, other criteria are related to high rates of HCV: HIV-positive patients, injection drug users, home living with infected family members, and sexual relationship between men. The recommendation of screening for HCV also refers to groups like chronic kidney disease patients under hemodialysis or patients under immunosuppressive therapy. Also, some recommendations refer to inmates, persons exposed to infected blood or fluids, persons with elevated risk behaviors, in the case of blood transfusion or organ donors. For these high risk categories, the *American Association for the Study of Liver Diseases* recommends the screening for HCV. The combination of factors mentioned above determines a more focused attention and more intensive counselling for screening. According to scientific studies, in the USA, being a man, black and aged about 30-39 are characteristics of infected persons (3).

Risk of chronic HCV is inversely correlated with age (90% of infants, 25% to 50% of children aged 1 to 5 years, under 5% of older children and adults (4). Regarding hepatitis C, it seems that there are 10 times more people living with HCV than HIV (Joint United Nations Programme on HIV/AIDS (5).

There is a need for studies targeting HCV children and adolescents' psychological aspects and the effect of treatment on quality of life. Few studies included children and several studies used a broad range (e.g. age cohort 0-20) to describe prevalence in younger populations. A literature review targeting HCV infection among children identified that the highest rate is in low-income countries and the lowest, in high-income countries (6). HCV affects 0, 2-2% of the general population under 18 years old. Some statistical data prove 6600 cases in Canada (7) and 23.000-46.000 cases in USA (8).

In Romania, the prevalence of HCV infection is about 3.23%, significant differences being recorded between geographical regions: Moldova (4.25%), Muntenia and Dobrogea (3.35%), respectively, Transilvania and Banat with 2.63%. (9). Another study focusing on 17 counties proves a prevalence of 4.6%, with different scores for Bucureşti (3.05%), Craiova (3.27%) and a high rate of prevalence in south-eastern Romania (6.37%) (10).

The aim of the study is to identify psychological problems in children infected with HCV, after one year of treatment.

MATERIAL AND METHODS

A number of 32 children (20 boys and 12 girls) registered with the diagnostic of hepatitis C in "Sf. Maria" Hospital for Children, Iasi, Romania, were evaluated in terms of psychological and behavioral aspects, one year after the beginning of their treatment with interferon, between 2012-2015, in a children's hospital in north-eastern Romania. None of them was identified as suffering from a psychiatric disease.

The variables taken into consideration were: age, gender, the presence of asthenia, irritability, agitation, sadness, somnolence, fatigability, insomnia and behavioral problems.

Data were recorded in the medical paper, after clinical investigation (observational evaluation, self-evaluation and parents' evaluation). Statistical data were processed using the software *SPSS 17.00*.

RESULTS

The age mean is 12.03 ± 3.81, with a minimum of 6 years old and a maximum of 18 (the average for boys is 11.75 ± 3.93 and 12.50 ± 3.77 for girls). All children are enrolled in schools and 59.38% of them live in urban areas. They come from 8 counties in north-eastern Romania: Iași (53.13%), Suceava (6.25%), Galați (6.25%), Botoșani (9.38%), Vaslui (15.63%), Neamț (3.13%), București (3.13%) and Bacău (3.13%).

The distribution of subjects according to the age at which they started their treatment is presented in Table I.

Behavioral problems were identified neither in girls, nor in boys.

Insomnia was also registered, 6 patients (18.75% of the questioned subjects) claimed that they had sleep-related problems - 5 boys (age of 9, 10, 15 and 2 boys of 18 years old) and one girl aged 18. Four out of 6 patients identified with insomnia are older than 15 and have a college education.

Fatigability was registered in 31 cases (96.88%) and somnolence was identified in 8 patients (25%), 6 boys and one girl. The presence of somnolence by gender is presented in Figure 1.

TABLE I.

The distribution of children considering their age at the beginning of the treatment

Age	6	7	8	9	10	11	12	13	14	15	16	17	18
N	4	1	2	4	2	3	3	1	2	4	1	4	1
%	12.50	3.13	6.25	12.50	6.25	9.38	9.38	3.13	6.25	12.50	3.13	12.50	3.13

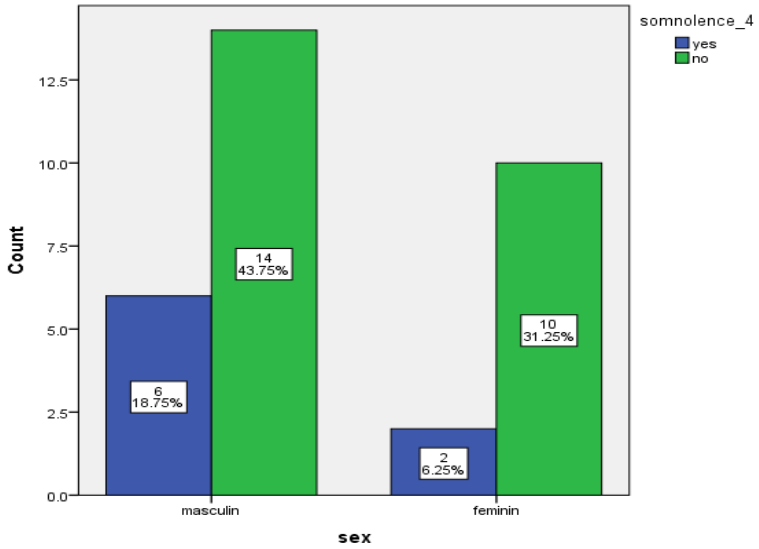

Fig. 1. The frequency of insomnia considering the gender of the patients

A number of 30 patients (93.75%) claimed that they felt sad at the time of the evaluation.

In the case of 10 children (31.25%) agitation was identified. The distribution according to the age and gender is presented in Figure 2. Only 4 of all evaluated patients (12.50%) presented irritability.

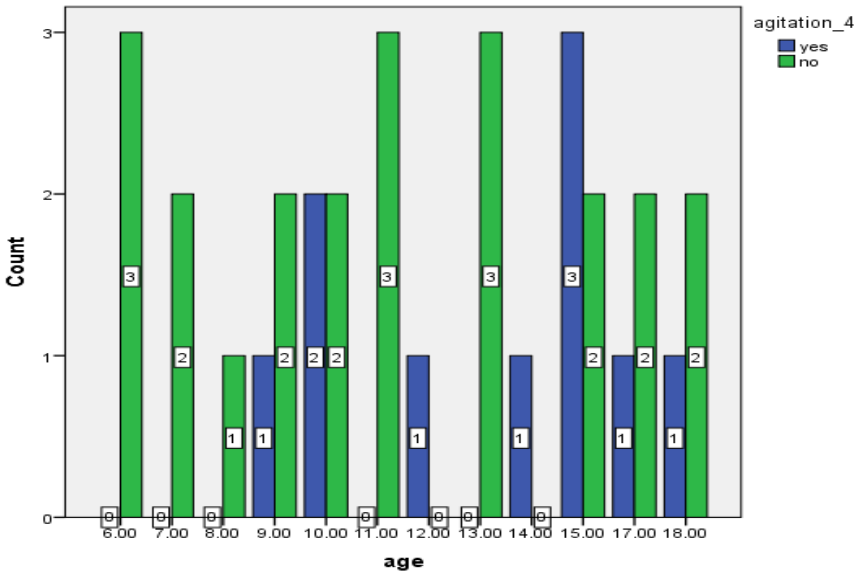

Fig. 2. The distribution of variable agitation considering age and gender

DISCUSSION

The correlational analysis proved that there is a positive relationship between variables:

- age and somnolence (.464**, p = .007),
- irritability and agitation (.357*, p = .045)
- irritability and insomnia (.545**, p = .001).

A significant negative correlation was identified between agitation and somnolence (-389*, p = .028), meaning that the more agitated the person is, the less somnolence is present.

The results could be explained by the fact that the older the patients are, the more scholastic and extra-school activity they are involved in.

The more irritability they feel, the more frequent the presence of sleep related-problems and agitation is.

Treatment and its effects on the psychological well-being of the pediatric patient

For the children, any opportunity should be taken to intervene earlier and potentially clear the infection and halt the progression of disease before there is significant impact on quality of life. A few studies point to psychological problems because of HCV treatment.

Depression. Teenagers using injected drugs run high risks of being infected with HCV. In most of the cases, depression and drug addiction are correlated: depressed people try to cope with the disease looking for drugs' effect. Consequently, this population becomes more vulnerable to psychiatric disorders during HCV treatment. The treatment of psychiatric disorders among drug users is a must. Regarding the management of HCV infection, the National Institute of Health recommends the diagnosis and treatment of depressive problems before the start of HCV treatment. The rates for depressive disorders among injecting drug

users are higher compared to other patients suffering from HCV, some researches identifying rates among adults of about 56% to 75% (11, 12, 13). The use of interferon increases psychiatric disorders; therefore, for patients already suffering from one, the risk of major depression is very high.

Cognitive deficit. In adults, HCV is associated with a low level of quality of life and cognitive impairments. In children, less scientific data are available, compared to adult patients. Data show that there are some cognitive dysfunctions. In its early stage, HCV treatment does not have important negative effects on QoL or on cognitive, behavioral or emotional levels. At older ages, emotional, cognitive and behavioral impairments diminish after the treatment is finished. A study of Rodrigue et al. found that symptoms of stress, depression, and anxiety are not pervasive in infected children and adolescents (14). The researchers proved that almost 20% of HCV-infected children had executive function impairments, preventing them from planning, organizing, and inhibiting their own behaviors. Children with HCV do not present a global impairment in quality of life, or in cognitive, behavioral, or emotional functioning, but children with HCV infection have worse cognitive functioning than the normal comparative group.

Self-esteem. Researches on the impact of hepatitis C on the level of self-esteem are poorly represented in the scientific literature. Studies prove that the internalized stigma is associated with low levels of self–esteem (15).

Family members' and caregivers' support for the quality of life of HCV pediatric patients.

The information regarding quality of life, psycho-behavioral changes or cognitive and emotional impairments during interferon treatment in children should be evaluated considering age and the relationship with parents (since, during adolescence, parent-child relationships could worsen and the tremendous changes during this stage of life could be exaggerated by parents). Teachers and caregivers should be warned about these changes and both must take into consideration the interferon effects, especially when the treatment is initiated, and should offer support. Also, teenagers should be carefully supervised considering the possibility to have risky behaviors: sharing razors for boys, nail files for girls, or the risk of drug consumption.

A research of Iorio et al. (16) identified in children that during the treatment for HCV with interferon irritability increased and the quality of life decreased. Also, some other psychosocial problems were identified by Nydegger et al. (17), proving that significantly lower physical and mental QoL is identified for children who acquired HCV in the first year of life.

In the case of children, the vertical transmission has an impact on parents' psychological well-being. Approximately 60% of children are infected this way. If the virus is transmitted by the mother, she will feel guilty. All caregivers of HCV-infected children reported higher stress and concern over their child's health, believing that it would deteriorate, and reported that HCV infection had a negative impact on the overall functioning of the family (18). In this situation, family support is very important for the quality of life of the patient and parents (especially mothers).

CONCLUSIONS

Managing psychological aspects during treatment for hepatitis C must be one of the

goals of the healthcare strategy for children and adolescents.

Almost all patients accused sadness and fatigability and the high rates imposed close psychological assistance.

A quarter of them, especially adolescents, accused somnolence.

A psychosocial intervention must be taken into consideration for all patients under treatment with interferon for 12 months and to train parents how to offer support to their children.

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OSTEOGENESIS IMPERFECTA–ACQUISITION OF BONE MASS AND GROWTH UNDER BISPHOSPHONATE AND rhGH-TREATMENT PERSONAL EXPERIENCE

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OSTEOGENESIS IMPERFECTA-ACQUISITION OF BONE MASS AND GROWTH UNDER BISPHOSPHONATE AND rhGH-TREATMENT. PERSONAL EXPERIENCE (Abstract) **Aim**: To verify the efficacy of bisphosphonates (Bis) in combination with recombinant human GH (rhGH) in children with osteogenesis imperfecta (OI). We also aimed at demonstrating a possible improvement in bone mineral density (BMD) and growth velocity when combined treatment of Bis and rhGH is used. **Material and methods:** Retrospective observational study on eight children with OI type IA. The treatment consisted in the administration of Risedronate for 24 months. The children weighing under 30 kg received 35 mg Risedronate/14 days. The children weighing over 30 kg received 35mg Risedronate / weekly. Lumbar spine and whole body bone mineral density (BMD) were assessed at baseline and after 24 months. The DXA-BMD results were compared with the reference data for bone density; in all cases the score at baseline was higher than -2.5. Two of the children with significant growth disorders and in which over the past year growth velocity was less than 2 cm/year, were also treated with rhGH at a dosage of 0.3 mg kg/week. **Results and discussion:** An increase in lumbar spine and whole body BMD was recorded both in girls and boys after two years of Bis therapy. After the study ended, the children have continued their treatment with Bis. The combined treatment resulted in improved linear growth. **Conclusions:** Our experience suggests that the use of combined therapy of growth hormone and bisphosphonates resulted in improved linear growth, increased growth velocity and, increased bone mass acquisition. **Keywords:** OSTEOGENESIS IMPERFECTA, BONE MASS, GROWTH VELOCITY.

Osteogenesis imperfecta (OI), also known as brittle bone disease, can be defined as a heterogeneous group of diseases characterized by bones that fracture easily, often from little or no apparent trauma, with variable severity and presumed or proven defects in collagen type I biosynthesis. In addition to fractures, people with OI often have muscle weakness, joint laxity, skeletal malformations, and other connective tissue problems. Genetic disorders involving the skeletal system arise through disturbances in the complex processes of skeletal development, growth and homeostasis and remain a diagnostic challenge because of their variety. Almost 400 different forms of skeletal dysplasia have been described. ***The prevalence*** of OI is approximately 1 in 25,000 to

50,000 people in the United States. Because some infants die at birth and would not be included in these surveys, the birth incidence is slightly higher, perhaps 1 in 15,000 -18,000 births (1). ***Etiology.*** With rare exceptions, OI results from mutations in the type I collagen genes and is a dominantly inherited disorder. There are very unusual forms of OI that seem to be inherited in a recessive fashion, which means that each parent is a carrier and contributes one altered gene each to their child, who is then affected with OI. Type I collagen is the primary structural protein of bone and skin. In 1979, Sillence (2) divided OI into four types based on clinical, radiographic, and genetic distinctions. In 2010, Warman (3) reported additional types that do not involve a defect of type I collagen. Most OI cases are caused by heterozygosity of dominant mutations in one of the two genes encoding the chains of type I collagen (COL1A1 and COL1A2) (4), with quantitative or qualitative defects in the synthesis of type I collagen. Cartilage-associated protein, prolyl 3-hydroxylase 1, and cyclophilin B form an intracellular collagen-modifying complex that 3 hydroxylates proline at position 986 (P986) in the a1 chains of collagen (types I, II, and V); mutations in these essential cofactors cause alterations in the posttranslational chain modification and collagen folding, which are responsible for autosomal-recessive lethal or severe OI (5,6). Life expectancy varies per the severity of the underlying disorder and ranges from very brief to average. Because there is no cure for OI, its management or ***treatment*** currently focuses on minimizing fractures and maximizing mobility and independent function. Regarding the pharmacological approach, Bis are now indicated for children with OI (7). Administered orally or parenterally, these form a strong, rapid bond to hydroxyapatite crystals in bone and, by decreasing osteoclast activity and number, inhibit bone resorption and reduce bone turnover, although effects on bone formation occur as well. The positive effects of Bis on pain and mobility and on decreasing long bone fractures have been demonstrated. *Pamidronate* was the first Bis described, and authorized for use in OI children. A randomized controlled clinical trial with a different oral Bis, found that *Risedronate* increased areal BMD and reduced first and recurrent clinical fractures in children with OI (8,9). *Zoledronic acid*, a newer Bis with a longer half-life, greater potency, and more convenient dosing, is being studied in children with OI. Questions that remain to be answered include long-term side effects as well as impact on bone quality and bone health, the dosing schedule that maximizes benefits and minimizes detrimental effects, and the time to end or cut back therapy, as well as the benefit to patients with milder OI. Patients with OI should receive an appropriate contribution of calcium intake, phosphorus and vitamin D (10).

GH has a positive effect on bone growth and bone turnover by stimulating osteoblasts, collagen synthesis, and longitudinal bone growth (11); GH stimulates the expression of insulin-like growth factor 1 (IGF1) and IGF-binding protein 3 (IGFBP-3), which in turn regulates the synthesis of type I collagen. The positive height growth and an increase in skeletal volume and BMD reduce the number of fractures, this being the main significant therapeutic benefit. Animal studies, in a mouse model of OI with bone phenotype comparable to a mild form of OI in humans, showed that systemic rhGH injections (12) increased spine and femur length, produced significant changes in densitometry parameters and ameliorated the biomechanical structural properties of

bone. Despite rhGH action on bone turnover and bone mineralization resulting in an increase in BMD and on the growth velocity rate, few human studies have been performed using rhGH in patients with OI (13, 14, 15). There are no many controlled trials on combined rhGH and Bis treatment in OI patients.

MATERIAL AND METHODS

Retrospective study on eight children, six girls and two boys, from five families with OI type IA.

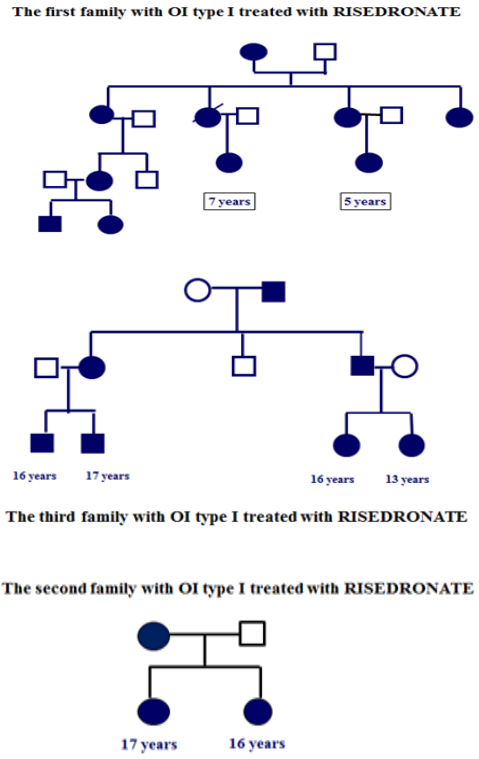

Fig.1. Family tree for each child included in the study

We have drawn the family tree for each child included in the study (fig 1). The X-ray exam confirmed the clinical diagnosis in all patients (fig. 2)

The children have been treated for 24 months with Risedronate. The children weighing under 30 kg received 35 mg Risedronate/14 days. The children weighing over 30 kg received 35mg Risedronate/ weekly. Two of the children (two girls) with significant growth disorders and in which over the past year growth velocity was under the 2 cm/year, have also received treatment with rhGH 0.3 mg/kg/ week. To monitor rhGH treatment compliance, the patients returned to the clinic every 6 months. The children treated only with Bis came every 12 months. All patients were evaluated for their auxological and clinical parameters-weight, height, body mass index, growth velocity, and Tanner stages-and the number of new peripheral or extra vertebral fractures. Bone metabolic parameters and bone mass measurements were determined at the time of enrollment after 12 and 24 months, and when the two girls started rhGH treatment, at every 6 months and finally at the end of rhGH treatment. Bone metabolic parameters were obtained/measured in the morning after an overnight fast. Serum Ca, P, alkaline phosphatase, parathyroid hormone (PTH), 25OHD, osteocalcin, C-terminal cross linking telopeptides of type I collagen, GH and IGF1 were determined; also measured were urinary Ca, P, and creatinine. Routine biochemistry samples, including creatinine and serum glucose, were collected. BMD was assessed at the lumbar spine and whole body using Hologic DXA-BMD with scans at baseline, and after 12 and 24 months of therapy. The DXA-BMD results were compared with the reference data for bone density; Z-score at baseline was in all cases over -2.5. Bone age (BA) was determined only in the patients on rhGH therapy.

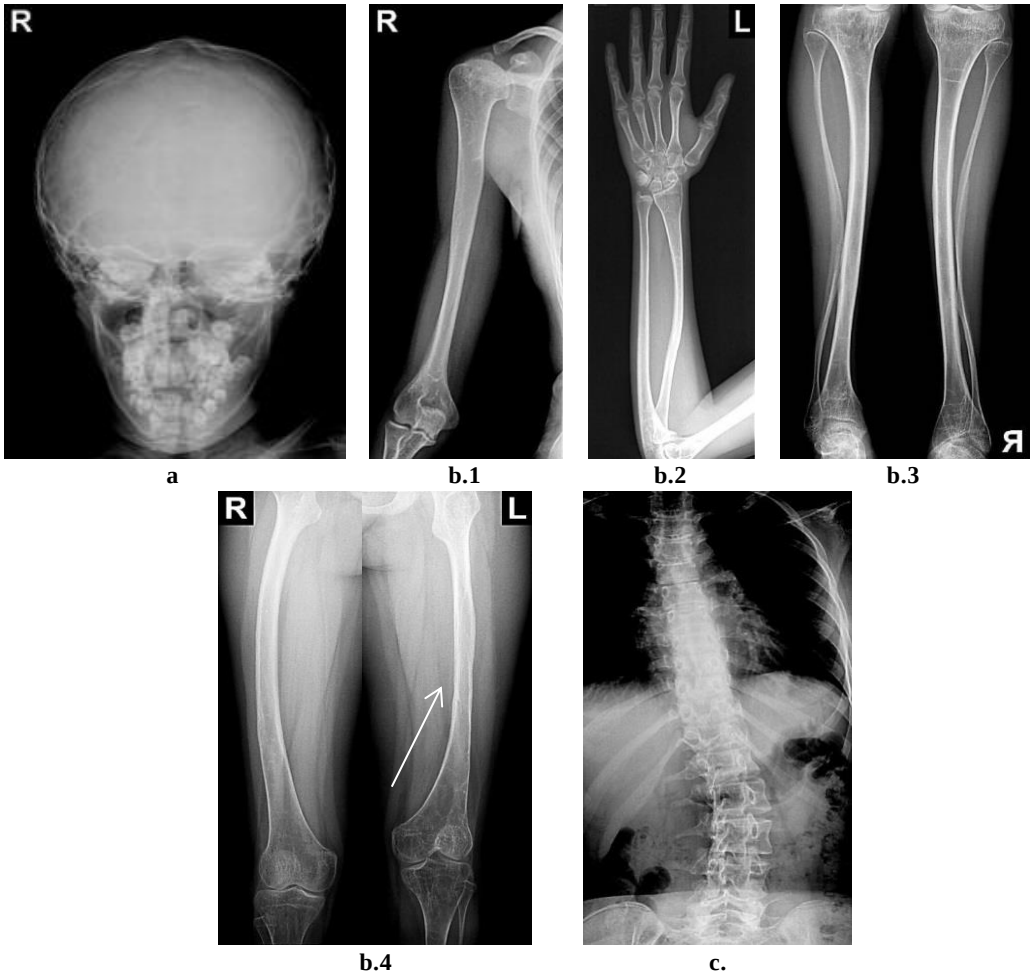

Fig.2. Patient AMB, 13 years old. Osteogenesis imperfecta type IA- X-ray appearance (Professor C. Galesanu- Personal collection)
a. The triangular skull, wormian bones. **b. 1-3.** Gracile long bones, thin dependent cortical **b.4.** recent fracture with unfit gag. **c.** Spinal deformities, multiple rib fractures.

RESULTS

At the beginning of the study, the mean lumbar BMD, expressed in SD, was lower than that of the normal population. Our results have indicated an increase in BMD of 13,2 % at girls and 22,6 % in boys after two years of therapy at the lumbar level. At the whole body the increase was 11, 5 % in girls and 25, 2 % in boys; this represented a reduction of the deficit of bone mass from -11 to -9 SDS in girls and from -9 to -4 SDS the boys (tab. I, II). No negative effects on the gastro-intestinal. Without new fractures under treatment. Children are still under the Bis therapy (14).

In our study, we followed the cases of

the two girls (cousins) which started the Bis treatment for OI type IA at the age of 7 and 5 years, respectively. In the girl aged 7, after the first 12 months of Bis treatment, the speed of growth was 2 cm/year; the speed of growth in the previous year being of 4.5 cm/year. In these circumstances, treatment with rhGH 0.3 mg/kg/week was initiated, which led to a speed of growth to 8.5 cm/year. The combined treatment was administered for 5 years, a final height of 160 cm being attained. Her cousin of 5 years, after a subnormal growth, at the age of 10 had a speed of growth of 2 cm/year and a growth retardation -2 SDS. Treatment with rhGH was initiated; her height after 5 years of treatment was 158 cm. In parallel we followed the acquisition of bone mass (whole body) which was very good: there was an increase in BMC of 478.19 g and BMD increased from 0.814 to 1.026 g/cm^2 reaching the normal ranges for age and sex (fig.3).

PTH, 25OHD and C-terminal cross linking telopeptides of type I collagen levels were in the normal range with no significant variations found during the study. Osteocalcin levels were in the low normal range at the beginning of the study, and remained at the same level. Serum Ca, P, alkaline phosphatase and urinary Ca and P have evolved within normal ranges. IGF1 levels were in the low normal range before therapy, and increased significantly after 12 months of rhGH therapy. After the study ended, the children have continued their treatment with Bis.

TABLE I.

Osteogenesis Imperfecta - Acquisition of bone mass under bisphosphonates at Lumbar Spine (LS)

Girls	Mean BMD LS (g/cm$^{2)}$	Mean BMD LS reference data (g/cm$^{2)}$	% deficit	% gain	SD deficit
Baseline	0.670	1.181	43.3	-	-9
After 24 months	0.751	1.236	-	13.2	-8
Boys					
Baseline	0.782	1.214	36.0	-	-6
After 24 months	0.959	1.271		22.6	-5

TABLE II.

Osteogenesis Imperfecta - Acquisition of bone mass under bisphosphonates at Whole body (HB).

Girls	Mean BMD HB (g/cm$^{2)}$	Mean BMD HB reference data (g/cm$^{2)}$	% deficit	% gain	SD deficit
Baseline	0.782	1.114	30.0	-	-11
After 24 months	0.872	1.152	24.6-	11.5	-7
Boys					
Baseline	0.882	1.200	26.5	-	-9
After 24 months	1.105	1.251	11.7	25.2	-4

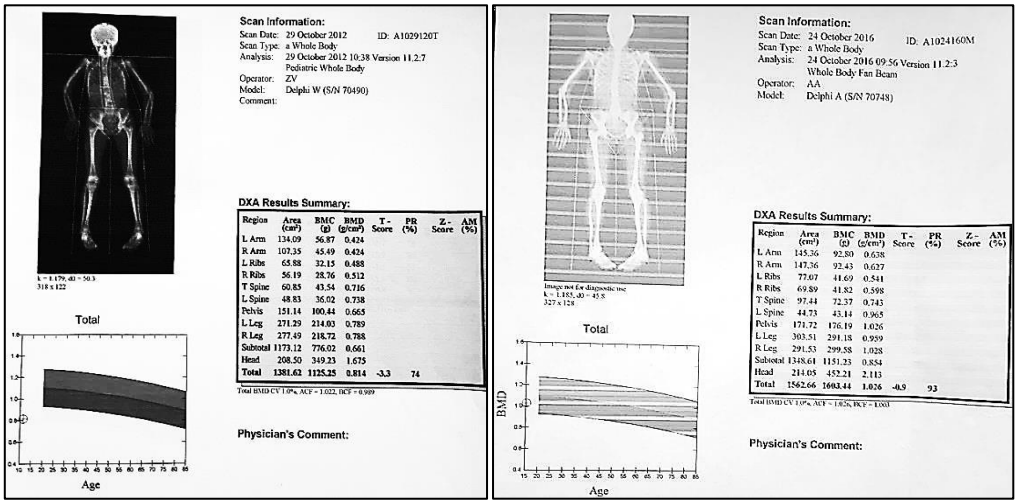

Fig. 3. DXA -BMD whole body in dynamics.
Patient GMP under treatment with bisphosphonates and rhGH: 4-year course.

DISCUSSION

The aim of this retrospective observational study was to clarify if rhGH can ameliorate bone metabolism in patients with OI who had already benefited from ongoing Bis treatment. The rationale behind Bis treatment in OI is based on various bone metabolic data (7, 9), and on the fact that Bis have achieved significant clinical improvements in infants, children, and adolescents suffering from moderate and severe forms of OI. Their results in BMD gain and reduced fracture incidence have been proven by clinical trials, whereas their action in decreasing pain, increasing mobility, and even their positive impact on growth (16) has been seen in observational trials. It is known that rhGH stimulates bone apposition, obtaining an improvement in well-being, especially concerning skeletal integrity, and that it has positive effects on BMD and growth velocity (12). In our experience, BMD and growth improved with Risedronate treatment in our OI patients, compared with pretreatment (8). We decided to couple the prevalent inhibition of bone resorption by Bis with the anabolic effects of rhGH to analyze the possible advantages on BMD, projected bone areas, growth, and fracture risks. Bone mass increased at our children as demonstrated by the results obtained, an analysis of BMC (g) and BMD (g/cm^2) relating to age. As expected, during the two years of study Bis treatment resulted in improvements in lumbar spine and whole body BMD when treatment was based on Risedronate alone. With the combined Bis–rhGH therapy, densitometry results showed a significant, further positive increase in DXA-BMD at the lumbar spine and whole body after five years. There is limited literature regarding the experience with recombinant human GH in OI. One of the first attempts to treat OI patients with rhGH was made by Kruse and Kuhlencordt; their patients had an increase in periosteal new bone formation and intracortical bone resorption (assessed using histomorphometric of bone biopsies), with relatively enhanced osteoblastic activ-

ity (17). Following these results, no further study was reported in the literature until 1993 when GH–somatomedin axis activity was studied in a limited number of non-selected OI patients (13). Even if other studies had shown that IGF1 serum levels are mostly in the low normal physiological range, Marini et al. (13) found hypo activity of this axis (without a true GH deficit) in approximately half of their OI patients and treated them with rhGH. In a further study by this group, the authors concluded that there is also a group of type IV OI children who would benefit from rhGH treatment in terms of linear growth, bone matrix synthesis, and bone histomorphometric parameters.

CONCLUSIONS

Our experience suggests that the use of combined therapy of growth hormone and bisphosphonates has improved linear growth, increasing growth velocity. Also, combined therapy, increased the acquisition of bone mass measured by dual-energy X-ray absorptiometry at the lumbar spine and whole body, and reduced the incidence of peripheral fractures.

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NEWS

MALDI-TOF ALONE VERSUS MALDI-TOF COMBINED WITH REAL-TIME ANTIMICROBIAL STEWARDSHIP INTERVENTIONS ON TIME TO OPTIMAL ANTIMICROBIAL THERAPY IN PATIENTS WITH POSITIVE BLOOD CULTURES

Introduction: Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) decreases time to organism identification and improves clinical and financial outcomes. The purpose of this study was to evaluate the impact of MALDI-TOF alone vs MALDI-TOF combined with real-time, pharmacist-driven, antimicrobial stewardship (AMS) intervention on patient outcomes. Methods: This single-center, pre-post quasi-experimental study evaluated hospitalized patients with positive blood cultures identified via MALDI-TOF combined with prospective AMS intervention, compared to a control cohort with MALDI-TOF identification without AMS intervention. AMS intervention included: real-time MALDI-TOF pharmacist notification, and prospective AMS provider feedback. The primary outcome was time to optimal antimicrobial therapy (TTOT). Results: A total of 252 blood cultures were included for final analysis: 126 in each group. MALDI-TOF + AMS intervention significantly reduced overall TTOT (75.17 vs 43.06 h, p <0.001), Gram + contaminant TTOT (48.21 vs 11.75 h, p <0.001), Gram - infection (GNI) TTOT (71.83 vs 35.98 h, p <0.001), and reduced overall hospital LOS (15.03 vs 9.02 days, p 0.021). TTOT for Gram + infection (GPI) was improved (64.04 vs 41.61 h, p 0.082). For GPI: reduced hospital LOS (14.64 vs 10.31 days, p 0.002) and length of antimicrobial therapy 24.30 vs 18.97 days, p 0.018). For GNI: reduced time to microbiologic clearance (51.13 vs 34.51 h, p <0.001), hospital LOS (15.40 vs 7.90 days, p 0.027), and ICU LOS (5.55 vs 1.19 days, p 0.035). Conclusion: In order to achieve optimal outcomes, rapid identification with MALDI-TOF combined with real-time AMS interventions is more impactful than MALDI-TOF alone. (Maya Beganovic, Michael Costello,Sarah M. Wieczorkiewicz. MALDI-TOF alone versus MALDI-TOF combined with real-time antimicrobial stewardship interventions on time to optimal antimicrobial therapy in patients with positive blood cultures. *Journal of Clinical Microbiology* 22 February 2017).

Cristina Tuchiluș

THE LINK BETWEEN QUALITY OF LIFE AND ECONOMIC ISSUES IN ANKYLOSING SPONDYLITIS

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THE LINK BETWEEN QUALITY OF LIFE AND ECONOMIC ISSUES IN ANKYLOSING SPONDYLITIS (Abstract): Ankylosing spondylitis (AS) is an inflammatory rheumatic disorder which mainly involves the axial skeleton. The most important clinical features are: inflammatory back pain and peripheral joint involvement, but extra-articular manifestations that occur most often in the eyes, heart and lungs may also be present. Joint stiffness, pain and a progressive loss of spinal mobility can lead to severe functional limitations with subsequent negative impact on patients' quality of life. These symptoms can interfere not only with physical health, but also with social life and psychological wellbeing. Many studies took into consideration these aspects and tried to analyze the relationship between disease activity, functional impairment, work ability, interpersonal relationships and total costs of this debilitating disease. After many years of studying, the authors concluded that AS has a socio-economic impact on patients, society and healthcare system. **Keywords***:* ANKYLOSING SPONDYLITIS, QUALITY OF LIFE, ECONOMIC IMPACT, COSTS.

Ankylosing Spondylitis (AS) is a chronic inflammatory rheumatic disease belonging to the large group of spondylarthrites (SpA) alongside psoriatic arthritis, reactive arthritis, inflammatory bowel disease-associated SpA (IBD-SpA) and undifferentiated SpA. It affects mainly the sacroiliac joints and the axial spine. The most important clinical features are low back pain, joint stiffness, progressive loss of spinal mobility and peripheral joint involvement. All these symptoms can lead to severe functional limitations due to a significant impact on patients' quality of life. The onset of AS usually occurs in the second and third decades of life, when the patients are still active, affecting two times as many men than women. The disease has a prevalence of 0.1% to 1.4% worldwide (1).

AS has a significant socioeconomic impact on both the patient and society. When we speak about disability we refer firstly to the degree of inflammatory activity which determines stiffness, pain, sleeping disorders and fatigue and, secondly, to the degree of bony ankyloses which leads to a progressive loss of spinal mobility (2). These items have a significant impact on patients' quality of life and health status, negatively affecting patient's physical and

psychological wellbeing as well as their social life (3).

QUALITY OF LIFE IN ANKYLOSING SPONDYLITIS

Quality of life in patients with ankylosing spondylitis has been the main subject of many studies because the disease has a significant impact on patients' daily activities. Symptoms like joint stiffness, constant pain and functional impairment have a huge effect on the physical, psychological and social function of the affected people.

The self-administered questionnaires most commonly used in most of the studies to evaluate the activity of AS and the quality of life of patients are:

Bath AS Disease Activity Index (BASDAI) - a six-item questionnaire which evaluates the AS disease activity one week prior to the examination. It consists of six visual analog scales (VAS) and measures fatigue, spinal and peripheral joint pain, localized tenderness and morning stiffness. Each symptom has a VAS score (0= best, 10= worst) and the last two scores pertaining to morning stiffness are calculated as a mean score. The final BASDAI score is reported on a 0-10 scale, the greater the score, the worse disease activity (4).

Bath AS Disease Functional Index (BASFI) measures the functional impairment due to the disease. It consists of ten questions designed to measure joint function in everyday activities. All questions are scored 0 to 10 on a VAS and the mean gives the final BASFI result (5).

Bath AS Metrology Index (BASMI) evaluates hip and spinal mobility referring to cervical rotation, tragus to wall distance, lumbar side flexion, lumbar flexion (Schober's index) and intermalleolar distance. The final score ranges from 0 to 10 (6).

Bath AS Disease Global Index (BASG-s) consists of two questions that measure the effect of AS on patient's general health over the last six months and the last week (7).

AS Quality of Life Questionnaire (ASQOL) comprises 18 questions, each with a "yes/no" response format, scored "1" and "0" respectively, about the effect of AS-related pain concerning the management of the disease, relationships, mood, sleep, motivation, activities of everyday living, independence and social life (8). A better quality of life is reflected by a lower ASQOL score.

Short-Form 36 Health Survey (SF-36) is a measure of generic health status which evaluates eight domains of physical and mental health over the previous 4 weeks: physical functioning, physical role, bodily pain, general health, vitality, social functioning, emotional role and mental health. These questions are summarized in the: Physical Component Summary (PCS) and the Mental Component Summary (MCS). A better quality of life is indicated by a higher score (9).

All these questionnaires, which are the main tools in evaluating the disease activity and its impact on patients' everyday life, are used in many studies on the quality of life and economic issues of patients with AS.

A Polish study including 54 patients with AS aimed at obtaining patients' opinion on the factors which influence their quality of life (10). The study relied on a 12-item questionnaire on: ability to work, family and social life, pain perception, frequent hospital admissions or impediments to optimal management of AS. The results showed that pain is the main risk factor for a poor quality of life. Others risk factors include: family problems, social status (job loss, lower income), limitations

in performing home-based exercises or sexual life impairment.

Based on the SF-36 health questionnaire, a cross sectional study examined the health status in 100 AS Tunisian patients compared with the general population (11). They also used others disease-specific questionnaires such as: BASDAI, BASFI, BASMI, BASG-s to assess the disease stage. The results revealed: a poor quality of life in patients with AS, a better status in women than in men, mental health affected more than physical health, worse quality of life in the patients with low educational levels and better physical functioning in employed persons. Like other studies (12, 13), this survey demonstrated the association between great impairment in quality of life and high scores of BASDAI, BASFI, BASMI and BASG-s, the most important influence being attributed to pain, disease activity, mobility limitation and functional impairment.

Another important study on 962 patients with AS evaluated the quality of life using the well-known questionnaires (BASDAI, BASFI, BASMI, BASRI- Bath Ankylosing Spondylitis Radiology Index, MASES-Maastricht Ankylosing Spondylitis Enthesitis Score, ASQOL, SF-36) (14). The authors demonstrated that the quality of life was impaired in all patients. This is firstly due to low scores on bodily pain, physical role, general health and vitality subscales. They also showed a correlation between ASQOL and BASDAI, BASFI, BASMI, BASRI, MASES, fatigue, disease duration, overall pain and nocturnal pain. The most important variables with a negative impact on quality of life were: fatigue, disease activity, bodily pain and functional status. This study, like other studies (15, 16) demonstrated that smoking and sedentary life can negatively affect the quality of life of these patients, while a higher educational level can improve it.

Based on all these studies, we can affirm that AS is characterized by an increased impairment of the quality of life, affecting both physical and mental health. An early diagnosis and a proper treatment can improve the disease parameters responsible for the deterioration of quality of life.

ECONOMIC ISSUES IN ANKYLOSING SPONDYLITIS

On the other hand, AS has a significant socioeconomic impact on society and healthcare system, work disability becoming a frequent consequence of AS (17). The decrease in physical mobility is the main reason for the increasing costs associated with this disease (18). We can talk about direct costs that include: reported ambulatory care visits, hospitalizations, diagnostic tests (blood and imaging tests), medications, assistive devices, non-allopathic treatments (hydrotherapy, acupuncture, massage), riding to visits - transportation fees and paid household help or indirect costs which are related to productivity loss due to annual sick leave, number of days of activity limitation, retirees and homemakers (19, 20, 21).

An important study in Hong Kong evaluated the costs for 145 AS patients (22) and demonstrated that indirect costs are 62% of total costs. Of the direct costs, technical examination and a visit to the physician are the costliest. Also, they found out that 59% of the patients had additional expenses for non-traditional therapy and health products. This retrospective cross-sectional study revealed that many male patients (30%) and female patients (43%) were unemployed because of AS-related

work disability. Among the patients who had a job, a high percentage (86%) had taken on average an 8-day sick leave in the past year.

In this study, the patients were evaluated using BASDAI, BASFI and SF-36 questionnaires which highlighted that BASFI was correlated with direct costs while BASDAI has no all cost implications. So, functional impairment is an important driver of annual costs.

Numerous studies have also demonstrated that indirect costs are the most important in the group of total costs (23, 24, 25) and that physical functional impairment (measured by BASFI score) due to high disease activity is decisive in terms of total costs.

A US cross-sectional survey in 591 patients suffering from AS evaluated the work and family life of these patients compared with general population (PSOAS - Prospective Study of Outcomes in Ankylosing Spondylitis) (26). It showed that 13% of patients were work disabled, with a greater prevalence among women, twice as high compared with the general population. Disease duration (≥20 years) was associated with nonparticipation in the work force and work disability as well as with patients' level of education; those who had finished only high school were more affected from this point of view. Also, this study highlighted that AS has a big influence on the social life of the patients. The patients were more likely to be divorced (~30%) or single (never married) (~50%) and women with AS had fewer children than women in the general population.

A literature review conducted in Italy included the most important studies published between 2002 and 2012 on the economic impact of AS (direct and indirect costs) (27). The authors concluded that, in AS, indirect costs are the main component of total costs and that functional disability played a decisive role. In their survey, Younes et al. (28) found that direct costs are influenced by disease duration; job absence is closely linked to patient's age (over 40), disease duration (more than 10 years) and to civil status (more frequent in married people). Boonen et al. evaluated work productivity loss due to presentisms (number of days with productivity ≤50% at work) and sick leave (19) and found out that 73% of cases of sick leave were AS-related. Also, the authors noticed that direct costs were related to the length of hospital stay, number of doctor visits, medication used for treatment (disease-modifying antirheumatic drugs, anti-TNF drugs) or physical rehabilitation treatment (hydrotherapy, physiotherapy). Regarding indirect costs, they noticed that patients treated with anti-TNF drugs have less work days lost, lower sickness compensation and fewer disability pension (21).

Ara et al. evaluated the direct costs associated with disease activity using the BASDAI and BASFI questionnaires (29). They concluded that AS severity determines the annual cost per patient which is strongly related to BASDAI and BASFI scores, meaning that if the disease is active, the costs are higher.

CONCLUSIONS

Our clinical experience supports that most AS patients are young and have a significant physical disability measured by BASDAI, BASFI, HAQ, SF-36. Due to pain and functional impairment, they cannot perform activities of daily living and require long periods of sick leave. Also, because of pain and social exclusion, our patients often exhibit signs of emotional

instability and depression.

In summary, we can affirm that AS is a systemic inflammatory disease which is characterized by a severely impaired quality of life of patients and has high social costs. Disease activity contributes to functional limitations over time, so BASDAI, BASFI, pain and fatigue are the most significant variables related to the quality of life in patients suffering from AS. Functional impairment has been demonstrated to be the most important cost driver, having an important influence on family life as well as on work ability. Work disability is strongly influenced by patients' age and disease duration. Indirect costs are dominant in AS suggesting that these should be included in any cost assessment of new treatments.

To improve the general health of patients while decreasing total costs, doctors should take into consideration: patient education, pharmacological treatment combined with physical treatment, proper management of pain and fatigue and adaptation to the working place.

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COLORECTAL CHEMOPREVENTION

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COLORECTAL CHEMOPREVENTION (Abstract). With over 1 million new cases diagnosed worldwide each year - incidence which seems to rise with the progressive westernization of lifestyles in Asian and African populations - colorectal cancer is the third most commonly diagnosed cancer in both men and women. Colorectal neoplasms and/or preneoplasms can be prevented by interfering with the various steps of oncogenesis, which begins with uncontrolled epithelial cell replication, continues with the formation of adenomas and eventually evolves into malignancy. The knowledge described herein will help to reduce and prevent this malignancy, which is one the most frequent neoplasms in some developed countries. Genetics, experimental and epidemiologic studies suggest that colorectal cancer results from complex interactions between inherited susceptibility and environmental factors. Primary prevention involves the identification of genetic, biologic, and environmental factors that are etiologic or pathogenic in the development of cancer, and subsequent complete or significant interference with their effects on carcinogenesis. K**eywords**: CHEMOPREVENTION, CARCINOGENESIS, COLORECTAL CANCER.

Colorectal cancer (CRC) is the second or third leading cause of cancer-related mortality in United States (1), incidence which seems to rise with the progressive westernization of lifestyles in Asian and African populations. In the past decade, therapeutic development has improved survival rates for patients with metastatic CRC, and many more treatment options exist for advanced disease. Many of these treatment options for advanced disease now include a combination of chemotherapy with targeted therapy. However, the fact that metastatic CRC remains incurable prompts investigators to explore a deeper understanding of the factors underlying cancer progression. The limited success achieved by only targeting tumor cells highlights the importance of understanding the role of the tumor microenvironment and its precise contribution to carcinogenesis. A major contributor to the tumor microenvironment is inflammation and inflammatory mediators. The recognition of chronic inflammation as the seventh trait acquired by tumor cells necessary for survival, growth, and metastasis (2-4) has intensified studies on the role of intratumoral inflammatory cells and proinflammatory cytokines in cancer progression. Chronic inflammation is now recognized as both a tumor initiator and promoter, and it has long been studied in relation to proinflammatory prostaglandins in colon cancer (5,

6). Clinical studies have shown that long-term use of nonsteroidal anti-inflammatory drugs (NSAIDS) reduces the risk of CRC by 40–50% (7), in part, by targeting cyclooxygenase-2 (COX-2), an enzyme required for prostanoid synthesis. COX-2 is overexpressed in the majority of CRCs (8, 9) and COX-2-derived prostaglandin E2 (PGE2) mediates various tumor promoting effects (6 - 8). PGE2 is the most abundant PG found in CRC tissue, and it has well demonstrated proneoplastic effects (12 - 14). PGE2 promotes tumor growth by inducing tumor cell proliferation, survival, migration/invasion (15 - 18) and by enhancing the development of a supportive tumor microenvironment. In CRC, the multistep process from normal colonic epithelium to an adenomatous polyp and ultimately to an invasive colon carcinoma is associated with or supported by the tumor microenvironment. The tumor microenvironment essentially consists of tumor-infiltrating cells, vasculature, extracellular matrix (ECM), and other matrix-associated molecules. Transformed epithelial cells modulate the functions of stromal cells with the overall purpose of facilitating their own growth, survival, invasion, and metastasis. This dependence or "addiction" of the cancer cells to the stromal components opens novel avenues for the development of potential therapeutic agents.

Cancer chemoprevention, as first defined by Sporn in 1976, is defined as the use of natural, synthetic, or biologic chemical agents to reverse, suppress, or prevent carcinogenic progression to invasive cancer (19). The success of several recent clinical trials in preventing cancer in high-risk populations suggests that chemoprevention is a rational and appealing strategy. Chemoprevention is divided into three groups. ***Primary prevention*** strategies seek to prevent malignancies (cancers) in an otherwise healthy population. These individuals may have a history that puts them at higher risk, such as a history of smoking or particular genetic mutations predisposing them to cancer development. ***Secondary prevention*** involves patients who have known premalignant (pre-cancerous) lesions utilizing medications or vitamins in an attempt to prevent the progression of these lesions into cancers. ***Tertiary prevention*** focuses on the prevention of new cancers in patients cured of an initial cancer or individuals who have been treated for premalignant lesions. Clinical trials of chemoprevention are based on the idea that interruption of the biological processes involved in carcinogenesis (the process of normal cells transforming into cancer cells) will inhibit this process and, in turn, reduce cancer incidence.

The ideal chemo preventive agent will not significantly alter quality of life, is inexpensive, safe, well tolerated, and effective. However, some chemo preventive drugs may have severe side effects in some patients, which is an issue when considering long-term administration of a compound to healthy people who may or may not develop cancer. Chemoprevention is thus of particular importance to genetically predisposed patients and to those patients who are especially susceptible to the environmental causes of colorectal cancer. Colorectal cancer is particularly suitable for prevention strategies because it is prevalent and associated with considerable mortality and morbidity (20). This cancer has a naturally history of transition from normal crypts through adenoma to overt adenocarcinoma occurring over an average of 10-20 years, thereby providing a window of opportunity for effective intervention

and prevention. Large bowel carcinogenesis occurs via a multi-step process, characterized by molecular and cellular alterations; interference with these processes forms the basis for chemoprevention, which attempts to target key molecular events along these pathways. Recent preclinical studies and clinical trials have provided data on the potential benefit of a number of nutritional or non-nutritional elements as well as drugs in the chemoprevention setting. The most important molecular markers/targets involved in signal pathways are polyamine 9ornithine decarboxylase), COX-2, iNOS, 3-hydroxy-3-methylglutatyl coenzyme A reductase, retinoid X receptor-alpha, estrogen receptor-beta, beta-catenin, 5-lipoxygenase, signal transducers and activators of transcription 3, NF-kB and hemeoxygenase-1. These can be also considered as biomarkers and/or surrogate endpoint markers in colorectal carcinogenesis and also enzymatic and molecular targets for colorectal malignancies (21,22). Currently, genetic information about cancer, molecular signaling and metabolic pathways has been translated into specific therapy that targets specific molecules for prevention. Developing new technologies to provide knowledge of the functions of non-coding RNA, such as small interfering RNA, microRNA and piwi interacting RNA, can form a basis for the development of novel chemo preventive agents that intervene at different steps during the multi-stage carcinogenesis process: preventing the initial mutation, blocking promotion to pre-malignant tumors, stopping progression from the pre-malignant state to in situ carcinomas or preventing invasion or metastasis. Because the early stages of tumor promotion and progression are rate limiting, successful targeting of molecular events during these stages can have a great impact on the outcomes.

Multiple lines of evidence indicate that aspirin and related NSAIDs are antitumorigenic and have the potential to prevent colorectal cancer. However, the mechanisms underlying this effect have yet to be fully elucidated. NF-κB is a ubiquitously expressed transcription factor that plays a critical role in cell proliferation and apoptosis. It was demonstrated that the RelA component of NF-κB is targeted to the nucleolus in response to NSAIDs and other pro-apoptotic stimuli of the NF-κB pathway and that nucleolar sequestration of RelA is required for the apoptotic effects of these agents. In present is currently studying the signaling networks involved in NSAID-mediated stimulation of the NF-κB pathway, nucleolar sequestration of RelA and the associated apoptosis. It was demonstrated that p38-mediated degradation of cylinD1/inhibition of CDK4 is important in this effect and so, this pathway is of particular interest. (19, 23, 24).

The disparity between the results of ACF formation and later tumor development by an agent was also documented previously on these parameters as a result of genistein administration. Genistein was thought to be a promising chemo preventive agent against colon carcinogenesis based on epidemiological observations that consumption of soybeans and soybean-based products containing a large amount of genistein might reduce the risk of colon cancer. While a short –term animal bioassay showed that genistein inhibits the development of azoxymethane-induced colonic ACF using tumors as end point demonstrated that dietary administration of this compound significantly enhances colon carcinogenesis. Celecoxib, a selective in-

hibitor of cyclooxygenase-2 and a promising chemo preventive agent against colon carcinogenesis, significantly inhibited BCAC in azoxymethane–treated rats, thus suggesting the possible application of BCAC as a biomarker.

CHEMOPREVENTIVE STUDIES

Despite promising data in epidemiologic studies, most dietary changes have not been successful in preventing colorectal cancer. Specifically, clinical trials have shown no benefit with fiber, beta carotene, vitamin A, C, and E interventions. Other studies suggest that calcium may prevent colorectal carcinoma by binding bile and fatty acids and inhibiting the proliferation of colonic epithelial cells. The Calcium Polyp Prevention Study evaluated calcium carbonate (3 g [1,200 mg of elemental calcium] daily) supplementation in 930 patients for four years and reported a decrease in the recurrence rate of colorectal adenomas. Although calcium supplementation led to a moderate reduction in risk of colorectal adenomas, it remains unclear whether this translates into prevention of invasive colorectal malignancies and a survival benefit.

Colon cancer prevention has now focused on novel targeted therapies, such as NSAIDs. Aspirin, an inhibitor of COX-1 and -2, has been studied in several large randomized studies, but the effect on colorectal cancer prevention is unclear. The US Physician's Health Study (25) which enrolled 22,071 physicians as participants, reported that aspirin had no effect on the incidence of polyps or colon cancer. However, Baron et al. (26) conducted the Aspirin/Folate Polyp Prevention Study, a randomized, double-blind, placebo-controlled trial of daily aspirin (325 mg and 81 mg) and daily folate (1 mg) in 1,121 patients with a recent history of colon adenomas. This trial demonstrated that the 81-mg dose of aspirin prevented recurrence of colorectal adenomas (47% placebo versus 38% aspirin 81 mg versus 45% aspirin 325 mg; $p = 0.04$). This translated into a relative-risk reduction of 19% in the 81-mg aspirin group and a nonsignificant reduction of 4% in the 325-mg aspirin group. This study also reported a relative-risk reduction of 40% in the 81-mg aspirin group for advanced lesions. Analysis of the folate intervention is ongoing. Also, Sandler et al. (27) reported the Colorectal Adenoma Prevention Study, which randomized 635 patients with prior colorectal cancer to 325 mg aspirin or placebo. Twenty-seven percent of the placebo group developed recurrent adenomas compared with 17% in the aspirin arm ($p = 0.0004$), for an adjusted relative risk of 0.65. Aspirin intervention delayed the development of recurrent adenoma and also decreased the number of recurrent adenomas.

Although the role of aspirin remains debated, the benefit of NSAIDs in chemoprevention has clearly been defined in certain high-risk subgroups. Aspirin and sulindac have been shown to reduce microsatellite instability in HNPCC cell lines carrying *h*MLH1, *h*MSH2, and *h*MSH6 mutations. In clinical trials of patients with FAP, sulindac (150 mg twice a day for nine months) was shown to decrease the number of polyps by 44% and decrease the diameter of the polyps by 35% ($p = 0.014$ and $p < 0.001$, respectively). In a study from the University of Texas MD Anderson Cancer Center and St. Mark's Hospital, United Kingdom (28) 77 patients with FAP (more than five polyps 2 mm in size) were randomized to receive placebo, 100 mg, or 400

mg of celecoxib twice daily. Celecoxib is a selective COX-2 inhibitor. Response to treatment was reported as the mean percent change from baseline. After six months, the 30 patients assigned to 400 mg of celecoxib had a 28% reduction in the mean number of colorectal polyps ($p = 0.003$) and a 30.7% reduction in the polyp burden ($p = 0.001$) compared with 4.5% and 4.9% in the placebo group, respectively. This positive result led to the FDA's approval of celecoxib in the treatment of patients with FAP. Other agents under investigation in colorectal chemoprevention include difluoromethylornithine (DFMO), which irreversibly inhibits ornithine decarboxylase and blocks cell proliferation. Ursodeoxycholic acid reduces the concentration of secondary bile acid deoxycholic acid in the colon and affects arachidonic acid metabolism. 3-hydroxy-3-methylglutaryl Coenzyme A reductase inhibitors are usually used in the setting of lowering cholesterol but also have antioxidant antiinflammatory properties and inhibit cell proliferation. Preclinical work in mutant APC murine models have shown that sulindac in combination with EGFR inhibitor EKI-785 can decrease intestinal polyps. Almost one-half the mice treated with the combination agents did not develop polyps. With the recent success of bevacizumab, an antibody to the VEGF-receptor in metastatic colorectal cancer, and cetuximab, an antibody to EGFR, further strategies will be applied to prevention. Studies have shown that individuals reporting a regular intake of aspirin and other (NSAIDs) have a reduced risk of developing colorectal polyps and cancer. It is thought that NSAIDs exert their chemo preventive effects in the colon by restoring a normal frequency of cell death. Because most colorectal cancers arise from adenomas (a type of colon polyp), it is believed that preventing adenoma formation can prevent cancer as well. In a study of 635 patients with a previous history of colorectal cancer who were randomized to receive either 325 mg of aspirin daily or placebo, there was a significantly reduced risk of development of an adenoma in the aspirin-treated group. However, adenomas still developed in some patients in the aspirin-treated group, and therefore aspirin should not be a substitution for surveillance colonoscopy. Another NSAID, celecoxib, has been shown to reduce the mean number of polyps by 28% in patients with the rare genetic disorder familial adenomatous polyposis. Because NSAIDS are associated with increased risk of gastrointestinal bleeding, renal impairment, and other toxicities, the United States Preventive Services Task Force (29) does not recommend NSAID use for the prevention of colorectal cancer.

CONCLUSIONS

Advances in delaying the development of colorectal carcinoma have been shown in patients with FAP with celecoxib treatment. However, the use of COX-2 inhibitors in the primary prevention of sporadic colorectal cancer is being studied in several ongoing trials. Current and future trials using celecoxib alone or in combination with chemotherapy and other biologic therapies are targeting several cohorts, including children with APC mutations, patients with FAP, HNPCC, prior colorectal adenoma, or prior history of sporadic adenomas. The use of celecoxib in the prevention of polyps has resulted in continued efforts to define a high-risk population and to implement a chemo preventive agent in the treatment of cancer. With regard to aspirin

use in the prevention of colon adenomas, two large randomized, placebo-controlled trials showed benefit. However, although the Aspirin/Folate Polyp Prevention Study and the Colorectal Adenoma Prevention Study reported positive results, a certain percentage of patients receiving aspirin intervention still developed colon adenomas. This suggests that aspirin use cannot be a substitute for colon surveillance and that further studies are necessary for effective colon cancer chemoprevention.

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NEWS

A 9- PROTEIN RISK SCORE CAN EVALUATE THE CARDIOVASCULAR RISK

Myocardial infarction, stroke, heart failure are major pathology which occur among patients with heart disease. In a prospective cohort study, researchers have assessed the cardiovascular risk by different causes by determining the level of plasma proteins. The study included patients with stable coronary heart disease divided in two cohort: outpatients from San Francisco which are followed ≤11.1 years and Norwegian patient which are followed 5.6 years. Researchers tracked the occurrence of heart attack, stroke, heart failure and determined plasma levels of 1130 proteins. After statistical analysis, Peter Gunz and colleagues have validated a 9-protein risk score for 4-year probability of myocardial infarction, stroke, heart failure, and all-cause death. The results was compared with the refit Framingham secondary event risk score in predicting cardiovascular events and showed that the 9-protein risk score performed better than the refit Framingham score. Authors suggested that additional studies are needed to determine the accuracy of this score depending on levels of cardiovascular risk in patients with stable CHD (Peter Ganz, Bettina Heidecker, Kristian Hveem, Christian Jonasson, Shintaro Kato, Mark R. Segal, David G. Sterling, Stephen A. Williams. Development and Validation of a Protein-Based Risk Score for Cardiovascular Outcomes Among Patients with Stable Coronary Heart Disease. *JAMA*. 2016;315(23):2532-2541).

Cătălina Luncă

MUSCULOSKELETAL IMPAIRMENT IN PRYMARY HYPOTHYROIDISM

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MUSCULOSKELETAL IMPAIRMENT IN HYPOTHYROIDISM (Abstract): This article contains bibliographical data concerning the rheumatic clinical manifestations in hypothyroidism: polyarthralgias, lack of recent skill of fine movements of the hands, carpal tunnel syndrome or tarsal, degenerative arthropathy or acute type (gout, chondrocalcinosis), adhesive capsulitis (frozen shoulder syndrome), generalized muscular stiffness, hypothyroid myopathy, secondary osteoarthritis, Dupuytren's contracture, "trigger finger" (also called as stenosing tenosynovitis or trigger thumb) etc. and data on the short history, epidemiology, of these disorders. Review include 60 bibliographical sources. **Keywords**: PRIMARY HYPOTHYROIDISM, MUSCULOSKELETAL IMPAIRMENT, RHEUMATIC DISEASES.

Thyroid hormones (THs) are vital role for maintaining the body's homeostasis. Thyroid hormones are among the key regulators of metabolism, adjusting the amount of proteins, carbohydrates, fats and minerals used by the body (1). Thyroid hormones play an important role in the development, maturation and maintaining morpho-functional integrity of the musculoskeletal structures (2). At the same time the decreasing synthesis of thyroid hormones leads to multiple negative effects on the entire system of the body functioning (1). Hormonal deficiency has unfavorable effects on bone formation and modeling, with consequences on the neuromuscular function and bone density. The relationship between musculoskeletal impairment and those of thyroid contains more aspects, among which, is, certainly, the imitation of the rheumatic diseases (2).

Currently thyroid gland pathology is considered one of the most common endocrine diseases in structure (3). A major endocrine pathology, involving various organs, frequently associated with musculoskeletal impairment presents, primary hypothyroidism (hT) (4, 5, 6, 7, 8, 9).

SHORT HISTORY

The relationship between arthritis and thyroid disease is known for a long time, in particular, the appearance of association with hypothyroidism (10). It is described for the first time in 1892, as the hypothyroid myopathy with endemic cretinism in children by E. Cocher, subsequently, defined as Kocher-Debré-Sémélaigne syndrome. In 1897, Johann Hoffman, for the first time, described a similar syndrome in adults, in a patient after thyroidectomy, which was later named in honor of this

researcher (11, 12). The relationship between thyroid disorders and shoulder pain was noticed in the first half of the 20th century (13). In 1960 was described the arthritis association with subclinical hypothyroidism, in the same time with the introduction of radioimmunoassay examination for thyroid stimulating hormone (TSH). The description of thyroid autoantibodies in the 50-s and 60-s of the last century forms a new stage in association of autoimmune thyroid disease (AIDT) with other diseases (14).

EPIDEMIOLOGY

In the Republic of Moldova, the thyroid pathology is ranked at the moment, the second disease as frequency among endocrine affections, leaving the first place only for diabetes. Comparing to year 2000, by 2006 the number of patients with thyroid pathology doubled (15). According to the data of specialized literature, 2-6% of the population suffer from hypothyroidism (16). The prevalence of subclinical hypothyroidism in the general population ranges between 1,3% -17,5%, depending on age and sex of the patient. The incidence of subclinical hypothyroidism is higher in women than in men, increasing with age and reaching 21%, in women, and 16% in men, aged over 74-years-old (17). Epidemiological studies indicate that the global prevalence of manifest hypothyroidism in population is 0,2-2%, subclinical 7-10% among women and 2,3% among men. In the group of older women, the prevalence of all forms of hypothyroidism can reach 12% or more (3). The estimates of the study conducted by NHANES (National Health and Nutrition Examination Survey) III highlight the prevalence of hypothyroidism in the US population which constitutes 4,6% in general population (0,3% clinical and 4,3% subclinical). In addition, this study found that prevalence rates increase with age and may approach up to 20% in women, from the general healthy population in the postmenopausal period (18). According to the results of a similar study conducted in Norway, the prevalence of diagnosed hypothyroidism is 4,8% in women and 0,9% in men (19).

According to I. Atroshi et al. (20) the prevalence of carpal tunnel syndrome (CTS) in the general population is 3,8%. However, M. Cakir and co-workers (6), reported that 9,5% of patients with thyroid disease have CTS, this phenomenon is more common among patients with hypothyroidism (30,4%). Women are affected more frequently (21). According to other studies, carpal tunnel syndrome occurs in approximately 30% of patients with hypothyroidism (22, 23).

Arthropathy occurs in 20-25% of patients with myxedema (8). Dupuytren's contracture, limited joint mobility and CTS are more common in patients with hypothyroidism, which are- 21,7%, 8,7% and 30,4%, respectively, and the "trigger finger "(TF), occurs in 10% of patients with subclinical hypothyroidism, which correlates with positive anti-thyroid peroxidase (anti-TPO) antibodies (6). Men are affected 7 times more often than women by Dupuytren's contracture, especially those aged over 40-years-old (24). The prevalence of fibromyalgia in AITD is determined at 30 to 40% of the patients (25, 26, 27).

CLINICAL MANIFESTATION

Rheumatic diseases are increasingly recognized as having an important role in de-

termining the health state of the population, and by diverse spreads, these diseases can be ranged from simple localized pain to the onset of disability movement (28).

Rheumatic clinical manifestations, produced by hypothyroidism are: polyarthralgias, lack of recent skill of fine movements of the hands (29), CTS (rarely tarsal), degenerative arthropathy or acute type (gout, chondrocalcinosis), adhesive capsulitis (frozen shoulder syndrome), generalized muscular stiffness, hypothyroid myopathy (2, 30), secondary osteoarthritis (OA) (31), Dupuytren's contracture, TF (also called as stenosing tenosynovitis or trigger thumb) (6), and of association with autoimmune diseases (vitiligo, rheumatoid arthritis (RA), Sjögren's syndrome, systemic lupus erythematosus (SLE), chronic active hepatitis, systemic sclerosis), which can dominate the clinical picture and serve as the only manifestation or symptom of the disease (32). According to V. Nasonova and N. Bunchyuk (33), in hypothyroidism, some patients may develop knee ligamentous weakness device, and Baker's cyst formation. Arthropathy is manifested by stiffness, joint swelling, sometimes the appearance of non-inflammatory joint effusion of articular cavity. Rheumatic manifestations can occur even in euthyroid patients, but more frequently in patients with hypothyroidism, in comparison with healthy persons without affecting the thyroid gland (34).

Carpal tunnel syndrome occurs when there is a compressing of the flexor tendons and the median nerve that travels through the carpal tunnel, patients usually present with pain, numbness or tingling in the median distribution (in the first two fingers and the side of the median third). These symptoms may worsen especially at night or at wrist flexion. In cases of prolonged physical activity**,** a weakness or atrophy of the thenar muscle may occur (35). Bilateral carpal tunnel syndrome has been reported in hypothyroidism by JC Jr. Chisholm (36), and an increased joint laxity, due to a hyaluronate deposits frequently found, this fact is reported by R. Ionescu (21).

Tarsal tunnel syndrome, with medioplantar nerve damage, is rarely observed (2). In these cases the patient complains pain such as burning and tingling in the big toe, planting area. Pains are nocturnal and may radiate to the back of the calf. The pain may improve while walking (24). The mechanism leading to compression is due to the accumulation of glycosaminoglycans in the surrounding tissues (2, 37).

Dupuytren's contracture is a painless condition that occurs marked thickening of the palmar aponeurosis, flexion contractures of the ring and little fingers (24, 38).

Stenosing tenosynovitis - is the most common cause of pain and disability at the level of hand. It affects flexor tendons of the fingers II-V and the long flexor of the thumb. Tenosynovitis occurs leading to fibrosis and constriction of the first fiber annular ring which covers the metacarpophalangeal (MCP) joint, being affected sliding tendon, causing pain and locking finger in flexion, especially in the morning, crepitations could be heard in the mobility (24).

Adhesive capsulitis is a painful condition, which is characterized by pain on movements and restriction in all dimensions, in the absence of joint damage. The progression of the disease has three phases:

• Painful phase- gradual onset of diffuse shoulder pain irradiated mobilization especially in the level of trapezius muscle. Sub-

sequently, the pain becomes nocturnal, waking the patient up from sleep for weeks - months;

• Adhesive phase (the stiffness). The pain becomes less severe, no longer appears in garaging or during the night, but still discomforting and feeling pain at the end of the movement. Limited movements of the gleno-humeral external rotation, internal rotation, and abduction becoming evident - up to one year;

• Resolution phase (thawing). The pain is less intense, the dominant symptom is limiting the movements of the shoulder.

A clinically useful indicator of the capsulitis presence is pain appeared during passive shoulder external rotation (21, 24).

Suffering pains. The simplest manifestations are the most common joint morning stiffness and eventual degenerative effusion. Accelerating speed of erythrocyte sedimentation rate for other reasons in some cases leads itself to confusion with rheumatoid arthritis (39). Rarely, the artropathy is monoarticulary or erosive, interestingly the interphalangeal joints can to mimic severe arthritis (40, 41). In some cases, calcium pyrophosphate dehydrate crystals were found in synovial fluid, but the condro-calcinosis association and its acute event (pseudogout) is not so frequent as in hyperparathyroidism and hemochromatosis (42). There are disputes over, whether or not there is random nature of the association between hT and chondrocalcinosis (39, 43, 44). However McCarty (45), a recognized researcher in chonrocalcinosis, says that the presence of calcium pyrophosphate dehydrate crystals in the joints is probably the most frequently associated with hT, and only then with hyperparathyroidism, hemochromatosis, and respectively, with hypophosphatasia. It was stated that in primary hT, in comparison with the general populations, there is indeed an increase in both the frequency of hyperuricemia and gout. In hT, it is affected kidney pool, by lowering the uric acid removal. hT was suspected as the trigger gout attacks (46). The symptomatology is alleviated through suppression of neutrophils (47). Hypothyroidism is linked to an increased incidence of fractures (48). It still remains controversial whether thyroid hormone replacement therapy also has adverse effects on bone. A review of 63 studies could not resolve the debate. Therefore, there is insufficient evidence on the effect of levothyroxine on the bone mineral density (BMD) (49).

After Bland et al. (50), in hT is usually affecting knees, MCP and proximal interphalangeal (PIP) joints, and as well as metatarsophalangeal (MTP), talocrural (TC) joints, without the presence of inflammatory synovitis. These impairments depend on TSH increase of the hyaluronic acid and the synthesis of proteoglycans in patients with hT (8, 50). Most cases of arthropathy change in hypothyroidism are found in adult, involving knees and hands, while the hip and femoral epiphysis occurs more frequently in children (7). Usually in hT, the syndrome of dolor palpation is missing. Generally, it affects symmetrically the knees, radiocarpal joint, small joints of the hands (PIP and MCP) and MTP joints. In some cases, there is a destructive arthropathy localized mainly in the PIP joints and less distal interphalangeal (DIP) joints (33).

In secondary OA, patients have "mechanical" type pain, stiffness from inactivity, limiting mobility, joint instability, crackling or grating sensation (crepitus)

highlighted by palpation or audible (31). In adults with hypothyroidism, joint pain can reveal an aseptic bone necrosis. Rubinstein and Brooks (51), described five patients with hypothyroidism, who were evaluated for a period of time ranging from 1 to 16 years-old, experiencing joint pain, which later were diagnosed with radiological and histological with aseptic bone necrosis. The bones involved were unilateral or bilateral femoral neck in three patients and two - to carpal bones (lunate bone or crescent) (51). Pretibial myxedema is presented in the form of painless nodules of pink or bright red color, of various sizes, being placed on the pretibial surface, which can have a similar aspect to *erythema nodosum* (21).

Muscle myopathy (hypothyroidism). In this condition, the most often reported symptoms by patients are: pain, cramps, stiffness, slight fatigue, and muscle weakness. On physical examination can be revealed muscular hypertrophy, proximal muscle weakness, delayed deep relaxation phase of tendon (52). At the initial examination, there is an increase in muscle mass of several groups of muscles, including the trapezius, deltoid, rectus capitis, and gastrocnemius (32). Stiffness and muscle weakness in myopathy are diffuse (53, 54). Generalized myalgias, when accompanied by painful trigger points may suggest this diagnosis, but these nonspecific symptoms may also be an initial hT phase (55, 56). Muscle pain and a feeling of "numbness" of the muscles are frequently and aggravated by cold exposure (57). A rare clinical disorder of myopathy in severe hT is Hoffman syndrome, which is manifested by severe muscle stiffness, increased muscle mass (pseudohypertraphy), variable muscle weakness, is accompanied by high levels of creatine kinase (58). Among of muscle symptoms of - muscle proximal myopathy (hypothyroid myopathy) occurs in 25-79% of cases in adult patients with primary hypothyroidism (22, 35, 59). According to Alakes's studies et al. (4), highlighted musculoskeletal manifestations in 120 patients with primary hypothyroidism constituted: bodily pain and myalgia - 83,33% of patients, muscle cramps and stiffness - 83,33%, back pain - 50%, arthralgia - 25%, adhesive capsulitis - 20,8%, limited joint mobility - 16,67%, myopathy - 8,33%, CTS - 6,6%, TF- 4,1% of Dupuytren's contracture - 3,33% and tarsal tunnel syndrome in 1,6% of patients. Associated rheumatologically manifestations were determined as follow: osteoarthritis - 66,7% of cases, mild inflammatory arthritis involving wrist joint - 50%, RA - 16,67%, SLE - 8,33%, fibromyalgia - 5%, Raynaud's phenomenon - 1,66% and the mixed connective tissue disease - at 1,66% of patients (4). A study conducted by Soy Mehmet et al. (60), in a group of 65 patients who were diagnosed with autoimmune thyroiditis, including 70% - with hypothyroidism demonstrated that the recorded rheumatic symptoms were as diverse as fibromyalgia - 34%, recurrent aphthous stomatitis - 18%, OA-12%, keratoconjunctivitis sicca and xerostomia - 10%, CTS - 10%, Raynoud's syndrome - 6%, inflammatory arthritis - 6%, autoimune - diseases 18%.

CONCLUSIONS

The relationship between hT and rheumatic diseases is significant and essential in the diagnosis and treatment of patients. The knowledge of spectrum of extra glandular clinical manifestations (including osteo-articular manifestations) is an im-

portant step in the management of patients with hT. However, thyroid dysfunction should be considered in the differential diagnosis in different musculoskeletal manifestations but the investigations of antithyroid antibodies should be a component part of screening the patients with joint problems, because they can serve as early signs at the presence and prognosis of the disease.

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ANEMIA IN INFLAMMATORY BOWEL DISEASE MORE THAN AN EXTRAINTESTINAL COMPLICATION

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ANEMIA IN INFLAMMATORY BOWEL DISEASE: MORE THAN AN EXTRAINTESTINAL COMPLICATION (Abstract). The most common hematologic complication of inflammatory bowel disease (IBD) - ulcerative colitis and Crohn's Disease is anemia. Anemia in patients with IBD may be a result of iron, vitamin B12 or folate deficiency; anemia of chronic disease and hemolytic anemia are other causes in these patients. Factors contributing to the development of anemia include chronic gastrointestinal blood loss, vitamin B12 malabsorption secondary to terminal ileitis, folate deficiency as a result of sulfasalazine therapy. Approximately 30% of patients with IBD have hemoglobin levels below 12g/dl. The risk of developing anemia relates to disease activity, given that blood loss and inflammatory anemia are triggered by intestinal inflammation. In the management strategy of IBD patients with anemia it is important to distinguish between the different types of anemia in order to decide an appropriate manner of treatment. **Keywords:** INFLAMMATORY BOWEL DISEASE, ANEMIA, IRON DEFICIENCY, INFLAMMATORY ANEMIA.

Crohn's disease and ulcerative colitis are chronic inflammatory diseases of the gastrointestinal tract, mainly affecting the small intestine and colon, which progress with relapse and remission (1). IBD is associated with extraintestinal manifestation in more than 25% of cases, including malnutrition and anemia (1, 2).

Anemia is a clinically significant state which could impair quality of life or work capability and it represents a serious comorbid condition associated with other diseases or even death (1, 3). There are two major types of anemia in IBD patients: iron deficiency anemia and inflammatory anemia called until recently anemia of chronic disease. Moreover, mixtures of the two types are commonly found in these patients (3, 4, 5).

Epidemiology of anemia in IBD. The reported prevalence of anemia in IBD is highly variable ranging from 15% up to 75% depending both on patient subgroup and definition of the disease. In hospitalized patients to prevalence is up to 70%, while in outpatients the reported prevalence is only 15% (4, 6).

Clinical manifestation. Patients may present with a diversity of symptoms and

sign among which the most relevant are weakness, fatigue, dyspnea, tachycardia, headaches. These signs overlap the sigs of the underlying disease (7, 8, 9) (tab. I).

Causes of anemia in IBD. The main types of anemia in IBD patients are iron deficiency anemia and inflammatory anemia or anemia of chronic disease (3).

TABLE I

Clinical consequences of anemia in IBD patients

Fatigue	**Motility disorder**	**Impairments in quality of life**
Dyspnea	**Nausea**	**Loss of libido / impotence**
Tachycardia	**Anorexia**	**Impairments in physical condition**
Headache	**Menorrhagia/amenorrhea**	**Reduce energy efficiency during physical activity**
Dizziness	**Weight loss**	**Impairments in cognitive function**

Iron deficiency anemia mainly results from chronic intestinal blood loss due to chronic inflammation of the intestinal mucosa and from reduced iron uptake from enterocytes (impaired absorption) (6, 7). Intestinal inflammation leads to a decreased serum iron level and therefore to a reduced availability of iron for erythropoiesis (6, 9). In addition, inflammatory status causes decreased iron uptake from the intestinal mucosa, hence, providing a multipart two-way pathophysiologic mechanism between iron deficiency and inflammation (3, 4).

In patients with ileal Crohn's disease, absorption of vitamin B12 and/or folate may be impaired and all these condition frequently overlap (6, 10). Similarly, anemia in IBD patients may be induced by specific therapy-thiopurines, 5-ASA derivatives and/or by hemolysis (6, 9, 11) (tab. II).

Anemia workup. Full blood count, serum ferritin and C-reactive protein (CRP), are minimum requisite to identify anemia, an inflammatory relapse or iron deficiency at an early stage (7). Diagnostic measurement of blood count and CRP has been part of screening recommendations in IBD patients (11, 12). Patients in remission should be followed-up every 6 to 12 month, while active disease needs measurements at least every 3 months (11). Patients with extensive ileal Crohn's disease, ileal resection or ileal-anal pouch need proper follow-up. Serum vitamin B12 and folic acid levels should be measured every 6 to 12 month (7, 8, 9) (tab. III).

TABLE II

Etiology of anemia in IBD patients

Iron deficiency	**Regular**
Anemia of chronic disease	
Vitamin B_{12} deficiency	**Common**
Folate deficiency	
Drug induced 5-ASA; Thiopurine	
Hemolysis	**Infrequently**
Myelodysplastic syndrome	
Aplastic anemia	

The initial workup of anemia starts with the evaluation of MCV in order to distinguish between microcytosis (iron restricted anemia - iron deficiency and/or inflammatory anemia) and macrocytosis (B12 or folate deficiency) and also normocytosis (anemia of chronic disease) (8).

Platelet and white blood cell counts should be measured to reveal pancytopenia (12).

Serum ferritin level reflects the iron stores. Iron deficiency anemia is present when serum ferritin level is below 30 μg/L and hemoglobin level below 12g/L for non-pregnant females and below 13g/L for males (8).

Therefore, serum ferritin level can be used to detect iron deficiency; a drawback is that ferritin is a marker of acute inflammation and has to be evaluated together with CRP, as elevated ferritin may be due to a flair of inflammation or a superimposed infection (8, 12).

TABLE III
Serum markers of anemia in IBD patients

Mean corpuscular volume (MCV)
CRP
Serum Vitamin B_{12}
Serum Folate
Ferritin
Transferrin
Transferrin saturation (T-Sat)
Hepcidin
Soluble transferrin receptor concentration (StFR)

The diagnosis of iron deficiency anemia in IBD patients may be difficult mainly when both iron and inflammatory anemia are present. In this case, laboratory measures of iron status may be unreliable, as the presence of intestinal inflammation affects the parameters of iron metabolism (12).

In IBD patients with anemia even transferrin concentration may not be elevated as in pure iron deficiency anemia, due to the low albumin levels found in patients with intestinal inflammation (13).

Active IBD determines elevated hepcidin levels. Hepcidin blocks intestinal iron absorption and also reduces the release of iron from stores resulting in inflammatory anemia (11, 14).

Thus, a serum ferritin level between 30 and 100 μg/L in the presence of anemia and high CRP level indicates a mixture of iron deficiency and inflammatory anemia (14). On the contrary, a serum ferritin level over 100 μg/L together with anemia and elevated CRP level indicates pure inflammatory anemia (14).

T-Sat is a measure of the iron content in the circulating transferrin. A T-Sat below 16% indicates a suboptimal source of iron for erythropoiesis (14). T-Sat level provides an indirect indication of the degree of iron use in the bone marrow but does not provide evidence of the iron stores (2). In IBD, T-Sat is used in the anemia workup in combination with ferritin and CRP, at a lower cut-off point <16% (along with ferritin level <30 μg/L suggests iron deficiency) (13).

Testing for increased **s**TfR distinguishes between these two types of anemia but it is not generally available yet. A high diagnostic power of sTfR or sTfR/log ferritin ratio in differentiating iron deficiency from inflammatory anemia has been suggested by several studies (13).

Drugs commonly used in IBD treatment may have myelosuppressive effects: direct (thiopurine) or indirect (antifolic effect of sulfasalazine) (11). Sulfasalazine inhibits erythropoiesis by different mechanisms: hemolysis, aplasia and/or folate absorption (12, 13). A mild anemia may be detected in patients treated with thiopurine drugs (aza-

thioprine or mercaptopurine) (8, 14).

Management of anemia in IBD. The correlation between disease activity and level of anemia is well known. Consequently, the most significant measure in the treatment of patients with IBD and anemia is the specific treatment of the underlying disease (12, 15).

Inflammatory anemia is a common cause of anemia among hospitalized IBD patients with active disease. Treatment of non-iron deficiency anemia may include correction of IBD specific treatment, nutritional supplements, treatment of infections and, in selected cases, erythropoiesis-stimulating agents (13, 14).

In IBD patients on biological treatment (infliximab, adalimumab) the response to therapy has been shown to determine improvement of erythropoiesis, by significantly increasing the serum transferrin level (2). Colectomy is reserved for intractable, anti-TNFα-resistant ulcerative colitis. Folic acid and vitamin B12 deficiencies, another cause of non-iron deficiency anemia, should be treated in order to avoid anemia especially in patients with Crohn's disease, ileal resection and ileal-anal pouch (14).

Red blood cell transfusion may be considered when hemoglobin is < 7g/dL or above if risk factors and/or fatal comorbidities are present (9, 15). Nowadays transfusions in IBD patients are restricted to the severe attack of ulcerative colitis with severe anemia and hemodynamic instability, surgery for Crohn's disease, in the presence of severe comorbidities (14, 15).

Iron replacement treatment should be started immediately after iron deficiency anemia is diagnosed. Treatment options for iron deficiency anemia are intravenous iron supplementation or oral iron administration (2, 16) (fig 1).

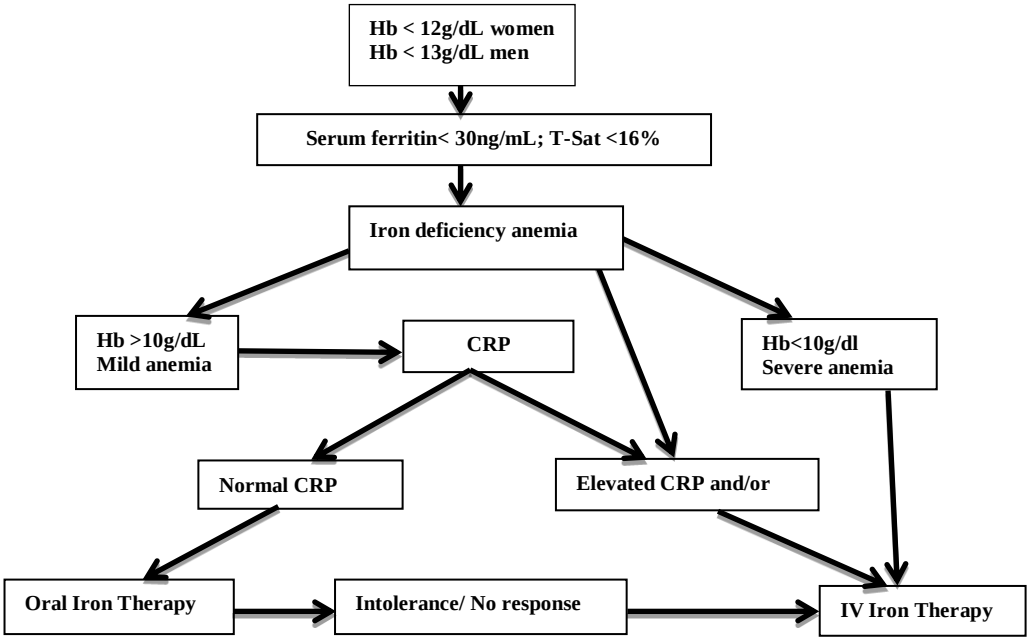

Fig. 1. Treatment strategies for IBD patients with iron deficiency anemia.

The therapeutic goals in iron deficiency anemia are to normalize hemoglobin, ferritin, and T-Sat levels, replenish iron stores (ferritin>100 μg/L) and improve quality of life (17). After the initiation of martial therapy, reticulocyte count will rise in the next 7-9 days and hemoglobin in one month (18).

Oral iron therapy is indicated in patients with mild anemia, hemoglobin > 10g/dL and remission in which oral iron absorption is not affected (17). There are many limitations of oral iron therapy: intolerance, noncompliance, worsens disease activity (14, 19).

Therefore, intravenous iron is the treatment of choice in iron deficiency anemia. According to the guidelines and consensus statement, in IBD patients intravenous iron supplementation is the preferred route. The advantage of intravenous iron therapy is better tolerance, compliance (usually single dose) and rapid reversal of anemia (18, 20, 21).

Ganzoni formula is useful to estimate iron requirements (2): Iron deficit (mg) = Body weight (kg) x [target Hb - actual (g/dL] x 2.4 + iron stores (500mg)

CONCLUSIONS

Our analysis of the literature studies revealed that anemia is the most common extraintestinal complication of IBD. It can increase the hospitalization rate in IBD patients and can affect quality of life and work capability.

An important goal for the IBD practitioner is to use anemia markers in order to accomplish the highest diagnosis yield of anemia in IBD patients. Anemia in IBD is usually a complex manifestation of a mixed type of iron deficiency and inflammatory conditions. The gastroenterologist should keep in mind other causes of anemia especially in small intestine involvement- vitamin B12 and folate deficiency. The pathophysiological causes of anemia in IBD include malabsorption, malnutrition, inflammation, intestinal resection, drug adverse events.

Iron deficiency anemia is common in IBD patients and is difficult to diagnose in the presence of inflammation. Intravenous iron therapy is a preferable option especially in active IBD patients and moderate-severe iron deficiency anemia.

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ENDOTHELIAL DYSFUNCTION ASSESSED BY LASER DOPPLER POST-OCCLUSIVE HYPEREMIA IN CHRONIC KIDNEY DISEASE PATIENTS

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ENDOTHELIAL DYSFUNCTION ASSESSED BY LASER DOPPLER POST-OCCLUSIVE HYPEREMIA IN CHRONIC KIDNEY DISEASE PATIENTS (Abstract): This study was intended to investigate the microcirculation endothelial dysfunction through a laser Doppler method (PORH post-occlusive reactive hyperemia) in patients with chronic kidney disease. **Materials and methods**: The study included 299 patients diagnosed with chronic kidney disease, which were divided into three groups: hemodialysis patients, peritoneal dialysis patients and patients with chronic kidney disease stages 3-4. Endotelial dysfunction was assesed with the Laser Doppler Perfusion Monitor type device (Periflux System Monitor 5001). **Results:** The values of the parameters we evaluated with postocclusive reactive hyperemia were altered in all patients with chronic kidney disease. In the dialysis groups there was no statistically significant correlation between parameters we tested by measuring PORH and the method of renal replacement therapy or dialysis duration. The same was observed in patients with pre-dialysis chronic kidney disease. **Conclusions:** Post-occlusive reactive hyperemia, a novel method for assessing endothelial dysfunction on the microcirculatory system and early marker of cardiovascular damage requires more studies to validate in patients advanced renal disfunction. **Keywords**: CHRONIC KIDNEY DISEASE, ENDOTHELIAL DYSFUNCTION, POST-OCCLUSIVE REACTIVE HYPEREMIA.

Chronic Kidney Disease (CKD) is characterized by structural or functional abnormalities of the kidneys, with a minimum duration of three months, which has serious consequences on the health of the individual, in the initial stages most patients being asymptomatic. Chronic kidney disease has long been under-diagnosed (1). Chronic kidney damage is a cardiovascular risk factor, which is associated with high morbidity and mortality, decreased quality of life and increased costs of health and social care (2). For this reason, in the last years, there was a significant increase in the interest of the early detection of kidney damage. The substrate of the initial changes in CKD, one of the most important cardiovascular risk factors, is represented by the endothelial dysfunction in close relationship with oxidative stress, inflammation and atherosclerosis (3,4). Endothelial dysfunction is an early phenomenon in atheromatosis, which precedes structural changes and clinical manifestations. For this reason, early detection of endothelial dysfunction is of major importance, since it

represents the initial event of atherogenesis (5,6). The detection and understanding of vascular changes may contribute to the development of therapeutic strategies to delay, stop or even regress vascular lesions and therefore have a particularly important impact on the clinical outcome of patients with renal impairment (7.8). The objective of the study was to assess endothelial dysfunction in advanced CKD patients using laser-Doppler post-occlusive reactive hyperemia (PORH).

MATERIALS AND METHODS

The study included 299 patients diagnosed with different CKD stages. The subjects were divided into 3 groups: 1) Stage 5 CKD patients, who were treated by hemodialysis (HD), 3 sessions / week, 4 hours/session, in "Dr. C.I. Parhon" dialysis center; 2) Stage 5 CKD patients, who were on peritoneal dialysis (CAPD – continuous ambulatory peritoneal dialysis); 3) patients diagnosed with CKD stages 3-4 who were periodically evaluated in the Nephrology Ambulatory of "Dr. C.I.Parhon" Hospital Iasi. The exclusion criterias were: patient's age <18 years; refusal to participate; psychiatric disorders that may affect patient compliance; dialysis duration <3 months; terminal stage malignancies; patients who had chemotherapy in the past 12 months; debilitating disorders (neurodegenerative diseases); systemic infections; acute coronary syndromes or strokes in the last 3 months. Each participant has completed a data acquisition paper.

Endothelial dysfunction was evaluated by a non-invasive laser Doppler method. In the study, we used the device Laser Doppler Perfusion Monitor (LDPM) type Periflux System 5001 (Perimed, Sweden). This device uses a low power laser signal with wavelength of 780 nm. For measurements we used the minimum value of the constant of time (0.2 seconds) and for the calibration, the manufacturer's calibration kit. Registration and data processing were done using the software offered by the manufacturer (Perisoft, Perimed). LDPM Monitor uses two optical fibers: one is the laser signal source for the skin, and the second fiber receives the reflected light from the tissue. The signal strength of the received laser signal depends on blood perfusion in that tissue.

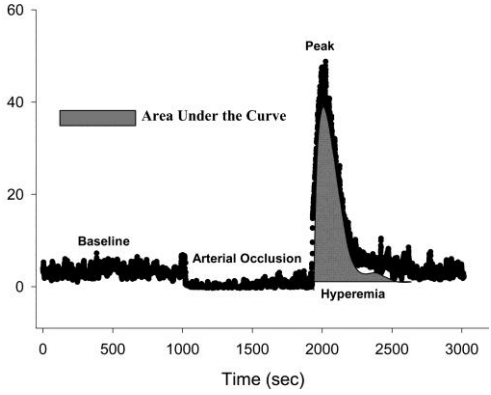

Fig. 1. PORH (Post Oclusive Reactive Hyperemia) measurement

PORH evaluation takes 9 minutes: the first 3 minutes we measure the baseline arterial blood flow in the forearm; next we practice the sudden occlusion of blood flow by inflating the sphygmomanometers' cuff, which is maintained for a further 3 minutes; then we immediately release the tensiometer's cuff measuring the blood flow for another 3 minutes. Parameters measured by this method are: RF (resting flow - basal arterial blood flow), BZ (biological zero - blood flow measured during obstruction), PF (peak flow - maximal arterial flow achieved after abrupt cessation of occlusion), TR (time to resting flow - the

time measured from the termination of the obstruction until the return of the blood flow to the basal value), TM (time to maximum flow- length of time from the release of the cuff until achieving maximal blood flow), TH (time to half recovery- length of time from the time of cessation of the obstruction until blood flow reaches the middle value between the maximum and the basal flow) (Fig. 1).

RESULTS

The average age of enrolled patients was 55.91+/-15.11 years; 46.5% (n = 139) were males. In the hemodialysis group (n = 168, 56.2% of subjects included in the study), mean age was 55.13 +/-14.27 years, the majority being males, 51.2% (n = 86). In peritoneal dialysis group (n = 73, 24.4% of all patients), mean age was 54.85 +/-15.06 years, the majority being females (64.4%). In the group of patients not on dialysis average age was 59.48 +/-17.17 years (Table I). There were no statistically significant differences between groups in terms of mean age (p = 0.132).

TABEL I

Description of the enrolled patients

		TOTAL	DIALYSED		NON-DIALYSED
			HD	PD	CKD
Number of patients		299(100%)	168(56,21%)	73(24,4%)	58(19,4%)
Average age		55,91+/15,11	55,13+/-14,27	54,85+/15,06	59,48+/17,17
Gender (%)	M	46,5% (139)	51,2% (86)	35,6% (26)	46,6% (26)
	F	53,5% (160)	48,8% (82)	64,4% (47)	53,4% (31)

The parameters of endothelial dysfunction in patients with chronic kidney disease

The detailed results of PORH parameters are presented in table II. In patients with peritoneal dialysis mean RF value was 32,33 significantly higher than in the other two groups (F = 7.44, p = 0.0007, 95% CI). There were no statistically significant differences between the RF values of hemodialysed versus non-dialysed patients (post-hoc LSD test, p = 0.904) (Table II, Fig.2).

We found statistically significant differences of BZ values between hemodialysed patients (5.44 ± 3.08) and peritoneal dialysed patients (6.88 ± 6.49) (post-hoc LSD test, p = 0.017) (Table. II, Fig. 3).

No statistically significant differences were found between PF values in hemodialysis, peritoneal dialysis and pre-dialysis patients (F = 0.709, p = 0.492) (Table II, Fig. 4).

We found statistically significant differences between the TR values in patients not on dialysis versus hemodialysis (post-hoc LSD test, p = 0.03) (Table II, Fig.5).

In non-dialysed patients TH values were significantly higher compared to TH values of patients on hemodialysis or peritoneal dialysis (post-hoc LSD test, p = 0.018). (Table II, Fig.6).

There were no statistically significant differences between the TM values in patients on hemodialysis or peritoneal dialysis compared to patients not on dialysis (F = 7.44, p = 0.0007) (Table II, Fig.7).

TABLE II

Post-oclusive reactive hyperemia parameters in all groups.

	Total	Hemodialysis patients	Peritoneal dialysis patients	Non-dialysed patients	ANOVA between groups comparisons	
	mean± SD (95%CI)	mean± SD (95%CI)	mean± SD (95%CI)	mean± SD (95%CI)	F	P
RF	18,1±37,63 (13,64-22,57)	13,1±6,58 (12,1-14,1)	***32,33±70,65 (15,84-48,81)**	12,27±6,06 (10,15-14,38)	7,44	<0,001
BZ	5,85±4,33 (5,34-6,36)	5,44±3,08 (4,97-5,91)	***6,88±6,49 (5,37-8,39)**	5,67±3,51 (4,45-6,9)	4,89	0,047
PF	60,66±154,03 (42,38-78,95)	59,79±184,35 (31,71-87,87)	74,09±103,75 (49,89-98,3)	36,15±22,41 (28,33-43,97)	0,709368	0,49
TR	2,02±13,2 (0,45-3,59)	1,07±3,93 (0,47-1,67)	2,14±9,34 (0,04-4,32)	***6,45±33,95 (5,39-18,3)**	9,895171	<0,001
TH	2,76±9,06 (1,69-3,84)	1,48±1,28 (1,29-1,68)	4,46±8,78 (2,41-6,5)	***5,46±21,98 (2,21-13,13)**	4,567447	0,01
TM	12,25±21,56 (9,69-14,81)	12,17±22,76 (8,7-15,64)	12,62±18,19 (8,37-16,86)	11,84±22,68 (3,93-19,76)	0,017638	0,98

*Significantly different from the other two groups

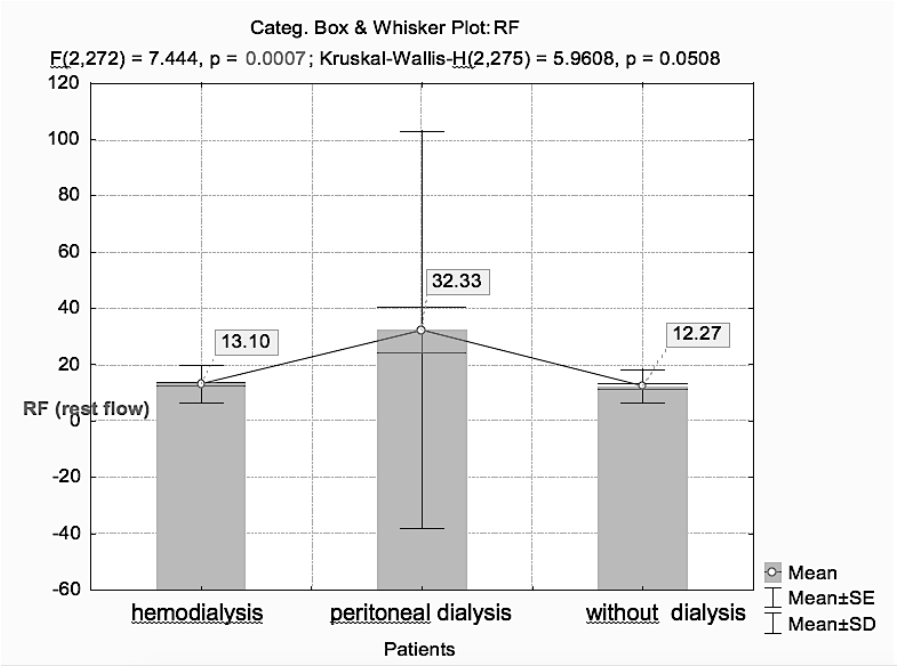

Fig. 2. RF (resting flow- basal arterial blood flow) values for CKD patients

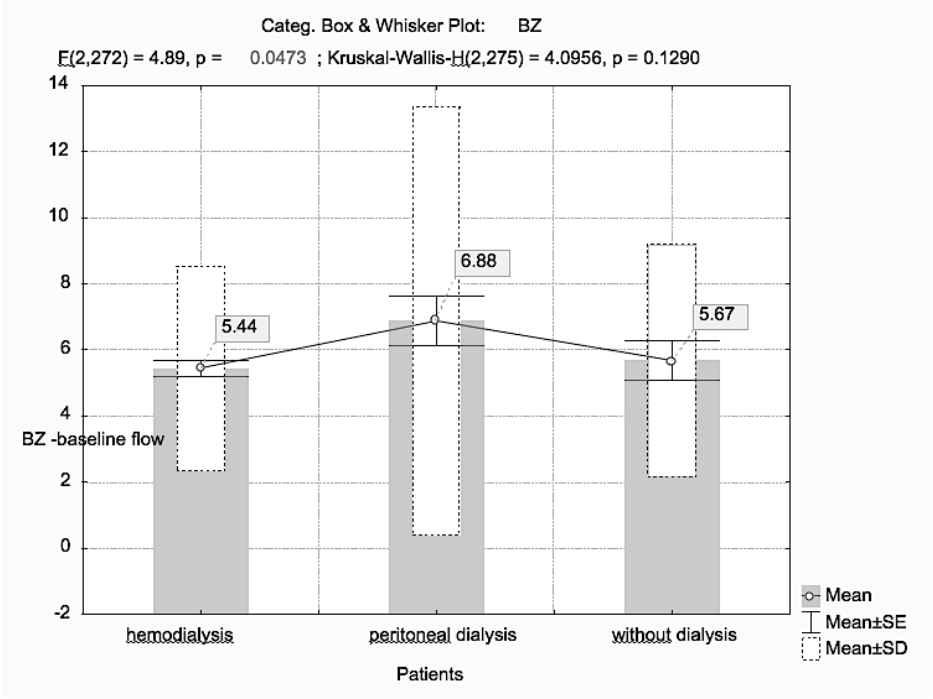

Fig. 3. BZ values of CKD patients

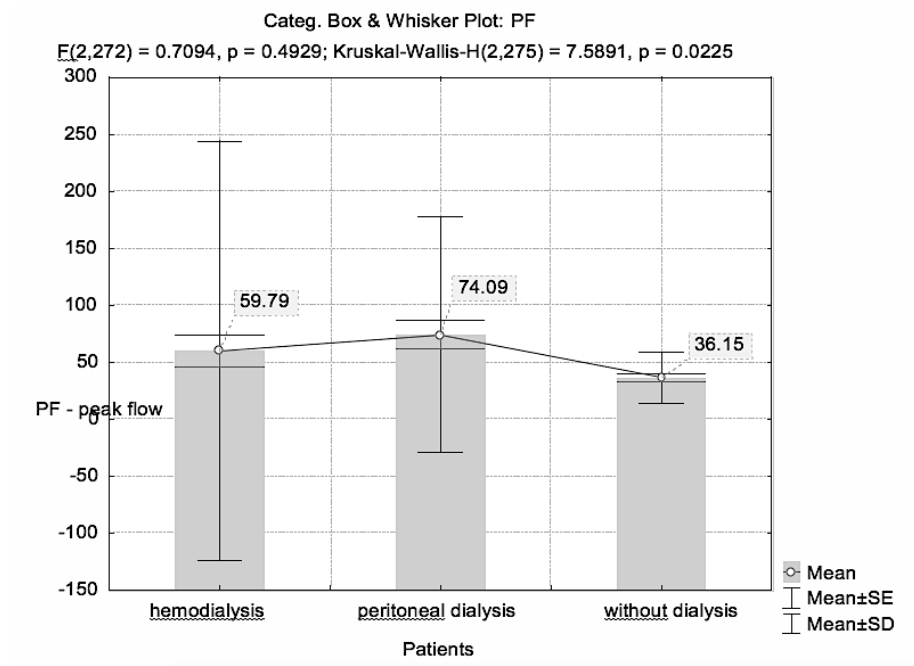

Fig. 4. PF values in CKD patients

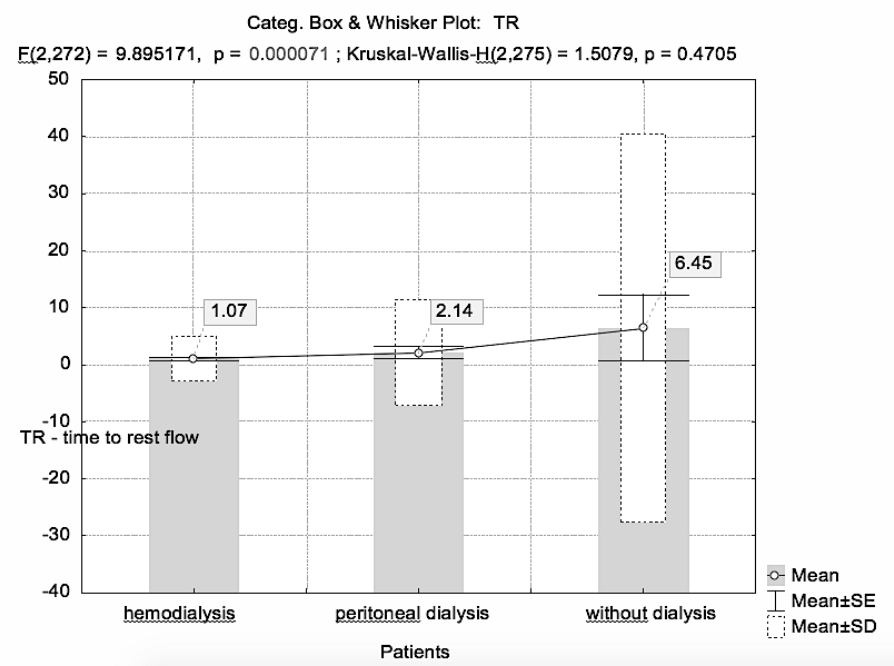

Fig. 5. TR values in CKD patients

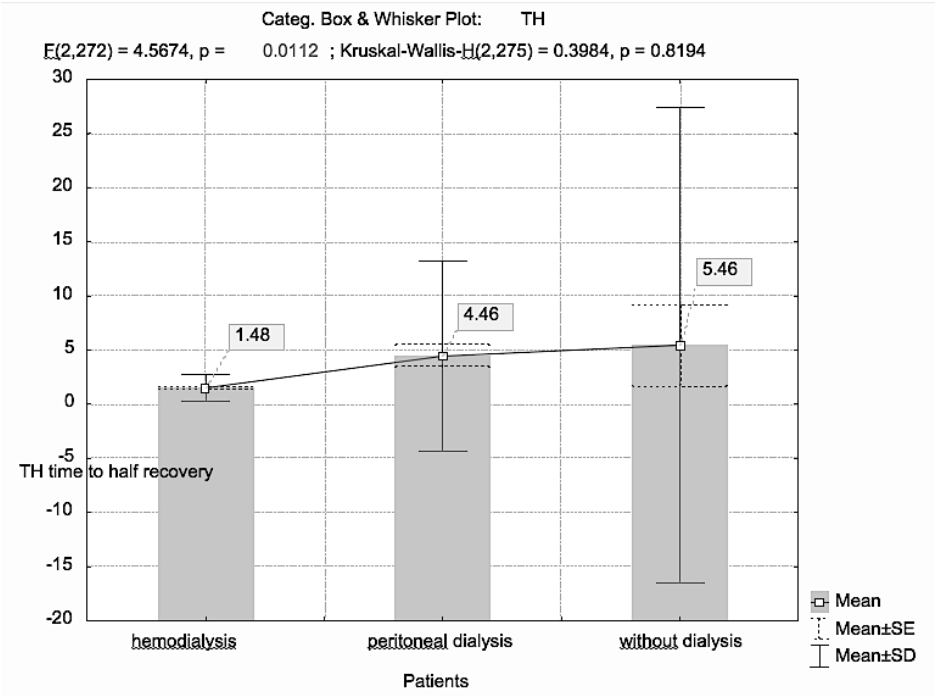

Fig. 6. TH values in CKD patients

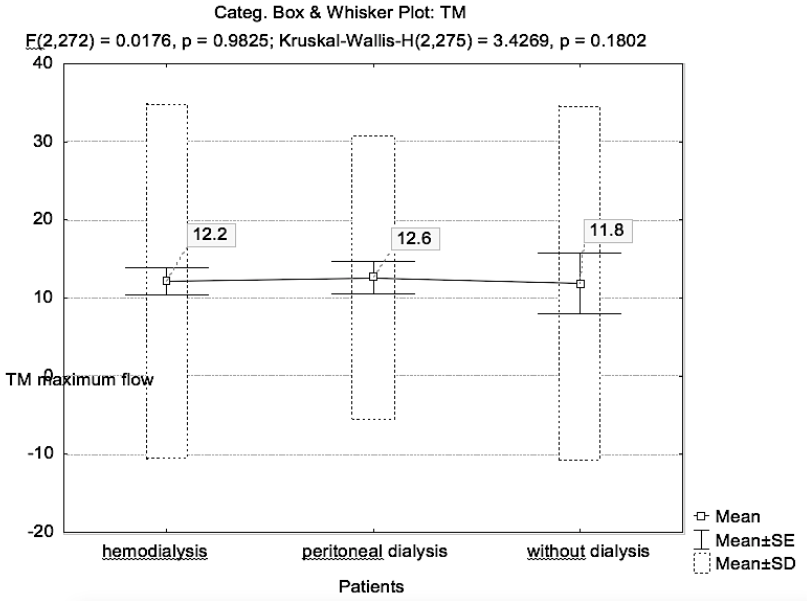

Fig. 7. TM values in CKD patients

The relationship between the duration of disease and post-occlusive reactive hyperemia (PORH)

Among dialysis patients (HD and CAPD) and non-dialysed patients there were no statistically significant correlations between PORH values and CKD or dialysis duration (Table III).

TABEL III

PORH values and CKD/dialysis duration

Spearman's rho		PORH			CKD duration			Dialysis time	
		HD	**DP**	**CKD**	**HD**	**DP**	**CKD**	**HD**	**DP**
BZ	N	168	73	58	168	73	58	168	73
	Corelation coef.	1,000	1,000	1,000	-,122	,166	-,032	-,122	,127
	p	-	-	-	0,115	0,161	0,807	0,116	0,285
PF	N	168	73	58	168	73	58	168	73
	Corelation coef.	1,000	1,000	1,000	-,034	-,003	-,019	-,052	-,093
	p	-	-	-	0,658	0,981	0,888	0,502	0,432
RF	N	168	73	58	168	73	58	168	73
	Corelation coef.	1,000	1,000	1,000	-,037	-,037	,033	-,061	-,010
	p	-	-	-	0,630	0,758	0,807	0,434	0,936
TM	N	168	73	58	168	73	58	168	73
	Corelation coef.	1,000	1,000	1,000	,026	-,026	-,127	-,031	-,114
	p	-	-	-	0,739	0,828	0,341	0,693	0,337
TH	N	168	73	58	168	73	58	168	73
	Corelation coef.	1,000	1,000	1,000	,097	-,088	,007	,050	-,035
	p	-	-	-	0,209	0,460	0,341	0,522	0,771
TR	N	168	73	58	168	73	58	168	73
	Corelation coef.	1,000	1,000	1,000	,065	-,084	,209	,000	-,021
	p	-	-	-	0,401	0,429	0,116	0,995	0,857

DISCUSSION

The link between chronic kidney disease and cardiovascular disease has already been demonstrated with chronic renal impairment beeing considered a major cardiovascular risk factor. Atherogenesis is one of the most important factors in this connection. Since endothelial dysfunction is a initiator phenomenon in atheromatosis and precedes structural changes and clinical manifestations, its early detection is crucial. Assessment of endothelial dysfunction can be done by invasive methods (determination of serum markers, plethysmography, termodilution method) or non-invasive methods done on macrocirculation (FMD – flow mediated dilatation) or microcirculation (PORH, iontophoresis) (9,10). The method used in this study has the advantage of being non-invasive, it can assess endothelial function in real time and it is also cheap and easy to be used in clinical medical practice.

In literature there are studies that investigated endothelial dysfunction on macrocirculation in patients with severe cardiovascular, metabolic and/or renal impairment. There are few data on the assessment of endothelial function on the microcirculation, mostly carried out on populations of diabetic patients and data are controversial (11, 14).

In a study on patients with chronic renal impairment, Stewart et. al, showed lower values of the parameters measured by PORH in subjects with renal pathology, but without being able to demonstrate the presence of significant differences between them and the group of subjects without renal impairment (12, 14).

Another study showed the presence of lower values for the parameters of post-occlusive reactive hyperemia in hypertensive patients with chronic kidney disease, and hypertensive patients with normal renal function, without being able to identify significant differences between these groups (13,14).

This study is one of the first to use Laser Doppler Post-occlusive Hyperemia in 299 patients in different CKD stages (predialysis and on dialysis). While no significant difference was found in “peak flow” and ”time to maximum flow” between the three groups, “time to half recovery” and “time to resting flow” were significantly higher in predialysis patients, while “resting flow” and “biological zero” parameters were higher in the peritoneal dialisys patients.

CONCLUSIONS

The present study showed that in patients with CKD there are changes in the values of the parameters evaluated by post-occlusive reactive hyperemia and these could be interpreted in the context of endothelial dysfunction in this population. For patients on hemodialysis and peritoneal dialysis, the correlation between the length of chronic kidney disease and dialysis time with the PORH parameters remained statistically insignificant, the same beeing observed in non-dialysed CKD patients, whose parameters were not statistically significant correlated with renal disease duration.

More studies are needed in CKD patients in order to validate this method of assessment of microcirculation endothelial dysfunction by PORH and to determine what parameter is a better predictor of future cardiovascular damage. The detection and understanding of early vascular changes in this population may contribute to the development of therapeutic strategies to delay, stop or even regress the vascular

lesions and therefore have a particularly important impact on the clinical outcome of patients with renal impairment.

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ASSESSING CARDIAC PERFORMANCE IN PATIENTS UNDERGOING CARDIAC RESYNCHRONIZATION THERAPY

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ASSESSING CARDIAC PERFORMANCE IN PATIENTS UNDERGOING CARDIAC RESYNCHRONIZATION THERAPY (Abstract). **Aim**. To analyze the effects (clinical, electrical and ultrasound) of cardiac resynchronization devices, with or without an implantable defibrillator (CRT-P or CRT-D) on the patients with heart failure in our clinic**. Material and Methods:** We retrospectively analyzed 56 patients with NYHA functional class II-IV heart failure who underwent CRT-P or CRT-D in our clinic in the interval January 1, 2010 – June 1, 2014. To evaluate the effects of cardiac resynchronization therapy (CRT) we focused on measuring the QRS complex duration on electrocardiogram, left ventricular ejection fraction (LVEF) and the NYHA functional class assessment. These parameters were assessed immediately before intervention and one month after device implantation. Patient characteristics were expressed as mean $^{+}/_{-}$ 1 standard deviation or as number and percentage. Variables were compared by paired t test. A p wave value less than 0.05 was considered statistically significant**. Results:** QRS complex duration decreased from an average of 155ms to 129ms, a statistically significant narrowing of 25.4ms ($p< 0.0001$). The LVEF increased from an average of 28% to 35% ($p < 0.001$). A statistically significant improvement ($p< 0.0001$) in NYHA functional class was recorded. **Conclusions:** CRT is an important therapeutic approach, in our study 82.56% of the patients being considered responders due to improvement in the NYHA functional class and at least a 5% increase in the LVEF. A narrowing of the QRS complex on electrocardiogram was obtained in all patients classified as responders, so this simple method for measuring QRS complex duration can be used to assess the response to biventricular stimulation. **Keywords**: HEART FAILURE, CARDIAC PACING, THERAPY, BIVENTRICULAR STIMULATION.

Cardiovascular diseases (CVD) in general and heart failure in particular are major health problems due to the large number of patients and the incurred implications, the socioeconomic ones included. For this reason, the diagnosis methods, treatment and prognosis were also of interest. Technical developments during the past decades had a tremendous impact in contemporary medicine leading to further improvement of the diagnostic and therapeutic approaches. Cardiology is one of the most privileged specialties in terms of technological advancements. Cardiac resynchronization therapy (CRT) plays an important role in the treatment of patients with NYHA functional class II-IV heart failure despite the optimal medical treatment. During the last decade, long-term clinical effects of CRT were evaluated by randomized multicentre trials, such

as MUSTIC-SR (Multisite Stimulation in Cardiomyopathy Study), MIRACLE (Multicenter InSync Randomized Clinical Evaluation Trial), COMPANION (Comparison of Medical Therapy, Pacing and Defibrillation in Heart Failure trial), CARE-HF (The Cardiac Resynchronization Heart Failure trial). These trials showed that CRT improves symptoms and exercise capacity and decreases morbidity and mortality from cardiovascular causes. Yet, despite the fact that the indications for CRT are well described, at present the parameters/criteria for patient selection are not well defined, so that studies revealed that up to 30% of the patients did not respond to CTR.

The response to CRT can be assessed by various methods, but there is no agreement on which method best predicts response to CRT. The most widely accepted method is the clinical criteria of the New York Heart Association (NYHA) functional classification. Echocardiographically, the most commonly used method remains the assessment of LVEF. Recently, much more confidence is granted to the comparatively simple method of measuring QRS complex duration.

The aim of our study was to analyze the effects (clinical, electrical and ultrasound) of CRT, with or without an implantable defibrillator (CRT-P or CRT-D) in patients with heart failure in our clinic**.**

MATERIAL AND METHODS

Patient population. This prospective observational study included 56 consecutive patients referred to the Cardiology Department of the "Prof. Dr. George I.M. Georgescu" Cardiovascular Disease Institute, Iasi, Romania for CRT between January 1, 2010 and June 1, 2014. All patients were in NYHA functional class III/IV on optimal pharmacological treatment and showed reduced left ventricular systolic function (ejection fraction [EF] ≤ 35%) and a QRS complex duration of > 120ms. Patients with permanent atrial fibrillation and recent myocardial infarction (< 3 month) were excluded.

All patients were on an optimal pharmacological treatment with the maximum tolerated doses of digitalis medication, beta blockers, diuretics and ACE inhibitors. Resting 12-lead EEGs (at a paper speed of 25 mm/s) were acquired immediately before and one month after CRT device implantation. Clinical evaluation included assessment of NYHA functional class before and one month after biventricular pacing.

Echocardiographic data were obtained using a conventional ACCUSON CV 70 System (Siemens, Germany). In all patients echocardiography was performed at rest, in the lateral decubitus position, at baseline and six months after implantation. A standard evaluation of left ventricular volumes was performed in the apical 4-chamber view according to Simpson's rule method. To minimize the variability in measurements, all patients were evaluated by the same physician.

Classified as responders were those patients who presented at six month follow-up improvement by at least one NYHA functional class, an increase in LVEF of ≥5%, and decrease in left ventricular end-systolic volume (LVESV) and left ventricular end-diastolic volume (LVEDV) of ≥15%.

Patient characteristics were expressed as the mean $^{+}/_{-}$ 1 standard deviation or as a number and percentage. Variables were compared by paired t test. The value of p wave below 0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS statistical software (SPSS v.21.0, Mac OS X).

RESULTS

Baseline characteristics. The study population consisted of 40 men (71.42%) and 16 women (28.58%) with a mean age of 68.2 ± 10.4 years. 36.42% of the patients were in NYHA functional class IV and the mean QRS complex duration was 178.8 ± 18ms before cardiac stimulation (tab. I).

TABLE I

Baseline characteristics

Baseline characteristics	Patients (n=56)
Age (years)	62.8±10.4
Gender (male)	71.42
Gender (female)	28.58
QRS duration(ms)	178±14
NYHA III class (%)	62.8
Ischemic CMD (%)	28.32
LVEF (%)	20.3±6
DTDVS (ml)	224.8±68.5
DTSVS (ml)	176.3±49
LAv (ml)	72.8±10.5

The evolution of heart failure expressed by NYHA functional class after biventricular stimulation After CRT functional capacity was improved, most patients presenting at one month follow up an improvement by at least one NYHA functional class. So, if the inclusion rate for NYHA class IV patients was of 37.2%, after one month it decreased to 21.4% (fig. 1).

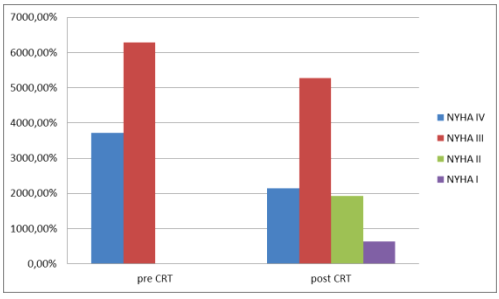

Fig. 1 .The evolution of heart failure expressed by NYHA functional class before and after CRT device implantation

The evolution of LVEF after CRT At baseline all patients had LVEF ≤35%. One month later, on echocardiographic assessment 82.26% of the patients showed an increase in LVEF of more than 5%, being classified as responders. 16.5% of them showed an LVEF improved by more than 15% - super-responders. 17.74% of the study patients showed no change in left ventricular systolic function - non-responders.

QRS duration on surface electrocardiogram decreased from an average of 178ms at baseline to 128ms, being a statistically significant correlation between QRS narrowing and the increase in LVEF. So, the patients with a narrowing of QRS complex of at least 30ms were classified as super-responders (16.5%), and those with a narrowing of 20 to 30ms as responders.

DISCUSSION

Heart failure is a syndrome characterized by cardiac remodeling; progressive left ventricular dilation, and consequently, reduced contractile function. Ventricular remodeling has been shown to be an independent negative prognostic factor. Atrial-ventricular and intraventricular delay further exacerbates left ventricular dysfunction and ventricular desynchronization favors mitral regurgitation and shortens left ventricular filling. Intraventricular delay is a pathophysiological process that directly depresses ventricular function, by causing ventricular remodeling and thus increasing morbidity and mortality. CRT has already been a major benefit in the treatment of heart failure. Numerous randomized trials have confirmed the important role of CRT on significant improvement in symptoms, increased exercise capacity, improved NYHA functional class (on average by 0.5-0.8 points) and increase by 20% in 6-minute walk distance. Quality of life was

significantly improved in all studies. Total mortality and hospitalization for heart failure were significantly reduced by CRT-P or CTR-D (COMPANION study).

The response to CRT can be assessed by various methods, but there is no agreement on which method best predicts response to CRT. The most widely accepted method is the clinical criteria of NYHA functional classification. Echocardiographically, the most commonly used method remains the assessment of LVEF. To these methods can be added the measuring of QRS duration on surface electrocardiogram as a simple but useful method of evaluation of clinical response to biventricular stimulation.

CONCLUSIONS

Cardiac resynchronization therapy is an important therapeutic option in patients with failure. In our study 82.56% of the patients were considered responders due to the improvement in NYHA functional class, increase in LVEF by at least 5% and narrowing of the QRS complex. The narrowing of the QRS complex on electrocardiogram was found in all patients classified as responders, so this simple method of measuring the duration of QRS complex can be used to assess the response to biventricular stimulation.

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NEWS

ANTI-INFLAMMATORY EFFECT OF *STAPHYLOCOCCUS AUREUS* ON AIRWAY EPITHELIAL CELLS

A study by Chekabab *et al* examined the way bacterial cells modulate inflammatory responses in the setting of polymicrobial infections. Two major respiratory pathogens, *Pseudomonas aeruginosa* and *Staphylococcus aureus* can simultaneously colonize the airways of patients with chronic obstructive diseases (e.g. cystic fibrosis). The mechanism by which airway epithelial cells integrate bacterial stimuli in the case of polymicrobial colonization or infection is unknown. Since epithelial cell signaling is crucial for the activation of innate immune responses, the study examined the inflammatory responses to *P. aeruginosa* and *S. aureus* co-stimulations. Immortalized airway epithelial cells exposed to bacteria-free filtrates from *P. aeruginosa* induced a high synthesis of IL-8, while bacteria-free filtrates from *S. aureus* showed a minimal effect. However, in the case of co-stimulation with bacteria-free filtrates from *P. aeruginosa* and *S. aureus*, *S. aureus* filtrates inhibited IL-8 production induced by *P. aeruginosa*, showing potent anti-inflammatory effects. It also decreased IL-8 production induced by the TLR1/TLR2 ligand Pam3CysSK4 and dampened TLR1/TLR2-mediated activation of the NF-κB pathway. These results demonstrate that *S. aureus* modulates inflammatory responses in the setting of polymicrobial infections (Chekabab SM, Silverman RJ, Lafayette SL, Luo Y, Rousseau S, Nguyen D. *Staphylococcus aureus* Inhibits IL-8 Responses Induced by *Pseudomonas aeruginosa* in Airway Epithelial Cells. PLoS One. 2015;10(9):e0137753. doi: 10.1371/journal.pone.0137753. eCollection 2015).

Teodora Vremera

QUALITY OF LIFE IN CROHN'S DISEASE PATIENTS

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QUALITY OF LIFE IN CROHN'S DISEASE PATIENTS (Abstract): **The aim** of the study is to assess the impact of Crohn's Disease (CD) on quality of life (QoL) patients with and to identify the significant modifications in QoL, depending on both the development particularities of CD and the epidemiologic parameters**. Material and methods:** a prospective study was conducted at the Institute of Gastroenterology and Hepathology Iaşi, between October 2011 and August 2014. The study enrolled 63 patients with CD. The CD diagnosis was based on clinical, biological, endoscopic and histological criteria. QoL was assessed by means of the IBDQ-32 questionnaire (*Inflammatory Bowel Disease Questionnaire*). **Results:** The total IBDQ score varied from 70 to 200, registering a slightly higher value in female patients. Parameters such as age, sex and smoking/ non-smoking status did not present significant differences in the IBDQ score. The values of the IBDQ score were in indirect, mild correlation with the CDAI score; over 49% of the subjects with higher CDAI had a lower IBDQ score. **Conclusions:** Except for the disease activity periods, the results of the studies investigating the impact of the various factors on the QoL in patients with CD are contradictory. Further research is needed in order to define the subgroups of patients likely to suffer from poorer QoL. **Keywords:** CROHN'S DISEASE, QUALITY OF LIFE, IMPACT.

Crohn's Disease (CD) is an inflammatory bowel disease (IBD) which may affect any part of the gastrointestinal tract, and may do so in a nonconfluent pattern. The inflammation in CD is typically transmural. The symptoms during the periods of flare-ups can be polymorphous, manifested through chronic diarrhea associated with blood and mucus, abdominal pain, chronic fatigue, fever and weight loss, often accompanied by extraintestinal manifestations (articular, cutaneous, hepatic, renal or ocular) (1).

The early onset and its chronic development, marked by periods of activity followed by remission, the pathologies associated, as well as the treatment of the disease, are all likely to affect the patients both physically and through the limitation of their social and emotional life, through their educational or professional activity (2).

Similarly to all other chronic disorders, this diagnosis is perceived as a verdict, a threat to their future, which is likely to inevitably affect their quality of life (QoL). Over the last period, there has been growing awareness of the psychosocial implications of chronic disorders, which have

previously been less known, or even ignored by the medical system (3). Although most of the times improvement in symptoms prevails over complete healing, the estimation of therapeutic success is essential through the assessment of the improvement in the patient's QoL. This is achieved through the use of questionnaires aimed at assessing QoL. Such methods are used especially in chronic disorders, in which the subjective improvement of the symptoms is one of the main objectives of the treatment (4).

Health-related quality of life (HRQoL) was defined as the functional effect of a disease and its treatment upon the patients, as perceived by the patients themselves. The need for HRQoL assessment emerged in order to achieve the objective of the medical act, more precisely, with a view to improve the patients' general state, respectively, their quality of life (6). HRQoL assessment contributes to quantifying the effectiveness of the medical act, taking into consideration its advantages and disadvantages (6).

The aim of the study is to assess QoL in patients with CD and to identify the significant modifications in QoL, depending on both the development particularities of CD and the epidemiologic parameters.

MATERIAL AND METHODS

A prospective study was conducted at the Institute of Gastroenterology and Hepathology Iasi, between October 2011 and August 2014. The study enrolled 63 patients with CD.

The CD diagnosis was based on conventional clinical features (diarrhea, associated or not with blood and mucus, abdominal pain, etc.), stool sampling in order to exclude infectious aetiologies, colonoscopy examinations by using the Mayo endoscopic score, biopsy sampling from the affected areas, and histopathological examinations. The disease activity was quantified by means of the CDAI score (*Crohn Disease Activity Index*).

QoL was assessed by means of the IBDQ-32 questionnaire (*Inflammatory Bowel Disease Questionnaire*), one of the questionnaires the most widely used in the assessment of HRQoL. The questionnaire contains 32 questions grouped into four fields: gastrointestinal symptoms (diarrheal stools, abdominal pain, rectal bleeding, and rectal tenesmus), systemic symptoms (fatigue, sleep disorders), emotional functions (depression, irritability, anger) and social functions (absenteeism, affected social status). The answers were marked on a scale from 1 (the worst) to 7 (the best). The total score ranged between 32 and 224. The lowest the score, the more affected the QoL (7).

Statistical analysis

The statistical treatment of the data was conducted by means of the SPSS 18.0 software as a perspective analysis, with no further adjustments for multiple testing, with a nominal significance defined as $p < 0.05$. The relationships between IBDQ-32 and the Mayo score are reported by the Pearson correlation coefficients. The qualitative differences were established by means of the Kruskal-Wallis test.

Ethical considerations

All the patients enrolled in the study signed an informed consent, through which they were given details on the aim of the study, its methodology, the risks and benefits incurred by the study, as well as information regarding the confidentiality of the results obtained.

RESULTS

The total IBDQ score varied from 70 to 200, registering an average value which was slightly higher in women (p=0.363) (tab I).

Gender distribution was approximately equal, the ratio of gender shares being 1/1. Both the average IBDQ score and its subgroups were slightly higher in the female patients (p>0.05). The patients' age did not influence the IBDQ score (p=0.987) (Fig.1).

Only 46% of the subjects were smokers, and the average IBDQ score did not present significant differences between the average values recorded in smokers (133.62±36.63) compared to non-smokers (143.47±27.44) (p=0.228) (fig.2).

The localisation and pattern of the disease did not influence the total IBDQ score (p=0.860, p=0.587). Nevertheless, slightly higher levels by subclass were highlighted in the patients with stenotic phenotype – B2 (p>0.05), (fig. 3). In the patients with *ileocolonic* location (L3), lower IBDQ score values were revealed, predominantly in the subgroups assessing QoL impairment by gastrointestinal symptoms, as well as impairment emotional function.

The individual values of the IBDQ score were in indirect, moderate correlation with the CDAI score; more than 49% of the subjects with a high CDAI score had a reduced IBDQ score (r= -0.494: R^2 =0.244; p< 0.001). The total IBDQ score was significantly lower in patients with moderate or severe flare (p=0.008). Assessed on subscales, gastrointestinal symptoms, systemic symptoms, emotional functions and social functions, the IBDQ scores were significantly lower in patients with severe flare (p<0.05) (tab. II).

TABLE I

Statistical indicators describing the IBDQ score by gender

Gender	N	Average	Standard deviation	Standard error	Confidence interval		Min	Max	p
					- 95%CI	+95%CI			
IBDQ									
female	32	142.59	31.260	5.526	131.32	153.86	76	195	0.363
male	31	135.16	33.053	5.936	123.04	147.29	70	200	
IBDQ-1 gastrointestinal symptoms									
female	32	30.66	7.998	1.414	27.77	33.54	14	46	0.397
male	31	28.94	8.029	1.442	25.99	31.88	15	46	
IBDQ-2 systemic symptoms									
female	32	33.50	8.527	1.507	30.43	36.57	16	48	0.315
male	31	31.39	8.015	1.440	28.45	34.33	17	50	
IBDQ-3 emotional functions									
female	32	38.84	8.941	1.581	35.62	42.07	22	58	0.396
male	31	36.87	9.383	1.685	33.43	40.31	16	54	
IBDQ-4 social functions									
female	32	39.25	7.565	1.337	36.52	41.98	24	49	0.393
male	31	37.52	8.422	1.513	34.43	40.61	22	53	

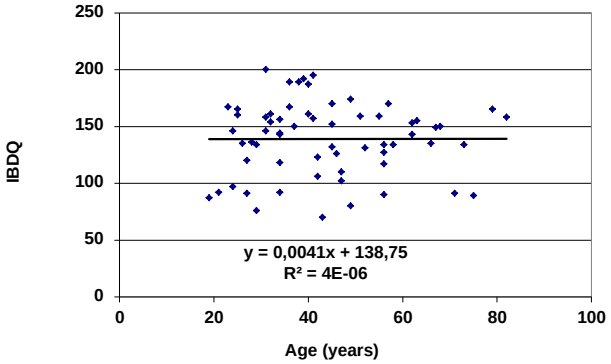

Fig. 1. IBDQ score correlation with age

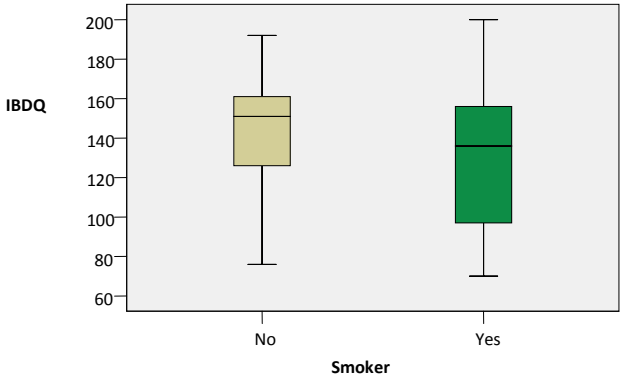

Fig. 2. Average IBDQ score values based on smoking status

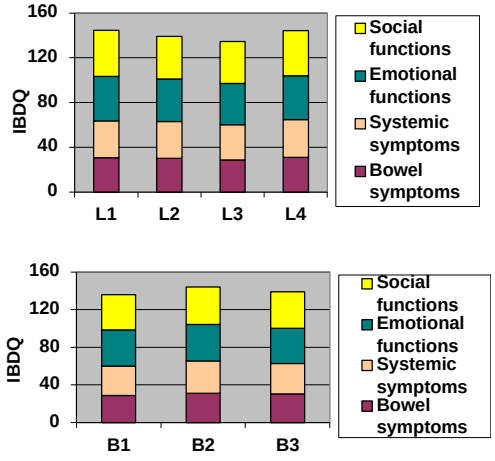

Fig. 3. IBDQ score based on disease localization (left) and disease pattern (right)

TABLE II

Total IBDQ score and subscales based on periods of activity

IBDQ	Remission (n=19)	Flare			p
		mild (n=19)	moderate (n=20)	severe (n=4)	
Total score	153.11±36.00	145.42±22.68	126.70±29.17	101.00±24.51	**0.008**
Bowel symptoms	33.47±9.19	31.11±5.95	26.90±6.95	21.50±7.00	**0.016**
Systemic symptoms	35.89±9.50	33.58±5.98	30.00±7.86	23.50±6.46	**0.033**
Emotional functions	41.58±9.92	40.00±6.84	34.10±8.63	27.75±5.32	**0.009**
Social functions	41.84±8.59	40.21±6.11	35.35±7.00	27.75±5.19	**0.003**

DISCUSSION

Similarly to the data obtained from the literature, the study confirms the fact that the disease flare is the factor likely to have the highest impact on QoL in patients with CD. To date, it has been proven that severe disease flare influences QoL negatively, both in the patients with CD and in those with UC (8). In the present study, CD had a negative impact on all aspects related to the patients' QoL, which may lead to depressive states and anxiety during and after periods of flare-ups. The total IBDQ and the scores of its subscales were significantly lower in the patients with moderate and severe disease activity (p=0.008).

In our study, the age, gender and the smoking status did not influence the IBDQ total score and sub-scores. Some studies have shown that QoL is more severely affected in women compared to men with CD (9). Nevertheless, this finding was not confirmed in the present study. As far as smoking is concerned, it was proven that smoking patients with CD underwent a higher risk of post-operative recidive, with lower response to medication. Recent data has suggested that smoking induces modifications at the level of both the inborn and acquired immune system. Moreover, smoking is associated with distinct modifications in the gut micro biota in both IBD and the healthy patients (10).

Although in our study, age did not influence the QoL score, previous research has shown significant differences in the QoL scores between young and old patients (11, 12).

Besides the disease flare-ups, the IBD patients' age and gender are likely to have a significant impact on their QoL. Another study has shown that patients aged >50 years old presented a significantly lower QoL score compared to younger patients ($p < 0.001$) (13).

On the other hand, other studies have shown that younger patients, even those with mild disease flare, presented a more influenced QoL, as well as a higher degree of chronic fatigue, compared to older patients ($p = 0.06$) (14,15).

Additionally, the disease localisation and pattern did not have a statistically significant influence on the IBDQ score. However, the patients with ileocolonic CD presented lower IBDQ scores.

CONCLUSIONS

The results of our study confirm the data in the literature regarding impaired QoL in patients with CD. The total IBDQ score varied from 70 to 200, registering a slightly higher value in female patients. Parameters such as age, sex or the smoking/ non-

smoking status did not present significant differences in the IBDQ score. The disease flare ups had the highest impact on the QoL in patients with CD. The values of the IBDQ score were in indirect, mild correlation with the CDAI score; over 49% of the subjects with higher CDAI had a lower IBDQ score. Except for the disease activity periods, the results of the studies investigating the impact of the various factors on the QoL in patients with CD are contradictory. Further research is needed in order to define the subgroups of patients likely to suffer from poorer QoL.

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THYROID FINE-NEEDLE BIOPSY: ASPIRATION VERSUS CAPILLARY

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THYROID FINE-NEEDLE BIOPSY: ASPIRATION VERSUS CAPILLARY (Abstract) Thyroid nodules are a common pathology of the thyroid gland. Thyroid fine-needle biopsy (FNB) is a technique used as the first step in the assessment of thyroid nodules. Some authors have demonstrated the superiority of nonaspiration compared with aspiration biopsy. **Aim**: The objective was to assess whether there are significant differences between the two thyroid biopsy techniques. **Material and methods:** The study group comprised 309 patients with thyroid nodules admitted to the Endocrinology Clinic of the Iasi "St. Spiridon "Hospital between 2005 and 2008 in which fine-needle nonaspiration or aspiration biopsy was performed. The slides were read by one pathologist. The smears were stained using the May-Grunwald-Giemsa method (MGG). The quality of smears obtained by the two sampling techniques was evaluated by using the scoring system developed by Mair. **Results and discussion:** No differences in smear quality between the two sampling techniques in terms of blood contamination, amount of cellular material, degree of cellular degeneration, degree of cellular trauma, and preservation of appropriate architecture were found. **Conclusion**: The results of this study show that the sensitivity and specificity of both techniques allow their use as standard screening methods for thyroid nodular lesions. **Keywords:** THYROID NODULE, THYROID CANCER, BIOPSY, THYROID.

Thyroid nodules are a common pathology of the thyroid gland. Fine-needle aspiration (FNA) is a technique used as the first step in the assessment of thyroid nodules (1). An alternative to this technique is fine-needle nonaspiration (FNNA) technique using capillary pressure to suck cells into the needle lumen. This technique was originally used for collecting cellular material from other organs such as liver or mammary gland. It uses a thin 25 gauge needle (0.50 mm outer diameter, 25 mm length) the cells being detached by the sharp edge of the needle and pushed into the needle lumen by the capillary pressure (2). Subsequently, the needle is removed and the cells are expelled on a slide. FNNA was for the first time used in the assessment of thyroid lesions by Santos in 1988 (3). Subsequent studies have given conflicting results on the effectiveness of the two methods (4, 5).Some authors support the superiority of FNNA compared with FNA. Among their arguments are less cellular and tissue trau-

ma and the reduced amount of background blood in smears. Also FNNA is less painful providing better patient compliance (6, 7, 8). Other authors have demonstrated that FNA is superior or equivalent to FNNA offering as arguments the reduced amount of cellular material obtained by FNA compared to FNNA (8).

The aim of this study was to assess whether there are significant differences between the two thyroid bioptic techniques.

MATERIAL AND METHODS

The study group comprised 309 patients with thyroid nodular lesions admitted to the Endocrinology Clinic of the Iasi "St. Spiridon" Hospital in the interval 2005-2008 in which one of the two techniques was performed. The slides were read by one pathologist. The smears were stained using the May-Grunwald-Giemsa method (MGG). The quality of smears obtained by the two sampling techniques was graded by using the scoring system developed by Mair and consisted in evaluation of blood contamination, amount of cellular material, degree of cellular degeneration, degree of cellular trauma and preservation of appropriate architecture. A score ranging from 0-2 was assigned to each of these criteria (tab. I).

TABLE I

Scoring system for the assessment of smear quality (4)

Criterion	Qualitative description	Point score
Blood contamination	Large amount – diagnosis greatly compromised	0
	Moderate - diagnosis possible	1
	Minimal- diagnosis easy	2
Amount of cellular material	Minimal or absent - diagnosis not possible	0
	Sufficient - diagnosis possible	1
	Abundant – diagnosis easy	2
Degree of cellular degeneration	Marked – diagnosis impossible	0
	Moderate -diagnostic possible	1
	Minimal – diagnosis easy	2
Degree of cellular trauma	Marked - diagnosis impossible	0
	Moderate - diagnosis easy	1
	Minimal –diagnosis obvious	2
Preservation of appropriate architecture	Minimal or absent - diagnosis impossible	0
	Moderate – diagnosis possible	1
	Excellent –diagnosis easy	2

Excluded from the study group were the patients in which thyroid biopsy was not completed with paraffin histology of surgical specimen obtained by the surgical removal of the thyroid gland. The cell samples obtained by fine needle biopsy and surgical specimens were processed and diagnosed in the Pathology Department of the "St. Spiridon" Hospital Iasi. The smears were assessed and classified according to Mair score, a score being assigned to every smear. The score (0-2) was considered C

(unsuitable for cytological diagnosis), (3-5) was considered B (suitable for cytological diagnosis) and (6-8) was considered and A (excellent for cytological diagnosis). The obtained scores were then compared between the two bioptic techniques, aspiration and capillary sampling. A total score ranging from 0 to 8 was assigned to each smear obtained by one of the two techniques. The obtained data were compared with the literature data and statistically processed using the statistical functions in *Excel* and *SPSS*. Because the variables did not have a normal distribution, for comparing the two methods the nonparametric chi-square test was used. Chi-square test is a nonparametric test that compares two or more frequencies in the same population. The accepted significance threshold was 95%, $p < 0.05$, but the lower this p value the stronger the significance. For the comparative evaluation we calculated the sensitivity, specificity, positive and negative predictive value of each of the two methods. The samples that could not be interpreted were excluded and the suspicious ones were considered malignant. The cytological diagnosis on each biopsy was compared with a diagnostically superior investigation, the "gold standard" in this study being the postoperative histopathological examination conducted in the Pathology Department of the "St. Spiridon" Hospital Iasi.

RESULTS AND DISCUSSION

A total of 309 patients were assessed by thin needle biopsy (287 women – 92.8 % and 22 men -7.2 %). In 34 patients (11%) biopsy was performed by aspiration (FNA) and in 275 patients (89%) by non aspiration (FNNA).

Cytologically, the massive blood contamination obscured the thyroid cell masses preventing microscopic examination, moderate blood contamination consisted of a moderate red blood cell mass mainly consisting of intact red blood cells together with intact thyroid cellular elements and minimal blood contamination was diagnosed in smears with isolated intact red blood cellular elements.

Of the smears obtained by fine-needle non aspiration (FNNA) 6.90%, confidence interval (CI): 4.46 to 10.53 showed massive blood contamination, diagnosis impossible, 66.54% (CI: 60.77 to 71.86) moderate blood contamination, diagnosis possible, and 26.54% (CI: 21.67 to 32.06) minimal blood contamination, diagnosis easy.

By aspiration technique massive background blood contamination, diagnosis impossible, was found in 2.94 % of the samples (CI: 0.52 to 14.91), moderate blood contamination, diagnosis possible in 58.23% (CI: 42.22 to 73.63), and minimal blood contamination, ideal for interpretation in 38.23% (CI: 23.90 to 54.95).

No statistically significant differences in the quality smears in terms of blood contamination were found between the two techniques ($p = 0.28$).

Amount of cellular material classified the smears into three categories: minimal, sufficient and abundant amount of cellular material.

The minimum amount of cellular material consisted of isolated thyroid cellular elements, less than 30 cells or free nuclei, sufficient amount of cell material was considered when the smear contained up to six groups of thyroid cellular elements, each group containing 10 thyrocytes, and the amount of cellular material was abundant when over six groups of thyroid cellular elements, each group containing 10 thyrocytes were visualized.

Of the FNNA smears 23.27% (CI: 18.66 to 28.61) showed minimal cellularity, diagnosis impossible, 47.63% (CI: 41.80 to 53.33) sufficient cellularity, and 29.02% (CI: 24.04 to 34.71) abundant amount of cellular material, ideal for diagnosis. Of the FNA smears 23.52% of cases (Cl: 12.43 to 39.99) showed minimal cellularity, diagnosis impossible, 41.17% of the cases (Cl: 26.36 to 57.77) sufficient cellularity, and 35.29% (CI: 21.48 to 52.08) abundant amount of cellular material, ideal for diagnosis.

No statistically significant differences in the quality smears in terms of the amount of cellular material were found between the two techniques (p = 0.71).

Degree of cellular degeneration: three categories were identified: marked, moderate and minimal degree of cellular degeneration.

In the marked degree of cellular degeneration the thyroid cellular elements are mainly represented by free nuclei and thyrocytes, the moderate degree of cellular degeneration is characterized by the presence of less than 6 cell groups of 10 thyrocytes showing intact cytoplasm, and the minimum degree of cell degeneration is considered in the presence of more than 6 groups of 10 thyrocytes with intact cytoplasm.

On FNNA smears the degree of cellular degeneration was marked, diagnosis impossible, in 24.36% (CI: 19.66 to 29.76), moderate in 58.9% (CI: 53.01 to 64.56) and minimal, ideal for diagnosis in 16.72% (CI: 12.78 to 21.59). With FNA the degree of cellular degeneration was marked in 23.52% (CI: 12.43 to 39.99), moderate in 47.05% (CI: 31.45 to 63, 26) and minimal in 29.41% (Cl: 16.83 to 46.16) of the samples.

No statistically significant differences in the quality smears in terms of the degree of cellular degeneration were found between the two techniques (p = 0.17).

Cellular trauma. Marked cellular trauma consisted of a marked degree of degeneration of cellular elements, most of them with free nuclei lysis and destroyed cytoplasm Moderated cellular trauma consisted of a moderate degree of degeneration of the cellular elements, most of them with free nuclei lysis and destroyed cytoplasm, the number of intact cell groups ranging for 1 to 6, and minimal cellular trauma was characterized by a low degree of degeneration of cellular elements, most of them with free nuclei lysis and destroyed cytoplasm, and over 6 groups of intact cells.

Of the FNNA smears 24.36% (CI: 19.66 to 29.76) showed a marked degree of cellular trauma, diagnosis impossible, 58.9% (CI: 53.01-64.56) a moderate degree of cellular trauma, and 16.72% (CI: 12.78 to 21.59) a minimal degree of cellular trauma, ideal for diagnosis.

In terms of cellular trauma 23.52% (CI: 12.43 to 39.99) of FNA smears showed marked cellular trauma, 47.05% (CI: 31.45 to 63.26) moderate cellular trauma, and 29.41% (CI: 16.83 to 46.16) minimal cellular trauma.

No statistically significant differences in the quality smears in terms of the degree of cellular trauma were found between the two techniques (p = 0.17).

Preservation of appropriate architecture. The smears were divided into 3 categories: minimal, moderate, and excellent preservation of appropriate architecture.

Smears with minimal preservation of appropriate architecture were those characterized by the presence of some cellular elements isolated, rare intact thyrocytes

arranged in large cell aggregates. Smears with a moderate preservation of appropriate architecture consisted of a single layer of thyrocytes in honeycomb or microfolicular arrangement. When some cellular elements were intact with well-preserved cellular border and intact cytoplasm the smear was considered as having an excellent preservation of appropriate architecture.

In 24.36% (CI: 19.66 to 29.76) of FNNA smears preservation of appropriate cellular architecture was minimal, non-diagnostic, moderate in 58.54% (CI: 52.64to 64.21) and excellent in 15.63% (CI: 11.82 to 20.39).

By FNA, preservation of appropriate architecture was: minimal, non-diagnostic in 23.52% (CI: 12.43 to 39.99), moderate in 50% (CI: 34.09 to 66.93) and excellent in 26.47% cases (CI: 14.80 to 43.11) of the smears.

No statistically significant difference in the quality smears in terms of the preservation of appropriate architecture was found between the two techniques ($p = 0.39$)

According to smear quality, 23.53% of FNA smears were categorized as C, unsuitable for diagnosis (score 0-2), 47.06% as B, diagnostically adequate (score 3-5) and 29.41% as A, excellent for diagnosis (score 6-8). Twenty-four percent of FNNA smears were categorized as C, unsuitable for diagnosis (score 0-2), 56.36% as B, diagnostically adequate (score 3-5), and 19.64% as A, excellent for diagnosis (score 6-8).

The difference in quality of the smear was not statistically significant ($p = 0.39$). When comparing the sensitivity and specificity of the two techniques we found that sensitivity was 71.43% (CI: 35.89 to 91.78) for FNA and 75% (CI: 56.64 to 87.32) for FNNA, and specificity was 91.67% (CI: 74.15 to 97.68) for FNA and 93.75% (CI: 89.65 to 96.33) for FNNA.

The differences in sensitivity and specificity between the two techniques were not statistically significant

Although cytology is less reliable than histopathology (9) thyroid biopsy is a minimally invasive investigation with a high sensitivity and specificity (10). There are several techniques of sampling cellular material, the most commonly used being fine-needle aspiration (FNA) and fine-needle non-aspiration (FNNA).

Both methods have at least theoretically advantages and disadvantages. FNA usually yields more material but also more blood contamination due to the applied aspiration which will affect the quality of the sample and implicitly the possibility of interpreting it, while FNNA may produce less material, but may cause less bleeding and produce a specimen of higher quality. However, both methods are equally used in practice.

What constitutes and adequate smear? Follicular cells are essential for a good interpretation, decreasing the number of false negative reports. Goellner et al (11) noted that the number of false negative results was lower or even eliminated by the presence of 5 or 6 groups of well-preserved follicular cells, with at least 10 cells per group.

Hamburger and Hussain (12) suggested the need of six cell groups, each containing an average of 16 cells while Nguyer et al. (13) required 10 cell groups each containing about 20 cells or 8-10 well preserved follicular fragments.

In our study approximately 24% of biopsy was considered diagnostically inadequate. There were no significant differences between FNA and FNNA in terms of the assessed parameters: background blood, amount of cellular material, degree of cel-

lular degeneration, degree of cellular trauma, and preservation of appropriate architecture. In studies conducted by Kamal and Santos a higher number of adequate smears were obtained by FNNA (8). Both studies found no significant differences between the two methods (14, 15).

CONCLUSIONS

Our study shows that the sensitivity and specificity of both techniques allow their use as standard screening methods for thyroid nodular lesions. The obtained results demonstrate that FNA and FNNA have similar efficacy.

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KEY FACTORS INFLUENCING THE LEVEL OF JOB SATISFACTION AMONG OBSTETRICS AND GYNECOLOGY PHYSICIANS IN ROMANIA

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KEY FACTORS INFLUENCING THE LEVEL OF JOB SATISFACTION AMONG OBSTETRICS AND GYNECOLOGY PHYSICIANS IN ROMANIA (Abstract): **Aim:** The purpose of this study was to identify the level of job satisfaction among obstetrics and gynecology physicians in Romania. **Material and methods**: Job satisfaction was measured using the Job Satisfaction Scale and personality traits were identified using the Big Five Inventory Socio-demographic variables were also considered to identify factors that can contribute to the level of satisfaction. The data analysis was performed using *SPSS Statistics v23.0.0*. **Results:** A low level of satisfaction was identified among obstetrics and gynecology physicians. 19.64% of doctors responded that they were satisfied or very satisfied with their job. Male doctors were more satisfied with their job than female doctors were (- t (110) = 2.00, p=0.05; 3.71 ± 0.82 vs 3.39 ± 0.59). Specialists were more satisfied than consulting doctors regarding overall job satisfaction (Mdif = 0.336, p=0.031). Statistical differences were identified among the variables insomnia, depression, and the use of pills to cope with stress. A high number of working hours and a high level of neuroticism were negatively correlated with job satisfaction. Extraversion and agreeableness were strongly related to job satisfaction. **Conclusions:** The results are important for obstetrics and gynecology physicians to explain the level of satisfaction related to their personality traits and for institutions to reconsider the important factors that could influence job satisfaction among doctors. **Keywords:** PERSONALITY TRAITS, JOB SATISFACTION, OB-GYN PHYSICIAN, NEUROTICISM, BIG FIVE INVENTORY, DEPRESSION.

The level of job satisfaction among medical specialties has varied over the years, because of health policies and changes in medical practice. Some studies show that high job satisfaction scores occur among dermatologists (1), geriatricians (2), pediatricians (3), forensic physicians (4), and ophthalmologists (5).

Job satisfaction for doctors is correlated with a high level of satisfaction among patients and increased compliance. Caring for patients, sense of accomplishment, continuity of care, autonomy, and having personal time are the most important factors associated with satisfaction with job (6). Satisfaction was found to be more linked to the level of autonomy than professional prestige (7).

Job dissatisfaction among physicians is associated with conflict, absenteeism, low

patient care rate, reduction in quality and quantity of work (8) and migration (9). Factors that influence doctors' satisfaction with their job are related to both environmental (institution, patients, activity, department) and psychological factors (personality traits, family issues).

Some statistics indicate that medical students and young doctors sometimes choose their specialty based on the quality of life assured by practicing it. For example, Lambert et al (10) found that younger physicians in England avoid surgical specialties and obstetrics and gynecology, because of the level of quality of life and the higher number of working hours. On the other hand, Schmidt (11) reported an 80% satisfaction rate among obstetricians and gynecologists in USA.

Bettes et al. (12) pointed out that obgyn doctors who are assisting delivery (vaginal or cesarean) are more prone to a low level of job satisfaction, because of the increased workload and decreased personal control. In the study of Kravitz et al. (13) higher career satisfaction was related to age greater than 65 years, academic activity, medical practice in small metropolitan areas, and the ability to obtain services. To increase satisfaction related to one's career and to help doctors cope with stress, providing training to junior physicians in this specialty has positive results on mental health and job satisfaction (14).

Personality traits in tandem with work values were found to predict specialty choice (15, 16) but some other researchers showed that personality does not have a strong influence on what individuals consider to be important in their work environment or on the level of their satisfaction with job (17).

The goal of this study was to identify the socio-demographic variables and personality traits (using Big Five Inventory measuring extraversion, agreeableness, conscientiousness, neuroticism, and openness) - that influence job satisfaction among obstetrics and gynecology physicians in Romania.

MATERIAL AND METHODS

Participants

The number of registered obstetrics and gynecology physicians who were working in public or private medical institutions was approximately 1,724 in 2016 (18). The subjects in our research study include 116 obstetrics and gynecology physicians, working in 10 departments across Romania. The number of doctors who answered the questionnaires and were included in the present study represents 6.72% of all registered doctors of this specialty in Romania.

Instruments

Physicians were asked to fill out a socio-demographic data sheet and to answer two questionnaires: *Job Satisfaction Scale* and *Big Five Inventory*. Before completing the questionnaires, the doctors had to sign an informed consent form that provided information about the research objective, data privacy, and the fact that the results were only being used for this study.

The *Job Satisfaction Scale* - JSS, (19) has 32 items and 3 dimensions: payment–promotion (14 items), management–interpersonal relationships (8 items), and organization–communication (10 items).

The personality traits were evaluated using the *Big Five Inventory* - BFI, (20,21). The instrument consists of 44 items corresponding to five personality factors: extraversion (8 items), agreeableness (9 items), conscientiousness (9 items), neuroticism (8 items), and openness (10 items). A Likert scale with 5 steps registered 1 as strong disagreement and 5 as strong agreement.

The other registered variables were age,

level of specialization (resident, specialist, or primary doctor), marital status (in a relationship or single), data about the partner (the partner is also a physician), number of children, number of years of experience in this specialty, teaching activity, number of hours of work per week, and number of shifts per month. Self-reported data about insomnia, the use of drugs to cope with stress, depressive periods in the past or present, and known chronic diseases were also reported.

Ethical approval

The research was approved by the Ethics Committee from the "Cuza-Voda" Obstetrics and Gynecology Clinical Hospital, Iasi, Romania. The present study is part of a larger study targeting professional and personal factors and their influences on ob-gyn doctors' wellbeing and their quality of life.

Statistical analysis

Statistical analysis of data was performed using *SPSS Statistics v23.0.0*. The *t* test for independent samples and One-Way ANOVA were used for the comparative analysis. Statistical difference was defined as $p<0.05$. For the correlational study, Pearson and Spearman correlations were used, and to identify the influence of personality traits and socio-demographic data on job satisfaction multiple linear regressions were used.

RESULTS

Socio-demographic and work-related data

A total of 35 men and 81 women were included in the study, Mage = 40.34 ± 12.11 (with a minimum of 26 and maximum of 70 years of age); 42 were residents, 11 were specialists, and 63 were physician consultants.

Regarding marital status, 31 declared that they were single and 95 that they were presently in a relationship. Almost 40% of them had a partner practicing the same profession.

TABLE I

Socio-demographic data

Variables		N (%)
Gender	male	35 (30.17)
	female	81 (69.83)
Age	≤ 30	40 (34.5)
	31-40	21 (18.1)
	41-50	31 (26.7)
	≥51	24 (20.7)
Level of specialization	resident	42 (36.21)
	specialist	11 (9.48)
	consultant	63 (54.31)
Marital status	In partnership	85 (73.28)
	Single	31 (26.73)
The partner is a doctor		45 (39.47)
Having children		76 (65.22)
Experience as O/G doctor (M years)		12.94 ± 10.94
Weekly work hours		56 ± 21
Monthly number of shifts		4.5 ± 2.2
Having academic activity		24 (20.69)
Number of simultaneous jobs	1	83 (71.6)
	2	25 (21.6)
	3	8 (6.8)

The average number of years working in this medical field was 12.94 ± 10.94. Regarding hours worked per week, we identified M = 56 ± 21 hours/week. The number of shifts per month was M = 4.5 ± 2.2 shifts (tab. I).

Psychological data

All subjects answered the BFI questionnaire, and 112 of 116 completed the JSS questionnaire.

Cronbach alpha scores for the JSS subscales are: 0.632 for payment–promotion, 0.786 for management–interpersonal relationships, and 0.727 for organization–communication. For overall job satisfaction, alpha Cronbach score is 0.837. For BFI, we obtained the following results for Cronbach's alpha coefficient: 0.802 for extraversion, 0.467 for agreeableness, 0.772 for conscientiousness, 0.769 for neuroticism, and 0.743 for openness to experience

Overall satisfaction score was 3.49 ± 0.68, which corresponded to a low level of job satisfaction. For the first factor, payment – promotion, the score of 2.78 ± 0.89 showed a very low level for this subscale, which means that they are not satisfied with the material rewards, other financial benefits, professional recognition, and promotions. Regarding the second subscale, management–interpersonal relationships, the score was 3.95 ± 0.86, which corresponds to a very high level of satisfaction. This score indicates that subjects were very pleased with the social climate within their institution and the relationships developed on both horizontal and vertical levels. The third subscale targets the way the tasks are defined and organized and the effort that is involved in accomplishing them. It also refers to communication and feedback. On this subscale, our subjects obtained a score of 4.15 ± 0.71, corresponding to a medium level of satisfaction (tab. II).

TABLE II

Results for *Job Satisfaction Scale* and *Big Five Inventory*

Instruments		Total	Male	Female
JSS	Payment - promotion	2.78 ± 0.89	3.10 ± 1.18	2.65 ± 0.70
	Management – interpersonal relationship	3.95 ± 0.86	4.12 ± 0.97	3.87 ± 0.81
	Organization - communication	4.15 ± 0.71	4.40 ± 0.79	4.04 ± 0.66
	Overall satisfaction	3.49 ± 0.68	3.71 ± 0.82	3.39 ± 0.59
BFI	Extraversion	3.71 ± 0.71	3.73 ± 0.72	3.69 ± 0.71
	Neuroticism	2.79 ± 0.71	2.67 ± 0.69	2.84 ± 0.73
	Openness	3.60 ± 0.57	3.60 ± 0.61	3.61 ± 0.56
	Conscientiousness	3.99 ± 0.59	4.05 ± 0.50	3.97 ± 0.63
	Agreeableness	3.85 ± 0.83	3.80 ± 0.55	3.87 ± 0.93

Self-reported information about having a chronic disease, depression, insomnia, and administration of drugs to cope with stress were also registered. A number of 46 (39.7%) of subjects declared that the suffer from a chronic disease, 15 (12.9%) had or have depressive symptoms, 41 physicians (35.3%) mentioned sleep-related problems and 12 of

them (10.3%) sustained that they use medicines to better adjust to stressful factors.

Gender

A significant difference was found for the gender variable regarding overall satisfaction (- t (110) = 2.00, p = 0.05). This indicates that men are more satisfied with their job than women are (3.71 ± 0.82 *vs* 3.39 ± 0.59). Significant differences were also found for the first and third subscales of JSS. Male doctors were more satisfied than female doctors regarding payment–promotion (3.10 ± 1.18 *vs* 2.65 ± 0.70; *t* (110) = 2.03, p = 0.049) and organization–communication (4.40 ± 0.79 *vs* 4.04 ± 0.66; *t* (110) = 2.44, p = 0.016). No significant difference was identified between men and women regarding the second subscale (management–interpersonal relationship).

The level of specialty

To analyze the differences among various medical specialties (residents, specialists, and consulting physicians), univariate analysis of variance (ANOVA) was used. We found that specialists are more satisfied overall with their job than consulting doctors were (Mdif = 0.336, p = 0.031) and management–interpersonal relationship subscale (Mdif = 0.459, p = 0.009). For the other two subscales, no difference was identified between the three specialty levels. No significant differences were found regarding all subscales and general satisfaction between residents and specialists or consultants (tab. III).

TABLE III

Comparative analysis regarding job satisfaction considering the level of specialty

JSS	Residents	Specialists	Consultants
Payment–promotion	2.84 ± 0.81	2.92 ± 0.51	2.72 ± 1.00
Management–interpersonal relationship	3.98 ± 0.78	**4.31 ± 0.26**	**3.85 ± 0.97**
Organization–communication	4.04 ± 0.67	4.43 ± 0.49	4.17 ± 0.77
Overall satisfaction	3.48 ± 0 .10	**3.77 ± 0.20**	**3.44 ± 0.08**

Insomnia, depression and use of medicine to cope with stress

The independent *t* test analysis revealed that significant differences between the subjects who self-reported sleep disorders regarding job satisfaction for all subscales and for overall job satisfaction, so we determined that doctors who experienced insomnia obtained lower scores. We also found that self-reported depression also produces differences in overall job satisfaction and the last subscale, organization–communication (tab. IV).

The results are important, because they indicate that sleep disorders determine significant differences in what determines satisfaction with professional activity. Also, it can be assumed that depression plays an important role in job satisfaction reported by obstetrics and gynecology physicians.

To analyze whether there are differences between doctors who use pills to cope with stress and those who do not self-administer stress medications. The results proved that significant differences only occurred with the third subscale, meaning that doctors who do not use pills to manage stress had higher levels of satisfaction regarding organization – communication: t (110) = - 2.08, p = 0.04 (Mwith = 3.75 ± 0.64 and Mwithout = 4.20 ± 0.71). For the other two subscales and for overall job satisfaction, no significant differences were found.

TABLE IV

Comparative analysis regarding job satisfaction considering *insomnia* and *depression*

JSS	Insomnia	Depression
Payment–promotion	t (110) = - 3.84, p = 0.000 (Mwith = 2.41 ± 0.57 and Mwithout = 2.98 ± 0.97)	t (109) = - 1.15, p = 0.24 (Mwith = 2.53 ± 0.61 and Mwithout = 2.83 ± 0.92)
Management–interpersonal relationship	t (110) = - 3.29, p = 0.001 (Mwith = 3.59 ± 0.75 and Mwithout = 4.14 ± 0.87)	t (109) = - 1.77, p = 0.07 (Mwith = 3.58 ± 0.61 and Mwithout = 4.01 ± 0.88)
Organization–communication	t (110) = - 4.38, p = 0.00 (Mwith = 3.77 ± 0.57 and Mwithout = 4.35 ± 0.70)	t (109) = - 2.23, p = 0.028 (Mwith = 3.76 ± 0.53 and Mwithout = 4.21 ± 0.72),
Overall satisfaction	t (110) = - 4.23, p = 0.00 (Mwith = 3.14 ± 0.50 and Mwithout = 3.67 ± 0.69)	t (109) = - 2.84, p = 0.009 (Mwith = 3.16 ± 0.42 and Mwithout = 3.54 ± 0.70)

The influence of other variables

The other variables analyzed were involvement in a didactic activity, chronic disease, marital status, and whether the partner was in the same profession. For these variables, we found no significant differences between obstetrics and gynecology doctors.

Correlational analysis between variables and JSS dimensions

The Spearman correlation was used when at least one of the variables in the correlation was not distributed normally, and Pearson correlation was used when both variables had a normal distribution (tab. V).

TABLE V

The results for the correlational analysis between variables and JSS dimensions

Variables	Payment and promotion	Management and interpersonal relationship	Organization and communication	Overall satisfaction
Age	R = - 0.134 p = 0.16	R = - 0.090 p = 0.34	R = 0.07 p = 0.46	R = - 0.059 p = 0.53
Years of experience	R = - 0.158 p = 0.09	R = - 0.085 p = 0.37	R = 0.09 p = 0.34	R = 0.062 p = 0.51
Children	R = 0.015 p = - 0.87	R = 0.062 p = 0.51	R = 0.075 p = 0.43	R = - 0.071 p = 0.46
Number of jobs	R = - 0.092 p = 0.33	R = - 0.034 p = 0.71	R = 0.066 p = 0.49	R = - 0.043 p = 0.65
Number of working hours/week	**R = - 0.191 p = 0.05***	R = - 0.154 p = 0.11	R = - 0.173 p = 0.07	**R = - 0.22 p = 0.02***
Number of swifts/month	R = - 0.005 p = 0.95	R = 0.018 p = 0.85	R = - 0.018 p = 0.84	R = 0.012 p = 0.90
Extraversion	R = 0.066 p = 0.49	**R = 0.225 p = 0.01***	**R = 0.272 p = 0.00****	**R = 0.222 p = 0.01***
Agreeableness	**R = 0.192 p = 0.04***	**R = 0.268 p = 0.00****	**R = 0.192 p = 0.04***	**R = 0.259 p = 0.00****
Conscientiousness	R = 0.098 p = 0.30	R = 0.116 p = 0.22	R = 0.171 p = 0.07	R = 0.129 p = 0.17
Neuroticism	**R = - 0.408 p = 0.00****	**R = - 0.428 p = 0.00****	**R = - 0.388 p = 0.00****	**R = - 0.458 p = 0.00****
Openness	R = - 0.046 p = 0.63	R = 0.100 p = 0.29	R = 0.169 p = 0.07	R = 0.034 p = 0.72

Among socio-demographic variables, only the number of working hours per week was negatively correlated with payment and promotion and overall satisfaction. Regarding the BFI traits, the statistical analysis identified a positive correlation between:

- agreeableness and all dimensions of JSS
- extraversion and two JSS dimensions (management–interpersonal relationships and organization–communication) and overall satisfaction

A strong negative correlation was identified between neuroticism and all dimensions of JSS.

These results prove that the more hours per week doctors work, the less satisfied they are with payment and promotion and the less overall satisfaction they experience regarding their profession.

The personality traits evaluated with BFI, conscientiousness–openness, had no correlation with the JSS dimensions. Extraversion seems to have no influence on payment–promotion, but it correlated with JSS overall satisfaction, management–interpersonal relationships, and organization–communication dimensions. Therefore, extraverts are more satisfied with interpersonal relationships–communication aspects of their job within their institution. The other trait, agreeableness, was positively correlated with all dimensions of JSS and overall satisfaction, proving that the more compassionate and cooperative a person is the higher level of job satisfaction he/she experiences. Agreeableness is a measure of trust and is helpful to a person's nature.

The correlational analysis proved that personality factors identified with BFI interact with some of the dimensions of job satisfaction and overall satisfaction identified with JSS. To identify which is the most effective prediction model for job satisfaction, we used multiple linear regressions with the hierarchical method (tab. VI).

TABLE VI

Regression analysis

	Models		Payment and Promotion	Management and Interpersonal Relationship	Organization and Communication	Overall Satisfaction
Step 1	Extraversion	R^2adjusted	- 0.002	0.042	0.065	0.040
		ΔR^2	0.007	0.050	0.074	0.049
		F	0.734	**5.841***	**8.770****	**5.677***
Step 2	Extraversion, agreeableness	R^2adjusted	0.002	0.053	0.082	0.062
		ΔR^2	0.013	0.019	0.025	0.030
		F	1.086	**4.090***	**5.960****	**4.657***
Step 3	Extraversion, agreeableness, conscientiousness	R^2adjusted	- 0.007	0.044	0.076	0.054
		ΔR^2	0.001	0.000	0.002	0.001
		F	0.755	**2.702***	**4.035****	**3.110***
Step 4	Extraversion, agreeableness, conscientiousness, neuroticism	R^2adjusted	0.098	0.156	0.139	0.183
		ΔR^2	0.110	0.116	0.069	0.133
		F	**4.004***	**6.120*****	**5.461*****	**7.222*****
Step 5	Extraversion, agreeableness, conscientiousness, neuroticism, openness	R^2adjusted	0.104	0.150	0.132	0.190
		ΔR^2	0.014	0.002	0.002	0.014
		F	**3.567****	**4.908*****	**4.386****	**6.201*****

*** p<0.001, ** p<0.01, * p<0.05

For the payment and promotion dimension, only the last two models of prediction proved to be significant. Of these two, the fourth model, which has extraversion, agreeableness, conscientiousness, and neuroticism as predictors, is providing the most significant contribution, 9.8% of the variance of payment and promotion. Also, among the four predictors of this model, only neuroticism had a significant statistical influence on payment and promotion (p = 0.000), which explains a lot of the variance in the dependent variable, in this case, payment - promotion (b = -0.480, beta = -0.388). The effect of this predictor is a negative one, indicating that the more present this personality trait is, the lower the payment and promotion satisfaction level will be.

For the management and interpersonal relationships factor, all the prediction models were significant. Also, the fourth model, which has extraversion, agreeableness, conscientiousness, and neuroticism as predictors, makes the most significant contribution (15.6% of the variance of management and interpersonal relationships). Of the four predictors of this model, only the personality trait neuroticism had a significant statistical influence (p = 0.000) on the dependent variable management–interpersonal relationships (b = -0.481, beta = -0.399). Its effect was also negative. This suggests that the more present neuroticism is, the less satisfaction a person will experience regarding management–interpersonal relationships.

In the case of organization communication, all the prediction models were statistically significant. The strongest prediction models were the first model with extraversion as a predictor and the fourth with extraversion, agreeableness, conscientiousness, and neuroticism as predictors. Of those two, the fourth had the greatest degree of variance for the dependent variable organization–communication (13.9%). Our results also show that of the four predictors of this model, neuroticism is the only one that has a significant influence (p=0.004) on the organization–communication variable (b = -0.305, beta = -0.307). The effect of this predictor is also a negative one, meaning that the more neuroticism is present in a person's personality structure, the less satisfaction he/she will experience for organization–communication.

Concerning overall job satisfaction, all the prediction models were significant. In this case too, the fourth model with extraversion, agreeableness, conscientiousness, and neuroticism as predictors was the strongest, (18.3% for overall job satisfaction). As with the other variables, only neuroticism had a significant statistical influence (p = 0.000) on overall job satisfaction (b = -0.404, beta = -0.427), its effect being a negative one too meaning that the more neuroticism is present as a personality trait, the less the subject will feel satisfied with professional activities. Based on the results, we can conclude that neuroticism plays the most important role in our participant's personality structure when it comes to predicting job satisfaction.

DISCUSSION

This study confirms some results reported in the literature. Satisfaction about professional life seems to have lower scores for obstetrics and gynecology doctors. Among medical specialties, ob-gyn

physicians obtained the lowest scores for job satisfaction compared with physicians in all other medical specialties (22).

The results prove that 19.64% of subjects are very satisfied or satisfied with their work (overall satisfaction score). Also, considering the domains, 31.25% are satisfied or very satisfied about payment and promotion, 79.46% are satisfied with management and interpersonal relationships, and 52.67% are satisfied or very satisfied with organization and communication. The rate of overall job satisfaction identified by our study is lower than that in other studies: 74 - 80% in USA identified by Leight, Tancredi, and Kravitz (22) and Kravitz et al. (13). One of the reasons could be the fact that no study has been conducted that includes obstetrics and gynecology doctors working in Eastern European countries. The few research studies conducted were in wealthy countries where the professional satisfaction is assured by different factors (income, medical settings, and professional opportunities, among other factors).

The low rate of overall job satisfaction and low level of satisfaction on the subscale related to payment and promotion proven by our research can be explained by two factors: first, the low income of physicians in Romania. This issue is proven by two arguments: the highest rate of "brain drain" among physicians in European Union countries occurs in Romanian physicians (9). The second argument is proven by our research; Romanian physicians have an increased number of working hours per week and a high number of shifts per month, influencing the level of professional exhaustion (33, 34). Secondly, the healthcare policies and Romanian malpractice laws are not encouraging for sustaining professional activity. Most of the medical institutions offer no legal or financial support to physicians in case of a medical error. In this situation, ob-gyn physicians are taking greater precautions when using new methods or procedures involving in risky medical interventions, which is a cause of professional dissatisfaction.

The results for gender differences regarding job satisfaction among doctors are ambiguous in the scientific literature. For example, McMurray et al. (23) found that women are more dissatisfied than men. Leight, Tancredi, and Kravitz (22) found no differences between genders, and Keeton et al (24) found that male obstetricians/gynecologists were less satisfied than women. Our results indicate that women are less satisfied with their job than men are. This fact has an important influence on one of the most important consequences of job dissatisfaction: migration. Statistical data prove that a significantly higher number of female doctors have migrated to Eastern European countries, with some research reporting 80% of the all specialties doctors left Romania to work abroad. In most of the cases, women doctors left together with their children, meaning that the decision to leave was determined to be for the long term or forever (9, 25). In the present study, male doctors were more satisfied than female doctors regarding payment–promotion and organization–communication subscales.

Variables like didactic activity, chronic disease, marital status, and having a partner with the same profession. For these variables, we found no significant differences

between obstetrics and gynecology doctors. The results are different from those reported in the literature; for example, regarding didactic activity, in a study in the US, gynecologists and obstetricians with teaching and research activity obtained higher scores in overall job satisfaction (26).

Among personality traits, agreeableness and neuroticism were proved to impact the most on the level of satisfaction with job and its dimensions. Neuroticism is an indication of emotional instability. Strong negative correlations between neuroticism and all dimensions of job satisfaction proved the importance of this trait in professional life. The results are conforming to some other studies on the influence of neuroticism on professional life. Regarding agreeableness, this personality trait seemed to be more specific to tight and collectivistic societies (27). Considering the history of the country, this personality traits could be explained by the fact that over 40% of ob-gyn physicians experienced for almost half of their lives a collectivistic type of society.

The results are important for ob-gyn physicians and for medical students choosing this specialty. Some comparative studies focusing on medical students revealed that students who enter obstetrics and gynecology score significantly higher on working with people (28). So, more vocational counseling is needed to be provided during their medical studies to help them discover, explain and develop their skills in concordance with their personality traits. Job dissatisfaction will be later in the professional life related to burnout and negative behaviors such as consumption of drugs, malpractice or suicide thoughts (29-31). Making medicals students be aware about the psychological issues of their professional life will help them to better adjust to the professional challenges.

Strengths and limitations of the study

The strengths of the study. Our research included a higher number of physicians from this specialty registered in Romania, compared with other studies (32). So, the results could be generalized. No other study measured the level of satisfaction with job and its relationship with other variables for this medical specialty in an Eastern European country. The results are important for adjusting health-policies for physicians because Romania is the main country with the brain-drain in Europe, high levels of migrations among physicians are registered in the last decade.

The limitations of the study. The research targets physicians working in Romania. The specificity of the ob-gyn profession (rules regarding the working hours per week or the number of shifts per month) is not identical in all countries in the world. For this reason, we cannot say that the results are generally characterizing all physicians in this specialty. The second limitation is due to the number of specialist doctors included in the research and the male/female number of subjects. So, comparative analysis could not be considered generally available for all ob-gyn physicians. Even though the present study provides a lot of valuable information for a better understanding of the psychological factors involved in obtaining job satisfaction, converging results from larger, more generalizable, carefully controlled studies

is needed.

CONCLUSIONS

Based on the study results, the doctors in the study sample experienced a low level of job satisfaction. When it comes to the factors of job satisfaction, physicians were not satisfied with the rewards. Our results highlight the importance of a regular work schedule for doctors because the correlational analysis indicated that the more hours per week the subjects worked, the less satisfied they were about payment and promotions and with their overall work life. Personality traits play an important role in predicting job satisfaction. The presence of depression and insomnia strongly influenced overall job satisfaction.

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THE IMPORTANCE OF CLINICAL PREDICTION MODELS IN NON-FATAL PULMONARY EMBOLISM: AN ANALYSIS OF THE BEST KNOWN CLINICAL SCORES

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THE IMPORTANCE OF CLINICAL PREDICTION MODELS IN NON-FATAL PULMONARY EMBOLISM: AN ANALYSIS OF THE BEST KNOWN CLINICAL SCORES (Abstract): The clinical evaluation in pulmonary embolism (PE) is the first instrument used by practitioners in the management of this potentially fatal pathology. The necessity of developing certain valid and especially affordable practical instruments has led to the emergence of various clinical prediction models. The purpose of this paper is to analyze the main clinical scores, as a diagnostic or a prognostic tool, with their strengths and weaknesses. The PESI score, while relatively recent, remains the most investigated and validated prognostic score for the identification of the mortality risk and major adverse events, with economic implications of health services reduction costs through the accurate identification of patients with a low risk who are candidates of early hospital discharge. The simplified Geneva score (with a similar accuracy as the Geneva one) identifies a high or low PE probability, especially in combination with D-dimers, with a prognosis value as well. The Wells and simplified Wells scores identify the high or low probability, being improved by the level of D-dimers, having similar results with the Geneva score. The LR-PED score, conceived as an identification score for low risk, uses biochemical and electrocardiographic markers, but is less validated. The Vienna Prediction Model is another system for the evaluation of the recurrence in which the level of D-dimers is the main prediction factor. Other scores were evaluated with a statistically low significance. The Geneva and the PESI scores remain the most valuable instruments of diagnosis and clinical prognostic, respectively. **Keywords**: PULMONARY EMBOLISM, CLINICAL SCORE, PROGNOSTIC, MORTALITY

Clinical prediction models have become more popular to aid clinical decision-making.

These models were developed to deliver a probability of having (a diagnostic prediction model) or developing (a prognostic prediction model) a certain outcome in an individual.

Pulmonary embolism (PE) is a potentially fatal condition, which's diagnostic and treatment should be rapid, especially in the case of a massive PE. Both the European Society of Cardiology (ESC) guide and the American Heart Association (AHA)

recommends the stratification of patients in patients with a high risk of mortality (massive PE) (1, 2) and patients with non-fatal risk. The non-fatal group comprises 90% of the patients and shows an early mortality risk that varies from less than 1% (low risk) up to 15% (intermediate risk)(1, 2). Using researches dating back as early as the 1990s, but also recent imagistic and laboratory studies, the ESC guide regarding the PE management (2014) recommended that the patients without signs of shock and without increased biomarkers or echocardiographic aspects indicative of right ventricular dysfunction in sPESI score 0 be listed in the low risk group who benefits from early hospital discharge and home care (1). All these are recommendations and not clear indications due to the absence, until recently, of solid proof for selecting patients who may receive outpatient treatment (3). Unlike other diagnostic and prognosis instruments, these clinical scores are based on clinical data which may be applied in any medical unit. Thus, one can determine from the admission, using clinical scores, laboratory and imagistic tests, a profile of the low-risk patient who can receive outpatient care. Taking into consideration the more and more frequent evidence underlining the fact that the low risk subgroup and non-fatal PE benefit from outpatient treatment, British Thoracic Society confirms this recommendation, a measure which may lead to a substantial reduction of healthcare costs (4).

The identification of a valid and easy clinical score to the largest extent possible both from an economic point of view and from the perspective of access to various sophisticated diagnosis instruments, which can be a diagnostic and a prognostic tool at the same time, remains a challenge that this paper attempts to elucidate, by reviewing the main clinical prediction models. Multiple clinical prediction rules were discovered. Some of them, well-known as a diagnostic tool, now have a proved prognostic value, others were defined from the beginning as a prognostic tool. The patients with non-high risk PE can benefit from an accurate and relatively fast clinical examination in order to receive the best possible treatment.

Therapeutic strategies determined by the risk degree and the importance of clinical prediction models. The European PE guide divides patients into 3 risk groups: high, intermediate and low. High-risk patients are those diagnosed with elements of shock or hypotension (defined as systolic blood pressure under 90 mmHg or a decrease of the pressure by more than 40 mmHg in 15 minutes, which is not caused by the debut of an arrhythmia, hypovolemia or sepsis) and who receive thrombolytic therapy and/or hemodynamic and respiratory support (1). They are not covered by the current paper.

Patients with normal blood pressure with non-high risk fall into the intermediate risk group in the presence of dysfunction markers of the right ventricle (RV) (echocardiographic and/or positive BNP) and/or of myocardial lesion (positive I or T troponin). Hemodynamically stable patients without RV dysfunction elements or myocardial lesion show a low mortality risk (<1%). The injectable antigoagulant treatment is administered to all patients when there is a clinical suspicion of acute PE, even prior to the diagnostic confirmation, being followed by oral anticoagulants (1). Thus, the importance of clinical prediction scores is confirmed, as they may identify from the beginning a low mortality risk, the

patients being able to benefit from early discharge and outpatient care, with a direct impact on healthcare costs. Several early discharge criteria may be defined: absence of shock or hypotension, absence of RV dysfunction, absence of important co-morbidities (chronic heart failure, chronic pulmonary diseases, and chronic renal disease), low recurrence risk, exclusion of patent *foramen ovale*, absence of active bleeding and low hemorrhagic risk, absence of the necessity of intravenous analgesic administration and absence of oxygenotherapy indication (3). All of these provided there is a compliant patient who benefits from family support to continue proper treatment at home. But, taking into consideration that patients, not diseases, are treated, each with his/her own particularities, the determination of accurate and statistically well-documented clinical scores is a must. The controversial hypothesis of full treatment outside the hospital of certain well-selected patients has also been tested, provided there is an easy access to a hospital unit. The bleeding, recurrence or mortality risk was similar between the group which received full outpatient treatment and the hospitalized group (5). However, these studies failed to take into consideration less frequent PE complications, such as chronic pulmonary hypertension, arrhythmia or the necessity for inotropic support or thrombolysis, while rare in case of low-risk patients (6). Another argument for the early hospital discharge is the emergence of new oral anticoagulants which do not require laboratory monitoring or adjustment of doses for patients with venous thromboembolism. The potential of the new oral anticoagulants to treat low-risk patients and with early discharge criteria is to be evaluated in future studies. Antithrombotic treatment may have also a potentially beneficial role (7).

Economic impact. Early discharge of correctly evaluated low-risk PE patients is associated with a reduction of hospital costs, allowing the medical unit to use the saved funds for the more efficient and more complex treatment of serious cases. The cost-efficiency ratio of diagnosis and treatment in venous thromboembolism has been evaluated, but especially in deep thrombophlebitis (DVT) which has a similar anticoagulant treatment. A study revealed that the cost of hospital care for a DVT patient was higher for unfractionated heparin as compared to fractionated heparin and the cost of fractionated heparin administration in outpatient treatment decreased by almost a half (8). With regard to PE, it has been proven that the fractionated heparin treatment would be cost-saving if more than 8% would be eligible for early discharge or more than 5% of patients could receive outpatient treatment (9).

DIAGNOSTIC CLINICAL PREDICTION RULES

The Geneva and Wells score. The best known and most used are the Geneva and Wells scores. In a prospective study which comprised 296 PE diagnosed patients admitted at University Hospital of Geneva, followed up for a period of 3 months, six independent mortality predictors were identified as well as other adverse events (PE recurrence, major bleeding). These factors were validated in a study conducted in an emergency department, later to be named the Geneva prediction rule (10). The revised Geneva score comprises 8 variables with a different weight, which might be difficult to memorize and calculate in an emergency unit. The simplified Geneva

score assigns 1 point for each factor used. Low probability is between 0-1 points, intermediate probability between 2-4 points and high probability over 5 points. The accuracy of the diagnosis and the clinical utility are not modified in case of the simplified Geneva score, being able to exclude PE when combined with a normal D-dimers value. The PE prevalence is similar to the original Geneva score and to the Wells score (11). Recently, the Geneva score was investigated in a large study, which evaluated 9 clinical scores as prognostic assessment tools in patients with pulmonary embolism (12). Out of these, the PESI and Geneva scores identified the in-hospital mortality < 1% (low-risk) for the PE patients. The identification of low recurrence risk seems to be superior with PESI score than Geneva score. However, 3 months-mortality was comparable between the 2 scores, both for the low-risk group (1.1% at PESI vs. 0% at Geneva) and for the high risk group (11.1% PESI *vs.* 14.3% Geneva). The use of both scores improves the prognosis evaluation. For instance, the mortality of patients with greater Geneva probability increased from 14.3% to 25% when the high PESI risk was added (13).

The Wells score, published in 2000, is based on a multicenter Canadian study and was developed as a clinical selection pretest of patients with high PE probability. A Wells score higher than 2 is indicative of a high PE probability which requires imagistic explorations, while a score lower than 2 has a negative predictive value for PE of 95% (14). The simplified Wells score assigns 1 point for each variable, a score value higher than 1 suggesting PE probability (15). In the PIOPED II data, the CT positive predictive value for PE at patients with Wells scores lower than 2 was of 58 % indicating that a large portion of the results from these patients are false-positive. The use of D – dimers tests in combination with the Wells score was suggested as a means to help with the patients' selection. It was proved that a negative D-dimers result reduces the PE probability for any Wells score. The PE probability for patients with a score of <2, even with positive D-dimers, was of 7.0 %. This is comparable to the PE rate for patients with a negative CT result (14). The Wells score accurately predicts PE probability at hospitalized patients and this population had a greater PE prevalence than other cohorts. However, the score has no prediction value to exclude a potentially fatal evolution (16).

Given the similarities between the Geneva and the Wells scores, various comparative analyses were conducted. A study that compared 4 clinical scores (the two ones and their simplified versions) concluded that they are similar in their ability to differentiate patients depending on the PE probability and in the patients' proportion who required no imagistic investigation due to the low risk points of the scores with normal D-dimers. Although there were discrepancies at approximately 30% of the patients regarding the low or high probability, there were no differences in the failure rates when the clinic – D-dimers combination was used. The simplified Wells score had similar performances with the Wells score and the revised Geneva score (15).There are limitations, however. The Wells score includes clinical judgment, which is subjective and cannot be standardized (17). It also has no prognostic value. Moreover, it was suggested that the predictive value of this score derives precisely from its subjective component (18).

The Pulmonary Embolism Rule-out Criteria (PERC) rule. This score was obtained to exclude the PE diagnosis using anamnesis and the patient's physical exam. A negative value implies: the clinical judgment of the physician regarding the probability lower than 15% of PE to which the following characteristics are added: age < 50, pulse < 100 beats/min, oxygen saturation > 94% in room air, absence of hemoptysis, absence of estrogen use, no PE antecedents or deep thrombophlebitis, no unilateral edema (objectified by the clinician), no recent surgical interventions or traumas (in the last 4 weeks, which involved endotracheal or spinal anesthesia) (19). Of a greater significance is the fact that this rule applies to a population with a PE prevalence lower than 10%. At this population, the sensitivity was of 100%, the specificity varying between 16-33% with a negative predictive value of 100% (19, 20, 21). In addition, it needs to be applied to low-risk patients, therefore another clinical score, such as the Wells score, should be calculated first. A single study used this score for patients with high risk as well and proved that the PERC is highly sensitive (100%) and has good negative predictive value (20). In case of the population with high PE prevalence, the PERC score, although combined with the revised Geneva score, could not exclude PE without further tests (22).

PROGNOSTIC CLINICAL PREDICTION RULES

The Pulmonary Embolism Prognostic Index (PESI) score. Various comparisons between clinical scores are difficult to interpret but it seems that PESI is the most studied with a total number of 22127 patients. PESI identified 43% of the patients with inter-hospital mortality less than 1%, defined by both guides (ESC and AHA) as low-risk. It comprises 11 clinical parameters with various degrees of prognosis. Depending on the score, the patient falls into one of the 5 classes, with mortality at 30 days ranging from 1.1% to 24.5% (23). Patients in the risk class I or II are low-risk and those in classes III-V are high-risk. However, the original score uses 11 clinical parameters and the final value requires difficult calculations which are hard to make in the clinical practice.

The simplified PESI (sPESI) was developed in 2010 and includes 6 out of the 11 original parameters, dividing patients into high-risk (1-6 points) and low-risk (0 points) patients (24). Both original and simplified PESI were included in the last ESC guidelines on the diagnosis and management of acute pulmonary embolism for risk stratification besides laboratory parameters and imaging tests.

A recent meta-analysis comprising 21 studies has comparatively analyzed the PESI versus sPESI accuracy in predicting adverse events (general mortality and PE related mortality, PE recurrence, non-fatal bleeding)(25). The PESI model also identifies patients with high-risk, a PESI high-risk score is a risk factor for mortality of all causes (AUC 0.78 (95% CI: 0.77 to 0.80)), PE related mortality (AUC 0.82 (95% CI: 0.75 to 0.89)) and significant adverse events. In addition, the sPESI group showed a similar accuracy with the PESI group (similar OR and AUC for PE related mortality, mortality of all causes and significant adverse events) in several studies (25, 26). sPESI was validated in a subsequent study which confirmed its applicability to an independent population (23). This model accurately identifies PE patients who show a low risk of fatal and

non-fatal medical events: no patient from the class I died or showed PE recurrence or major bleeding at 90 days from the initial PE. Out of the class II patients, only 1% died and no patient showed adverse events. The negative predictive value for mortality among patients with low-risk (classes I and II) was of 98-99%. Thus, one can consider in these cases the possibility of early hospital discharge, depending on the patient's particularities (23)

The biological markers of risk in PE are associated with a high risk of fatal events, but one of the most important benefits is their negative predictive value. The association of troponin with PESI score was studied in order to improve the risk evaluation. A study proved the superiority of PESI as compared to troponin I in the identification of low-risk patients. The combined prognosis value PESI + troponin I was not superior to PESI (27). The use of high-sensitive troponin added prognosis value to the PESI score (28). A T troponin ≥ 14pg/ml associated to sPESI ≥1, in case of PE hemodynamically stable patients, was an independent predictor of early death and complications. T troponin < 14 pg/ml and sPESI =0 were identified at 24% of the subjects for whom no adverse effects were mentioned.

Although PESI and sPESI correctly identify low risk, certain patients initially identified as high-risk, after the initiation of the treatment may fall into a lower risk category, using the PESI calculation at 48 hours from the admission (PESI 48). It has been noticed that the decrease of PESI and sPESI at 48 hours after the treatment was associated with the decrease of mortality. Notable modifications were attributed to the improvement of heart frequency, temperature and oxygen saturation. Thus, 8% more patients fell into the low-risk group. Moreover, patients reclassified as high-risk showed a short-term increase of mortality of up to 50%. The modification of PESI and sPESI after 48 hours from the start of the treatment is correlated with subsequent mortality (29). Venetz et al. conducted a comparative study among patients with high and low risk using PESI, sPESI and 30 days mortality (30). Although sPESI accurately identified low risk of adverse events, PESI classified a higher proportion of low-risk patients, having a greater discriminating power than sPESI (6). A recent study has confirmed that the PESI score may also accurately predict long-term mortality (1 year) in case of patients without cancer. sPESI showed a similar accuracy at 3 and 6 months, but significantly lower at 1 year (31).

Compared to the **ESC prognostic model**, the proportion of patients classified as low-risk was smaller. sPESI indentified with more accuracy the low-risk group for whom outpatient treatment may be an option. sPESI is easy to apply, being routinely used in the clinical practice and takes into consideration both the PE severity and the associated co-morbidities without requiring complex laboratory or imagistic tests or other less affordable ones (32).

The **Shock Index** (the ratio between cardiac frequency and systolic blood pressure) is a simple method of predicting high risk of unfavorable events when its value exceeds 1. This value is related to inter-hospital mortality and reveals a negative prognosis in association with echocardiography (33). Analyzed in comparison with the Shock Index, sPESI showed greater prognosis accuracy in predicting 30 days mortality as well as in identifying low-risk patients, having a negative predictive value for death among those with higher risk (34).

The PESI and the Geneva scores are two clinical scores which identify both high risk of adverse events and low risk. PESI had a greater discriminatory power for predicting 30 days mortality than Geneva. Patients with PESI low-risk had a lower mortality and negative predictive value of death than the patients for whom the Geneva score was used (30-day mortality - PESI 0.9%; 95% CI, 0.3 to 2.2; vs. Geneva, 5.6%; 95% CI, 3.6 to 7.6 - $p < 0.0001$) (35). Uresandi et al. developed a prognostic clinical score (**Spanish score**) in a multicenter prospective study at PE patients (36). The score includes the following variables: recent severe bleedings, metastatic cancer, non-metastatic cancer, values of serum creatinine > 2 mg/dL, bed immobilization due to a recent medical condition, absence of surgical interventions in the last two months and age >60. The score classified 47.8% of patients as low-risk. Compared to the Spanish score, sPESI had a better sensitivity and negative predictive value, with a more accurate identification of low risk of adverse events (37). The Spanish score and PESI were formally applied in a randomized control trial aimed to select patients' disposition for the initial treatment of PE. The study was prematurely stopped because of an unexpected high rate of adverse outcomes in included patients at low risk according to Spanish score, even if results were in favor of short hospital stay.(12)

The Low Risk Pulmonary Embolism Decision (LR-PED) rule. This score, recently identified, destined initially to identify eligible patients for early discharge and outpatient treatment, includes: age, preexisting heart failure, atrial fibrillation, heart rate, creatinine, glycemia, troponin I and protein C reactive upon admission. Compared to the sPESI and the Geneva score, it showed a greater sensitivity and negative predictive value for detecting patients with low risk (38). Despite the small dimension of the study lot and the lack of validation in an independent lot which diminishes the clinical applicability, the LR - PED score directs attention on the importance of combining the analysis parameters (troponin I, creatinine, protein C reactive and glycemia) on the heart rate (6).

Vienna Prediction Model is another system for the evaluation of recurrence at patients with PE or idiopathic deep vein thrombosis at the first episode. Age, sex, location of the thrombus, body mass index, V Leiden factor, prothrombin mutation and D-dimers were preselected as relevant risk factors as previously demonstrated. Only sex, thrombus location and D-dimers were identified as relevant predictors of recurrence. It was noticed that the association of the male sex and the localization of the embolism with recurrence risk diminishes in time, while the association with the D-dimers level remains stable. This model allows the prediction of the recurrence risk at various time intervals. Thus, D-dimers were repeatedly measured on a 2 years' duration (39). The repetition of dosing D-dimers after the anticoagulants were stopped was studied before, proving that the persistence of a high level of D-dimers at 3 months from the cessation of the anticoagulant treatment was associated with a higher recurrence rate (40).

The Global Registry of Acute Coronary Events (GRACE) risk score is well-known for high diagnostic performance for adverse outcomes in acute coronary syndrome. Comparisons between GRACE and the other risk scores were performed and it has been shown that this risk score can accurately predict 30-day mortality in pa-

tients admitted for acute PE (41).

CONCLUSIONS

In the daily practice, the existence of scores made up of accessible clinical parameters determines the rapid initiation of optimum therapeutic measures, with the determination of the prognosis and possibly of the outpatient surveillance. The existence of multiple clinical scores may be confusing and time-consuming for a physician, so the large validation of one prediction model (with diagnostic and prognostic value) is required. The Geneva score meets the criteria for both a diagnostic and prognostic clinical rule. In the case of non-fatal PE, which may have sometimes an unpredictable evolution, the PESI and sPESI are the ones to assess the mortality risk and the risk of major adverse events. Certain scores failed to prove their efficiency fully until now, further studies being necessary. The availability of different clinical scores helps doctors to assess PE patients and establish immediate treatment (diagnostic scores) and longtime treatment (prognostic scores) with a favorable impact on the healthcare costs.

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THERAPEUTICAL CONSIDERATIONS IN ASSOCIATED ATRIAL FIBRILLATION AND HEART FAILURE

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THERAPEUTICAL CONSIDERATIONS IN ASSOCIATED ATRIAL FIBRILLATION AND HEART FAILURE (Abstract): Atrial fibrillation is a supraventricular tachyarrhythmia very common in medical practice, often associated with heart failure. Pathophysiological relationship between atrial fibrillation and heart failure is in the attention of numerous case studies, being incomplete elucidated. **Material and methods:** We made a retrospective study on patients with both diseases, hospitalized in Cardiovascular Rehabilitation Hospital, Iasi, during 01.01.2013 - 31.12.2013. The obtained data allowed the classification of patients according to gender distribution, age groups, area of origin, clinical aspects, and association with other diseases, instituted treatment and appreciation of CHADS2 score. Data interpretation was performed with appropriate statistical methods. **Results:** We found a higher frequency of the disease among male patients, male: female ratio being 2:1; the most of the patients lived in urban area. The pick of diseases incidence was in patients over 65 years with a total percentage of 70.84% of cases. We noted that the most common symptoms were exertional dyspnea (in all patients), palpitations, dizziness, headache, fatigue, asthenia, dyspnea at rest and pain / chest pressure. In our study, the majority of patients received the beta-blocker - digoxin combination (46 patients, 40 patients respectively). **Conclusions:** The coexistence of the two disorders could be explained by identifying common risk factors. Beta blockers should be the first therapeutic option in patients with chronic heart failure and atrial fibrillation because they have the effect of controlling heart rate and improve survival in patients with these disorders. Meanwhile, digoxin is a drug, only certain conditions of high accuracy monitoring; whose major clinical indications are heart failure and atrial rhythm disturbances. **Keywords:** ATRIAL FIBRILLATION, HEART FAILURE, DIGOXIN, BETA-BLOCKER

Atrial fibrillation (AF) is a supraventricular tachyarrhythmia very common in medical practice, which consists of a disorganized cardiac electrical activity and progressive deterioration of atrial electromechanical function (1, 2, 3).

Heart failure (HF) is symptomatic in about 30% of patients with AF, and AF is also found in approximately 30-40% of patients with symptoms of HF (4, 5, 6). Pathophysiological relationship between AF and HF was in the spotlight of numer-

ous studies, as it has not been fully elucidated. In patients with chronic HF and AF, beta blockers should be the first therapeutic option because their effect are related to heart rate control and the improvement of survival in patients suffering from these disorders (7, 8). Meanwhile, digoxin is an effective drug, safe only during an accurate monitoring, whose major clinical indications are HF and atrial rhythm disturbances (9, 10). In combination of the two, digoxin has electrophysiological effects which decrease the atrioventricular node level, making it potentially useful in controlling heart rate (11, 12, 13).

MATERIAL AND METHODS

We present a retrospective study on patients with associated pathology - AF an HF that aims to highlight clinical and laboratory aspects, the type of treatment instituted, the occurrence of side effects and the assessment of the CHADS2 score (Cardiac failure-Hypertension-Age- Diabetes-Stroke double), in order to evaluate the risk of stroke and thromboembolism in this class of patients. During 01.01.2013 - 31.12.2013, we recorded a number of 2279 hospitalizations at Rehabilitation Hospital - Cardiovascular Rehabilitation Clinic, Iasi. Within these, 72 were related to the above mentioned pathologies. Our data allowed the classification of patients according to gender distribution, age, area of origin, clinical aspects, association with other diseases, type of instituted treatment and appreciation of CHADS2 score. Data interpretation was performed with appropriate statistical methods.

RESULTS AND DISCUSSION

CHADS2 score. We conducted the evaluation of CHADS2 score in order to assess the risk of ischemic stroke events and thromboembolic phenomena, namely of the need for an anticoagulant therapy, considering the following criteria:

- 2 points for a history of stroke or ischemic transient pathology;
- 1 point for each of the following: age over 75 years, hypertension, diabetes, heart failure.

A score of 0 represents a low risk of stroke or thromboembolism, a score of 1-2 represents a moderate risk and a risk higher than 2 is translated into an increased risk of presenting these complications (fig.1).

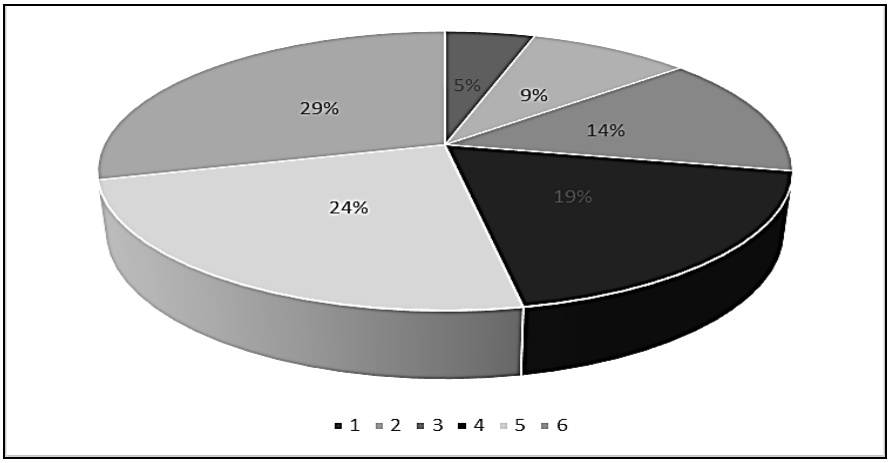

Fig. 1. Distribution of cases according to $CHADS_2$ score;

The distribution by area of origin. The classification of patients based on the area of origin was as follows: urban 54.2%, rural 45.8% (fig. 2).

Distribution of patients by gender. Analyzing the data from figure 3 we can see a higher frequency of the diseases among male patients, male: female ratio being 2:1. In terms of AF, the literature mentiones that men are affected more often than women, and in terms of HF, gender ratio is approximately equal (fig. 3).

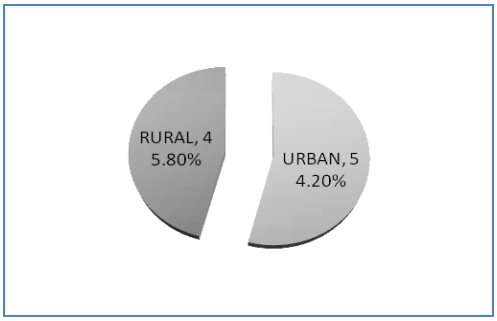

Fig. 2. Distribution of patients by area of origin

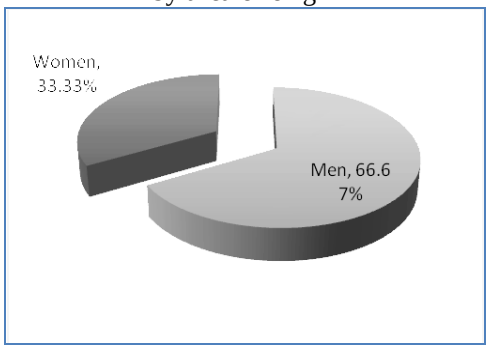

Fig. 3. Distribution of patients by gender

Distribution of cases by age groups. We calculated the mean age of incidence of the associated pathologies that was 69 years. We noted that our results were consistent with the literature data according to which the prevalence increases in patients older than 65 years (14, 15). Indeed, in our study the pick of incidence was in patients over 65 years, with a total percentage of 70.84% of cases, more precisely on the segment 65-75 years being registered the highest percentage of 37.50% of total cases. The lowest age recorded in this study was 53 years, and the highest was 83 years (fig. 4).

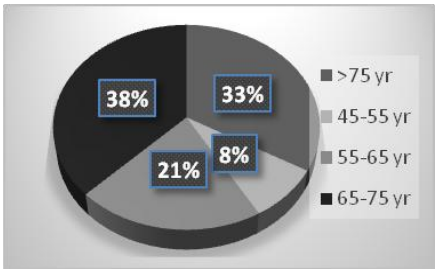

Fig. 4. Distribution of cases by age groups

Family history (HCA). HCA study revealed a number of 27 patients, 12 females and 15 males, who had a first degree relative suffering from heart disease (fig. 5).

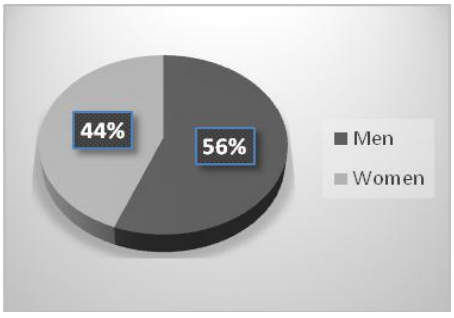

Fig. 5. Distribution of cases by AHC

Risk Factors. Regarding the usual risk factors, we noted a number of 15 patients affected by chronic alcohol consumption, a number of 24 smokers, and 12 who were smokers and affected by chronic alcohol consumption.

Distribution of patients by symptoms. We noted that the most common symptoms are exertional dyspnea (in all patients),

palpitations, dizziness, headache, fatigue, asthenia, rest dyspnea and pain/chest pressure (fig. 6).

Classification of patients according to the type of AF is shown in fig. 7, and according to NYHA class in fig. 8. Associated pathology is also an important element in calculating the risk, in assessing the prognosis of these patients and in choosing an appropriate treatment (fig. 9).

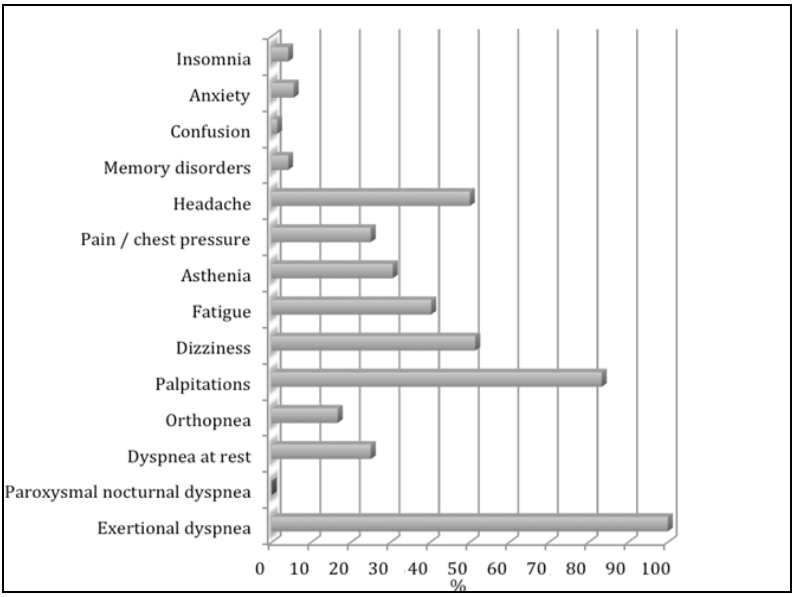

Fig. 6. Dominant symptoms in patients

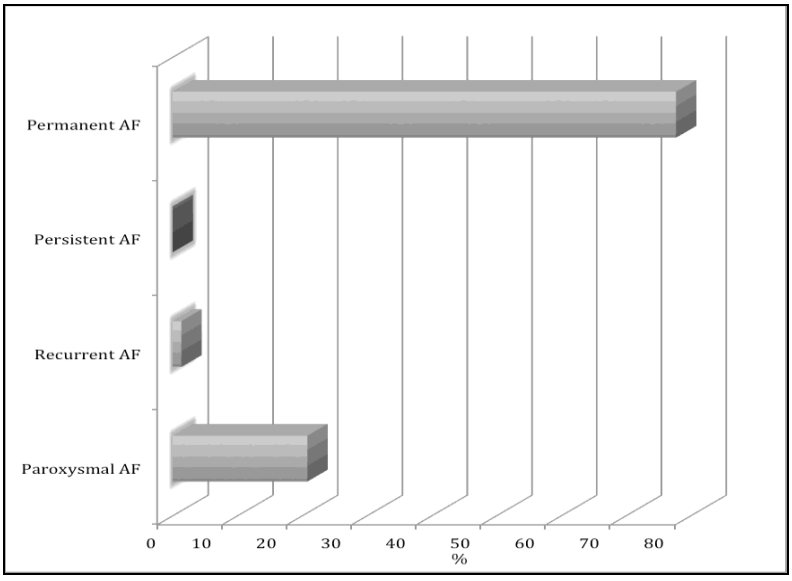

Fig.7. The presence of different forms of AF

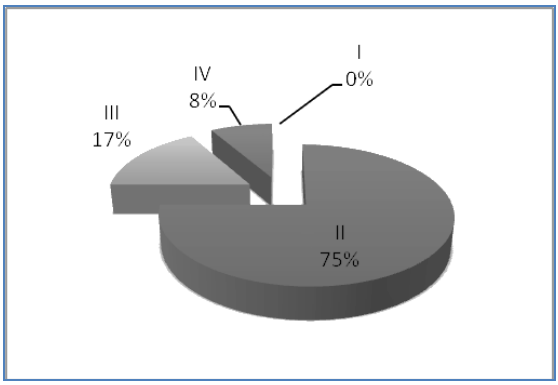

Fig. 8. Distribution of patients according to NYHA class

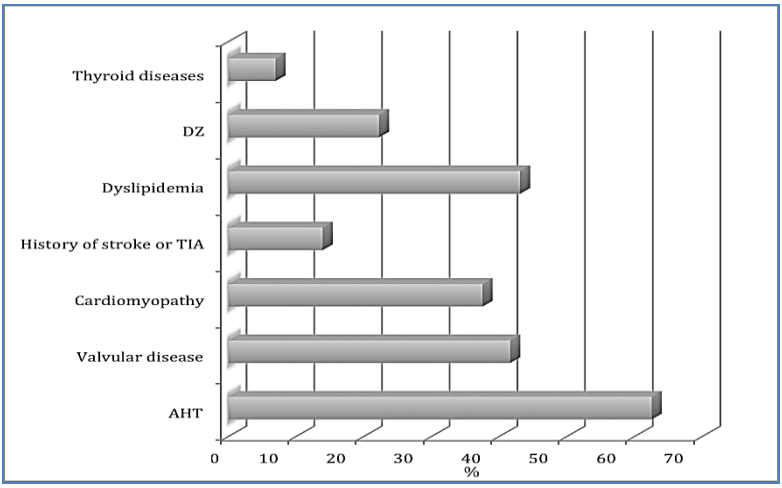

Fig. 9. Distribution of patients according to the associated pathology

The instituted treatment. The classes of drugs that were used for the treatment were diuretics, converting enzyme inhibitors, angiotensin receptor blockers, beta blockers, antiarrhythmics, anticoagulants and antiplatelet agents, nitrates, statins, fibrates and other cardiac preparations. Beta blocker class was represented by carvedilol (22 patients), bisoprolol (14 patients), nebivolol (6 patients), betaxolol (4 patients). Digoxin was administered intermittently 5 days per week, with two-day break, in maintenance doses ranging between 0.25 and 0.50 mg / day, *per os* in a single dose in most patients. Patients over 70 years, those with reduced muscle mass, those with variations in potassium, calcium or magnesium levels, patients with hypothyroidism required caution and special care regarding dose adjustment, more precisely a decrease to 0.125 mg / day to avoid toxic effects. In patients with HF or left ventricular dysfunction involving AF, digoxin can be used as first-line agent for heart rate control. We also know that treatment is made intermittent in patients with NYHA class I, first using other therapeutic classes such as diuretics, while in

those with NYHA class II or III we begin the initial treatment with digoxin, especially in cases with association of the two diseases (16).

We noted that 33 patients had an increased risk of ischemic stroke or thromboembolism, having a CHADS2 score higher than 2; most of them were male patients (18 versus 15). Most of the patients had a score of 2, thus having a moderate risk to develop these complications.

Guidelines ACC/AHA/ESC (American College of Cardiology/American Heart Association/European Society of Cardiology) recommends beta-blockers or non-dihydropyridine calcium blockers as first-line agents for frequency control in AF (17). These agents take action quickly and are effective in reducing heart rate at rest and during exercise in patients with AF. In addition, in AFFIRM study beta-blockers proved to be superior to calcium channel blockers (18, 19).

Studies have shown that beta-blocker carvedilol is effective in these patients, but sometimes we do not get a satisfactory response in heart rate control because of the fact that beta-blocker doses should be higher (20, 21). Digoxin is less effective in controlling ventricular rate compared with beta-blockers in patients with AF, both because it acts slowly, taking about 60 minutes, and because it has a weak action as a blocking agent of atrioventricular node. In addition, it is not effective in controlling heart rate during exercise and can be used only for sedentary patients (22, 23). Combining the two, beta blocker and digoxin, is therefore a solution to get the desired result both in sedentary patients and in those who exercise. In our study, patients received the beta-blocker - digoxin combination (46 patients, 40 patients respectively). We should mention that there were no adverse effects registered during hospitalization.

CONCLUSIONS

Patients who have AF and associated HF are a challenge for the treating physician due to the limitations imposed by their treatment, due to the intricate hemodynamic stress, due to the mechanism of the two diseases, and the unfavorable prognosis of this association. Our epidemiological data showed that patients with this pathology were male, from urban areas, older than 65 years. The major challenges in the treatment of these pathologies are getting sinus rhythm and heart rate control. Beta blockers should be the first therapeutic option in patients with chronic heart failure and atrial fibrillation because they have the effect of controlling heart rate and improve survival in patients with these disorders. Meanwhile, digoxin is a drug, very efficient in conditions of high accuracy monitoring whose major clinical indications are heart failure and atrial rhythm disturbances. In our study, the majority of patients received the beta-blocker - digoxin combination as a solution to get the desired results both in sedentary patients and in those who exercise.

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ASSESSING THE RISK OF DECOMPENSATION BY ASCITES AND SPONTANEOUS BACTERIAL PERITONITIS IN CIRRHOSIS

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ASSESSING THE RISK OF DECOMPENSATION BY ASCITES AND SPONTANEOUS BACTERIAL PERITONITIS IN CIRRHOSIS (Abstract): **Aim**. The current trend is to analyze predictive factors of transition from compensated to decompensated stage by the onset of ascites and later of spontaneous bacterial peritonitis (SBP), which would make possible an early diagnosis of liver cirrhosis at the compensated stage. The aim of the study is to evaluate patients with liver cirrhosis, assessing the rate and the risk factors of decompensation by ascites and SBP. **Material and Methods:** The prospective study included patients with cirrhosis of different etiologies admitted to a ward of the Institute of Gastroenterology and Hepatology of Iaşi in the period 1st January 2010-31st December 2010, which were reassessed clinically and in laboratory for 2 years. The essential criteria for the diagnosis of SBP were the presence of > 250 PMN / mm^3. Compensated cirrhosis was defined as the absence of ascites. The presence of ascites and / or upper gastrointestinal hemorrhage (UGH) marks the state of decompensated cirrhosis. To assess the severity of liver cirrhosis there were used Child-Pugh score and MELD score. Diagnostic paracentesis and ascitic fluid sampling were performed at admission to hospital and during hospitalization, in the event of signs and symptoms of SBP, after antibiotic treatment. Macroscopic, biochemical (albumin, protein), cytological (cellularity) and bacteriological (smear and culture) investigations of the ascetic fluid were performed. Lack of response to empirical treatment was considered in cases of general condition deterioration and decreased PMN <25 % of baseline. **Results:** By comparing the mean values of patients with and without SBP, it is noted that bilirubin and creatinine were significantly higher in patients with SBP, and total protein, albumin and prothrombin time were significantly lower in patients with SBP, these biochemical parameters correlate with the degree of hepatic impairment and may be considered risk factors for SBP. In relation to the mentioned elements, the most important predictors of PBS risk are low protein concentration in ascitic fluid under 1g/dl, increased levels of serum bilirubin and low platelet count. Impaired liver function, infectious complications, and previous episodes of SBP, UGH are risk factors for an episode of SBP. Empirical therapy of nosocomial SBP with third-generation cephalosporins is often inefficient due to the high prevalence of multiresistant (MR) bacteria. **Conclusions:** Assessment of clinically significant portal hypertension (PHT) and the degree of hepatic impairment may stratify patients with cirrhosis according to the risk of decompensation, making possible the identification of high risk patients. The knowledge of the risk factors in SBP is important not only to identify patients who could benefit from preventive therapy, but also in understanding the pathogenesis of the disease.
Keywords: SPONTANEOUS BACTERIAL PERITONITIS, RISK FACTORS, DECOMPENSATION, TREATMENT

The pathogenetic processes involved in cirrhosis are multiple and incompletely understood. Constant cell necrosis and subsequent fibrosis promote regeneration. The evolution towards decompensated cirrhosis is based on clinically significant portal hypertension and the degree of hepatic impairment (1, 2). Multiple studies in the literature have evaluated the predictors of decompensation, the most important being considered porto-hepatic venous gradient over 10 mmHg, MELD score over 10, albumin below 4 g/dL, and low concentration of sodium (3). Child-Pugh and MELD scores can stratify patients according to the severity of liver disease, identifying those in advanced stages of the disease and can also assess their prognosis. The assessment of clinically significant PHT and the degree of hepatic impairment may stratify patients with cirrhosis according to the risk of decompensation, allowing the identification of patients at high risk (4, 5).

Risk factors for decompensation include variceal bleeding, infections, alcohol, drugs, dehydration, and constipation. In addition, obese patients have a high risk of decompensation. In a recent study, decompensation occurred significantly more frequently in obese patients compared with patients who were overweight or who had a normal body mass index (6).

The current trend is to analyze predictor factors of transition from compensated to decompensated stage by the appearance of ascites and later spontaneous bacterial peritonitis (SBP), which would make the diagnosis as early as possible in cases of liver cirrhosis. The aim of the study is to evaluate patients with liver cirrhosis, assessing the rate and the risk factors of decompensation by ascites and SBP.

MATERIAL AND METHODS

The prospective study included 153 patients with cirrhosis of different etiologies admitted to a ward of the Institute of Gastroenterology and Hepatology of Iaşi in the period 1 January 2010-31 December 2010, which were reassessed clinically and in laboratory for 2 years. The essential criteria for the diagnosis of SBP were the presence of > 250 PMN / mm^3. Compensated cirrhosis was defined as the absence of ascites. The presence of ascites and / or upper gastrointestinal hemorrhage (UGH) marks the state of decompensated cirrhosis. To assess the severity of liver cirrhosis there were used Child-Pugh score and MELD score. Diagnostic paracentesis and ascitic fluid sampling were performed at admission to hospital and during hospitalization, in the event of signs and symptoms of SBP, after antibiotic treatment. Macroscopic, biochemical (albumin, protein), cytological (cellularity) and bacteriological (smear and culture) investigations of the ascetic fluid were performed. Lack of response to empirical treatment was considered in cases of general condition deterioration and decreased PMN <25 % of baseline.

RESULTS

Comparison of the frequency with which these complications occurred in patients classified as Baveno and Child (3), revealed significant increase of frequency from each stage of the study, presented in table I.

Risk factors of decompensation with ascites and SBP are analyzed, since the frequency of decompensated cirrhosis increases significantly during the study (fig. 1).

We also studied the evolution of each indicator separately in the three phases of the study (fig. 2, 3, 4, 5).

TABLE I

The frequency of cases according Baveno and Child classifications (phases comparisons)

Compared classes and phases	Phase I		Phase II		Phase III		P
	n	%	N	%	N	%	
Class I Baveno	21	**13.73**	7	*4.58*↓	3	*1.96*↓	**0.0029; 0.0001**
Class II Baveno	24	**15.69**	17	11.11	13	*8.50*↓	**0.027**
Class III Baveno	89	*58.17*↓	105	**68.63**	111	72.55	**0.0029**
Class IV Baveno	19	12.42	24	15.69	26	16.99	
Class A Child	33	**21.57**	15	***9.80***↓	4	*2.61*↓	**0.0025; 0.0048**
Class B Child	54	**35.29**	29	*18.95*↓	33	21.57	**0.0007**
Class C Child	66	*43.14*↓	109	**71.24**	116	75.82	<**0.00001**

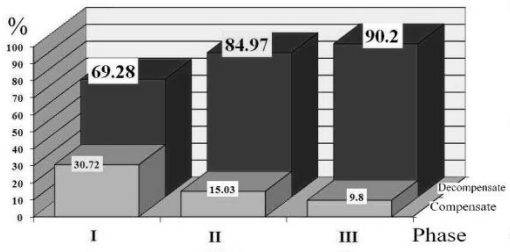

Fig. 1. Evolution of liver cirrhosis

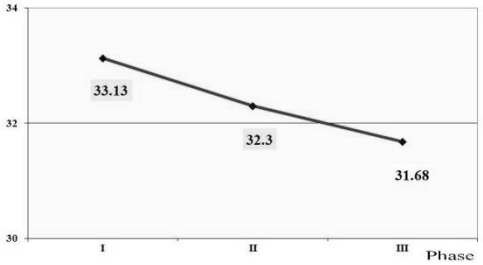

Fig. 2. Evolution of albumin

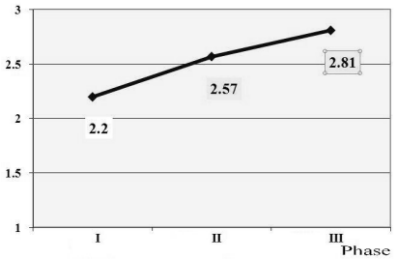

Fig. 3. Evolution of bilirubin

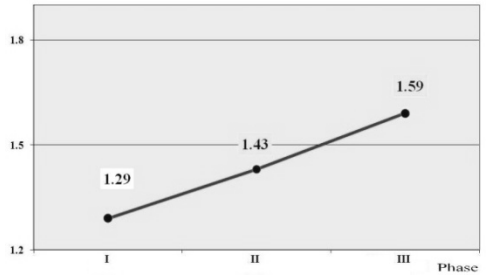

Fig. 4. Evolution of creatinine

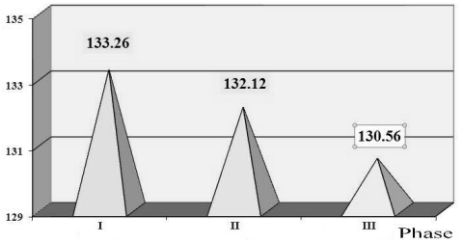

Fig. 5. Evolution of sodium

During the trial there were 58 episodes of SBP in 46 patients, of which 12 were relapses. Of all cases of SBP, 18 had positive cultures, the most common bacteria being Escherichia coli (50% of cases) and Enterococcus faecium (15.66% of cases) (fig. 6).

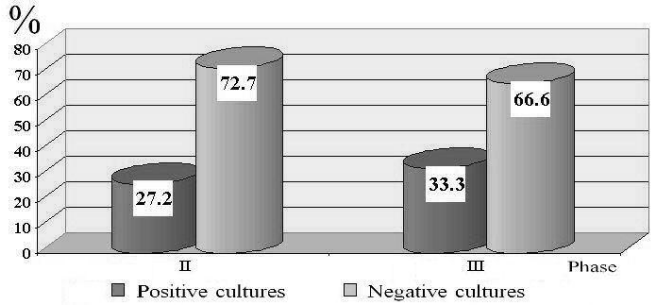

Fig. 6. The frequency of cases with SBP with positive and negative cultures

As shown in the table II, hepatic dysfunction, infectious complications, and previous episodes of SBP, as well as upper gastrointestinal bleeding are risk factors for a new episode of SBP. We compared the biological parameters in patients with and without SBP (tab. III, IV, fig. 7).

Empirical therapy was initiated in all 58 cases of SBP, in 39 cases with cefotaxime and in 19 cases with another antibiotic (Ciprofloxacin or Amoxocilin-clavulanate). Empiric treatment response was achieved in 41 patients, 28 who received treatment with cefotaxime and 12 who had another antibiotic. Treatment failure was recorded in 18 cases. In 7 cases there were isolated bacteria producing extended spectrum beta-lactamases (ESBL), but susceptible to imipenem. Recurrence was noted in 12 cases and mortality was recorded in 9 cases out of 58 patients with SBP. Multivariate analysis identified as predictors of failure of empirical therapy MELD score and SBP caused by bacteria ESBL (tab. V).

TABLE II

Frequency of association of SBP with some complications during hospitalization

Complications during hospitalization (risk factors for SBP)	Total SBP		SBP episode phase II		SBP episode phase III		SBP recurrence	
	N	%	N	%	n	%	N	%
Hepatorenal syndrome	17	36.96	5	22.73	7	29.17	5	41.67
Infectious complications	10	21.74	2	9.10	6	25	2	16.67
Signs of hepatic encephalopathy	4	8.70	2	9.09	2	8.33	0	0
Upper gastrointestinal bleeding	8	17.39	5	22.73	2	8.33	1	8.33
jaundice	8	17.39	2	9.09	4	16.67	2	16.67
Total SBP	46	100	22	100	24	100	12	100

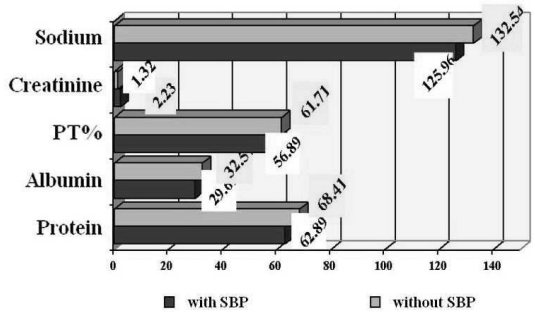

Fig. 7. Mean values of parameters in patients with and without SBP

TABLE III

The average platelet count in patients with SBP compared to the patients without SBP

Evaluations	With SBP			Without SBP			p
	n	Mean	Std. dev.	N	Mean	Std. dev.	
1 year	22	90822.7	60783.75	131	125174.8	89723.74	**0.0001**
2 years	36	92111.1	69757.06	117	123269.2	100206.0	**0.0001**

TABLE IV

Comparisons between the mean values of the proteins and neutrophils in the ascitic fluid, in patients with and without SBP

Variables		With SBP			Without SBP		P
	n	Mean	St. dev.	n	Media	St. dev.	
PROTEINS	57	1.2366	0.329	68	**1.90368**	1.82853	**0,0076**
PMN	57	436.2395	1085.885	68	74.20652	72.11888	**<0,00001**

TABLE V

Antibiotic resistance profile of isolated microorganisms

Bacteria	Total number	Resistant to			BLSE
		Cefotaxime	Ciprofloxacin	Ampiciline	
Escherichia coli	9	4	4	7	4
Enterococus faceium	5	2	2	3	2
Pseudomonas fluorescens	1	1	0	1	1
Streptococus pneumoniae	1	0	0	1	0
Enterobacter	1	0	1	0	0
Streptococus aureus	1	0	0	0	0

DISCUSSION

The study included 153 patients; the average age was 60.29 years±10.12 years, with a predominantly urban origin. There was a high prevalence of alcoholic cirrhosis compared with the virus etiology. Numerous studies have focused on the natural history of cirrhosis. To assess the severity of liver cirrhosis there were used Child-Pugh and MELD scores.

Survival statistics highlights the differences between compensated and decompensated cirrhosis (3). According to clinical and biological characteristics of patients on admission, most patients were enrolled in Child C and Child class B.

Results emphasize that most cirrhotic patients are in advanced stages of liver disease.

The current trend is to analyze predictors of transition from compensated to decompensated stage by the appearance of ascites and later SBP, which would make the diagnosis as early as possible in cases of liver cirrhosis and prevent decompensation.

The assessment of clinically significant portal hypertension and the degree of hepatic impairment may stratify patients with cirrhosis according to the risk of decompensation, making possible the identification of high risk patients (7).

After comparing the average values of patients with and those without SBP, it is

noted that bilirubin and creatinine were significantly higher in patients with SBP, and total protein, albumin, and prothrombin time mean values were significantly less in SBP, these biochemical parameters may be regarded as risk factors of SBP.

In relation to the mentioned pathogenic elements, the most important predictors of risk for SBP are low protein concentration in ascitic fluid, under 1g/dl, increased levels of serum bilirubin and low platelet count (8, 9).

The most common bacteria which have been isolated are *E. coli* (50% of cases) and *E. faecium* (15.66 % of cases). Cefotaxime resistance was recorded in 7 cases, ampicillin resistance in 12 cases and ciprofloxacin in 7 cases; mostly identified spread spectrum beta-lactamase producing bacteria (ESBL). Empirical therapy of nosocomial SBP with 3rd generation cephalosporin is often inefficient due to the high prevalence of multiresistant bacteria (9).

Epidemiological differences emphasize the need to know the local epidemiological features and to tailor the antibiotic therapy (10).

CONCLUSIONS

By comparing the mean values of patients with SBP and those without SBP, it has been shown that bilirubin and creatinine were significantly higher in patients with SBP, and total protein, albumin and prothrombin time values were in average significantly lower in patients with SBP, these biochemical parameters correlates with the degree of hepatic impairment and may be considered risk factors for SBP.

In relation to mentioned pathogenic elements, the most important risk factors for SBP are low protein concentration in ascitic fluid, under 1g/dl, increased levels of serum bilirubin and thrombocytopenia.

The therapy with 3rd generation cephalosporin is often inefficient due to the high prevalence of multiresistant bacteria; the epidemiological differences underline the need to be aware of the local epidemiological features and to tailor the antibiotic therapy accordingly.

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NEWS

SUBTYPING OF GLIOMAS OF VARIOUS WHO GRADES BY THE APPLICATION OF IMMUNOHISTOCHEMISTRY

Approximately 2% of all adult malignancies are brain tumours, and 80% of these are gliomas. They can occur at any age, regardless of gender or ethnicity. Currently, there is no cure for glioma, and, in spite of aggressive therapy, the morbidity is high. The most aggressive type of glioma, WHO grade IV, is also referred to as glioblastoma multiforme (GBM). According to the cell type, gliomas are divided into astrocytic, oligodendroglial, and ependymal. The neuropathological grading of gliomas is based upon the St Anne/Mayo system, including the assessment of features such as (i) cellular atypia (WHO grade II), (ii) mitotic figures (WHO grade III), (iii) endothelial proliferation, and/or (iv) necrosis (WHO grade IV), all visualized with haematoxylin and eosin (H&E) staining. In 2006, three molecular subtypes of GBM were defined on the basis of molecular profiles and the protein expression of tumour cells, i.e. proneural, proliferative, and mesenchymal. Glioblastoma multiforme (GBM) could be defined by molecular genetic analyses, as well. So, in 2010, independently of the type and grade, GBM were subdivided into four protein-based subtypes by IHC. The four GBM subtypes were classical, proneural, mesenchymal, and neural gliomas. The majority of the low-grade gliomas were of the proneural subtype, i.e. high p53 expression (63% of grade II). The classical subtype, with high EGFR and low p53 expression, was most common in GBMs (39%), followed by the proneural (29%) and mesenchymal (with high CD44 and MERTK expression) (29%) subtypes, a frequency that is in line with previously published data based on molecular genetics. The current strategy for diagnosis of a brain tumour incorporates assessment of the histological type (astrocytic/oligodendrocytic/ependymal) and assessment of the grade (WHO grading system). Recent observations have indicated that the molecular signature of the tumour also appears to be of significance. Assessment of the expression of the five proteins EGFR, CD44, MERTK, p53 and OLIG2 is sufficient for subtyping gliomas, and can be recommended for implementation in clinical practice for both low-grade and high-grade gliomas.(Svetlana N Popova, Michael Bergqvist, Anna Dimberg, et al. Subtyping of gliomas of various WHO grades by the application of immunohistochemistry. *Histopathology* 2014; 64: 365–379)

Doina Butcovan

PARKINSON'S DISEASE AND CAROTID INTIMA-MEDIA THICKNESS

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PARKINSON'S DISEASE AND CAROTID INTIMA-MEDIA THICKNESS (Abstract): Reports about the impact of cerebrovascular disease (CVD) on clinical status in Parkinson's disease (PD) are rather controversial. There have been a few studies and inconsistent results regarding the coincidence of Parkinson's disease (PD) and atherosclerotic diseases, such as cerebrovascular disease. Carotid intima-media thickness (IMT) is a known marker for subclinical atherosclerosis. **Aim:** This study was done to investigate the carotid IMT between PD patients and controls. **Material and methods**: A total of 54 PD patients and 50 controls were examined. The duration of Parkinson's disease, the severity of Parkinson's disease (the Hoehn–Yahr stage) and carotid IMT were examined. **Results:** The mean Hoehn and Yahr stage was 2.78 (range 2-4). Duration of disease had a mean of 7.59 ± 0.85 years. The left CCA mean IMT was 0.900 ± 0.147 in Parkinson group and 0.828 ± 0.118 in control group (p=0.007). The right CCA mean IMT was 0.891 ± 0.176 mm in the Parkinson group and 0.860 ± 0.164 in control group (p=0.360). No relationship between the Hoehn and Yahr stages or the duration of PD with the IMT were found by the Pearson's correlation test. **Conclusions:** The carotid IMT was higher in PD patients than in controls. **Keywords**: CAROTID INTIMA-MEDIA THICKNESS, PARKINSON'S DISEASE, SUBCLINICAL ATHEROSCLEROSIS

Parkinson's disease (PD) is one of the most frequent neurodegenerative disorders, characterized by progressive degeneration not only of the dopaminergic, nigrostriatal system, responsible for the core motor symptoms but also by involvement of many other neuronal systems and organs.There are multifocal neurodegenerative lesions affecting the central, peripheral, and autonomic nervous systems and many other organs (eg, adrenals, retina, heart, skin). The resulting biochemical deficits cause the heterogenous clinical picture including the variegated and often early presenting nonmotor deficit of this multisystem disorder (1). There have been a few studies and inconsistent results regarding the coincidence of Parkinson's disease and atherosclerotic diseases, such as cerebrovascular disease (CVD). Some authors reports that the pathology of vessels supplying the brain contributes to disease severity in PD patients, even without clinically manifest CVD (2). Studies have shown that carotid intima-media thickness (IMT) is an indicator of early generalized atherosclerosis and

that arterial wall thickness is associated with cardiovascular disease and stroke. The carotid IMT can be measured using Doppler ultrasound (3,4). As increased thickness in the intima media complex of the carotid artery has a clinical significance to cardiovascular comorbidity, we examined the hypothesis that patients with PD have an increased thickness of the intima-media complex of the carotid artery (5, 6).

MATERIAL AND METHODS

A prospective, descriptive study was carried out on 54 patients with PD, diagnosed according with United Kingdom Parkinson's Disease Society Brain Bank Diagnostic Criteria for Parkinson's Disease. None of the patients had vascular parkinsonism or was severely impaired by any other comorbidity. Other exclusion criteria were clinical features consistent with a diagnosis of atypical parkinsonism, such as multiple system atrophy, progressive supranuclear palsy, and corticobasal degeneration. The control group was composed of 50 healthy age- and sex-matched subjects. Extracranial carotid IMT measurements were performed according to a predetermined, standardized scanning protocol for the right and left comune carotid arteries. To evaluate carotid atherosclerosis, high-resolution B-mode ultrasonography was performed with a 7.5-MHz linear type probe (Acuson X 300, Siemens, Germany). The IMT was evaluated as the distance between the luminal intimal interface and the medial-adventitial interface, and it was measured using an electronic caliper on the frozen frame of a suitable longitudinal image. Three measurements of far-wall IMT were taken manually in each image using ultrasound calipers, and the average values of these readings were used in the analyses. The mean of left and right IMTs were used in the analyses. Statistical analysis was performed with the SPSS 13.0 statistical package program. Mean IMT of both groups, the duration of PD, and the Hoehn&Yahr scores of both groups were compared. Student's t test and Pearson's correlation analysis were used. Significance levels were set at $p<0.05$ in all cases and 95% confidence intervals (CI) were estimated.

RESULTS

No significant differences in the ages or gender were observed between the two groups. The age of parkinsonian patients ranged between 32 and 85 years with a mean of 66.37±8.90 years while the mean age of controls was a little smaller 63.92±12.63 years. The mean Hoehn and Yahr stage was 2.78 (range 2-4). Duration of disease varied from 1 to 32 years with a mean of 7.59 ± 0.85 years (tab. I**).**

TABLE. I

Demographic data of patients with Parkinson's disease and controls

	Parkinson (n=54)	**Controls (n=50)**	**P**
Age, years	66.37±8.90	63.92±12.63	0.253
Sex, male	61%	56%	0.121
Duration of disease	7.59 ± 0,85		
Hoehn–Yahr stage	2.78±0.79		

Data are mean ± SD or number (%).

Analyses of the left CCA revealed a mean IMT of 0.900 ± 0.147 in Parkinson group and 0.828 ± 0.118 in control group while the right CCA was found to have mean IMT of 0.891 ± 0.176 mm in the Parkinson group and 0.860 ± 0.164 in control group. The difference was statistically significant only for the left side (tab. II, III, fig.1).

TABLE II

Descriptive data of IMT on study groups

Interval	N	Average	Std. Deviation	Std. Error	Confidence interval		Min	Max
					- 95%CI	+95%CI		
IMT right								
Parkinson	54	0.891	0.176	0.024	0.843	0.939	0.5	1.4
Control	50	0.860	0.164	0.023	0.813	0.907	0.5	1.2
IMT left								
Parkinson	54	0.900	0.147	0.020	0.860	0.940	0.6	1.2
Control	50	0.828	0.118	0.017	0.795	0.861	0.7	1.1

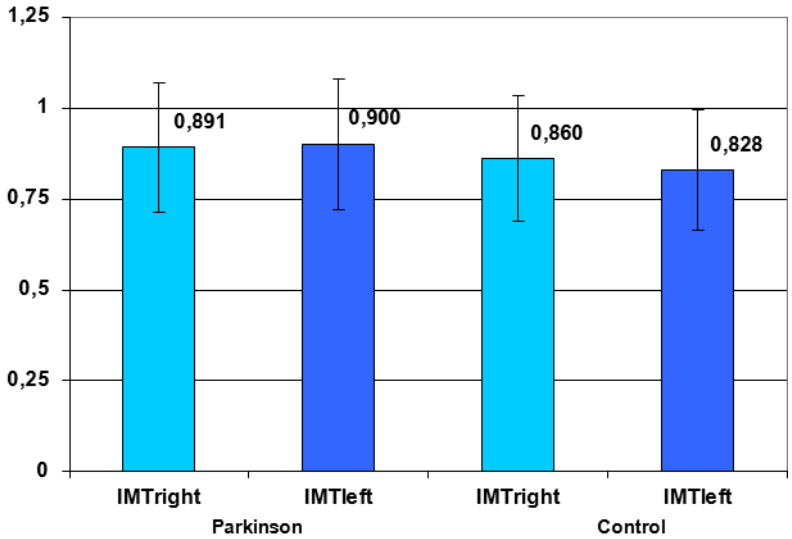

Fig. 1. Average values of IMT (mm) between study groups

TABLE III

Correlation of mean values of IMT between study groups

IMT	Control group Mean ± SD	Parkinson group Mean ± SD	*P* value Two-tailed probability
Right	0.860 ± 0.164	0.891 ± 0.176	0.360
Left	0.828 ± 0.118	0.900 ± 0.147	0.007*

*Significant (P<0.05)

No relationship between the Hoehn and Yahr stages or the duration of PD with the IMT were found by the Pearson's correlation test. (tab. IV)

TABLE IV

Pearson's correlation between IMT. duration of disease and Hoehn and Yahr stage

Correlations		Duration of disease	Hoehn Yahr stage
IMTright	Pearson Correlation	-.175	-.015
	Sig. (2-tailed)	.206	.914
	N	54	54
IMTleft	Pearson Correlation	-.144	-.130
	Sig. (2-tailed)	.298	.350
	N	54	54

** Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

Increased carotid intima-media thickness is a noninvasive marker of systemic atherosclerosis and relates to the risk of future cerebrovascular disease attacks (7).There have been some previous studies of carotid IMT in PD (8.9.10). One reported that the IMT in PD patients was significantly greater than those in controls (4). Others reported that carotid IMT did not correlate with PD duration or severity and others proved that IMT is thinner in parkinsonian patients (2). In our study we founded that IMT is thicker in parkinsonian patients on both carotids arteries but only for the left side statistically significance was reached. We did not find any relationship between Hoehn and Yahr stage and IMT. or between duration of Parkinson disease and IMT of the carotid artery. It is not clear whether increased IMT of the carotid artery is a cause or a result of the severity of Parkinsonism. One possible mechanism by wich subclinical vascular impairment can accelerate the evolution of Parkinson disease is the deleterious effect of otherwise subclinical hypoperfusion on regions made vulnerable by the degenerative process (13.14.15). On the other hand. other mechanisms may link PD with a higher risk of atherosclerosis. L-dopa treatment may promote secondary hyper- homocysteinemia. which may induce atherosclerosis but these hypertrophic changes were observed primarily in patients with a Hoehn-Yahr stage of 3-5 (4). In our study patients were Hoehn-Yahr stage of 2-4 and not all of them had treatment with L-dopa.

CONCLUSIONS

We conclude that patients with PD are at an increased risk for thickening of the intima media of the carotid artery. Due to the small sample size of this study we weren't able to say if this concomitant vascular pathology may contribute to a less favorable course in PD patients. Larger studies are needed to precisely establish the role of vascular factors in PD.

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A DIMENSIONAL APPROACH OF SUICIDAL PERSONALITY

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A DIMENSIONAL APPROACH OF SUICIDAL PERSONALITY **(**Abstract)**:** Although the last decade research has shown that personality disorders (PD) represent a major risk in suicidal behavior, the role of personality dimensions in determining this type of behavior hasn't been yet studied thoroughly. **Aim:** we intend to highlight some personality traits that may induce a suicidal behavior in patients diagnosed with PD. **Methods**: the sample consisted of 131 patients with PD, 46 of them presenting both a personality disorder and suicide behavior (suicidal ideation/suicide attempt). The personality dimensions were studied using the Temperament and Character Inventory (T.C.I.) developed by Robert Cloninger. Suicide risk was assessed using the Mini-International Neuropsychiatric Interview (M.I.N.I.), Module C - Suicide Ideas. **Results:** although the sample was analyzed in Clusters DSM type A, B, C and an analyze of co morbidities with the Axe I was also made, the analyze was taken further in our attempt to find a common factor that would differentiate the non-suicidal patients from the suicidal ones. The evident result appeared in two character dimensions that are highly related with suicide attempts. **Conclusion**: self-conducting and cooperativeness could be considered marker-dimensions in a quick evaluation of suicide risk. **Keywords**: SUICIDE BEHAVIOUR, PERSONALITY DISORDERS, TEMPERAMENT AND CHARACTER INVENTORY (TCI).

The last decade research has shown that personality disorders (PD) represent a major risk in suicidal behavior (suicidal ideation, suicide attempt, accomplished suicide), as severe as the risk of suicidal behavior manifested in bipolar disorders and schizophrenia (1). Although the non-fatal suicidal behavior including the suicide attempt is one of the criteria for diagnosis in borderline PD and the incidence of the suicide attempts among the persons who meet BP diagnostic criteria is well recognized, the association between the PD or personality traits and suicide hasn't been fully documented. In spite of medicine great progress in studying normal or pathological personality, the role of personality traits or dimensions in determining suicidal behavior hasn't been completely established during the last decades. I would like to mention that Chapter III of the present DSM-V manual introduces modifications in diagnosing PD, namely the dimensional approach in determining a personality disorder as specific trait.

General hypothesis of the research: highlighting some predisposing factors for suicidal behavior in patients with personali-

ty disorders. *Specific hypothesis of the research*: highlighting the particular dimensions of personality in PD patients with suicidal behavior as compared to PD patients without suicidal behavior. Evaluating the relationship between the personality dimensions scores and suicide risk in PD patients.

MATERIAL AND METHODS

In the present research, the *Targeted population* was patients with any PD type. A special interest was given to patients with active suicidality, i.e. patients who have made a suicide attempt a little while before the hospitalization and have manifested an intense suicidal ideation and who were diagnosed with personality disorder. The available population for the present study was the PD patients from a medical center from Cluj (Transylvania central region). The sample consisted of 131 patients diagnosed with PD of any type who solicited medical evaluation and treatment from The Psychiatric Hospital III Cluj-Napoca between 2004-2009 and signed an acknowledged consent.

Selection criteria: patients diagnosed with PD according to DSM-IV-TR (Axe II); age between 18-65 years; patients who signed a consent for participating in this study. Selection criteria of the population included in this study comply with the international methodology and took into account the type of clinical expression (the personality disorder in the present study) and the type of event (the suicidal behavior in the present study) ().

Exclusion criteria: organic personality disorder; mental retard; acute and chronic psychosis.

Comparison groups: *patients with personality disorders and suicidal behavior* (N=46), the suicidal behavior refer to the index episode, i.e. the episode that generates the target symptoms: recent suicidal attempt (cause of hospitalization or presentation of the patient to a clinical consult); present intense suicidal ideation with or without a suicidal plan; present fluctuating suicidal ideation; *patients with personality disorders without suicidal behavior* (N=85).

Work instruments consisted of: The Mini-International Neuropsychiatric Interview (M.I.N.I.) including Module 3/Suicide ideas, Structured Clinical Interview for Axis II Personality Disorders (SCID II) and Temperament and Character Inventory – (TCI) (1, 2, 5).

The categorial tests for evaluating psychopathology and PD were: Mini-International Neuropsychiatric Interview (M.I.N.I.) to identify the co morbid disorders on Axis I and to assess the suicidal risk: low, moderate, high, as in the M.I.N.I. Module C - Suicide ideas; Structured Clinical Interview for Axis II Personality Disorders (SCID II) was applied for the categorial diagnoses of PD on Axis II diagnosis level (1, 2, 5).

Dimensional tests for personality evaluation - The Temperament and Character Inventory (TCI) is a battery of tests under the form of an auto evaluation questionnaire with 240 items of right/wrong possible answers which evaluates 7 high descriptors of personality or behavior, including 4 descriptors of temperament and 3 of character. Each of those descriptors has multiple facets and is composed of several inferior level components (tab. I, II, III).

TABLE I

Descriptors of personality or behavior

The four dimensions of human temperament	The three character dimensions
➢ Novelty seeking ➢ Harm avoidance ➢ Reward dependence ➢ Persistence/perseverance	➢ Self-directedness ➢ Cooperativeness ➢ Self-transcendence

TABLE II

Multifaceted temperament dimensions

NS	**Novelty seeking:**
NS1	Exploratory excitability *vs.* Stoic rigidity
NS2	Impulsiveness *vs.* Reflection
NS3	Extravagance *vs.* Restriction
NS4	Disorderliness *vs.* Discipline
HA	**Harm avoidance**
HA1	Anticipatory worry and pessimism *vs.* Disinherited optimism
HA2	HA2 Fear of uncertainty *vs.* Certainty
HA3	Shyness *vs.* Gregariousness
HA4	Fatigability and asthenia *vs.* Vigor
RD	**Reward dependence**
RD1	Sentimentality *vs.* Stubbornness
RD3	Attachment *vs.* Detachment
RD4	Dependence *vs.* Independence
P	**Persistence**

TABLE III

Multifaceted character dimensions

SD	**Self-directedness**
SD1	Responsibility *vs.* Blame
SD2	Purposeful *vs.* Absence of purpose
SD3	Resourcefulness *vs.* Inertia
SD4	Self-acceptance *vs.* Self-criticism
SD5	Enlightened second nature *vs.* Bad habits
C	**Cooperativeness**
C1	Social acceptance *vs.* Social intolerance
C2	Empathy *vs.* Social lack of interest
C3	Helpfulness *vs.* Refusal to help
C4	Compassion *vs.* Revenge
C5	Pure-hearted conscience *vs.* self-favoring behavior
ST	**Self-transcendence**
ST1	Creative self-forgetfulness *vs.* Conscious individual experience
ST2	Transpersonal identification *vs.* Individual identification
ST3	Spiritual acceptance *vs.* Rational materialism

Quick interpretation of TCI: all types of personality disorders would be characterized by low scores in self-directedness which means low impulse control and low inner force, irresponsibility, deficiency in formulating and pursuing purposes, incapacity to auto help and low self-acceptance. The PD patients also present low cooperativeness manifested in a bad interpersonal functioning, they are intolerants, selfish, hostile, unfriendly, vindictive and time-serving. The more severe personality disorders (e.g. borderline, antisocial PD) show low scores in self-transcendence meaning instability of affects and self-image. *Suicidal behavior analysis - severity of actual suicidal risk* was made using Module 3 - Suicide ideas of Mini-International Neuropsychiatric Interview (M.I.N.I.) thus allowing the classification of patients according to suicidal risk level: low, mild, high (fig.1). *Statistical data analysis* was performed using SPSS 15.0 and the variance analysis (ANOVA) or the Mann-Whitney tests were used for exploring the differences between the comparison groups. Distribution of patients' scores in personality dimensions tests according to suicidal risk categories scored in MINI was analyzed using Tamhane and Turkey SHD post-hoc tests in analysis of variance.

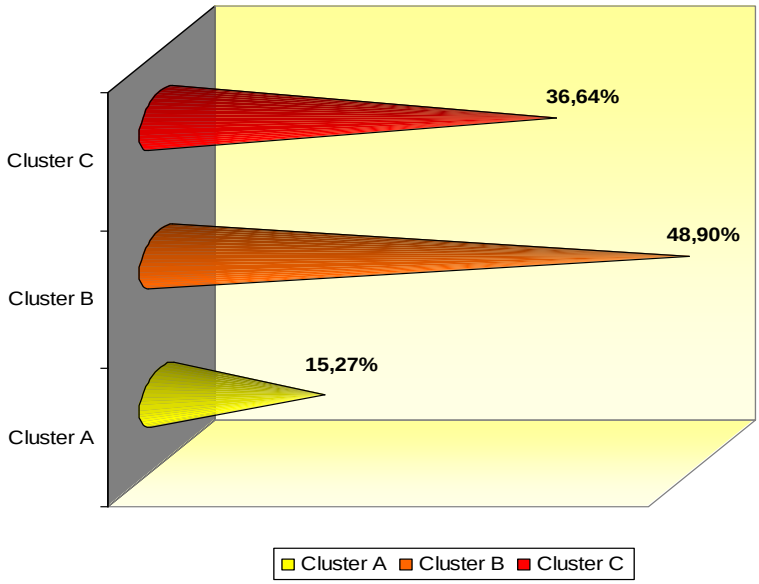

Fig. 1. Suicide risk severity in personality disorder clusters

RESULTS AND DISCUSSION

In our study sample (N=131) the highest percentage of patients belonged to Cluster B DSM -IV-TR personality disorders (48.90%), followed by Cluster C (36.40%). The less represented were the patients with Cluster a personality disorders (36.40 %) (paranoid, schizoid, schizotypal). Cluster B personality disorders is best represented in number and percentage in the analyzed group of personality disorders (N=131) by the increased number of patients with borderline personality disorder (22.14%, practically almost a quarter of the entire lot).

Also we noted a significant presence in the sample group of the narcissistic personality disorders (16.79%). It shall be noted the significant number of cases with obsessive-compulsive personality disorder (19.80 %) that were included after diagnosing with the above mentioned analysis tools (fig. 2).

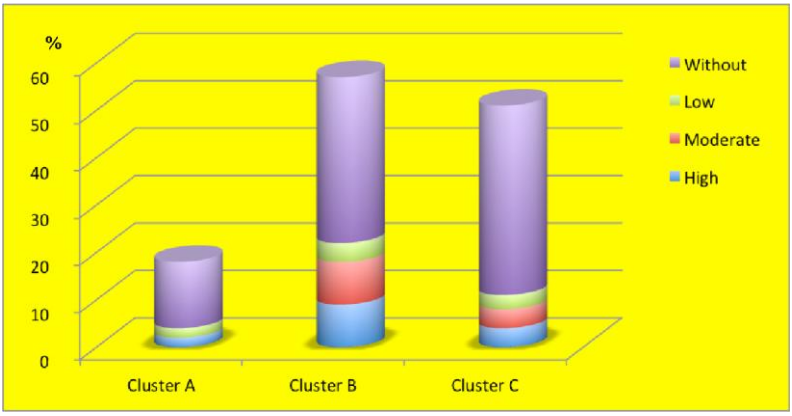

Fig. 2. Discussion of suicidal risk for personality disorder clusters

In our sample the highest percentage was found in Cluster B personality disorders, namely in borderline, narcissistic, histrionic and antisocial patients, in that order of frequency. Distribution of patients in our sample is not surprising, as the incidence rates of personality disorders is worldwide known. Although a more substantial percentage of patients in Cluster A had been preferable, it should be noted that the population of Transylvania by the incidence of personality disorders in the group subjected to observation confirm the existing international epidemiological data. In the present study an increased suicide risk has been reported in cluster B of personality disorders. Clinical evaluation of the borderline patient in a crisis stage is complicated. Those patients often present a history of many autolytic attempts and the impact of a short period of hospitalization on the current suicidal risk is unclear. Chronic suicidal risk in their case is significantly higher than the suicidal risk in general population, because of their history of multiple attempts which increase with self-aggressive behavior (1, 2). Stanley (2) found that patients with a self-mutilation history undergo a risk of suicide because of their increased level of depression, hopelessness, impulsivity and because they are mistakenly perceiving and hence are underestimating the lethality of their suicidal behavior. The chronic risk level can be estimated by a careful case history focused on a previous suicidal behavior and targeted on most severe suicidal attempts considered as such from a medical and intentionality point of view. Evaluation of suicide risk in patients with narcissistic personality disorder is also a unique challenge. Ronningstam and Maltsberger (3) conducted an intellectually provocative description of narcissists undergoing a suicidal risk even when not depressed. The same authors described the possible meanings of suicidal behavior in those patients: attempt to increase self-esteem by feeling in control, trying to protect themselves against anticipated narcissistic threats - "death before

dishonor", act of revenge after a narcissistic trauma, false sense of indestructibility, expressing a desire to attack or destroy an imperfect self. Although we had a small percentage of antisocial personality disorder patients in our sample as the increased suicide risk was found in cluster B patients, those patients require a special discussion, since patients with behavior disorder or antisocial personality present an unparalleled challenge for the clinician. When evaluating them during a crisis, besides the suicidal risk estimation has to be done of the potential risk of violence in those patients particularly. For example, Martine et al. (4) reported that 10 out of 23 patients with antisocial behavior had a history of violent acts on others. We also identified some avoidant and dependent personality disorders in our sample, but in a rather small percentage. Suicidal risk for Cluster C personality disorders in the sample was moderate. Even if cluster C personality disorders were present in our sample those patients had mostly obsessive-compulsive personality disorders, while the presence of Cluster A personality disorders (schizoid, schizotypal, paranoid) was insufficient to produce a statistical difference. I considered being difficult to evaluate the severity of suicidal risk using categorial diagnostic approach as in DSM -IV-TR personality disorder clusters, therefore I favored careful observation of the relationship between personality dimensions of patients and their suicidal behavior (fig. 3).

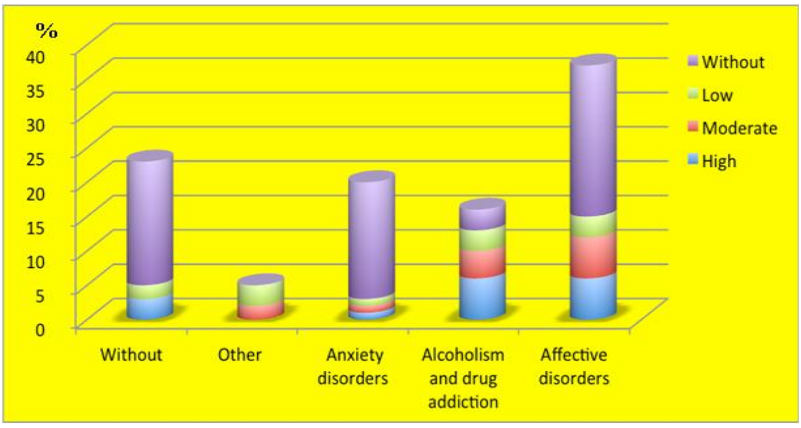

Fig. 3. Severity of suicide risk in PD sample group (N=131), with consideration of the psychic co morbidity type in Axis I

Suicide risk also depends on co morbidity with Axis I disorders and was found to be high in case of mood disorders and addictions - alcoholism and drug addiction. It is well known that a major factor in the escalation of suicide risk level is the presence of Axis I disorders co-occurrent with personality disorders. In our sample group 90% of the patients with personality disorder had co morbid Axis I disorders. Studies on suicidal behavior in personality disorder patients imposed as a necessity an evaluation of Axis I-Axis II co-occurrence. Several authors have identified as a rule the presence of Axis I psychiatric disorders in relation to co morbid Axis II personality disorders (5, 6, 7, and 8). Many studies indicate high prevalence of major depression co morbidity in subjects diagnosed with borderline personality disorder (6, 9,

10, and 11). There is also evidence of addiction co-occurrence within the same personality disorder; the most intensively studied one, i.e. borderline PD (12). In our study suicide risk depended with a high probability on personality disorder co morbidity with Axis I disorders and proved to be higher for affective disorders - major depressive episode and for addictions - alcoholism and drug addiction. Other studies conducted in previous years by the author (in collaboration) identified similar results (13, 14).

In 2002 Hawton (5) stated the lack of studies that explained the escalating suicidal risk phenomenon in PD co-occurrent with Axis I psychiatric disorders. After extended studies on mono- and dizygotic twins, Kendler (15), a recognized specialist in psychiatric genetic research, divided PD in internalized disorders, characterized by latent internalized predisposition, reflected in an internalized experience of suffering as it happens in affective and anxiety disorders and externalized disorders, reflected in an acting-out externalized predisposition as it happens in addictions with psychoactive substances and antisocial behavior. Studies of the same author have shown that personality latent traits are the basis for Axis I disorders and that they remain stable over time and between symptomatic episodes.

Nastase in 2009 cites Markon (16) who in 2005 in an attempt to establish a connection between studies on personality and studies on psychopathology used 52 studies in a meta-analysis and made correlations with scales derived from dimensional personality inventories which included DAPP-BQ, NEO-PI-R and Temperament and Character Inventory (TCI) developed by Cloninger (17). Markon's results lead to a hierarchical model of personality based on superordinate structures called dimensions of personality. Consistent with the idea of hierarchical structure of personality is the opinion of Cloninger (17) who supports the existence of a fundamental multidimensional architecture of psychiatric co morbidity, suggesting that co morbid disorders share common components of vulnerability. Thus, individuals with high scores in avoiding trauma are at increased risk for developing affective and anxiety disorders, that is internalized psychiatric disorders. Individuals with a high level of novelty seeking are at increased risk for drug addiction, suicidal behavior, bulimia, cluster B personality disorders, that is externalized psychiatric disorders. Relationships are rather non-linear (i.e. not one-to-one) with multidimensional configurations of personality traits, presenting multiple alternative completions within Axis I disorders and each and every Axis I syndrome having multiple possible configurations of personality history. Cloninger argues, therefore, that mental disorders are clinically and etiologically heterogeneous being characterized as manifestations of complex adaptive systems described multidimensionally, that these mental disorders have a complex epigenesis implying nonlinear interactions between multiple genetic and environmental influences. While there may be rare cases of discrete disorders with clinical and precise causal limits, those are exceptions. Therefore efforts to describe psychopathology in terms of categories result in extensive co morbidity (17). This would be a possible explanation for the high rate of personality disorders co morbidity with Axis I disorders, as in the present study (87.86 %). As the above mentioned studies highlighted the importance of psychological characteristics and basic multidimensional structures in determining suicidal

behavior stating them as common denominator in suicidal behavior analysis, we chose to make an in-depth analysis by observing and quantifying the personality traits of our patients who presented a high comorbidity with axis I psychiatric disorders such as depression, addictions and anxiety disorders. Therefore, personality dimensional analysis of the patients diagnosed with PD has become a necessity in the present study as category analysis of DSM clusters: A, B, C and the impact of co morbidities in these clusters on the occurrence of suicidal behavior has not brought further clarifications to the research. Cloninger's (17) Temperament and Character Inventory (TCI) was chosen for a dimensional analysis of the patients' personality structures (fig. 4). We decided to use this tool due to the great amount of evidence showing the connection between temperament dimensions and serotonergic, dopaminergic and GABAergic neurotransmitter systems and because we wanted to confirm or deny whether personality or its dimensions are endophenotypes in suicide.

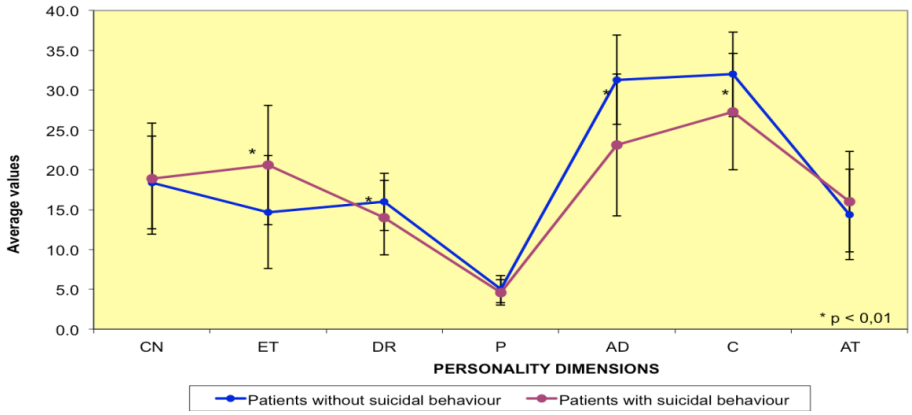

Fig. 4. Differences scored in personality dimensions (temperament and character) Patients with personality disorder and suicidal behavior scored significantly higher in "Harm avoidance" (fig.5) and significantly lower in "Reward dependence" ($p < 0.01$ - ANOVA). They also obtained significantly lower scores in "Self-directedness" and "Cooperativeness" ($p < 0.01$ - ANOVA with Welch correction).

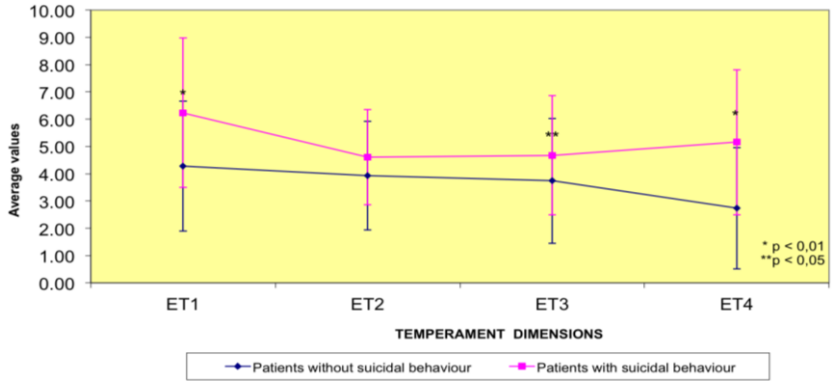

Fig. 5. Differences scored in temperament dimensions (harm avoidance)

Patients with suicidal behavior scored significantly higher in HA1 ($p<0.01$ - ANOVA), HA3 ($p< 0.05$- Mann Whitney U test) and HA4 ($p< 0.01$ - Mann -Whitney U test). Thus, patients diagnosed with PD and suicidal behavior are tensed, apprehensive, insecure, discouraged, pessimistic even when others have no worry, their personality profile is reflected by significantly higher HA1 scores. They also tend to be inhibited and shy in most social contexts, they need encouragement and validation, and they are more sensitive to criticism and punishment than patients with PD without suicidal behavior, as scored in HA3. Patients with suicidal behavior scored significantly low in Self-directedness (SD total) (fig. 6). They obtained significantly low scores in character traits that define this important dimension called self-directedness, namely Responsibility *vs.* Blame (SD1), Purposeful *vs.* Absence of purpose (SD2), Resourcefulness *vs.* Inertia (SD3) and Enlightened second nature *vs.* Bad habits (SD5) as compared with patients without suicidal behavior.

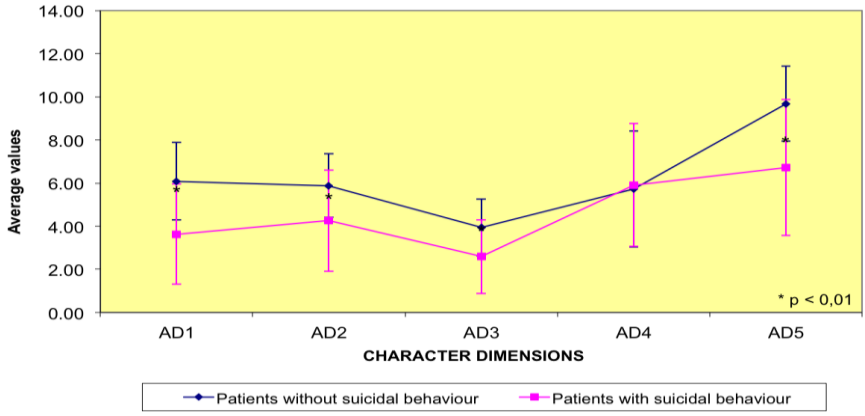

Fig. 6. Differences scored in character dimensions (self-directedness)

Patients with suicidal behavior feel excessively guilty and think that their attitudes, behavior and choices are determined by influences beyond their control and against their will and they do not assume responsibility for their actions as shown by SD1 score. Significantly low SD2 scores reveal their uncertainty about long-term goals and their impulse to respond to immediate needs and present circumstances. Those patients with PD proved unable to delay reward in order to achieve their goal. Moreover, significantly low SD3 (AD) scores mean helplessness, hopelessness, inefficiency. Those individuals have not developed skills and self-confidence for solving problems and thus they feel unable and incompetent to face obstacles. They usually expect others to take the lead and solve problems. Patients with PD and suicidal behavior (suicidal ideation and suicide attempt in the index episode) scored significantly lower in Cooperativeness (total score). They obtained significantly lower scores in those character traits that define Cooperativeness (C1-C5) as compared to the patients with PD without suicidal behavior (fig. 7).

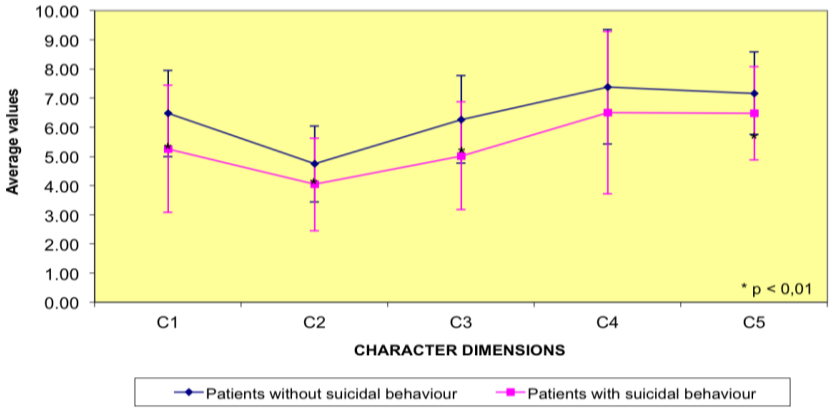

Fig. 7. Differences scored in character dimensions (cooperativeness)

Thus, consistent with significantly low scores in "Social acceptance *vs.* Social intolerance" (C1) suicidal patients are unfriendly and socially intolerant. In our sample group we found they exhibit less patience with others and are judgmental, especially with those who have goals and values other than theirs, as compared to the standard group of patients with personality disorder without suicidal behavior. Statistically significant lower scores at C2, C3 and C5 for suicidal patients portray them as persons with no concern for other people's feelings, unable to sympathize with other people's emotions, sufferings or difficulties, they lack of consistency and honesty in social relations. Those patients are less likely to seek help as they always hide their real suicidal intentions. Therefore, control is no longer effective in case of human individuals with personality disorder because of the low scores in Self-directedness and Cooperativeness which represent the diagnostic specific indicator of a PD. When the control gate disappears, negative emotionality is very high, making human individuals vulnerable to mental disorders that could lead to escalation of suicide risk.

CONCLUSIONS

Suicide risk, according to the present study, has both biological and genetic determinism. This conclusion is supported by validated correlations between increased suicide risk and high, respectively low scores in temperament dimensions: harm avoidance and reward dependence. Social learning has an equal impact on individual's maladaptive behavior, a conclusion supported by intense positive correlations between increased suicide risk and low scores in character dimensions as self-directedness, cooperativeness. Self-directedness and cooperativeness could be considered marker-dimensions for a quick evaluation of suicidal risk, as they are highly correlated with suicide attempt. Dimensional evaluation performed using the Temperament and Character Inventory developed by Robert Cloninger revealed the suicide risk stronger than categorial clinical evaluation, highlighting a high prognostic potential of evaluating suicide risk.

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IMPACT OF METABOLIC SYNDROME ON THE DEVELOPMENT OF CARDIOVASCULAR DISEASE

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IMPACT OF METABOLIC SYNDROME ON THE DEVELOPMENT OF CARDIOVASCULAR DISEASE (Abstract): In recent years, the metabolic syndrome represents a major health problem worldwide, with a great impact in population, and also at individual level, by an increased prevalence of cardiovascular risk. **Material and methods:** We conducted a descriptive study on a lot of 131 patients hospitalized in Cardiovascular Clinic of Rehabilitation Hospital in Iasi, in the period April 2012 - April 2013. **Results:** In the analyzed group, the largest number of metabolic syndrome cases was recorded in the 50-59 years age group. There were no significant differences between the rural / urban areas ($t = 0.113$, $p = 0.910$). Frequency of ischemia was significantly higher in subjects with essential hypertension of IInd and IIIrd degree (Pearson Chi-square test = 3.955, df = 1, $p < 0.05$). Significant differences were observed on females related to obesity and ischemia, meaning that women with higher obesity are predisposed to ischemic events. Regarding the degree of exercise testing and the origin area, no significant differences were registered (Pearson Chi-square test = 13.526, $p = 0.004$, df = 3). **Conclusions:** Our study offers new information on metabolic syndrome, analyzing epidemiological and clinical criteria. **Key words:** METABOLIC SYNDROME, EPIDEMIOLOGY, CLINICAL CRITERIA.

Metabolic syndrome, also known as X syndrome, insulin resistance syndrome, hypertension syndrome or catecholamine excess syndrome, is an important health problem worldwide by an increased prevalence of cardiovascular risk (1, 2). Clinical identification of the metabolic syndrome is based on the evaluation of specific parameters: hypertension, obesity, glucose, dyslipidemia and hyper-insulinemia, all modifiable beyond those non-modifiable like age and gender (3, 4). The metabolic syndrome seems to increase modestly the cardiovascular risk, whereas in non-diabetic individuals it predicts diabetes much more efficiently. Different studies have compared the performance of the classical cardiovascular evaluation methods (the Framingham risk score, the SCORE charts, the Progetto CUORE score) and metabolic syndrome in cardiovascular disease prediction. At people with high risk the presence of the metabolic syndrome usually does not improve the risk, whereas at people with lower risk its presence increases significantly the chances of cardiovascular disease (5, 6).

MATERIAL AND METHODS

The study included a lot of 131 patients hospitalized in Cardiovascular Clinic of Rehabilitation Hospital Iasi, in the period April 2012- April 2013.

Parameters evaluation

• demographic data: gender, age, residence

• data on lifestyle: smoking, daily physical activity regime

• anthropometric data: weight, height, body mass index, waist circumference

• family and personal history, with particular interest for cardiovascular and metabolic disease: myocardial infarction, stroke, hypertension, type 2 diabetes

• clinical data: blood pressure

• biochemical data: glucose, total cholesterol, HDL-cholesterol, triglycerides

• exercise test

RESULTS AND DISCUSSION

The study group consisted of 59 female patients (20 from 39 patients from rural and urban) and 72 male patients (31 patients from rural and 41 from urban areas). Most cases were registered in the age group 50-59 years with an average of 55.89 (fig. 1). Regarding the distribution by rural/urban area, there were no significant differences ($t = 0.113$, $p = 0.910$) (fig. 2). Also, there were no significant differences related to age and sex.

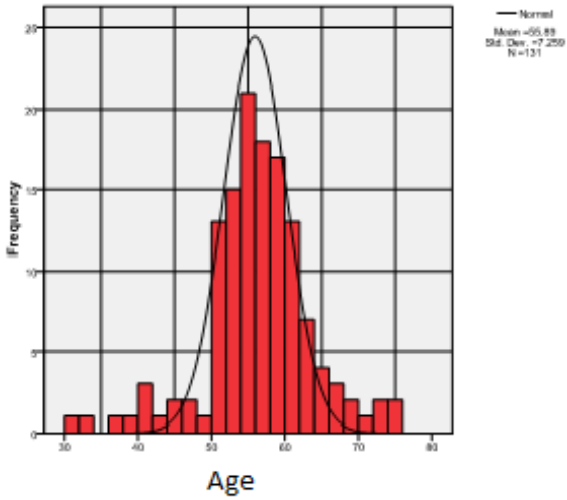

Fig. 1. Distribution by age

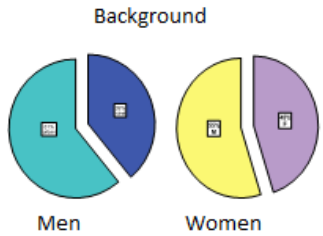

Fig. 2. Distribution by area of origin

A main factor analyzed in the study was the blood pressure. A similar increasing trend was observed in hypertension degree in both females and males, most of hospitalized patients having blood pressure values above maximum. From the statistical point of view, no significant differences were found (fig. 3). An inadequate blood pressure control and poor adherence to treatment remain among the major limitations in the management of hypertensive patients, particularly of those at high risk of cardiovascular events. Many studies have shown that an intensive, protocol-based, management approach to achieving blood pressure control in hypertensive patients in

family practice is effective and works even when there is flexibility built into the algorithm to allow family physicians to use their judgment in individual patients. The results obtained in our study are consistent with results from the literature (7, 8).

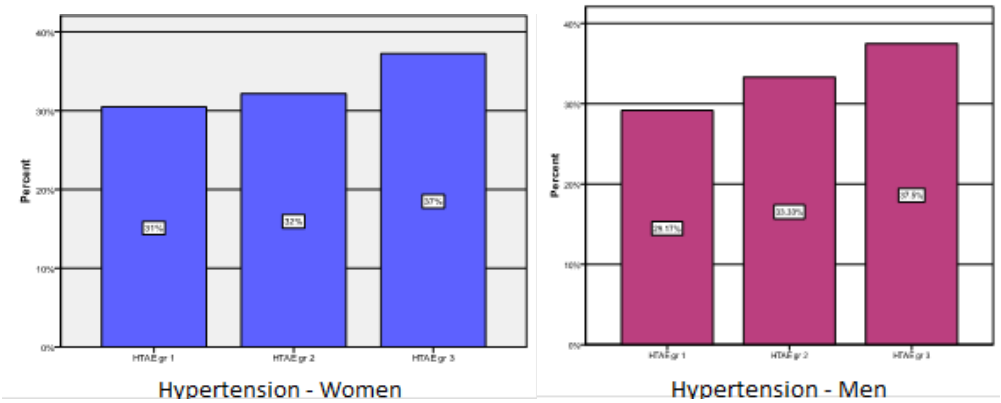

Fig. 3. Sex distribution of hypertension

The literature mentions that area of origin and lifestyles influences the evolution of cardiovascular disease (9). This is found in our study, in urban being more patients with hypertension IIIrd degree, possibly due to stress or poor nutrition. In figure 4, there is a distribution of patients according to the environment, with statistically significant differences between urban / rural (fig. 4).

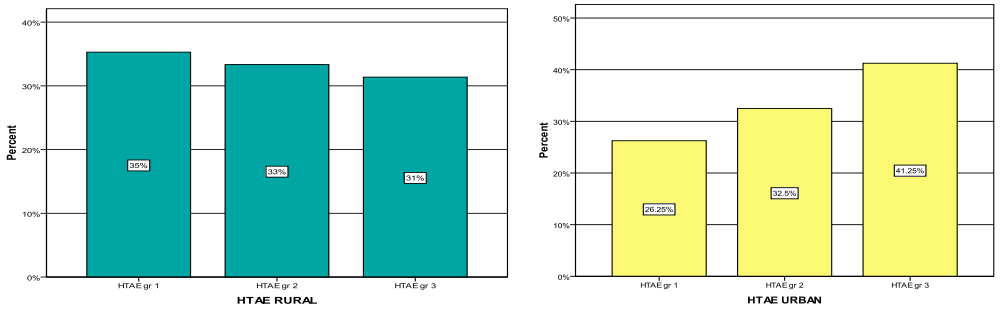

Fig. 4. AHT distribution by grade and area of origin

Although in the graph we observe an increase in positive ischemia frequency from patients with hypertension of I[st] degree to patients with hypertension of IIIrd degree, though not significantly increase in frequency of ischemia for hypertension grade I, II and III was present at individual level. There is a significant difference between ischemia (positive, negative) if we compare the hypertension II and III degree cases together with those with hypertension I degree (Pearson Chi-square test = 3.955, df = 1, p <0.05) (fig. 5, 6). The frequency of ischemia is significantly higher in subjects with hypertension IInd and IIIrd degree, according to data from literature (7, 8).

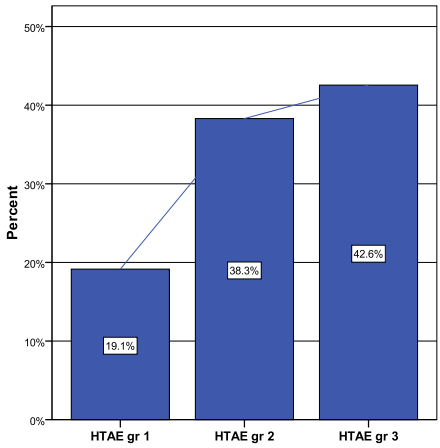

Fig. 5. Hypertension-ischemia relation

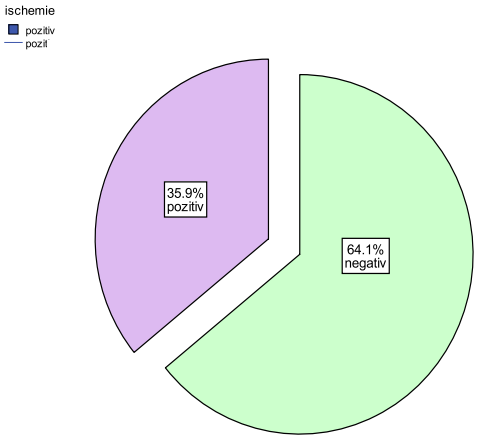

Fig. 6. Graphic representation of ischemia

Comparing the different degrees of obesity and the area of origin there were not significant differences found and no significantly higher risk, but there are significant differences related to sex (Pearson Chi-square test = 14.437, df = 3, p = 0.002, p <0.001). Current researches have shown that the number of obese people is higher in urban areas, possibly due to improper diet and a higher stress (10, 11).

Significant differences were observed for females in relation to obesity and ischemia, meaning that women with obesity have often significantly higher ischemia (fig. 7).There are no significant differences between different degrees of obesity and ischemia, but if we group the overweight and obesity Ist degree in a single category and obesity IInd degree in another category, there are significant differences (Pearson Chi-Square test, p = 0.052). Regardless of gender, the overweight and obesity I degree patients are more likely to develop ischemic phenomena.

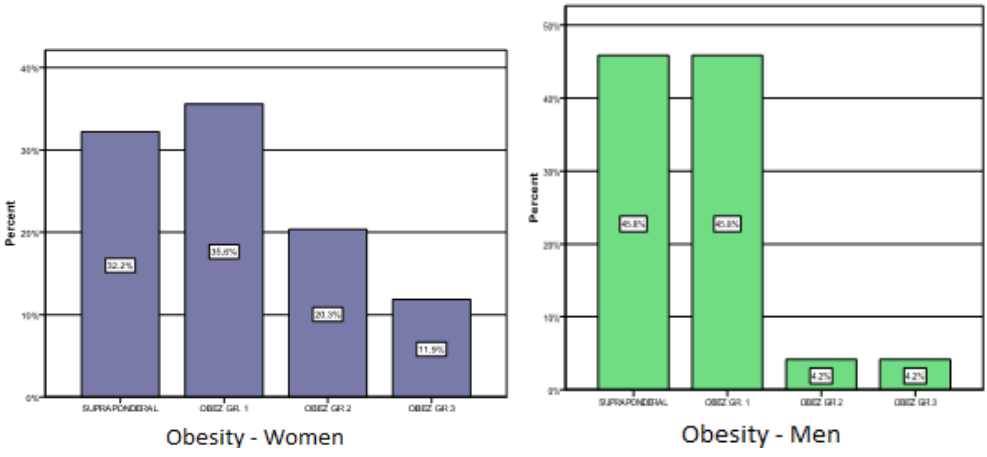

Fig. 7. Obesity-ischemia relation according to gender

Obesity is a consequence of many risk factors, as a reduced physical exercise or increased energy consumption. In the interplay between obesity and metabolic complications are involved many chronic degenerative disorders, including atherosclerosis, commonly associated with hypertension and a chronic low grade inflammation (12).

Numerous observational studies have shown that dietary patterns similar to the Mediterranean diet, rich in fruit and vegetables and high in monounsaturated fats and fiber, resulted in decrease prevalence of the metabolic syndrome (13). Interventional studies also revealed a decrease in markers of inflammation in subjects with metabolic syndrome consuming national dietary guidelines (14).

Another parameter studied was the cholesterol, known as a major risk factor involved in cardiovascular diseases pathogenesis (15). Although the number of patients with dyslipidemia is high, they do not correlate with the presence of ischemia. In our study, there is no significant differences and no significantly higher risk between sex and area of origin in relation to different forms of dyslipidemia (fig. 8).

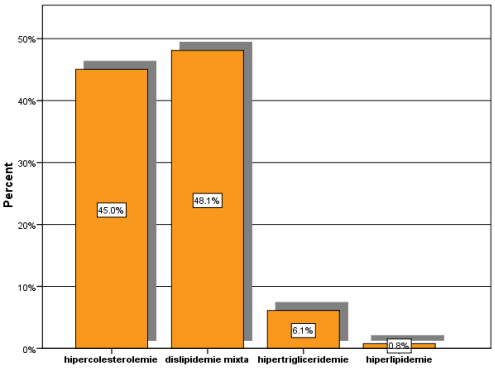

Fig. 8. Dyslipidemia – ischemia relation

Many data suggest that low intensity exercise training, a widely recommended practice, is particularly effective for treating the metabolic, functional and morphological disorders triggered by metabolic syndrome (16). In our study, all patients with metabolic syndrome were tested for effort. The highest percentage of tested patients had submaximal results with statistically significant differences to those with maximal test. Comparing the degree of exercise testing and the area of origin, as well as the sex of the patient, no significant differences were registered (Pearson Chi-square test = 13.526, p = 0.004, df = 3)(fig. 9).

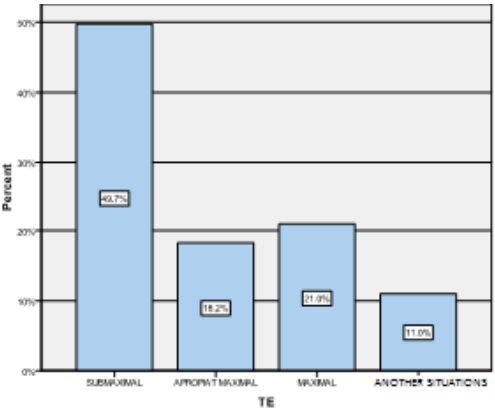

Fig. 9. Exercise testing

Thus, a healthy lifestyle based on a diet low in saturated fat, rich in fruits, vegetables, daily physical activity, no alcohol and no smoking, prevents or slows the metabolic syndrome.

CONCLUSIONS

Our study results provide new information on metabolic syndrome, analyzing epidemiological and clinical criteria. In the studied populational group, the lifestyle modification is the first option of treatment, but in people with high risk drug therapy is necessary. The age group most commonly affected was 50-59 years,

as in the literature. There were no significant differences by age group in relation to sex or area of origin, or between sex and area of origin related to the different degrees of hypertension. Ischemia frequency was significantly higher in subjects with hypertension IInd and IIIrd degree, and reporting various degrees of obesity and the area of origin were not found significant differences and no significantly higher risk, but there are significant differences related to sex, women with high obesity being significantly more susceptible to ischemia.

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QUALITY OF LIFE OF PATIENTS WITH ESOPHAGEAL REPLACEMENT FOR CONGENITAL AND ACQUIRED ESOPHAGEAL ANOMALIES

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QUALITY OF LIFE OF PATIENTS WITH ESOPHAGAL REPLACEMENT FOR CONGENITAL AND ACQUIRED ESOPHAGEAL ANOMALIES (Abstract) **Aim:** To assess the long-term quality of life (QoL) and symptoms experienced by patients who underwent esophageal replacement for congenital and acquired children esophageal pathology. **Material and methods:** The study group comprised 71 patients divided into 2 groups, group I: 34 patients who underwent gastric tube esophagoplasty, and group II: 37 patients who underwent colic tube esophagoplasty. QoL score was assessed using a 24-item original questionnaire covering aspects of eating, disease-specific symptoms, physical and psychological development, and social integration. **Results:** QoL scores based on patient and parental responses were very close. Long-term postoperative functional outcomes for the two groups were comparable. **Conclusions:** The QoL scores and general life status were not influenced by the used technique and this pathology should be approached according to the particularity of each case and surgeon's personal experience and preference. **Keywords:** QUALITY OF LIFE, ESOPHAGEAL REPLACEMENT, ESOPHAGEAL ATRESIA, CAUSTIC LESSIONS

The most important criterion for assessing the long-term outcome for patients requiring of pediatric surgical procedures is quality of life (1, 2, 3).

The aim of this paper was to assess the long-term quality of life of a group of patients that have undergone esophagoplasty for congenital or acquired esophageal lesions. The study group comprised 71 patients who underwent esophageal replacement in the Pediatric Surgery and Orthopedics Unit of "Sf. Maria" Emergency Children Hospital, Iasi. The surgical procedures for esophageal replacement in both esophageal atresia and postcaustic esophageal stenosis were: gastric tube esophagoplasty and colic tube esophagoplasty. Since 1992 the authors' procedure of choice for esophageal replacement has been colic tube esophagoplasty (left colon graft vascularized by the left colic artery) because of the lower incidence of complications and the advantages related to this technique. The long-term functional outcome appears to be clinically and radiologically favorable, but the assessment of the quality of life after such a major surgical procedure based on patients and/or their parents perception has not been investigated so far (4, 5).

MATERIAL AND METHODS

The authors have developed an original questionnaire comprising 24 items covering aspects of eating, disease-specific symptoms, psychological and physical status, and level of social integration.

Responses given by the patient/his parents for each item were scored according to the frequency of each symptom occurrence as follows: score 0 – all the time; 1 - most of the time; 2 –sometimes/quite often; 3 – rarely; 4 – never.

The total score of the questionnaire ranged from 0 to 96, with the highest score representing the best quality of life. Depending on the age of the patients at the time of assessment, the questionnaire was completed by them or their parents.

The study population was divided into 2 groups: group I-gastric tube esophagoplasty: 34 cases and group II- colic tube esophagoplasty: 37 cases.

All patients underwent a full investigation protocol with clinical and imaging evaluation (contrast barium study and esophageal-gastric endoscopy) in order to assess the postoperative functional outcome.

RESULTS

The mean time since esophagoplasty was 12.7 years with a range of 8 to 25 years: 14.8 years for group I and 10.9 years for group II.

The mean number of surgical interventions for each case was 4 (group I- 4 surgical procedures/case and group II- 5 surgical procedures/case). The mean age at esophagoplasty was 5.6 years, ranges 2 to 17 years; the mean age of the patients participating in this assessment was 14.1 years, ranges 8 to 37 years.

The social status of the patients was as follows: educational level: primary school 25 patients; secondary school 25 patients; professional school 9 patients; high school 9 patients; post-school qualification 9 patients, and university 4 patients.

Of the adult patients 12 were qualified employees and 14 were doing odds jobs.

The following aspects of eating have been followed: unrestricted diet; size of the meals as compared to other family members; need of water intake during meals; dysphagia; total number of meals per day; episodes of coughing associated with meals.

The questionnaire on the quality of life was completed by the family in 24 cases (patients aged 8 to 13 years) and by the patient in 47 cases (ages between 14 and 37 years).

The mean score of the questionnaire when completed by the patient was 79 (ranges 60 to 90) and when completed by the family was 81 (ranges 70 to 91).

A slightly higher score by 2 points was obtained based on parental perception as compared with patient perception. For the two study groups the mean score was 80 for the gastric tube esophageal replacement (group I), and 81 for the colic tube esophageal replacement (group II).

DISCUSSION

Given the great diversity of treated anomalies, the different ages of patients at the time of surgery, the particularities of each surgical intervention (graft in retrosternal position or in the “bed of the old esophagus”, with or without esophagectomy), an accurate long-term assessment of postoperative outcomes is perfectly justified and opportune (6, 7). The aim of the present paper was to measure and compare some aspects of the quality of life for

patients who underwent colic tube or gastric tube esophageal replacement at pediatric age. As far as the authors are aware, in Romania there are no other studies that have investigated the long- term quality of life in patients with such surgical interventions. As to the functional and imaging outcome, over the time there have been frequent attempts to compare the two used techniques, but all have failed to establish which technique is superior in terms of postoperative results (8,9). In the two groups, the QoL scores were quite similar (80 and 81, respectively) and showed that there were no important patient/parents differences in perception of quality of life in relation with the surgical technique used (1).

Because we did not use a standard questionnaire that could have been applied to healthy individuals as control group, we could not make a comparison of health-related quality of life in our patients with the quality of life in healthy population. We noticed that patients tended to perceive their QoL as being poorer than their parents' perception, and this trend was more marked as adults' age increases and they become aware of the problems related to surgical intervention (1, 2).

CONCLUSIONS

Generally speaking, the patients who underwent esophageal replacement live a normal life although their psychosocial integration and their emotional independence is somehow reduced. The two most frequently used techniques of esophagoplasty have comparable postoperative outcomes in terms of patients/ family perception and socio-professional and emotional integration.

The choice of a specific surgical technique for esophageal replacement in patients with congenital or acquired esophageal lesions should be based on the particularities of each patient because there are no objective criteria to guide the surgeon.

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BIOLOGICAL MONITORING AS PROFICIENT ASSESSMENT METHOD IN CHRONIC EXPOSURE TO IRRITATING GASES AND VAPORS

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BIOLOGICAL MONITORING AS PROFICIENT ASSESSMENT METHOD IN CHRONIC EXPOSURE TO IRRITATING GASES AND VAPORS (Abstract): Air pollution and its adverse health effect represent a global issue. Long term or permanent exposure at low doses of toxic material may increase the number of new appeared severe respiratory diseases and may aggravate most of the existing chronic cases. **Aim of study**: In the present study, the main toxic aerosols released by the greatest chemical factory in Transylvania were analyzed and their health impacts were measured. **Material and methods:** We have used in our trial biologic monitoring 1786 employees from a chemical factory in a transversal descriptive study. In the same time, we have noticed environmental determination of air quality and also, we have checked the health status and other 90 cases from a nonchemical factory. In this factory there are also pneumotrop harms. **Results** of our study give useful information regarding workers health status, with possibility to make pollution-medical condition association: there was no significant association between occurrence of decreased lung function tests and exposure of chemical irritants compared to the second investigated factory. **Conclusions**: Exposure to pneumotrope hazards imposes safeguards to protect health and biological monitoring has proven absolutely necessary to do so. Last but not least, lifestyle monitoring is to be effective. **Keywords**: IRRITATING GASES AND VAPORS, RESPIRATORY DISEASE, BIOLOGICAL MONITORING.

Fertilizers' production involves a series of chemical reactions such nitrogen capture, nitration, nitratation, during which some intermediate/final chemicals occurs, for example ammonia, nitrogen, nitric acid and others less important. These chemicals are well known to cause mucosal and skin irritation. Also, these chemicals cause irritation of upper and lower airways causing chronic lung diseases (1).

Nitrogen oxides (NOx), especially nitrogen monoxide and nitrogen dioxide which are the "smog's" constituents and give to this a chocolate brown color, also occur in other industrial conditions, like emission gases as result by burning of different fuels; the reaction of nitrogen dioxide with chemicals produced by sunlight leads to the arise of nitric acid, which is a major constituent of acid rain (2). Because of their insolvency in water, NOx tend to not cause immediate upper

respiratory tract irritation. Subsequent respiratory symptoms will be related to the concentration of NOx inhaled, the duration of exposure, the way of exposure and the health of the exposed person (3). Irritation effects already occur after an exposure of 3 ppm, this concentration being able to produce an altered lung test even after a short time exposure, as shown in American studies (1).

Ammonia (NH_3) is produced mainly in fertilizer's industry (about 80%), but also in pigments' industry, plastic and oil industry (4, 5). The ammonia can causes alkaline chemical skin burns, eye burns and lung burns (5). Light exposures affect in the first instance the upper airway, but many severe exposures tend to affect entire respiratory system. The histological damage due to the alkalinity is a liquefaction necrosis with deeper destruction than those produced by an acid. At respiratory tract this leads to cilia and mucosal destruction and finally to lung infections. In addition, secretions, cellular debris, edema and its reaction – smooth muscle contraction causes a significant airway obstruction (6). Airway epithelium may recover within 6 hours after exposure, if the basal layer remains intact. However, the damaged epithelium is often replaced by granular tissue, which is the underlying etiology of chronic lung disease (7, 8). The injuries of respiratory system are proportional to the amplitude of breath, duration of exposure, pH, concentration of gases and liquids (5).

Purpose: monitoring employees for an effective screening, establishing an association between impaired lung tests / chronic lung diseases incidence and professional exposure to irritant vapors and gases, respectively nitrogen oxides and ammonia.

MATERIAL AND METHODS

The study group includes workers exposed to nitrogen oxides and ammonia in a chemical factory. For a long time they had been exposed intermittently to the highest allowed concentration of these irritants (>14 mg/m3 for 8 h-MAC, and >5 mg/m3 for 8 h-MAC (9)) and particles (> 10 mg/m^3). The study group consisted in 1786 workers.

It was performed a professional anamnesis – the 21 items, with special attention at workplace symptomatology, professional age and life conditions / smoking, alcohol, sport, past medical history / especially previous lung diseases. The next stage was to perform function lung tests. The previous stages were studied also in the control group, which consisted in a group of workers from textile industry without chemical respiratory irritant hazards but with the well known organic and inorganic particles exposure, outside of the studied factory. The type of the study is a descriptive transversal study.

RESULTS

Since lower ventilator function test parameters are characteristic to chronic lung diseases with various etiologies we have analyzed the smoking habit of workers as a cofactor in triggering of the specified lung conditions. A number of 53% of the cases were non-smokers. Another important factor in triggering professional lung diseases was the duration of exposure at respiratory hazards (fig. 1).

As shown, over half of employees had more than 15 years at the same workplace. The average is 16.5 years. A number of 48 employees had more than 40 years at the same workplace. A single employee had the highest period: 44 years. It's about a man, non-smoker, with an episode of work-

ing accident in 1972, caused by inhaling a massive concentration of ammonia, but in the present with normal lung function tests.

Interesting is that the average age of employees is 43.6 years, over half of those being over 40.

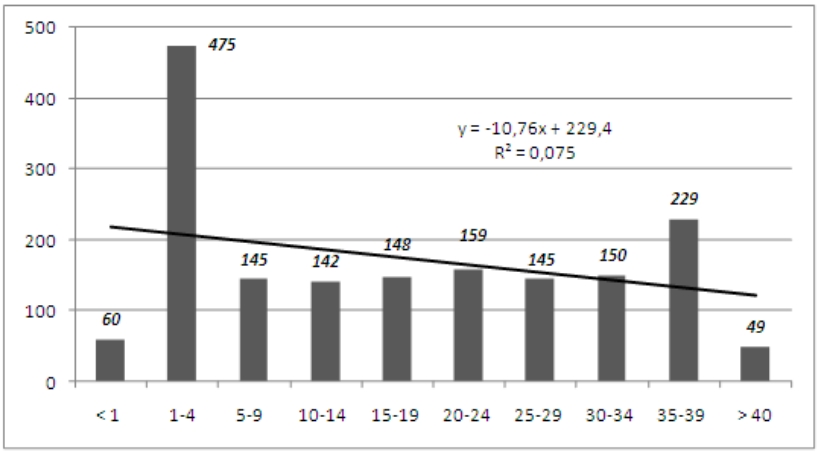

Fig. 1. Total period (years) at present workplace

Since according to the literature, the action of the gases and vapors is to decrease the lung tests parameters, we have performed the cheapest and simplest method – the spirometry, which is also very sensitive to detect a lung disease in its early stage. We have performed this test both in the study group and in the control group (fig. 2, 3, 4).

Surprisingly, a relatively high percentage of workers - almost 70% of the study group, had normal spirometry values, still dominated the obstructive syndromes without decrease FEV1 in 17.34% causes. Because FEV1 value is an accurate indicator of chronic obstructive pulmonary disease, still we wanted fellow it (fig. 3, 4), correlate it with professional history. So we followed up FEV1 according to years of exposure.

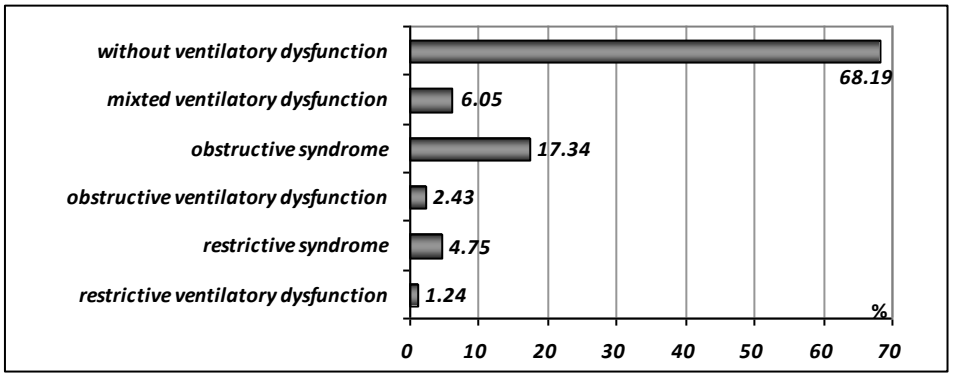

Fig. 2. Functional diagnosis in the study group

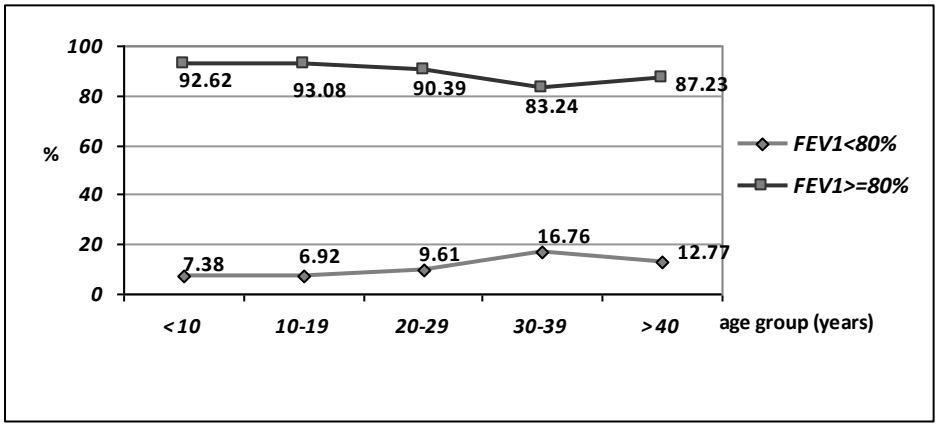

Fig. 3. Evolution of FEV1 values with the years in workplaces

Length professional activity certainly contributed to the decline of FEV1 value. (Correlation coefficient (Spearman r) = -0.06447; 95% confidence interval: -0.1278 to -0.00063; p=0.0415) Than we followed the FEV1 value with workers age (fig. 4).

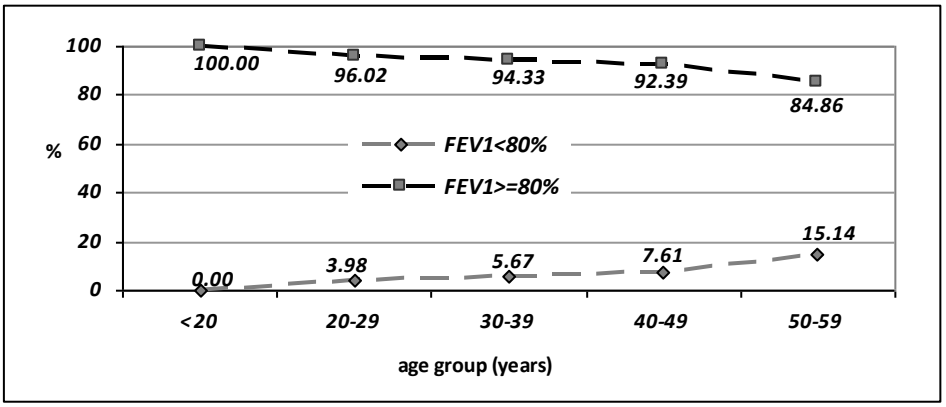

Fig. 4. Evolution of FEV 1 values with the workers ages

Statistically significant negative correlation between FEV1 value and advance in age is evident. Correlation coefficient (Spearman r) = -0.1518; 95% confidence interval: -0.2136 to -0.08874; p<0.0001). Continuing to evaluate data from medical history, and because smoking is a contributory factor in altering the function lung tests we have analyzed functional parameters by this habit (fig. 5).

We founded no significant differences between smokers and non-smokers functional parameters. As the value of FEV1 is a very sensitive parameter, which shows us very confident the different stages of lung obstruction – typical in chronic lung diseases caused by irritant gases and vapors (according to the literature) we have analyzed separately this parameter (fig. 6).

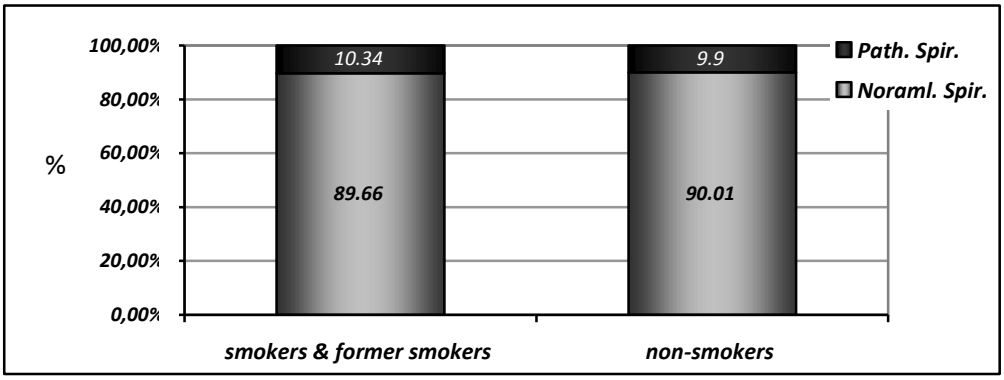

Fig. 5. Smoking habit and spirometry values

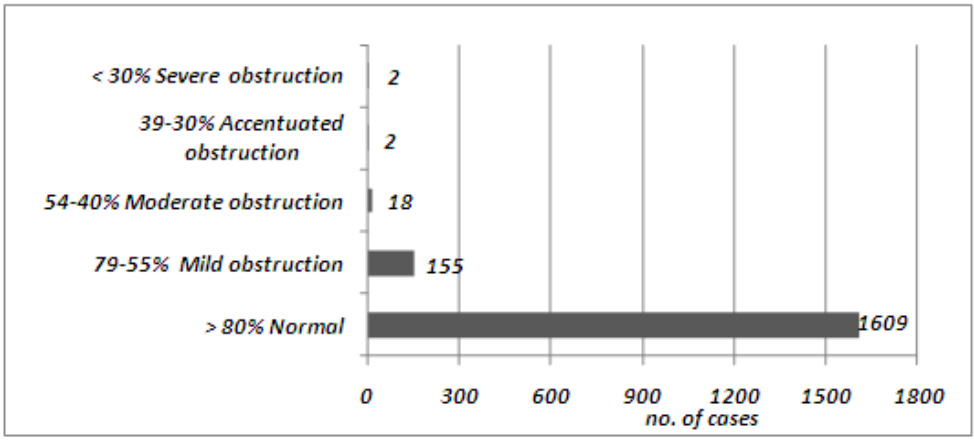

Fig. 6. Severity of FEV1 in the study group

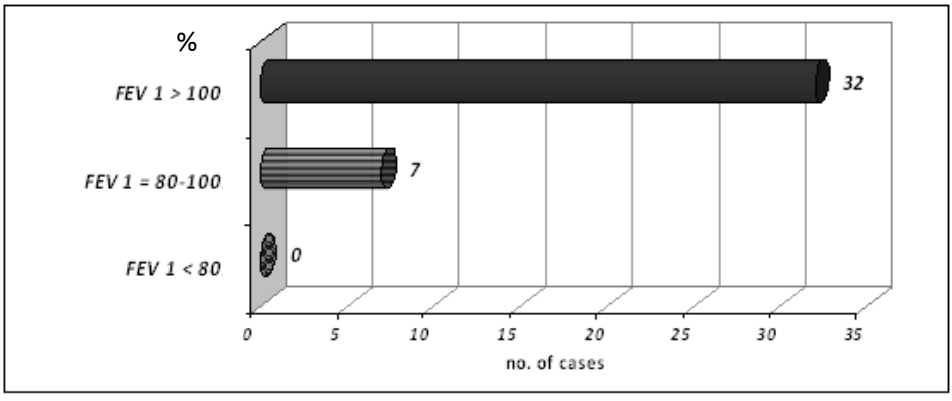

Fig. 7. FEV 1 values in sport practitioner workers

Ventilatory dysfunction grades were mostly mild. Filling in the questionnaires related to their life style, some workers stated sport habits with exercise at least 2-3 times a week. FEV 1 for these workers surprised us: 39 workers had FEV 1 over 100%.

Analyzing overall the chemical platform, especially FEV1 of workers exposed to irritant gases and vapors, according to the FEV1 of the control group with the Fisher test with 95% confidence interval, we have calculated the value of p (tab. I).

TABLE I

Analysis of FEV 1 values in the study group versus the control group

Patients	FEV < 80%	FEV = 80 -100%
STUDY GROUP (exposure to NOx, NH_3, P)	172	1598
CONTROL GROUP (exposure to P)	6	84

The results showed that $p>0.05$, $p=0.4610$; relative risk = 1.458 for 95% confidence interval: 0.6641 to 3.199. That means that there is no significant statistic association between decreased FEV 1 values in the two studied groups.

As a *recommendation*, that it is forbidden for all the workers who have lung infections, even though these are just mild infections to work in an environment with NOx and ammonia, because nasal obstruction, the inability to fell the smell of small amounts of ammonia in the air can lead to dramatic consequences. Even though those with allergic rhinitis or chronic rhinosinusitis. The intoxicated ones can achieve sequels for a lifetime. The workers with previous chronic lung diseases (asthma, COPD, bronchitis) are not allowed to work in such occupational environment.

CONCLUSIONS

We did not find a significant statistical difference between the FEV 1 values between the study group and the control group. The investigated workers have a prolonged exposure to NOx and ammonia in average 16.5 years. 68% of workers had normal values of functional lung tests and the ventilator dysfunctions were mostly mild ones. The age of workers and the years of exposure contribute on diminution of FEV1 and other spirometric parameters.

Smoking habit has not decreased significantly spirometric values among workers with irritating gas exposure. Sport is a good factor for maintaining functional lung tests in normal range (most cases having FEV1 over 100%).

Appealing to biological monitoring of workers in this study, we concluded that there are no significant difference between workers with particles exposure and workers with particles and irritating gases exposure. Biological monitoring of workers in exposure is very important in screening of chronic lung diseases, to diagnose them in time and stop the exposure as soon as possible.

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NEWS

EVODIAMINE - ANTIBACTERIAL AGENT AGAINST DRUG-RESISTANT *KLEBSIELLA PNEUMONIAE*

Nowadays, clinical bacteria are becoming resistant to conventional antibiotics, and multi-drug resistant Gram-negative bacteria confer the greatest risk to public health. The broad spectrum β-lactamase mutations have given rise to extended-spectrum β-lactamases, which hydrolyze oxyimino β-lactams; their emergence is a worldwide public health concern because few antibiotics provide effective anti-extended-spectrum β-lactamases activity. DNA topoisomerases regulate the topological state of DNA that is crucial for initiation and elongation during DNA synthesis. Topoisomerase inhibitors have been developed for antitumor, antiviral, and antibacterial applications. Certain molecules have been reported to enhance DNA cleavage and inhibit relaxation activity of bacterial topoisomerase I, thereby providing antibacterial activity. Evodiamine, an alkaloid isolated from *Evodia rutaecarpa* (Juss.) (Lauraceae), has been reported to exhibit numerous beneficial physiological effects including vasorelaxation, antiobesity, anticancer, and anti-inflammatory effects. The study investigated the bactericidal effect of evodiamine against multidrug-resistant *Klebsiella pneumoniae,* also possessing the extended-spectrum β-lactamases gene type. Evodiamine showed an inhibitory effect against the clinical isolate of *K. Pneumoniae* with a MIC value of 128 μg/mL, significantly lower compared with antibiotics such as cefotaxime, aztreonam, gentamicin, cefazolin, and cefutoxime (> 512 μg/mL). The results suggest that evodiamine acts as a bacterial topoisomerase I inhibitor and it could be used as a potential agent against drug-resistant bacteria (Wu JY, Chang MC, Chen CS, Lin HC, Tsai HP, Yang CC, Yang CH, Lin CM. Topoisomerase I inhibitor evodiamine acts as an antibacterial agent against drug-resistant *Klebsiella pneumoniae. Planta Med* 2013; 79: 27–29).

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NUTRITION IN PEDIATRIC INFLAMMATORY BOWEL DISEASE: IS IT USEFUL FOR PREVENTION AND THERAPY?

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NUTRITION IN PEDIATRIC INFLAMMATORY BOWEL DISEASE: IS IT USEFUL FOR PREVENTION AND THERAPY? (Abstract): Countless studies aiming to discover the cause and cure of inflammatory bowel disease (IBD) have been conducted over the years worldwide. Food nutrients were believed to play a certain role in the causation of the disease, in conjunction with genetic, immunologic, environmental and other factors (especially intestinal microbiota). Various nutrients were found to be involved, sometimes with controversial results, possibly pertaining to geographic regions. Nutrition has to be especially considered in children as disease itself and/or the medication can severely impair normal growth and development, sometimes with permanent long-term sequels. We reviewed the data on the possible roles of diet in preventing the disease and relapses. We emphasize the crucial importance of enteral nutrition in inducing and maintaining the remission of Crohn's disease, according to the existing consensuses (benefits, indications, routes of administration, types of formula, duration, results). We also present data on parenteral nutrition in pediatric IBD, with current updates from literature. **Keywords:** NUTRITION, CROHN'S DISEASE, THERAPY, CHILDREN

Despite numerous studies performed worldwide, the etiopathogenesis of inflammatory bowel disease (IBD) remains largely unknown. It is considered to be the result of the interactions between genetic, immunologic and environmental factors, closely related to intestinal microbiota. The role of various food nutrients in favoring IBD has been extensively studied over the years. Possible mechanisms include direct effect of dietary antigens, alteration of gene expression, alteration in the composition of the enteric microflora, and the effect on gastrointestinal permeability (1). Nutrition has to be considered especially in children, also since the disease itself and/or the medication (including steroids) could severely impair normal growth and development, sometimes with definitive long-term sequels.

Undernutrition and particularly growth failure are less common in children with ulcerative colitis (UC) compared to those with Crohn's disease (CD) (2). These aspects should be especially considered, since 20% of IBD cases occur before the age of 18 years (3). While in adults therapy aims especially at inducing and maintaining remission (ideally with mucosal healing), in children, recovery of nutritional deficiencies and restoring the somatic and psychological development need to be also

considered (4).

Nutrition in IBD refers to: enteral nutrition (elemental, semielemental and polymeric liquid formulas), parenteral nutrition, and diet (both during the flares and remissions). The new ECCO guidelines in 2010 (3) and especially NASPGHAN in 2012 (5) have brought important specifications regarding enteral nutrition in CD.

Are any food components directly involved in IBD etiology?

Many nutrients have been considered in various studies, sometimes with controversial results, possibly pertaining to geographic regions. In a recent Italian study, high cheese intake was associated with CD and that of pasta, sweets, butter, and margarine with IBD. Conversely, high fish and potato intake significantly reduced the risk of IBD, vegetables and tuna reduced the risk of CD and eggs decreased the risk for UC (6). In a recent Danish research, low fiber diet and high sugar intake were significantly associated with development of IBD (7). In a recent systematic review of previous studies in adults, published in March 2012, it was shown that a diet high in protein from meat and fish has been associated with a higher risk of IBD. Intake of n-6 polyunsaturated fatty linoleic acid (from red meat and some cooking oils and margarine) may confer a risk for UC, whereas n-3 polyunsaturated fatty acids may be protective. No effect has been found in intake of dietary fibers, sugar, macronutrients, vitamin C, D, E, carotene, or retinol on risk of UC (8). A comprehensive analysis of possible roles and mechanisms of dietary components in determining IBD has been recently published. It has been shown that there could exist some ‘‘proinflammatory’’ eating habits in genetically susceptible individuals, playing a role in IBD. However, *there is currently insufficient evidence to support a direct relationship between diet and IBD (1),* larger prospective studies being required, including young subjects.

Recently, the role of hypovitaminosis D (with connotations in food intake) in determining IBD (especially CD) has been pointed out (9); higher predicted plasma levels of 25(OH)D significantly reduce the risk for incident CD and insignificantly reduce the risk for UC in women (10).

What is the nutritional status of children with IBD?

Undernutrition and especially growth retardation are more often encountered in CD than in UC. A body mass index (BMI) < 5th percentile at the diagnosis was seen in 22-24% of children with CD, compared with UC - 7-9% (2). At the time of diagnosis, up to 85% of children with CD and 65% of those with UC have weight loss (11). In the 2nd Pediatric Clinic in Cluj-Napoca (1998-2010), all children with CD had weight loss until the diagnosis, compared with 74.1% with UC (average of 4.8 kg and 3 kg, respectively). Height deficit at presentation has been found in 46% of children with CD (12). Decreased height velocity is the most sensitive parameter of an impaired linear growth (could appear years before the symptoms) (13) and has been detected in about half of children with CD (12). Despite the therapy, a quarter of children with CD may not achieve full adult height potential (12). Conversely, children with UC (without excessive steroid use) reach their expected adult height (13). Moreover, children with IBD (especially BC) are particularly prone to disturbed bone health (due to the disease itself and/or steroids) and severe osteopenia is detected in 12-18% of those with CD *vs.* 3-6% in UC (14).

On the other hand, recent studies carried out in US have shown that overweight/obesity does exist in children with IBD. In 783 children with newly diagnosed IBD, a BMI consistent with overweight or risk for overweight was present in 10% of those with CD and 20-30% with UC (2). In other study on 1598 children, the prevalence of overweight/obese status was 20% for CD and 30% for UC (15).

The etiopathogenesis of undernutrition is multifactorial. Increased digestive losses (diarrhea, bleeding, protein-losing enteropathy), increased requirements, and malabsorption (due to inflammation, fistulas, resections) can be responsible, but the most important factor is the suboptimal food intake, secondary to anorexia (due to pro-inflammatory cytokines and adipokines, alterations in hypothalamic serotonin levels), symptoms, diet and medication (Rev. in 11,16). Malnutrition, pubertal delay, steroid therapy and pro-inflammatory cytokines (by direct effect on bone metabolism, promoting anorexia and interference with growth hormone/insulin-like growth factor-1 axis) contribute at the growth failure (11, 16).

It is beyond the aim of this paper to detail the methods of assessing the nutritional status. We only highlight the importance of assessing (both at the diagnosis and after, every 3-6 months) the following items: weight, height, BMI and the pubertal Tanner staging. Bone mineral status should be assessed using dual-energy X-ray absorptiometry (4, 16). Consequently, the therapy could be adjusted to provide a normal growth and development.

WHAT IS THE ROLE OF NUTRITION IN IBD?

Since there is yet no cure for IBD, there is only pathogenetic therapy, the following methods being used (sometimes in combination): medication, surgery, nutrition and psychotherapy. A successful program based on nutrition requires, besides the pediatric gastroenterologist, a dietician, a dedicated nutrition nurse, a psychologist and, especially, financial support (5).

1. ENTERAL NUTRITION (EN)

EN refers to the administration of liquid formulas, containing the nutrients necessary for growth and development, providing also a high caloric intake. The differentiation between the types of formulas is made by the degree of protein hydrolysis. Accordingly, there are polymeric (whole proteins), semielemental (oligopeptides) and elemental (only amino acids) formulas. EN can be exclusive (EEN), as the sole dietary source and partial (PEN), in addition to a normal diet (5).

1.a. EEN is considered the *first-line therapy in induction the remission in children with CD* (3, 4, 11, 17), however it is not effective and should not be used in UC (4). EEN has many important *advantages:* bowel rest, modification of the gut flora, decrease of the antigenic load to the gut, reduction of the intestinal inflammation with mucosal healing, down-regulation of the pro-inflammatory cytokines, normalization of the biological inflammatory markers, improvement of growth, nutritional status and of bone health, as well as of the quality of life, with minimal adverse effects (3, 11, 17, 18, 19). *Disadvantages* concern especially the difficulties in compliance and the high costs (5, 11, 17). The *duration* of the EEN is usually 6-8 weeks, however increasing the interval until 12 weeks could have additional benefits (5). *Remission* through EEN appears in up to 85% of chil-

dren, being as effective as steroids in the most recent meta-analysis on 204 children (3,17,20), but without the adverse effects of steroids. Contrary to previous opinions, EEN is effective *irrespective of the enteral or colonic locations* of the disease, being recommended also in those with *perianal* involvement, according to the recent consensuses (3, 5, 17, 21, 22). When choosing the type of formula, we have to consider that very recent studies have shown that *polymeric formula* is not inferior to the elemental or semielemental ones (3, 4, 5, 23, 24), being used in more than 90% of centers in Europe, North America and Pacific Asia (17). Among the advantages of the polymeric formula, better weight gain (25), better taste and lesser cost have to be mentioned (5). In the 2nd Pediatric Clinic in Cluj-Napoca, as in many other gastroenterology centers across the world, we have used Modulen IBD®, containing TGF-β (transforming-growth factor β), with many functions: anti-inflammatory activity, growth improvement, regulation of immune response and tissue repair. As regards the *route of administration* – oral, nasogastric tube or gastrostomy, obviously the oral route is preferred, whenever possible, with better compliance (4). A recent study at Necker Hospital in Paris, on 106 newly-diagnosed children with CD using EEN with Modulen IBD® for 8 weeks, showed that fractioned oral feeding is as effective as the continuous enteral feeding to induce remission (75 *vs.*. 85%, respectively), with similar compliance rates (87 *vs.*. 90%, respectively) (26). *The estimated energy requirement* can be calculated using the Schofield equation (27), considered the best instrument (28); other factors (related to activity, fever) could be added (5); most children need approximately 120% of reference nutrient intake (4, 5). Quantities should be introduced gradually, over 3-6 days, until the full volume is reached (5). *Time to remission* has been reported between 11 days and 2.5 weeks (20); however, 3-4 weeks have to be sometimes allowed in order to decide upon the efficacy (5); inflammatory markers improve in about 1 week (29). *Duration of remission* after EEN is controversial in the literature. In some studies, the relapse appeared earlier than in those with remission induced by steroids, with 3 (30) – 4.58 months (31); duration of remission was 7 months after EEN *vs.* 10 months after steroids (30). In another study, duration of remission was longer after EEN (32). Ten months after EEN, 39% present a relapse (33). Return to normal food can be made either by beginning with a low-fiber diet or by gradually reintroducing food, over 1-3 weeks, while gradually reducing the formula (4,5).

1.b. PEN is less efficacious in *inducing remission in CD and should not be recommended* (3). In a comparative study in children, EEN induced remission in 42% *vs.* PEN – only in 15%; in children on PEN, half of the requirements were provided by formula (34). Some pediatricians allow the addition of various foods, with a maximum of 10% of the whole energy intake, appearing as not decreasing the efficacy of EN as induction therapy (5).

PEN could be, however, continued after the first 8 weeks of EEN, significantly increasing the rate of *maintaining the remission* (35) and also improving *growth and nutritional status* (especially when associated to other therapies) (5). PEN could be provided in various forms: overnight nasogastric formula in conjunction with normal daytime food (PEN *vs.* normal food, 1 year later: relapse 43 *vs.* 79%,

p<0.02) (36); cyclically – every 1 month out of 4 with EEN (1 year later: reduced activity of CD and of steroids use, with significant height and weight gains) (37); oral supplements of EN in addition to oral eating throughout the day, the preferred option whenever possible (recent study in Israel, using Modulen IBD®, with significant decrease of the activity of CD and of serologic inflammatory markers, as well as improvement of the BMI) (38). Long-term results concerning remission in 44 CD children, using EN over 1-7 years show the following: majority of those continuing PEN did not relapse after remission was established, approximately 50% did not require steroids after the diagnosis and in those who necessitated steroids, their introduction was delayed with a median period of 68 (6-190) weeks; also, the weight Z-scores were improved after 12 months, with no improvement in height Z scores (22).

Recent surveys have evaluated the use of EN in children with CD, across the world. In 2003, EN was used by 62% of pediatric gastroenterologists in Europe *vs.* only 4% in North America (39). In 2011, pediatric gastroenterologists from North America reported that 31% have never used EN, 55% have used it rarely and only 12% do use it regularly. EN has been used significantly more in Canada than in US, including maintaining of remission (40). In the 2nd Pediatric Clinic in Cluj-Napoca, we have used Modulen IBD® in 80% of cases (associated to various therapies), with good results.

PEN has no therapeutic role in UC, however it could be used in those with malnutrition (4, 13).

2. TOTAL PARENTERAL NUTRITION (TPN)

In CD, TPN indications are restricted to: severe malnutrition, complications (fistulas) and nutritional support pre- and postoperatively (4,5). TPN has similar efficacy to EEN, however with much higher costs and more complications (38).

TPN is not effective as a primary therapy for UC, and may even deprive colonocytes of short-chain fatty acids (41). It is restricted only to: severe malnutrition, acute severe colitis, with imminence of toxic megacolon (42) and nutritional support pre- and postoperatively.

3. WHAT TO KNOW ABOUT THE DIET OF THE IBD PATIENT?

Intriguingly, even if no food component has been directly involved in causing IBD, the patients' perception is different. A very recent survey on 244 French adults has shown that 15.6% believed that a diet could initiate the disease and in 47.5% the disease changed the pleasure of eating. Also, 57.8% believed that food can play a role in causing a relapse; moreover, 40% identified food as a risk factor for relapse and 66.8% avoided certain foods, in order to prevent a relapse. One fifth of the patients considered that dietary beliefs and behavior had an impact on their social life. Excluding food was associated with refusing outdoor dining and not sharing the same menu with the family (43).

A systematic review, of existing dietary guidelines written by experts of world / international/ national gastroenterology and nutrition organizations, clearly mentions that *no diet could prevent the relapse* and, therefore, it is not required (41). Diet should be, however, adapted to the individual tolerance to different foods, especially considering other associated medical conditions (41). Foods high in fiber, such as popcorn, nuts, and seeds may result in

cramping, bloating or diarrhea; high fat foods may not be completely absorbed by the digestive tract and could result in gas and diarrhea; milk, cheese, or dairy products may cause abdominal discomfort, bloating, gas and diarrhea in those with lactose intolerance; raw fruits and vegetables may lead to bloating, gas or diarrhea; alcoholic beverages may not worsen IBD, but determine some symptoms (41).

Obviously, when deficiencies are detected, vitamin/mineral supplements should be given: iron (better tolerated and more effective in intravenous administration) (44), vitamin D and calcium – in some opinions also with role in decreasing the relapse (45), vitamin B_{12} (41).

The following rules and food restrictions are recommended during severe relapses, in order not to worsen symptoms: small amounts of food throughout the day; decrease excess saturated fat (fried food, sausages, salami, hot dog, omelet, sauces, margarine, butter, heavy cream), excess sugars and high-fiber foods (cereals, nuts, most raw fruits – except grated apple, banana and melon, most vegetables – beetroot, broccoli, cabbage, cauliflower, spinach, mustard, turnip tops, mushrooms, beans, onion, pepper, pumpkin), avoid dairy products (if lactose intolerant), as well as coffee, alcohol, sweet juice and coke (41).

In conclusion, nutrition is a useful therapy in CD: exclusive enteral nutrition induces remission, partial enteral nutrition decreases the relapse rate, improving growth and nutritional status, total parenteral nutrition has similar efficacy to the enteral one, but with much more adverse effects and higher costs. Nutrition is not effective and should not be used as a primary therapy in UC. As of yet, no diet can prevent the development/relapse of IBD. Some food restrictions are recommended during flares, in order not to worsen symptoms.

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ELECTROLYTE DISTURBANCES IN PATIENTS WITH CHRONIC HEART FAILURE – CLINICAL, EVOLUTIVE AND THERAPEUTIC IMPLICATIONS

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ELECTROLYTE DISTURBANCES IN PATIENTS WITH CHRONIC HEART FAILURE – CLINICAL, EVOLUTIVE AND THERAPEUTIC IMPLICATIONS (**Abstract**): Patients with heart failure are, by definition, hemodynamic unstable. Often, this condition may be accentuated by medication (digitalis, diuretics, antiarrhythmics), so that they become more sensitive to electrolyte disturbances, reacting sometimes violently with severe and life threatening rhythm troubles. A**im:** the evaluation of the incidence of electrolyte disturbances in patients diagnosed with chronic heart failure in order to establish a correlation with the evolution, prognosis and therapeutic implications. **Material and methods:** We analyzed retrospectively 100 patients diagnosed with chronic heart failure NYHA II-IV classes, admitted in Cardiology Clinic during 2009 – 2011. We analyzed electrolytic disturbances occurred during different strategies of therapy. **Results:** 100 patients with heart failure were admitted in Cardiology Clinic during 2009-2011, 75 males and 25 females. Diagnosis was established by classical criteria. Evaluation was very complex and included: complete clinical examination, electrocardiogram, echocardiography, chest ray examination and biochemical analyses especially hepatic, renal function and electrolyte status. **Conclusions:** The obtained data showed that electrolyte disturbances are frequent in patients with chronic heart failure, irrespectively of NYHA class. Hyperkalaemia, hypokalaemia and hyponatraemia are associated with diuretic therapy and may play a very important role in subsequent development of life-threatening arrhythmias. **Key words:** ELECTROLYTE DISTURBANCES, HYPOKALAEMIA, HYPERKALAEMIA, HYPONATRAEMIA.

The patient with heart failure is by definition a hemodynamically unstable patient in which any therapeutic gesture initially beneficial, may have unexpected side effects, some of them life-threatening (1). This type of patient usually has electrical instability related to the underlying disease and potentiated under medication (diuretics, digitalis, antiarrhythmics etc.) (1, 2, 3) In these conditions, they become more sensitive to even minor electrolyte balance disturbances, sometimes reacting violently through serious arrhythmias or conduction disturbances. The medication of the patient with heart failure involves complex therapeutic schedule, adapted to the etiopatho-

genic and hemodynamic features of each case. In these schedules the diuretic medication is essential and it is pathophysiologically addressed to the salt and water retention (3). Although well tolerated by most patients, diuretic medication acting at renal level disrupts the electrolyte balance so that, depending on the type of mechanism, on the patients' condition at the time, on the associated medication and diet, there may appear other electrolyte and fluid disorders some of which could be life-threatening (1, 2, 3). Hyponatraemia is the most common electrolyte disorder encountered in intensive care services (its frequency is estimated at 30-40% of the hospitalized patients) (4, 5).

The aim of the study is to evaluate the incidence of electrolyte disorders encountered in patients with heart failure development and to establish a correlation between these disorders and the apparition of certain complications, and the entailed prognosis and therapeutic implications.

MATERIAL AND METHODS

The study is retrospective and included a group of 100 patients, 75 men and 25 women, aged 48-85 years, hospitalized in the 1st Cardiology Clinic between the years 2009-2011 and diagnosed with chronic heart failure of the NYHA II-IV classes.

The classification of patients in the NYHA class of severity of heart failure was as follows (fig. 1):

NYHA Class II - 15 patients: 10 men, 5 women;

NYHA class III - 72 patients: 55 men, 17 women

NYHA Class IV - 13 patients: 8 men, 5 women.

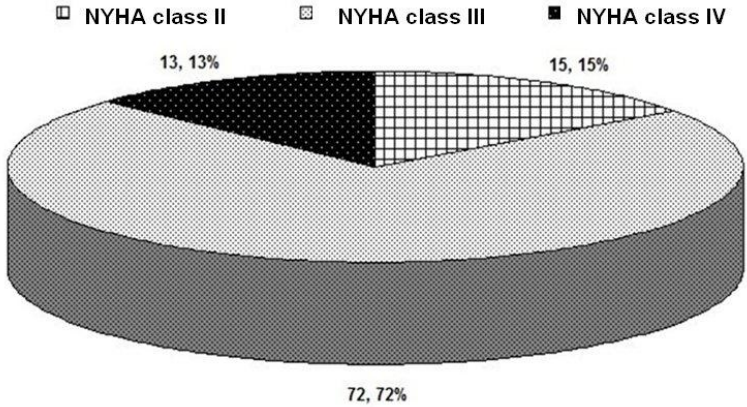

Fig. 1. Distribution of patients according to the NYHA class of heart failure severity.

The diagnosis of heart failure was based on the known and classical clinical and paraclinical data:

- Symptoms and signs of left ventricular failure (shortness of breath of different degrees, cardiac cough, tachycardia with / without gallop rhythm, presence of organic heart breaths and pulmonary rales stasis);

- Signs of right myocardial failure: hepatomegaly, edema in the legs (cardiac type), turgid jugular (level I-III), hepatojugular reflux, with or without ascites.

Paraclinical exploration of the patients included:

- Chest radiography - objectified cardiomegaly (ICT over 0, 55) with or without

signs of pulmonary stasis or pleural effusions.

- Echocardiography (2D, M mode, Doppler) objectified cardiac cavities dilation with decreased contractile function parameters (ejection fraction, shortening fraction), and other aspects: valvular regurgitation, intracardiac thrombus, presence of spontaneous contrast and signs of hypertension pulmonary artery. Echocardiography was essential for identifying the etiology of heart failure.

- Peripheral venous pressure was elevated in most patients with right heart failure component.

- 12 leads surface ECG. There were sought: rhythm or management disturbances, ischemo-lesional changes, overstress of certain cavities, changes due to electrolyte disturbances.

- Biochemical balance included: hepatic function (transaminases, bilirubin, GGT), renal function (urea, creatinine, uric acid, creatinine clearance, RA), serum electrolytes (Na +, K +, Ca + +, Mg + +);

- Paraclinical investigation was complemented with complete blood count, spirogram and determination of blood gases and pH.

Patient evaluation was performed first at the time of admission and subsequently, depending on each particular case, was performed daily during hospitalization.

The etiology of heart failure was as follows (fig. 2):

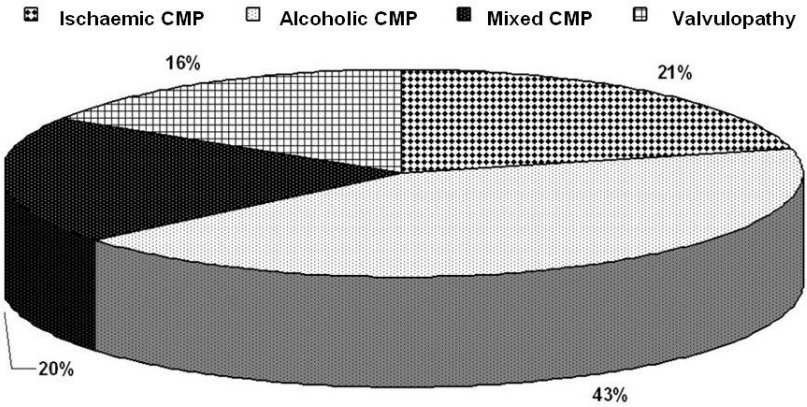

Fig. 2. Etiology of heart failure in the studied group.

- Ischemic dilated cardiomyopathy - in 20% of cases;

- Ethanolic dilated cardiomyopathy - in 45% of cases;

- Mixed dilated cardiomyopathy (ischemic, toxic, diabetic) - 20% of cases.

- Valvular heart disease - 15% of cases.

Etiology was based on history, physical examination and echocardiographic data resulted from examination. In some cases, to these was added the presence of the biochemical markers that supported chronic alcoholism.

Patients' treatment was strictly individualized according to the haemodynamic status at the time of hospitalization, the etiological features, the presence or absence of arrhythmias and/or conduction disturbances and included, besides the

appropriate hygienic-dietary measures, the following (fig. 3):

- Diuretic: loop - 15 patients (15%), spironolactone + loop diuretic combination - 85% of patients;
- Nitrate type vasodilator - 75% of patients;
- Converting enzyme inhibitor (ACEI) - adjusted doses of TA- was administered to 85% of patients (65% received perindopril, 20% received ramipril);
- Beta blocker - 90% of patients (55% carvedilol, bisoprolol 35%);
- Digoxin - 70% of patients;
- Amiodarone - 30% of patients;
- Anticoagulant - 60% of patients;
- Antiplatelet - 75% of patients received aspirin (in 50% of the cases it was associated with anticoagulant), 25% of patients receiving clopidogrel.

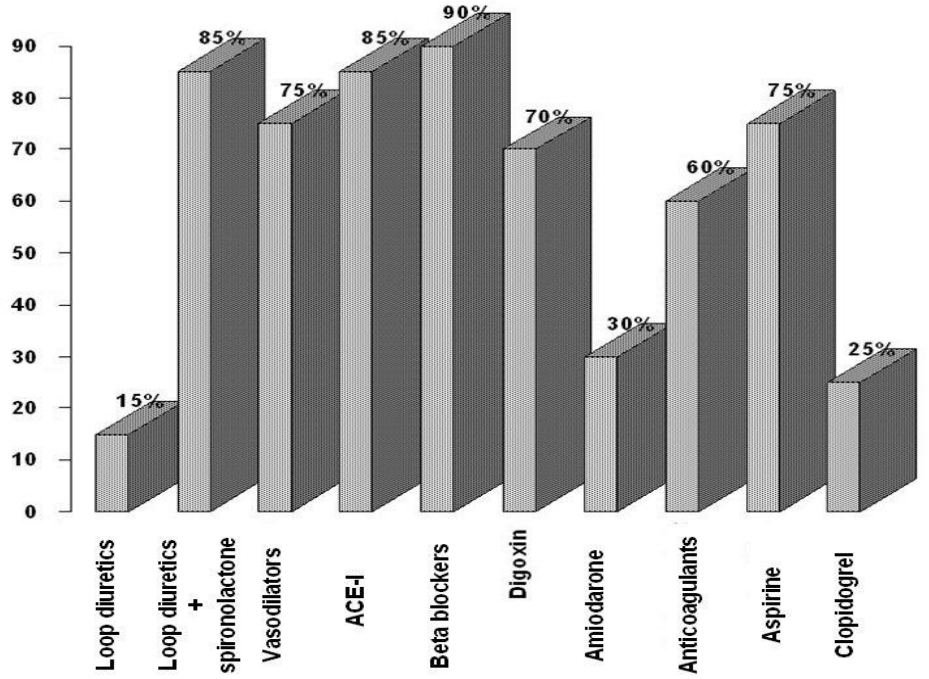

Fig. 3. Treatment of patients in the studied group.

Patients received therapeutic regimens that included combinations tailored to each individual case, as follows:

- Loop diuretic + vasodilator (nitrate) + beta blocker + ACE inhibitor - 10% of cases;
- Loop diuretic + spironolactone + ACE inhibitor + beta blocker + vasodilator (nitrate) - 40% of cases;
- Loop diuretic + spironolactone + ACE inhibitor + beta blocker + digoxin + vasodilator - 20% of cases;
- Loop diuretic + ACE inhibitor + amiodarone + vasodilator - 30% of cases.

All patients received an antiplatelet regimen and in 60% of the cases an anticoagulant administration was necessary.

Introducing the anticoagulant regimen was imposed by the presence of echocardiographic intracavitary thrombus or spontaneous contrast, by the presence of dilated right cavities and other clinical signs suggestive of thromboembolism associated phenomena. Throughout the immobilization in bed there were also injected prophylactic doses of low molecular weight heparin.

The nitrate type vasodilator was used with preference in patients with signs of left ventricular failure considered to be due to ischemia and in patients with evident pulmonary stasis.

RESULTS AND DISCUSSION

The electrolyte disturbances encountered in patients of the group were the following:

Hyperkalaemia (serum K values between 5.8 to 6.5 mmol/l) - present in 65% of patients receiving loop diuretic combination with spironolactone associated with ACE inhibitors. 51% of patients with hyperkalaemia showed electrocardiographic changes suggestive of this type of electrolyte disorder: high T beams, in precordial (5%), ventricular extrasystoles systematized in bi- form and trigeminy ventricular doublets (45% of cases). 35% of these cases were associated with elevated urea and serum creatinine with functional significance of renal failure. The therapeutic conduct consisted of stopping potassium saving diuretic, replacing it only with loop diuretic, infusion of glucose 5% buffered with serum insulin to normalize serum potassium. In patients receiving concomitant digoxin, presence of ventricular extrasystolic arrhythmia imposed the replacement of digitalis with beta blocker.

Hypokalaemia (serum potassium values between 2.1 to 3 mmol/l) was present in 8% of patients that had only loop diuretic therapeutic regimen. 2% had diarrhea as additional contributing factor. Clinically, the patients had asthenia, fatigue, muscle cramps. 5% of the patients receiving concomitant digoxin showed on the electrocardiogram ventricular bigeminy and in 2% of them QT prolongation was found compared with the initial values. The therapeutic conduct consisted of temporarily stopping the diuretic, more potassium (orally or intravenously if necessary) until normalization of serum potassium. The presence of ventricular extrasystoles imposed reconsidering the therapeutic regimen and removing digoxin where present. Lidocaine or beta blocker was used to control arrhythmias.

Hyponatraemia (serum sodium values between 128-110 mmol/l) was present in all patients with NYHA IV class heart failure receiving diuretic therapy. Clinically, these patients presented generalized edema at admission. In 5% of the patients hyponatraemia persisted after stopping diuretic, so it was considered dilution hyponatremia and significantly correlated with increased mortality in this population. Correction of hyponatraemia was difficult, with partial response and limited to fluid restriction and administration of sodium chloride, while continuing the diuretic.

In 15% of cases, these electrolyte disturbances have been associated with inflection of the renal function, that is, increased urea and creatinine values understood as renal functional failure and whose main cause was the diuretic abuse with subsequent reduction in pressure renal perfusion. In 10% of the cases, increased levels of uric acid could be seen (between 9-11 mg%), correlated with the loop diuretic doses. In 7% of cases, renal failure was corrected by

temporary withdrawal of the diuretic and proper hydration. In 8% of cases, it was necessary the administration of vasodilator dose dopamine (2-3 mcg/kg/min).

CONCLUSIONS

Electrolyte disturbances are common in patients with heart failure development, regardless of NYHA functional class. In most cases this is due to medication, especially to the diuretic therapy. From this perspective, certain drug combinations as loop diuretics with digoxin, potassium saver diuretics with ACE inhibitors present a more important risk. Correcting these imbalances is a major emergency as they often are life-threatening, representing the substrate for different arrhythmias including the ventricular which can represent a vital threat.

Hyponatraemia is an independent negative prognostic factor especially in patients enrolled in the NYHA IV functional class and it is often associated with increased mortality.

Knowing these electrolyte disturbances that can occur at any time in the evolution of patients with heart failure imposes attention to the choice of therapeutic regimen, caution in dosing and combination of drugs, which are, in fact, absolutely necessary.

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ACIDUL URIC, EVENIMENTELE CARDIOVASCULARE ŞI DISFUNCȚIA RENALĂ - O LEGĂTURĂ CONJUNCTURALĂ?

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URIC ACID, CARDIOVASCULAR EVENTS AND RENAL DYSFUNCTION: A CIRCUMSTANTIAL CONNECTION? (Abstract): **Aim**: The relationship between asymptomatic hyperuricemia, cardiovascular events and renal dysfunction was studied (affirmed or challenged) long time ago. To investigate this relationship we conducted this study. **Material and methods**: We analyzed 163 patients consecutively admitted in the Intensive Cardiac Care Unit of the 1st Cardiology Clinic, County Hospital "St. Spiridon" Iasi. The cut-off value of serum uric acid was defined as 7 mg%. There were recorded anthropometric, anamnesis, clinical and paraclinical data. **Results:** We compared the normal uricemia group (108 patients) with the hyperuricemia group (55 patients). The serum uric acid value was 5.22+/-1.16 mg% in the normouricemia group and 8.58+/-1.86 mg% in the hyperuricemia group. The statistically significant differences between the two groups relate to sex, smoking as cardiovascular risk factor, diastolic blood pressure and ejection fraction (lower in the hyperuricemia group were the dilated cardiomyopathy prevail). If the patients are grouped according to creatinine *clearance* less than or greater than 60 ml / min we found statistically significant differences between the two groups in terms of serum uric acid value ($p < 0.001$), age ($p < 0.001$), renal dysfunction and dyslipidaemia (p 0.001). **Conclusions:** The analysis performed doesn't demonstrate the role of uric acid as independent risk factor in a group that includes patients with different heart diseases (dilated cardiomyopathy, ischemic heart disease or hypertension). **Key words:** URIC ACID, CARDIOVASCULAR, RENAL DYSFUNCTION

Relația dintre hiperuricemia asimptomatică, evenimentele cardiovasculare şi disfuncția renală a fost analizată, afirmată (1) sau contestată (2) de mult timp, începând cu semnalul princeps lansat în 1879 de către Mohamed (3). Datele recente indică asocierea pierderii evolutive a uricazei la om (proces inițiat, se pare, în urmă cu aproximativ 24 milioane de ani) (4) cu o serie de avantaje evolutive: o capacitate antioxidantă înaltă şi longevitate sporită, menținerea valorilor tensiunii arteriale în cazul unei diete hiposodate (5), sporirea inteligenței şi neuroprotecție. Pe de altă parte, sunt dovezi în favoarea rolului potențial de toxină cardiorenală a acidului uric (6), hiperuricemia fiind asociată cu numeroase comorbidități: obezitate, sindrom

metabolic, diabet zaharat, hiperlipoproteinemie, hipertensiune arterială, insuficienţă cardiacă sau boală cronică de rinichi (7, 8, 9). Caracterul de factor de risc independent al acidului uric este însă un subiect de controversă, unii autori susţinând idea că până nu apar noi date, pacienţii hiperuricemici ar trebui să fie evaluaţi în direcţia prezenţei altor factori de risc deja validaţi (10). Intenţia de a investiga relaţia dintre nivelele acidului uric, evenimentele cardiovasculare şi disfuncţia renală a stat la baza realizării acestei lucrări.

MATERIAL ŞI METODE

Au fost incluşi în studiu 163 de pacienţi internaţi consecutiv în Unitatea de Supraveghere şi Tratament Avansat al pacienţilor Cardiaci Critici (USTACC) din cadrul Clinicii I Medicale Cardiologice "C. I. Negoiţă", Spitalul Clinic Judeţean de Urgenţă "Sf. Spiridon" Iaşi, în perioada iunie – decembrie 2011, 106 (65,03%) bărbaţi şi 57 (34,97%) femei cu vârsta medie 64,92 +/- 11,32 (limite 36-89). Au fost consemnate datele antropometrice (talie, greutate, indicele de masă corporală), anamnestice (antecedente personale coronariene, cerebrale şi periferice; factorii de risc cardiovascular: fumat, dislipidemie, hipertensiunea arterială, diabetul zaharat), clinice (valorile tensiunii arteriale, frecvenţei cardiace, diagnosticul principal) şi biochimice referitoare la funcţia renală (acidul uric seric, urea serică, creatinina serică, *clearance*-ul de creatinină calculat conform formulei Cockcroft-Gault), dislipidemie (colesterolul seric, trigliceridele serice, HDL-C, LDL-C) şi generale (hemoleucograma şi glicemia). S-a efectuat o electrocardiogramă (s-a consemnat ritmul şi descrierea traseului) şi un examen ecocardiografic transtoracic utilizând un ecocardiograf Esaote MyLab™ 30 GOLD Cardiovascular cu un transductor *phased-array* de 2-5 MHz (cu măsurarea diametrelor şi dimensiunii pereţilor cordului, aprecierea funcţiei sistolice şi diastolice ventriculare, diametrul venei cave inferioare) în cazul fiecărui pacient. Analiza statistică s-a realizat utilizând *Microsoft ® Office Excel 2003* şi *SPSS 14,0 for Windows*. Analiza corelaţiilor s-a efectuat cu ajutorul coeficientului de corelaţie Pearson. Semnificaţia statistică a fost luată în consideraţie pentru un nivel $p < 0,05$.

REZULTATE

Analiza lotului s-a efectuat în funcţie de nivelul acidului uric seric. Deoarece în literatură valoarea discriminativă pentru normo- şi hiperuricemie este variabilă (5,12 mg% în studiul Framingham, 1987 şi diferenţiată peste 6 mg% la femei şi peste 8 mg% la bărbaţi în studiul lui Filippatos et al) (9) în acest studiu s-a considerat valoarea cut-off peste 7 mg%. În funcţie de această variabilă lotul a fost analizat comparativ: normouricemici (n = 108) *vs.* hiperuricemici (n = 55) (tab. I).

Singurele diferenţe statistic semnificative între cele două grupuri se referă la sex, la prezenţa tabagismului cronic ca şi factor de risc cardiovascular şi la valorile tensiunii arteriale diastolice. S-au evaluat valorile medii ale acidului uric în cele două grupuri, cu evidenţierea diferenţelor semnificative care ţin de disfuncţia renală (tab. II şi III).

Toate celelalte variabile biologice sunt similare în cadrul grupului cu sau fără hiperuricemie. Există o diferenţă statistic semnificativă între fracţia de ejecţie, mai redusă în grupul cu hiperuricemie în care au predominat însă şi cazurile prezentând cardiomiopatie dilatativă (fig. 1).

TABELUL I

Caracteristicile lotului studiat

n (%) sau medie +/-DS (mediana)	Normouricemie (n = 108)	Hiperuricemie (n = 55)	Valoarea p
Vârsta	63,45+/-10,50 (68)	67,86+/-12,57 (68)	0,147
Sex feminin	49 (45,37)	8 (14,54)	0,007
AP coronare	64 (59,26)	29 (52,73)	0,596
AP cerebrale	18 (16,67)	13 (23,64)	0,504
AP periferice	8 (7,41)	1 (1,82)	0,216
FR tabagism	41 (37,97)	37 (67,28)	0,033
FR HTA	64 (59,26)	29 (52,73)	0,596
FR HLP	46 (42,60)	13 (23,64)	0,143
FR Diabet zaharat	36 (33,34)	11 (20,01)	0,281
Talie (cm)	170,02+/-12,54 (170)	170,52+/-8,04 (170)	0,869
Greutate (kg)	78,72+/-16,82 (80)	77,14+/-15,77 (77)	0,721
IMC	27,76+/-8,88 (26,9)	26,41+/-4,39 (25,43)	0,515
TAS (mmHg)	143+/-23,70 (140)	138,81+/-27,56 (130)	0,524
TAD (mmHg)	83,81+/-13,19 (80)	75,48+/-16,11 (70)	0,032
Frecvenţa cardiacă (bpm)	82,0+/-15,10	81,31+/-15,51	0,580

Legendă: n număr; DS deviaţia standard; AP antecedente personale; FR factor de risc; HTA hipertensiune arterială; HLP hiperlipoproteinemie; IMC indice de masă corporală; TAS tensiune arterială sistolică; TAD tensiune arterială diastolică..

TABELUL II

Analizele de laborator ale pacienţilor din lotul studiat

n (%) sau medie +/-DS (mediana)	Normouricemie (n = 108)	Hiperuricemie (n = 55)	Valoarea p
Acid uric seric (mg%)	5,22+/-1,16 (5,45)	8,58+/-1,86 (7,9)	0,001
Uree serică (mg%)	39,95+/-16,72 (37)	67,33+/-33,96 (53)	0,002
Creatinina serică (mg%)	0,9+/-0,22 (0,93)	1,7+/-0,9 (1,34)	0,001
Clearance creatinină (ml/min)	82,98+/-27,49 (85,6)	56,33+/-36,84 (42,5)	0,002
Colesterol seric (mg%)	194,17+/-65,50 (176)	181,81+/-85,23 (159)	0,533
Trigliceride serice (mg%)	140,22+/-97,43 (108)	115,19+/-51,16 (99)	0,276
HDL-C (mg%)	48,7+/-18,57 (43,9)	32,17+/-6,79 (32)	0,014
LDL-C (mg%)	101,20+/-46,84 (87)	116,44+/-46,64 (105)	0414
Glicemia la internare (mg%)	134+/-57,65 (113,5)	139+/-69,48 (115)	0,778
Hemoglobina (g%)	13,96+/-1,74 (14)	14,29+/-2,71 (14,2)	0,566
Hematocrit (%)	41,73+/-6,09 (42,5)	43,65+/-1,17 (43,7)	0,298

Legendă: n număr; DS deviaţia standard.

TABELUL III

Variabile ecocardiografice ale pacienților din lotul studiat

n (%) sau medie +/-DS (mediana)	Normouricemie (n = 108)	Hiperuricemie (n = 55)	Valoarea p
Prezența HVS.	57 (52,78)	31 (56,37)	0,726
Fractia de ejectie (%)	61,2+/-3,1	55,9+/-4,1	0,036

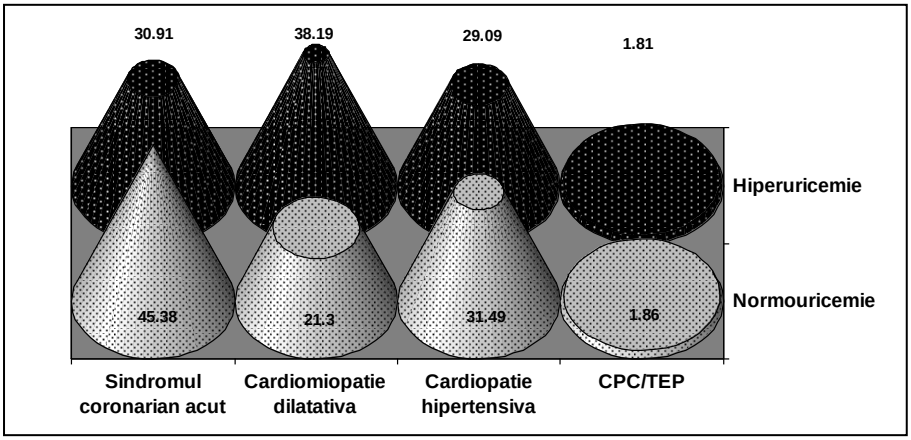

Fig. 1, Distribuția afecțiunilor cardiace (%) în cadrul celor două grupuri cu normo- şi hiperuricemie.

Nu s-au evidențiat diferențe semnificative statistic în ceea ce priveşte diagnosticul principal al afecțiunii cardiace care a determinat internarea pacienților. În cazul grupul pacienților cu hiperuricemie dar cu o valoare la limită s-au administrat mai frecvent sartanii (tab. IV).

Grupând pacienții lotului studiat în funcție de valoarea *clearance*-ului de creatinină mai mic sau mai mare decât 60 ml/minut am constatat existența unor diferențe semnificative statistic între cele două grupuri atât în ceea ce priveşte valoarea acidului uric seric ($p < 0,001$), vârsta ($p < 0,001$), disfuncția renală (valorile ureei şi creatininei serice, cum era de aşteptat) şi prezența HLP (p 0,001), a valorilor colesterolului seric (p 0,003) dar şi a trigliceridelor serice (p 0,010).

TABELUL IV

Medicația cardiovasculară administrată pacienților din lotul studiat

n (%)	Normouricemie (n = 108)	Hiperuricemie (n = 55)	Valoarea p
Betablocante	44 (40,74)	26 (47,28)	0,596
IECA	51 (47,23)	26 (47,28)	0,136
Sartani	10 (9,26)	1 (1,81)	0,048
Anticalcice	39 (36,12)	19 (34,55)	0,855
Diuretice	77 (71,30)	39 (70,91)	0,123

Legendă: IECA inhibitori ai enzimei de conversie a angiotensinei.

Pentru o grupare în funcţie de un alt nivel de *cut-off* al *clearance*-ului de creatinină (30 ml/min) diferenţele între valorile acidului uric nu mai sunt semnificative, menţinându-se diferenţa în ceea ce priveşte prezenţa disfuncţiei renale (uree serică p 0,001, creatinina serică p < 0,001), precum şi a antecedentelor cerebro-vasculare (p 0,043) şi a tratamentul cu IECA (p < 0,001).

DISCUŢII

Acidul uric, rezultat al catabolismului purinelor prin intervenţia xantinoxidazei, este un acid slab (pKa 5,8) care se găseşte în compartimentul extracelular sub formă de urat de sodiu, fiind supus unor multiple mecanisme reabsortive şi secretorii la nivelul tubilor contorţi renali şi doar un procent de 8-12% din acidul prezent iniţial în ultrafiltrat este excretat (8). Acest model operaţional generează o serie de întrebări: când şi de ce am pierdut în cursul evoluţiei uricaza (urat oxidaza), enzimă care cu excepţia umanoizilor şi primatelor mari este responsabilă de metabolizarea acidului uric în alantoină, un produs mult mai solubil şi în consecinţă de ce păstrăm concentraţia serică a acidului uric la valori de 3,7-7 mg% la om faţă de 0,5-1,5 mg% la celelalte animale, reprezintă această modificare un avantaj evolutiv sau hiperuricemia s-a transformat treptat într-un dezavantaj fiind implicată în calitate de factor de risc încă neprobat a fi independent (10) în geneza unor afecţiuni cardio-vasculare şi renale severe. Analiza este şi mai rafinată dacă se iau în consideraţie intervale distincte ale nivelelor serice ale acidului uric, unele în zona considerată a fi normală (3,03-5,04 mg% *vs.* 5,05-5,88 mg% *vs.* 5,89-9,58 mg) (11). Relaţia potenţială dintre acidul uric şi patologia cardiovasculară ar putea genera atitudini terapeutice specifice: administrarea de uricozurice (probenecid, benzbromarona, sulfinpirazona şi chiar losartanul şi fenofibratul), de inhibitori ai xantinoxidazei (febuxostat, oxipurinol) sau chiar a unei uricaze sintetice (rasburicasa) (12, 13).

În studiul efectuat, hiperuricemia nu s-a dovedit asociată cu prezenţa sindromului coronarian acut ci doar cu prezenţa disfuncţiei renale moderate (conform clasificării KDOQI - *Kidney Disease Outcomes Quality Initiative*), relaţie care nu a fost evidenţiată în cazul afectării renale severe. Studii recente (9) au arătat similar că această asociere este semnificativă doar când hiperuricemia este un marker al creşterii activităţii xantin-oxidazei şi nu când aceasta este determinată de către alterarea eliminării renale a acidului uric. Acest studiu nu evidenţiază corelaţii între nivelurile acidului uric şi tipul afecţiunii cardiace a pacienţilor incluşi în lot.

Limitele studiului sunt reprezentate de către mărimea redusă a lotului, natura observaţională a analizei fără urmărirea în evoluţie a ratei de apariţie a evenimentelor cardiovasculare sau de progresie a disfuncţiei renale.

CONCLUZII

Analiza efectuată nu a demonstrat diferenţe semnificative între cele două grupuri cu normo sau hiperuricemie. Valoarea uricemiei constatată în cazul evaluării unei disfuncţii renale medii, nu se menţine în cazul asocierii unei disfuncţii renale severe.

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NOUTĂȚI

REFACEREA VEDERII DUPĂ TRANSPLANTUL DE CELULE CU ROL DE FOTORECEPTORI

Transplantul celular ar putea reprezenta o soluție terapeutică în cazul orbirii provocate de pierderea fotoreceptorilor. Deși precursorii transplantați ai celulelor cu bastonașe pot migra la nivelul retinei adulte, unde se diferențiază pentru a dobândi caracteristicile morfologice speciale ale celulelor fotoreceptoare mature, întrebarea fundamentală care persistă este cea legată de posibilitatea ca un transplant de celule cu potențial de fotoreceptori să poată îmbunătăți performanța vizuală. Există o serie de dovezi recente care relevă o ameliorare a vederii funcționale mediate de celule cu bastonașe la șoarecii $Gnat1^{-/-}$, la care funcția celulelor cu bastonașe s-a pierdut, ei reprezentând un model de orbire congenitală staționară nocturnă. S-a putut astfel evidenția faptul că precursorii transplantați al acestor celule formează triada clasică a conexiunilor sinaptice cu neuronii bipolari la nivel retinian. Celulele fotoreceptoare nou integrate sunt responsabile de percepția luminoasă, cu o cinetică similară celulelor fotoreceptoare adulte.Se demonstrează astfel fezabilitatea acestui tip de transplant ca o strategie terapeutică pentru refacerea vederii după degradări retiniene.(Pearson R.A., Barber A.C. Rizzi M. et al. Restoration of vision after transplantation of photoreceptors. *Nature* 2012; 99-103)

Carmen Lăcrămioara Zamfir

PARTICULARITĂȚI EVOLUTIVE ALE TUMORILOR DE TRUNCHI CEREBRAL LA COPIL

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FEATURES OF BRAIN STEM TUMORS IN CHILDREN. (Abstract). Brain stem tumors account for about 10-20% of childhood brain tumors. Peak incidence for these tumors occurs around age 6 to 7 years. **Aim:** Despite their severity and poor prognosis, brain stem tumors remain an area of intense research with regard to their diagnosis and management. **Material and methods:** In the interval 2003-2010, 8 children (4 girls and 4 boys) aged 2-13 years (mean age 6.82), diagnosed with brain stem tumors were followed up. Disease history, onset symptoms, complete physical, laboratory and imaging investigations, and individualized therapeutic approach have been reviewed. Family history was considered to be of particular clinical importance. Monitoring the disease progression was possible until the time of death (when it occurred in hospital) or by information provided by the family and family physician in cases where death occurred at patient's home. **Results**: Clinical signs and symptoms depend on tumor location, its aggressiveness, and patient's age. Progressive neurological deficits, signs and symptoms caused by increased intracranial pressure, visual disturbances, behavioral disorders, seizures, endocrine disruption, failure to thrive may occur in various combinations. In only 50% of our cases the tumor could be removed. Imaging proved highly suggestive for a brain stem tumor. Histopathological examination diagnosed one pilocytic astrocytoma (grade I), one fibrillary astrocytoma (grade II), one anaplastic astrocytoma (grade III), and one glioblastoma multiforme (grade IV). In the remaining 4 cases imaging was suggestive for glial tumors. Multimodal therapy was used in 2 patients, 7 received adjuvant chemotherapy, and in 1 case no therapy was administered because the tumor rapidly progressed to death. Seven of our patients died on an average of 6.28 months after the diagnosis (range 2 to 9 months). A family history of brain tumors in 2 of our cases supports the hypothesis of genetic factors involvement. **Conclusions**: Brain stem tumors are still difficult to investigate, and the results on their long- and medium-term survival remain uncertain.
Key words: TUMOR, BRAINSTEM, SURVIVAL, CHILD

Tumorile sistemului nervos central reprezintă a doua malignitate a copilăriei, fiind cea mai frecventă tumoră solidă din pediatrie(1, 2). Clasificarea histo-patologică este complexă şi se bazează în principal pe tipul histologic al celulei de origine, coroborată cu dezvoltarea embriologică (3). Semnele şi simptomele clinice depind de localizarea tumorală, de agresivitatea sa şi de vârsta pacientului. Deficitele neurologice progresive, semnele şi simptomele determinate de creşterea presiunii

intracraniene, tulburările de vedere, tulburările comportamentale, convulsiile, tulburările endocrine, stagnarea creşterii şi întârzierea achiziţiilor psiho-motorii sau chiar involuţia acestora pot apare izolat sau în diverse asocieri (2). Durata evoluţiei poate varia de la câteve zile până chiar la câţiva ani, în funcţie de agresivitatea celulei tumorale şi de ritmul creşterii neoplasmului. Este deja bine statuată legătura între tumorile cerebrale şi anumite sindroame genetice: neurofibromatoza tip I, sindromul Li-Fraumeni, scleroza tuberoasă, sindroamele Von Hippel-Lindau, Turcot şi Gorlin (4). Evaluarea imagistică include computer-tomografia (CT) şi rezonanţa magnetică nucleară (RMN) care se completează reciproc şi descriu localizarea tumorală, extensia locală, compresiunea pe structurile de vecinătate, hidrocefalia supraiacentă. Tomografia cu emisie de pozitroni poate diferenţia recidiva tumorală de necroza, cicatricile postoperatorii sau edemul local postterapeutic (1). Intervenţia chirurgicală are drept scop îndepărtarea unei cât mai mari porţiuni tumorale, obţinerea probei necesare examinării histopatologice şi rezolvarea hidrocefaliei. Intervenţia chirurgicală este urmată de chimio-radioterapie în funcţie de tipul histopatologic tumoral. Datorită toxicităţii neurocognitive a radioterapiei, în prezent sunt preferate radioterapia hiperfracţionată, radiochirurgia stereotaxică sau terapia cu protoni, mult mai puţin agresive pe ţesutul nervos nobil decât radioterapia standard conformaţională (5, 6, 7). Supravieţuirea cazurilor cu grad redus de malignitate (I şi II) este de aproximativ 80% după o rezecţie tumorală de bună calitate. Spre deosebire de acestea, tumorile cu grad mare de malignitate au un prognostic mult mai rezervat, cu o supravieţuire la 5 ani de sub 20% (1, 2). Tumorile trunchiului cerebral reprezintă 10-20% din totalul tumorilor cerebrale din pediatrie şi au per se un prognostic extrem de rezervat datorită structurilor nobile şi a centrilor vitali de la acest nivel. Incidenţa maximă se situează la vârsta de 6-7 ani. Simptomatologia diferă în funcţie de tipul dezvoltării tumorii (infiltrativ sau nodular) şi se poate manifesta sub forma deficitelor neurologice diverse, a hipertensiunii intracraniene, a ataxiei, hemiparezelor, nistagmusului, a tulburărilor senzoriale în diverse asocieri. Diagnosticul histo-patologic este de cele mai multe ori doar suspectat prin descrierea imagistică (leziuni focale sau infiltrative, cu structură mixtă) deoarece intervenţia chirurgicală implică riscuri majore. Marea majoritate a acestor tumori sunt glioame cu grad diferit de anaplazie dar se pot întâlni şi tumori neuroectodermale primitive (PNET) mai ales în cazul tumorilor focale (2). Planul terapeutic este individualizat pentru fiecare pacient în parte, urmând caracteristicile clinice, imagistice şi histologia suspectată sau chiar certă (în cazurile care au permis biopsia tumorală); terapia poate asocia radioterapie (posibilă în funcţie de vârsta pacientului şi de preferat cu modalităţi radioterapice care să minimalizeze efectele adverse pe ţesuturile nervoase nobile de vecinătate) şi chimioterapie antitumorală adjuvantă (rezervată cazurilor cu diagnostic histopatologic de astrocitom cu grad mare de malignitate sau PNET). Supravieţuirea medie variază între 4 şi 15 luni şi doar 6-10% supravieţuiesc la 2 ani de la diagnostic (1, 2). O rată de supravieţuire mai mare (de 94% la 4 ani de la diagnostic) s-a observat în cazurile de tumori focale care au beneficiat de o exereză chirurgicală de bună calitate urmată de radioterapie (2).

MATERIAL ŞI METODĂ

În perioada 2003-2010 în cadrul Clinicii a IV-a Pediatrie, Secţia de Hemato-Oncologie Pediatrică a Spitalului Clinic de Urgenţă pentru Copii „Sf. Maria" Iaşi au fost urmăriţi un număr de 8 pacienţi diagnosticaţi şi trataţi pentru tumori ale trunchiului cerebral. Studiul prezent a analizat dosarele medicale ale acestor pacienţi din punct de vedere al istoricului bolii, al simptomatologiei de debut şi al examenului clinic complet precum şi al investigaţiilor paraclinice, imagistice şi al conduitei terapeutice individualizate de la caz la caz. S-au notat şi antecedentele heredo-colaterale considerate a avea o importanţă clinică deosebită. Monitorizarea evoluţiei a fost posibilă pînă la momentul decesului (când acesta s-a produs în spital) sau prin informaţiile furnizate de către familie şi de către medicul de familie în cazurile când decesul s-a produs la domiciliul pacientului.

REZULTATE ŞI DISCUŢII

Într-o perioadă de timp de 8 ani în cadrul Clinicii a IV-a Pediatrie, Secţia de Hemato-Oncologie Pediatrică a Spitalului Clinic de Urgenţă pentru Copii „Sf. Maria" Iaşi au fost urmăriţi 8 pacienţi diagnosticaţi cu tumori de trunchi cerebral, 4 fete şi 4 băieţi deci o repartiţie egală pe sexe. În medie a fost diagnosticat un caz nou pe an. Vârsta de debut a variat de la 2 la 13 ani, cu o medie de 6,82 ani, ceea ce se încadrează în media de vârstă citată de literatura internaţională.

Debutul afecţiunii a fost reprezentat de manifestări de hipertensiune intracraniană, pareze ale nervilor cranieni, nistagmus, hemipareze şi tulburări de deglutiţie instalate brutal sau insidios şi care au determinat adresarea către serviciile de sănătate la intervale de timp variabile în funcţie de brutalitatea instalării simptomatologiei. Durata debutului a variat de la câteva zile la 5 luni.

Examinarea clinică a relevat prezenţa deficitelor neurologice diverse, a parezelor de nervi cranieni, a nistagmusului şi a fost urmată de investigaţia imagistică prin computer-tomograf (CT) şi rezonanţă magnetică nucleară (RMN) care au descris formaţiunile expansive cu structură mixtă şi aspect neomogen la nivelul trunchiului cerebral; în 7 cazuri diagnosticul imagistic a fost de tumoră infiltrativă la nivelul punţii cu extensie în pedunculii cerebrali şi la nivelul ventriculului IV şi doar într-un singur caz a fost descrisă o tumoră cu aspect nodular la nivel bulbo-pontin.

În doar 50% dintre cazurile studiate a fost posibilă intervenţia chirurgicală de exereză tumorală: 1 caz cu ablaţie tumorală parţială, 2 cazuri cu ablaţie tumorală subtotală şi 1 caz care a beneficiat doar de biopsie tumorală. Dealtfel biopsia tumorală nu este obligatorie în cazurile în care descrierea imagistică este înalt sugestivă pentru o tumoră de trunchi cerebral. Examinarea histopatologică a diagnosticat în aceste cazuri 1 astrocitom pilocitic (grad I de malignitate), 1 astrocitom fibrilar (grad II), un astrocitom de grad III anaplazic şi 1 caz de glioblastom multiform cu grad maxim de anaplazie. În celelalte 4 cazuri descrierea imagistică a fost sugestivă pentru tumori gliale.

Terapia multimodală a inclus radioterapia la 2 pacienţi, doza aplicată fiind de 34 Gy divizată în 17 fracţiuni. Aceste 2 cazuri sunt reprezentate de glioblastomul multiform şi de un caz fără biopsie tumorală. 7 din 8 cazuri au fost incluse în protocolul de chimioterapie adjuvantă (după obţinerea consimţământului informat al familiei) şi au primit Temozolomidă în doză de 150 mg/m^2/zi, timp de 5 zile consecutiv, repetat la 28 de zile interval. Cel de-al 8-lea caz nu

a primit chimioterapie datorită evoluţiei rapide a simptomatologiei care a impus transferul în Clinica de Neurochirurgie unde a şi decedat. Asociat chimioterapiei pacienţii au primit şi terapie antiedematoasă cerebrală şi simptomatice.

Evoluţia sub tratamentul menţionat a inclus scurte perioade de ameliorare urmate de recrudescenţa şi agravarea simptomatologiei neurologice, cu decesul a 7 dintre pacienţi în medie la 6,28 de luni de la momentul diagnosticului. Durata supravieţuirii la aceşti pacienţi a variat de la 2 luni la 9 luni. Un pacient este în viaţă la 18 luni de la diagnostic.

O menţiune specială este reprezentată de prezenţa în antecedentele heredo-colaterale a doi dintre pacienţi a rudelor de gradul I şi respectiv III cu tumori cerebrale: pacienta R. G. de 13 ani cu astrocitom anaplazic are un frate diagnosticat cu astrocitom pilocitic recidivant de Ventricul IV (şi care se află în prezent în chimioterapie cu Temozolomidă) iar pacienta D. I. de 5 ani decedată prin tumoră pontomezencefalică nebiopsiată a avut bunica maternă decedată tot prin tumoră cerebrală. Aceste cazuri susţin ipoteza implicării unor factori genetici în etiopatogenia tumorilor cerebrale, chiar dacă acest lucru nu a putut fi dovedit la aceste paciente.

În evoluţie 2 cazuri au prezentat manifestări rar citate în literatură: o pacientă a dezvoltat tulburări de personalitate şi comportamentale, cu elemente de heteroagresivitate iar un al doilea pacient a dezvoltat în perioada postoperatorie tulburări majore de deglutiţie, cu pneumonie de aspiraţie, ceea ce a impus traheostomia de necesitate.

În cele ce urmează prezentăm cazul pacientului de sex masculin, M. L. C., în vârstă de 2 ani şi 9 luni, care s-a internat în luna martie 2010 în Secţia Hemato-Oncologie a Spitalului de Urgenţă pentru Copii „Sf. Maria" Iaşi pentru investigarea unui deficit neurologic progresiv, instalat cu o lună anterior internării. La internare prezenta stare generală relativ bună, afebril, asimetrie facială cu ptoză palpebrală dreaptă, asimetria comisurilor bucale, tulburări de echilibru, reflexe osteo-tendinoase vii, tetrapareză spastică, retard psihomotor. Investigaţiile biologice (hemoleucogramă, sindrom inflamator, probe hepatice şi renale) au fost în limite normale. Traseul electroencefalografic nu a decelat modificări. Evaluarea CT a descris prezenţa unei leziuni heterogene la nivel bulbar şi parţial pontin, imprecis delimitată, cu minimă priză de contrast periferică şi dimensiuni de 1,9⁄2,7⁄2,8 cm, asociată cu o zonă de 4⁄6 mm pontină cu densitate sugestivă de hemoragie recentă; leziunea determină efect de masă asupra parenchimului bulbo-pontin adiacent şi asupra arterei vertebrale stângi pe care o deplasează lateral. S-a completat evaluarea imagistică prin efectuarea RMN care a descris o leziune mixtă, multiloculată situată pontin inferior până la nivelul medullei oblongata, cu dimensiuni de 2,6⁄2,7⁄3,2 cm şi care amprentează ventriculul IV fără a determina însă hidrocefalie supraiacentă (Fig. 1 şi 2).

Descrierea imagistică era sugestivă pentru o tumoră de tip astrocitar cu localizare bulbo-pontină, motiv pentru care s-a iniţiat terapie antiedematoasă cerebrală şi chimioterapie cu Temozolomidă, 200 mg⁄m2⁄zi, 5 zile, repetat la 28 de zile interval. A primit 4 cure de chimioterapie, fără a dezvolta toxicitate hematologică majoră dar cu instalarea unui sindrom Cushing iatrogen corticoterapiei. În luna iulie 2010 s-a intervenit neurochirurgical cu ablaţia tumorală subtotală (debulking intracapsular cu urmărirea planului de clivaj de la nivelul polului inferior

până în vecinătatea protuberanței unde tumora infiltrează parenchimul). Examinarea histo-patologică a descris un astrocitom pilocitic (grad I de malignitate) cu histochimie: GFAP pozitiv difuz, VIM pozitiv difuz, NFT negativ, SYN negativ, Neu N negativ, Ki 67 pozitiv în 1-2% din celulele tumorale. Evoluția postoperatorie imediată a fost favorabilă, cu ameliorarea tetraparezei dar cu menținerea disfagiei; în evoluție disfagia s-a accentuat şi a fost urmată de complicații, cu pneumonie de aspirație care a impus traheostomia (menținută 10 zile), antibioticoterapia intravenoasă cu spectru larg, terapia simptomatică şi kinetoterapia. După remiterea simptomelor respiratorii ameliorarea clinică a permis suspendarea traheostomei. Pacientul a continuat kinetoterapia recuperatorie cu ameliorarea evidentă neurologică şi a rămas în supraveghere oncologică (dat fiind gradul I mic de malignitate al celulei tumorale). Menține tulburările de deglutiție minore şi sindromul Cushing iatrogen. Monitorizarea imagistică repetată a evidențiat un minim rest tumoral (nodul ovalar gadolinofil de 9⁄9⁄7 mm anterolateral dreapta la nivelul punții precum şi un chist bulbocervical bilobat de 8⁄8⁄14 mm), fără modificări ale sistemului ventricular. Consultul neurochirurgical a opiniat pentru temporizarea reintervenției şi urmărirea iterativă clinico-imagistică.

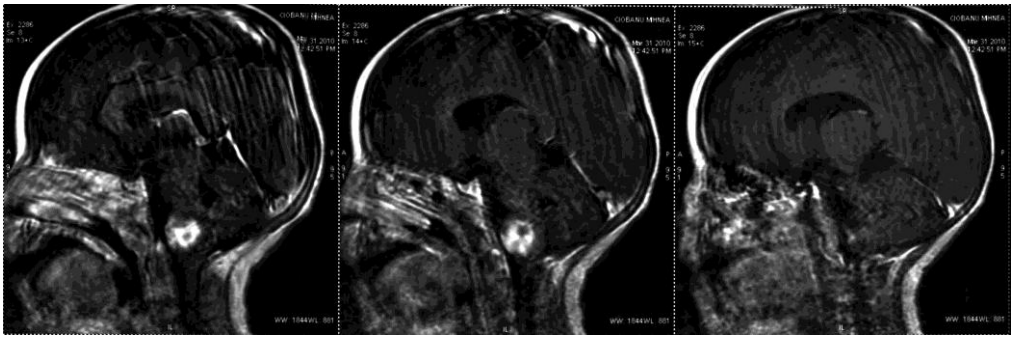

Fig. 1. RMN preoperator postcontrast intravenos cu secțiuni sagitale care descriu leziunea preponderent pontină cu structură mixtă.

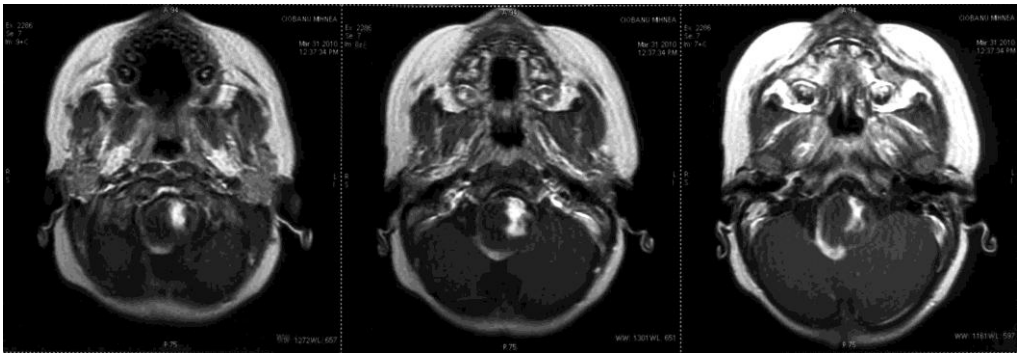

Fig. 2. RMN preoperator postcontrast intravenos cu secțiuni transversale care descriu leziunea preponderent pontină cu structură mixtă.

CONCLUZII

Datele prezentate susțin incidența scăzută a tumorilor de trunchi cerebral în pediatrie, în cazul acestui studiu fiind diagnosticat un caz nou pentru fiecare an de monitorizare. Manifestările de debut şi examenul clinic au orientat diagnosticul spre o patologie tumorală de trunchi cerebral care a fost confirmată prin evaluarea imagistică (CT şi RMN). Doar jumătate dintre pacienți au beneficiat de diagnostic histopatologic prin biopsie tumorală. Conduita terapeutică a asociat radio-şi chimioterapie conform protocoalelor internaționale în vigoare dar evoluția a fost progresivă şi a dus la decesul a 87,5% dintre pacienți în mai puțin de 12 luni de la diagnostic. Singurul caz al unui pacient supraviețuitor la 18 luni de la diagnostic este al unui băiat în vârstă de 2 ani la momentul diagnosticării; a fost confirmat cu astrocitom pilocitic nodular pontin prin rezecție tumorală subtotală şi menține un minim rest tumoral pentru care necesită evaluare în dinamică. Tumorile trunchiului cerebral rămân un capitol al patologiei tumorale pediatrice dificil de explorat şi cu rezultate incerte în ceea ce priveşte supraviețiurea pe termen mediu şi lung.

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DEPRESSION AND ZUNG SELF-RATING DEPRESSION SCALE IN PARKINSON'S DISEASE

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DEPRESSION AND ZUNG SELF-RATING DEPRESSION SCALE IN PARKINSON'S DISEASE (Abstract): Patients affected by Parkinson's disease (PD) experience many non-motor symptoms, including depression. Depression has a negative impact on everyday activities and quality of life. Still, depression is underrecognized and undertreated overall. Among the instruments used to investigate depression in PD, Zung Self-Rating Depression Scale (ZSRDS) is a widely-used questionnaire for screening depressive symptoms and for measuring progress following treatment. In this article, we review the literature concerning depression associated with PD, paying specific attention to ZSRDS as a tool that investigates depression to guide treatment decisions. **Keywords:** DEPRESSION, ZUNG SELF-RATING DEPRESSION SCALE, PARKINSON'S DISEASE.

Parkinson disease (PD) was first described in 1817 by the British doctor James Parkinson (1) and is currently ranked as second to Alzheimer's disease as the most prevalent age-related complex, idiopathic neurological disorder. It emerges from deterioration of the dopaminergic neurons from *substantia nigra* and is characterized by bradykinesia, tremor (especially in the hands and/or arms) and muscle rigidity along with impaired gait, and posture (2-5).

Although PD is known especially for the debilitating motor symptoms, it also determines multiple non-motor deteriorations (6). Non-motor symptoms may appear before motor symptoms and include depression, anxiety, sleep disturbances, cognitive dysfunction, dementia, olfactory deficits and constipation (2-6).

Depression and Parkinson's disease

Depression is a common psychiatric disorder and global public health problem. It is the third leading cause of years lived with disability worldwide and is associated with a high risk of early mortality from suicide (7,

8). As with Parkinson's disease, its prevalence is influenced by both genetic factors and environmental exposures (9, 10).

Due to the influence on neurotransmissions, depressive disturbances may occur in up to 50% of patients diagnosed with PD which negatively impact the quality of life, mobility, cognition, functionality and other comorbidities (11). Depressed PD patients experience faster cognitive and motor decline, lower quality of life and enhanced mortality compared to non-depressed PD patients (12).

Depression can manifest several years before the onset of motor symptoms, but it can appear at any phase during PD. General risk factors for depression in PD include longer duration of the disease, motor disability, akinetic-rigid variant of PD, other neuropsychiatric symptoms, sleep disorders, restless legs syndrome, higher daily doses of levodopa, female gender and younger age (13).

In patients diagnosed with PD, depression is underrecognized, neurologists failing to identify its presence in more than 50% of the cases, while the calculated diagnostic accuracy is 35% (14). Similar symptoms like reduced facial expression, fatigue, problems with sleeping, diminished appetite and psychomotor retardation contribute to the underdiagnosis of depression in patients with PD (15).

In a recent review article, Santiago et al concluded that "the precise mechanism underlying the association between depression and PD remains unknown" although some hypothesis (e.g. serotonin hypothesis, inflammation hypothesis) are investigated. Throughout the world PD affects 7-10 million people and considering that it is an incurable progressive neurodegenerative disorder and the absence of completely validated biomarkers for establishing the early stages of the disorder, therapeutic intervention is challenging (16).

Goodarzi *et al.* showed in a systematic review that tricyclic antidepressants (TCAs), specifically amitriptyline, proved to be beneficial for mood, yet this was counterbalanced by the adverse effects. Also, it was mentioned that selective serotonin reuptake inhibitors (SSRIs) exhibited some benefit, but the SSRIs could worsen PD symptoms of restless legs, periodic limb movement and REM sleep behavior disorder. They concluded that there is not enough evidence to support one antidepressant over another and the therapy must be individualized considering adverse effects and drug interactions (17).

In terms of antiparkinsonian medication, monoamine oxidase type B inhibitors have demonstrated beneficial effects for patients with treatment-resistant depression and early therapy with rasagiline has been associated with neuroprotective effects (16).

To ensure correct diagnosis and establish a suitable therapy, several rating instruments are typically used: a) clinician-administered scales (like Montgomery-Asberg Depression Rating Scale, Hamilton Depression Scale and Young Mania Rating Scale) and b) self-assessment scales (like Zung Self-Rating Depression Scale, Patient Health Questionnaire, Beck Depression Inventory and Mood Disorder Questionnaire). These questionnaires are not specifically formulated for PD and they are combined with psychiatric/psychological interviews (18-20)**.**

Zung self-rating depression scale (ZSRDS) and Parkinson's disease

ZSRDS was developed by psychiatrist William W.K. Zung (1929-1992) and takes about 10 minutes to complete (21).

It is a 20-item scale, as shown in Table 1, with 4-point severity (from 1 = none, or a little of the time; to 4 = most, or all the time). 10 items show negative experience and 10 items show positive experience and are reverse scored (2, 5, 6, 11, 12, 14, 16, 17, 18 and 20). The interpretation of the result is: <50 = normal; 50-59 = minimal-

mild depression; 60-69 = moderate-marked depression; ≥70 = severe depression (22-24). Correlations with other depression scales include .68 with the Beck Depression Inventory and Mood Disorder Questionnaire and .41 with the Hamilton Depression Rating Scale (24).

ZSRDS evaluates psychological and somatic symptoms of depression and has been validated in many languages, including English, Italian, Japanese, Brazilian, Finnish and Chinese.

Somatic comorbidities confer some additional risk of morbidity in people with depression (25) thus it's important to identify somatic elements in patients with PD and depression and to allow appropriate treatment to improve patients' quality of life.

It has been extensively used to screen for and measure severity of depression in depressive patients with or without PD (26-30).

TABLE I

Zung Self-Rating Depression Scale (ZSRDS)

1. I feel down hearted and blue.
2. Morning is when I feel the best.
3. I have crying spells or feel like it.
4. I have trouble sleeping at night.
5. I eat as much as I used to.
6. I still enjoy sex.
7. I notice that I am losing weight.
8. I have trouble with constipation.
9. My heart beats faster than usual.
10. I get tired for no reason.
11. My mind is as clear as it used to be.
12. I find it easy to do the things I used to.
13. I am restless and can't keep still.
14. I feel hopeful about the future.
15. I am more irritable than usual.
16. I find it easy to make decisions.
17. I feel that I am useful and needed.
18. My life is pretty full.
19. I feel that others would be better off if I were
20. I still enjoy the things I used to do.

In a systematic literature review, Scrag *et al*. revealed that ZSRDS "has good internal consistency and test–retest reliability" and is more easily comprehended than other scales (e.g. Beck Depression Inventory and Mood Disorder Questionnaire). After analyzing several rating instruments, the authors concluded that ZSRDS is more suitable "for measurement of severity of depressive symptoms" than for the screening process (26).

However, recent years' research has successfully used ZSRDS for screening purposes.

In an electronic survey among patients diagnosed with PD, in patients with depression according to ZSRDS, almost 51% of them consider themselves not being depressed, and from those that do not have depression, almost 15% consider themselves depressed (31).

Olfactory dysfunction, anhedonia and depression have been evaluated in PD patients, and to assess the latter non-motor symptom of the disease the authors of the study relied on ZSRDS (32). Just as other researchers did when analyzing the correlation between depressive symptoms and nocturnal disturbances in PD patients (33,34).

This questionnaire also demonstrated its effectiveness in a recent study about the association of decreased serum brain-derived neurotrophic factor with depression in patients with PD (29).

Pramipexole is a non-ergot dopamine receptor agonist indicated for treating PD (both motor and non-motor symptoms). Different studies were conducted to assess the antidepressant effect of pramipexole, within which depressive symptoms have been measured by ZSRDS (35, 36).

One of the ergot alkaloids, bromocriptine is an agonist of dopamine receptors used in parkinsonism and endocrine disorders. Przuntek *et al*. developed two trials with concomitant administration of bromocriptine to levodopa therapy to quantify the beneficial impact of the combination therapy on the adverse effects of long term levodo-

pa treatment. Also, in this case ZSRDS has been used for measuring depressive symptoms (37, 38).

Chagas et al used it alongside other instruments, like the Patient Health Questionnaire-9 and the Geriatric. Depression Scale, and it proved to be a valid tool for screening depression in patients diagnosed with PD without dementia (30, 39).

The quality of life could influence the adherence of patients. Therefore, some questionnaires are used to identify and measure the adherence of PD patients with depression versus PD patients without depression (40).

CONCLUSIONS

Depression has a meaningful impact on patients' and their caregivers' lives, as well as on the health care system, therefore it is important to adequately screen for and correctly treat depression in patients affected by PD.

Although the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) from the American Psychiatric Association's is the golden standard for diagnosing depressive disorders, ZSRDS is efficient and easy to manipulate. Early recognition of depression is beneficial for the care of PD patients and the use of questionnaires can be useful to identify and to follow the severity of depression.

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CERCETĂRI CLINICO-EPIDEMIOLOGICE RETROSPECTIVE ASUPRA INFECȚIILOR INVAZIVE LA BOLNAVII DIABETICI ÎN SPITALUL CLINIC DE BOLI INFECȚIOASE IAŞI, ÎN PERIOADA 2008-2010

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EPIDEMIOLOGY AND CLINICAL CHARACTERISTICS IN DIABETIC PATIENTS WITH INVAZIVE DISEASE ASSISTED AT THE CLINICAL HOSPITAL OF INFECTIOUS DISEASES BETWEEN 2008-2010 (Abstract): Infections in the diabetic population can be severe and life threatning at least for two reasons: clinical signs are often torpid, masqued by chronical complications of diabetes leading to late recognition and medical adresability and also because of the inability to control established infection due to complex cell-mediated and humoral immunity deffects. The aim of the study was to evaluate the etiology, clinical features and outcome in diabetic patients with invazive disease. **Material and methods**: A retrospective study was conducted between January 2008 and December 2010 at The Clinical Hospital of Infectious Diseases Iaşi among 75 diabetic patients with sepsis of microbiologically confirmed etiology (positive cultures from normally sterile sites) and sepsis with clinically suspected etiology (positive cultures from pus). Cases with positive urocultures and coprocultures only, were excluded. **Results**: From 75 diabetics, 56% were males, 90,7% being over 50 years. Severe cases of sepsis (33/75, 44%) were associated more often with insulin-treated diabetes than non-insulin treated group (40% vs. 4%, $p<0,005$) probably because of multiple comorbidities associated as suggested by a high Charlson score (6,09 vs. 4,09, $p<0,05$). There were 64 cases with confirmed etiology and 11 cases with clinically suspected etiology. *Staphylococcus aureus* and *Escherichia coli* were the most common agents isolated, being involved in 16 (21,3%) cases each, followed by coagulase negative *Staphylococci* (14/18,66%), *Enterococcus* spp.(6/8%), group B *Streptococcus* (3/4%), *Streptococcus pneumoniae*, *Enterobacter* spp., *Salmonella* spp. (2/2,66% each), *Aerococcus viridans*, *Streptococcus equi*, *Klebsiella pneumoniae*, *Neisseria meningitidis*, *Sphyngomonas paucimobilis*, *Pseudomonas aeruginosa*, *Proteus mirabilis* (1/1,33%). Multiple septic disseminations occured in 17(22,6%) cases and meningeal involvment was doccumented in 10(15,6%) cases. Meticillin resistance was noted in 53,3% for invasive isolates of *S. aureus*. Among 16 invazive strains of *E. coli*, 25% were resistant to 3rd generation cephalosporins , 17% to ciprofloxacin and 6,6% to aminoglycosides and colimycin. There were no *E. coli* strains resistant to imipenem, piperacillin-tazobactam and aztreonam. The mean mortality rate was 14,66%. **Conclusions**: The high level of resistance of *S. aureus* and *E. coli* to commonly used antibiotics and meningeal involvement imposes a multidisciplinary approach of the diabetic patient. Proper knowledge of the pathogens involved in the potential invazive

diseases is an important tool for successful treatment with appropriate empirical broad-spectrum antimicrobial agents. **Key words:** DIABETIC INVAZIVE INFECTIONS, METICILLIN RESISTANCE, MENINGITIS, UROSEPSIS.

Persoanele cu diabet zaharat (DZ) pot contracta orice infecție ce afectează populația generală, dar, evoluția acesteia spre forme severe de sepsis are cel puțin două substraturi: imunitatea umorală şi celulară deficitară reprezintă un factor important în limitarea infecției iar manifestările clinice, adesea torpide, mascate de complicațiile cronice ale diabetului reprezintă un impediment în diagnosticul şi inițierea precoce a terapiei. Sepsisul sever asociat cu DZ constituie o cauză de morbiditate şi mortalitate ridicată, cetoacidoza diabetică fiind indusă de variate infecții în 28% din cazuri. Cunoaşterea agenților patogenilor implicați, cel mai frecvent, în infecțiile cu potențial invaziv favorizează o abordare terapeutică adecvată.

MATERIAL ŞI METODĂ

Pentru a efectua corelații clinico-epidemiologice asupra infecțiilor invazive la bolnavul diabetic am realizat un studiu retrospectiv în Spitalul Clinic de Boli Infecțioase "Sf. Parascheva" Iaşi în perioada ianuarie 2008-decembrie 2010, prin evaluarea foilor de observație şi a documentelor anexate. În studiu au fost incluşi 75 pacienți cu diabet zaharat internați cu diagnosticul principal de sepsis cu etiologie precizată (prin culturi pozitive din hemoculturi şi/sau alte produse biologice normal sterile) şi sepsis cu etiologie suspectată clinic (culturi pozitive din colecții purulente cu diferite localizări). Au fost excluse cazurile de sepsis cu etiologie neprecizată şi cele la care s-au obținut doar uroculturi şi coproculturi pozitive. Au fost evaluate datele demografice (vârstă, sex, mediu), perioada spitalizării, tipul diabetului, etiologia şi severitatea infecției. Pacienții diabetici au fost împărțiți în două loturi în relație cu gradul de severitate al sepsisului, conform criteriilor standard (10): sepsis a fost definit ca infecție suspectată sau dovedită însoțită de sindrom de răspuns inflamator sistemic (cel puțin 2 criterii –febră/hipotermie, tahicardie, tahipnee, leucocitoză/leucopenie). Sepsisul sever este definit ca sepsis cu disfuncție organică acută (insuficiență renală, insuficiență respiratorie, coagulare intravasculară diseminată sau şoc). Comorbiditățile au fost identificate şi calculate utilizând scorul Charlson (9). Produsele biologice obținute au fost prelucrate în laboratorul de bacteriologie prin examen direct, culturi, latex aglutinare. Testarea rezistenței la antibiotice a izolatelor bacteriene a fost realizată prin metoda standard difuzimetrică (CLSI) şi E-test. S-a constituit o bază de date în Excel care a fost prelucrată ulterior cu ajutorul programelor *SPSS 16.0* şi *Epi Info 3.5.1*. Variabilele studiate au fost analizate descriptiv şi analitic şi s-au considerat semnificative statistic valorile $p< 0,05$.

REZULTATE

În perioada studiată, din cei 75 de pacienți incluşi în studiu, 56% au fost de sex masculin, cu vârste cuprinse între 31 şi 83 ani, 90,7% având peste 50 ani.

Din 55 (73,3%) pacienți cu DZ insulino-tratat, antecedente de DZ tip 1 au fost documentate la 9,3%; DZ tip 2 insulinotratat 58,7%; 5,3% au necesitat conversia terapiei hipoglicemiante orale în insulinote-

rapie; 26,7% au fost pacienți cu DZ tip 2 tratați cu antidiabetice orale (ADO) (tab. I). Pe baza criteriilor de selecție a gradului de severitate al sepsisului au fost documentate 42 cazuri de sepsis şi 33 cazuri de sepsis sever şi/sau şoc septic, vârsta medie în sepsisul sever fiind mai mare (70,19 *vs.* 59,25; p<0,001). Nu s-au înregistrat diferențe semnificative din punct de vedere statistic între cele două grupuri legate de sexul şi mediul de proveniență al pacienților. În lotul de pacienți cu sepsis sever scorul de comorbiditate Charlson a fost mai mare (6,09 *vs.* 4,04; p<0,05).

Investigațiile microbiologice au confirmat etiologia la 64 cazuri de sepsis, majoritatea microorganismelor (82 tulpini) fiind identificate în hemoculturi (HC) sau concomitent din HC şi alte produse patologice (lichid cefalorahidian, puroi, urocultură). În 11 cazuri de sepsis cu etiologie suspectată clinic, agentul patogen a fost izolat din puroi provenit din diferite situsuri care au fost identificate ca poartă de intrare posibilă.

TABELUL I

Unele caracteristici ale lotului studiat

Caracteristici	Sepsis Nr./% (42/56%)	Sepsis sever /Şoc septic Nr./% (33/44%)	P
Sex M F	 26 (34,7%) 16 (21,3%)	 16 (21,3%) 17 (22,7%)	0,353
Vârstă (31-83 ani)	59,25	70,19	**0,001**
Mediu U R	 28 (37,3%) 14 (18,7%)	 19 (25,3%) 14 (18,7%)	0,570
DZ IT DZ tip 2 non IT	25 (33,3%) 17 (22,7%)	30 (40%) 3 (4%)	**0,005**
Nr. cazuri confirmate microbiologic[a]/etiologie suspectată clinic[b]	33 (44%) 9 (12%)	31 (41,3%) 2 (2,7%)	0,124
Nr.mediu zile spitalizare pacienți cu evoluție favorabilă/tranferati	15/0	18,4/5,14	**0,05**
Nr. mediu zile spitalizare pacienți decedați	-	4,72	**0,001**
Index de comorbiditate Charlson	4,09	6,09	**0,05**
Mortalitate	0	11	**0,0002**

[a]Confirmare microbiologică prin culturi pozitive din HC/alte produse biogice normal sterile,
[b]culturi pozitive din puroi , IT=insulino-tratat

Dintre cazurile de sepsis cu etiologie certă, în 38 (59,3%) cazuri au fost implicate bacterii Gram pozitiv, 24 (37,5%) bacterii Gram negativ, 2 (3,1%) cazuri fiind cu etiologie mixtă. Pacienții cu sepsis cu *Staphylococcus aureus* au prezentat criterii de sepsis sever/şoc septic sau sepsis în 9 (14,06%) respectiv 4 (6,25%) cazuri.

Determinări multiple în cadrul sepsisului stafilococic au survenit la 7 pacienți. Pe lângă infecția cutanată (la 8 din 13 paci-

enţi) au fost obiectivate clinic şi paraclinic următoarele localizări: bronhopneumonie şi meningită (3), artrită, meningită şi epidurită (1), articulare (1), pneumonie şi pleurezie (1), bronhopneumonie (1). Stafilococi coagulază-negativ au fost izolaţi din HC la 12 pacienţi diabetici (4 cu determinare respiratorie, 7 doar cu bacteriemie).

TABELUL II

Etiologia infecţiilor şi determinările din cadrul sepsisului la pacienţii diabetici din lotul studiat

Microorganism (nr.tulpini)	Izolat din	Nr. cazuri sepsis sever/sepsis cu determinări						Total[3] ss/s	D
		Multiple	Mg	Resp.	Cutanată	HC+	S.posibil		
S. aureus (21)	$8^{1}/4^{2a}/2^{3}/1^{4}/6^{6b}$	5^{a}ss/2s	-	1ss	2ss	1ss/2s	3s	11/4	3
Stafilococ coN (14)	$12^{1}/2^{6}$	-	-	3ss/1s	1s	7s	$2ss^{c}$	3/9	1
Streptococ grup B (5)	$2^{1}/1^{1a}$ $1^{4}/1^{6b}$	$1ss^{4}$	-	1ss	-	1s	-	2/1	1
Enterococcus spp.(6)	$5^{1}/1^{5}$	-	-	1ss	-	5s	-	1/5	1
Streptococcus pneumoniae(2)	2^{7}	1ss	1ss	-	-	-	-	2/0	-
Aerococcus viridians (1)	1^{7}	-	1ss	-	-	-	-	1/0	-
Streptococcus equi (1)	1^{1}	-	-	-	-	1s	-	0/1	-
Escherichia coli (18^{a})	$8^{1}/7^{5}/1^{7}/1^{2a}/1^{6d}$	5ss	-	-	-	2ss/9s	-	7/9	3
Salmonella grup B/C (2)	2^{1}	-	-	-	-	2s	-	0/2	-
Pseudomonas aeruginosa (1)	1^{1}	1ss	-	-	-	-	-	1/0	1
Proteus mirabilis (1)	$1^{1`}$	-	-	1ss	-	-	-	1/0	1
Klebsiella pneumoniae (2^{e})	$1^{4}/1^{6e}$	1ss	-	-	-	-	-	1/0	-
Enterobacter spp. (3)	$1^{1}/2^{6}$	-	-	1s	-	-	1s	0/1	-
Sphyngomonas paucimobilis(1)	1^{1}	-	-	-	-	1s	-	0/1	-
Neisseria meningitidis grup B(1)	1^{7}	1ss	-	-	-	-	-	1/0	-
Etiologie mixtă	$2^{2a}/5^{6bde}$	2^{2a}ss	-	-	-	-	5s	0/5	-

a=2 cazuri *S. aureus* –în asociere cu *E. coli*, respective Streptococ Grup B; b=3 cazuri *S. aureus* associate cu Streptococ Grup B, cu *K. oxytoca*, respectiv *Morganella morganii*; c=puroi otic; d=1 caz *E. coli* în asociere cu *M. morganii*; e=1 caz Enterobacter spp. în asociere cu *K. pneumoniae*; 1=HC; 2=HC+puroi; 3=HC+LCR+puroi; 4=LCR; 5=HC+UC; 6=puroi; 7=HC+LCR; s=sepsis; ss=sepsis sever; *= Nr.total cazuri ss/s etiologie cunoscută, Stafilococ coN=stafilococ coagulază negativ; Mg=meningită, D=decese.

Infecţii streptococice au fost identificate la 13 (20,31%) pacienţi. *S. pneumoniae* a fost izolat atât din HC cât şi din LCR la 2 pacienţi cu sepsis sever cu determinare meningiană. Infecţii invazive cu streptococ grup B au survenit în 3 (4,6%) cazuri. În 5

din cele 6 cazuri de sepsis cu enterococ tulpinile au fost izolate din HC, fără o poartă de intrare obiectivată clinic şi un caz a fost interpretat ca urosepsis. *Aerococcus viridans* a fost identificat în HC şi LCR la un singur caz de sepsis sever cu determinare meningiană, secundară unui episod de otomastoidită. *Streptococcus equi zooepidermidicus* a fost izolat din HC la un pacient cu sepsis.

Au fost izolate 18 tulpini de *E. coli*, 7 din acestea fiind implicate în urosepsis. Dintre cazurile de sepsis sever 5 au avut determinări multiple. Alte microorganisme Gram negativ identificate ca agent etiologic la pacientul diabetic cu meningită au fost *Klebsiella pneumoniae* şi *Neisseria meningitidis* grup B (câte un caz fiecare). În restul cazurilor de sepsis au fost identificate în HC, Salmonella grup B respectiv grup C (2), *Pseudomonas aeruginosa*, *Sphyngomonas paucimobillis*, *Proteus mirabilis* şi Enterobacter spp. (1) (tab. II).

Din cele 7 (9,33%) cazuri de sepsis de etiologie mixtă, cu punct de plecare cutanat, la doi pacienți cu sepsis sever s-au izolat *S. aureus* şi *E. coli* respectiv Streptococ grup B din HC şi puroi; la restul pacienților au fost identificate asocieri dintre *S.aureus* şi Streptococ grup B (1); *Klebsiella oxytoca* şi *Morganella morganii* (1), *K. pneumoniae* şi Enterobacter spp.(1), respectiv *E. coli* şi *M. morganii* (1).

DISCUȚII

Invazia sangvină poate fi determinată de variate specii bacteriene, dar în mod particular pacienții cu diabet sunt receptivi la agresiunea stafilococică cu o incidență de 8-36% (4). Într-un studiu condus de Artero et al. (6) pe o perioadă de 7 ani, au fost evaluate caracteristicile clinice şi microbiologice ale pacienților diabetici cu bacteriemie şi sepsis sever sau şoc septic, comparativ cu nondiabetici, spitalizați într-un serviciu de terapie intensivă. Bacteriemia de origine nosocomială s-a înregistrat frecvent la pacienți cu DZ având ca etiologie tulpini de stafilococi coagulază negativ (21.6%), *Acinetobacter baumannii* (15%), *Staphylococcus aureus* (11.6%), *Escherichia coli* (8.3%), *Enterococcus* spp. (8.3%). La pacienții cu infecții invazive cu diabet s-a constatat o prevalența crescută a sepsisului sever şi a şocului septic.

În studiul nostru majoritatea pacienților (73,3%) erau cu DZ tratat cu insulină, în această categorie fiind incluşi şi cei la care a fost necesară introducerea insulinei pentru controlul dezechilibrelor metabolice induse de episodul infecțios. Un numar semnificativ mai mare de cazuri de sepsis sever a fost diagnosticat la acest subgrup comparativ cu bolnavii diabetic tratați cu ADO (40% vs. 4%, $p<0,005$). Acest fapt este datorat în special comorbidităților multiple secundare diabetului sau a unor imunodepresii asociate DZ, după cum este sugerat de scorul Charlson (6,09 vs. 4,09, $p<0,05$).

Spectrul etiologic al sepsisului la pacienții diabetici incluşi în studiul nostru a fost caracterizat de preponderența stafilococului (40%), fiind incluse şi cazurile cu etiologie suspectată clinic. *S. aureus* a fost implicat în cele mai multe cazuri de sepsis sever, cu determinări multiple. Dintre tulpinile invazive, 8 din 15 (53,3%) au fost *S. aureus* meticilino-rezistent. Deşi numărul de cazuri studiate este mic şi nu pot fi făcute extrapolări la întreaga populație diabetică, este de remarcat faptul că, procentul de rezistență al *S. aureus* este mult mai mare decât valorile de 19,7%, raportate de EARSS-Net în 7 din 22 țări din Europa, tendințe în scădere în perioada 2002-2009 (11). Rezistența la meticilină a *S. aureus* a fost de 48-50% în cele 2 studii desfăşurate în regiunea de NE a României care au analizat caracteristicile

infecţiilor stafilococice în perioada 2006-2008 (13,14). Prevalenţa enterococilor rezistenţi la vancomicină variază în Europa de la 1-40% (12). În 5 (6,6%) cazuri de infecţii invazive a fost documentată etiologia cu *Enterococcus* spp., toate tulpinile fiind sensibile la vancomicină, două rezistente la ampicilină, trei la ciprofloxacină şi una, la ambele antibiotice.

Infecţiile cu streptococ grup B constituie un risc major pentru pacienţii cu DZ şi alte grupuri cu imunitate scăzută (nounăscuţi, gravide), având un spectru larg de manifestări clinice, de la infecţii cutanate necrozante la sindromul şocului toxic (2,3). Contrar datelor din literatură în studiul nostru au fost izolate doar 5 tulpini (6,09%) de streptococ grup B (4).

Bacteriemia cu bacili Gram negativ rezultă de obicei din infecţii ale tractului urinar, infecţii gastrointestinale, ale veziculei biliare sau la pacienţii spitalizaţi cu catetere intravenoase. La pacientul diabetic gangrenă sinergică şi pneumonia cu bacili Gram negativ sunt alte două manifestări clinice responsabile de diseminare hematogenă. În studiul nostru dintre infecţiile invazive determinate de bacterii Gram negativ, mai mult de jumătate (66,6%) au fost cauzate de *E. coli*. Din 16 cazuri de sepsis cu *E.coli,* 43,75% au avut originea în afecţiuni ale aparatului urinar. Referitor la sensibilitatea la antibiotice a tulpinilor de *E. coli* invazive se remarcă o rată a rezistenţei la cefalosporine de generaţia aIII-a de 25% (4/16), ciprofloxacină 17,64% (3/17) şi 6,66% (1/15) la aminoglicozide, respectiv colimicină (1/11). Doar o tulpină de *E. coli* invazivă a fost decelată cu betalactamaze cu spectru extins. Datele obţinute în studiul nostru sunt similare cu cele raportate de Popescu et al. (8) în cadrul unui studiu multicentric în România, referitor la fluoroquinolone, dar diferite în ceea ce priveşte rezistenţa la cefalosporinele de generaţia a 3-a (CG3), la pacienţii diabetici fiind constatată o rată a rezistenţei mai mare de 25% vs. 14,08%. Nu s-au decelat tulpini rezistente la imipenem, piperacilină-tazobactam şi aztreonam. În literatură este notată o sensibilitate în creştere la piperacilină-tazobactam şi nitrofurantoin (7,8).

Afectarea meningiană a fost documentată la 10 (15,6%) cazuri de sepsis sever. Agenţii etiologici implicaţi în meningită, la bolnavii diabetici din lotul studiat, au fost *S. aureus*, pneumococul, *A. viridians*, streptococul grup B, *E. coli*, *K. pneumoniae* şi meningococul. Evoluţia a fost nefavorabilă la 11 pacienţi, rata mortalităţii în populaţia diabetică fiind de 14,66%. Microorganismele implicate în evoluţia nefavorabilă au fost: *S. aureus* şi *E. coli* (3 cazuri), stafilococ coagulază negativ, *P. mirabilis*, streptococ grup B, *Enterococcus* spp. (câte un caz).

CONCLUZII

Majoritatea infecţiilor invazive la bolnavii diabetici internaţi în Spitalul Clinic de Boli Infecţioase (2008-2010) au survenit la cei insulino-trataţi, cazurile de sepsis sever având vârsta peste 70 ani. Etiologia sepsisului a fost dominată de *S. aureus* şi *E. coli*, determinări multiple, în cadrul sepsisului sever, fiind cauzate cel mai frecvent de etiologia stafilococică. Nivelul crescut al meticilino-rezistenţei (53,3%), rezistenţa în creştere la CG3 şi la fluorquinolone a bacililor Gram negativ, cât şi afectarea meningiană frecventă reprezintă un semnal de alarmă şi impun o abordare multidisciplinară a pacientului diabetic. Considerăm că sunt necesare studii extinse pentru optimizarea terapiei la această categorie de pacienţi pe baza cunoaşterii profilului de rezistenţă regională al germenilor implicaţi şi a particularităţilor evolutive comparativ cu pacienţii non-diabetici.

MULȚUMIRI

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ANTIBIOTIC PROPHYLAXIS CONSIDERING PATIENT OUTCOME FROM PERCUTANEOUS ENDOSCOPIC GASTROSTOMY

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ANTIBIOTIC PROPHYLAXIS CONSIDERING PATIENT OUTCOME FROM PERCUTANEOUS ENDOSCOPIC GASTROSTOMY (Abstract): **Objectives:** The main indications for PEG insertions are the following: stroke/CVA/diffuse cerebral vascular disease, neurological conditions, e.g. head injury, motor neurone disease, Multiple Sclerosis etc. Peristomal infection can sometimes complicate PEG placement. Antibiotics, either prophylaxis or concurrent, can reduce the incidence of peristomal wound infection after PEG placement. **Material and method**: Seventy five patients outcome from Percutaneous Endoscopic Gastrostomy (PEG) were analysed in the period: September 1999 and October 2007 to the West Cumberland Hospital, Whitehaven (WCH) United Kingdom. This paper was done to evaluate the practice in WCH and the outcome has helped us to implement the changes successfully to conform to the guidelines set out by the British Association of Gastroenterologists. Our main objectives were to evaluate the antibiotic prophylaxis, the procedure and post procedure complications and the deaths after PEG tube insertion. **Conclusions**: No immediate procedure complications; Infection was the most common postoperative complication; Antibiotics, either prophylaxis or concurrent, reduce the incidence of peristomal wound infection after PEG placement; Early PEG placement facilitates their transfer to a non-acute care environment. **Key words:** PERCUTANEOUS ENDOSCOPIC GASTROSTOMY, ANTIBIOTIC PROPHYLAXIS.

Percutaneous Endoscopic Gastrostomy (PEG) was first described in 1980. This descriptive study was initially conducted in 75 patients, who have undergone PEG tube placement during September 1999 and October 2007 with an overall average of 70 years of age. Medical records were analysed a well as getting ourselves familiar with various local and national guidelines for PEG insertion and removal.

OBJECTIVES

The main indications for PEG insertions are the following: stroke/CVA/diffuse cerebral vascular disease, neurological conditions, e.g. head injury, motor neurone disease, Multiple Sclerosis, cerebral palsy, myotonic dystrophy, head and neck malignancy, gut dysmotility, oesophageal involvement in AIDS, psychiatric patients with eating disorders, therapeutic, e.g. tethering of gastric volvulus. Peristomal infection can sometimes complicate PEG placement. Antibiotics, either prophylaxis or concurrent, can reduce the incidence of peristomal wound infection after PEG

placement (1,2,3).

RESULTS

Figure **1-a** presents antibiotic prophylaxis given at time of PEG procedure for 62 patients (82%), and if no antibiotic treatment already in use for the seven patients (8%) (fig.1-b). All patients should receive prophylactic antibiotics before the procedure, such as: 1,2g Co-amoxyclav (Augmentin) IV, or 1.5g Cefuroxime (penicillin allergy) (fig. 2).

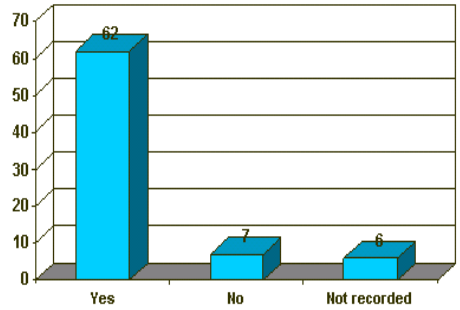

Fig.1-a Antibiotic prophylaxis given at time of procedure.

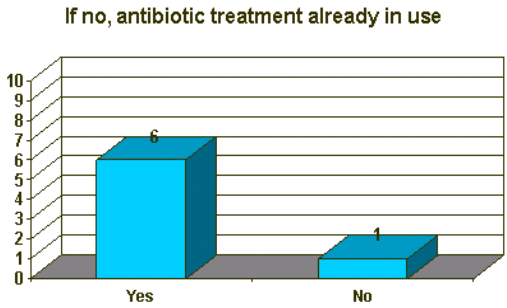

Fig.1-b If no, antibiotic treatment already in use.

Revised patients received the following antibiotics: Co-amoxyclav (Augmentin) IV for 59 patients, Cefuroxime for 3 patients, Teicoplanin for 2 patients, 1 patient received Gentamicin and for 9 patients had no antibiotics administered (fig. 2).

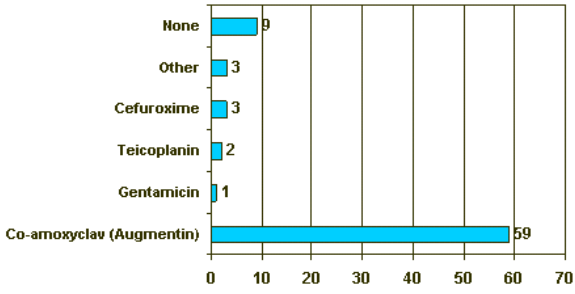

Fig.2 Antibiotics given.

All patients require IV access and they should be kept NBM (Nil by mouth) prior to the procedure. Recent U&Es, FBCs, full clotting results (abnormal or no result will lead to cancellation of the procedure for that day). Medication or therapies used during the PEG procedure are: Xilocaine throat spray, Midazolam and Buscopan (fig. 3).

Antibiotic prophylaxis is to be recommended as a general measure in Percutaneous Endoscopic Gastrostomy (7,8). PEG tubes were inserted by specialized physicians in all cases. One patient required reversal agent after sedation, 51 patients (68%) do not required reversal agent and 23 patients (31%) were not recorded (fig. 4**).**

Complications were classified as severe (bleeding, peritonitis, tube dislocation which needed intervention) and in non-

severe (local infection, pain which were treated by conventional means) (9,10). No cases of bleeding or failure were recorded during PEG procedure for all the patients included in the present study (fig. 5).

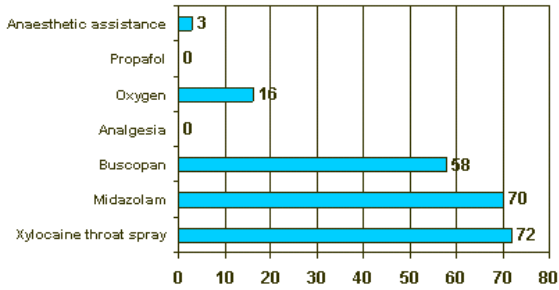

Fig. 3. Medication/therapies used during procedure

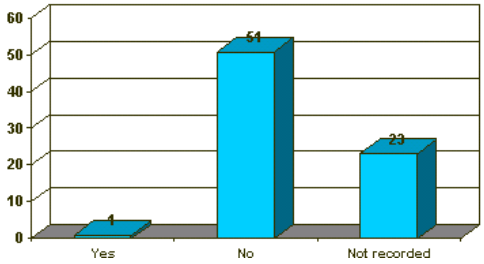

Fig. 4. Reversal agent used

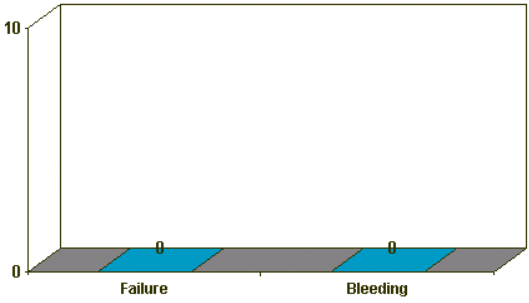

Fig. 5. Procedural complications

LONG TERM COMPLICATIONS

Infection at the insertion site, oesophageal reflux resulting in aspiration pneumonia, tube occlusion, accidental or intentional removal of PEG tube by the patient, gastric perforation, gastrocolic fistula, internal leakage, dehiscence, peritonitis, subcutaneous abscess, buried bumper syndrome (migration of the internal bumper of the PEG tube into the gastric or abdominal wall) are considered the most common complications (11,12,13).

In our analysis the post procedure complications within 30 days (fig. 6**)** showed an overall complication rate of 21%, including chest infection for 9 patients (12%), wound site infection for 4 patients (5%), 1 patient (1.4%) peritonitis

and 2 patients haemorrhage (2.6%). Eight patients presented multiple co-morbidities. Indications for PEG procedure should therefore be carefully reviewed. The rate of complications seems to be determined by post procedure care rather than by the indication or the procedure itself (14, 15,16). Figure 7 shows the number of patients who required treatment with antibiotics.

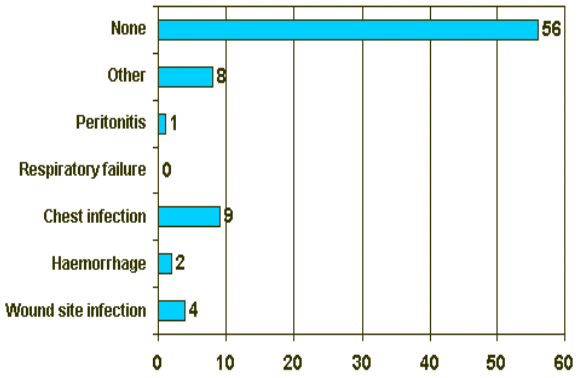

Fig. 6. Post procedure complications within 30 days

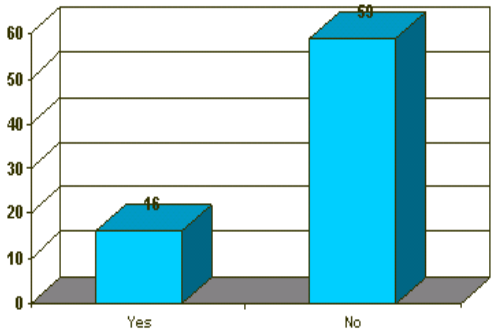

Fig. 7. Post procedure information; Patients receive antibiotic treatment

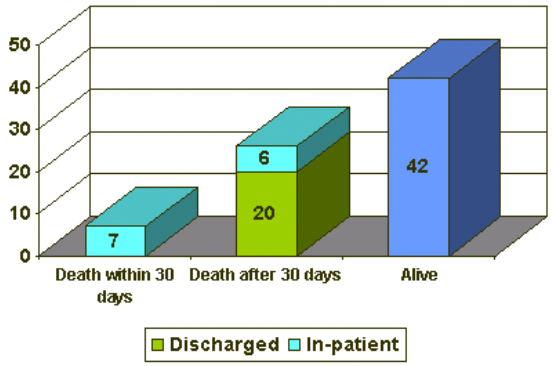

Fig. 8. Post procedure information; Outcome

The post procedure information regarding the outcome of the patients is presented in figure 8. Only 7 patients (9%) died within 30 days, 26 patients (34%) died after 30 days, and 42 patients (57%) are still alive. Non-severe complications occurred in 57% of the cases and severe complications were recorded in 9% of the cases.

All severe complications developed within 30 days led to the death of 7 patients (9%) are presented in fig.8. Death was due to Cardiovascular disease, Cardiac impairment for 5 patients, severe renal impairment disease for 2 patients, Alzheimer severe dementia for 2 patients, COPD for 3 patients, and other diseases for 6 patients (fig. 9).

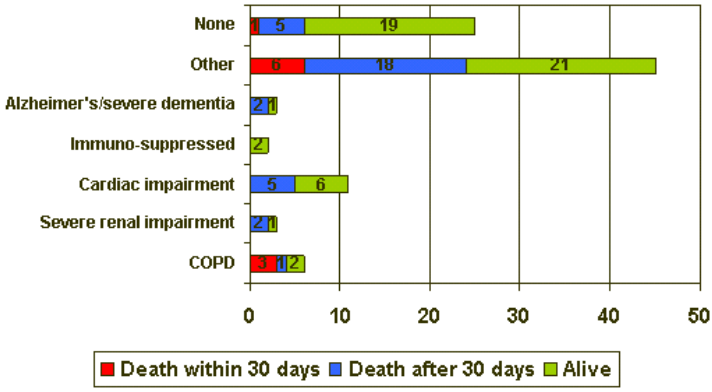

Fig. 9. Post procedure information; Co-morbidities

Mortality and morbidity rates after PEG tube insertion are not insignificant for the majority of the cases but, more observations and research on a larger number of patients susceptible to PEG method are needed as well as a multidisciplinary team assessment must be performed on all patients being referred for PEG tube insertion (17).

RECOMANDATION TO PEG PROTOCOL: Establishing consent in all patients pre operatively, achieve 100% antibiotic prophylaxis and follow the Trust PEG protocol.

CONTRAINDICATIONS TO PEG PROTOCOLS: Current chest infection, ascites, portal hypertension (relative contraindication), peritonitis, bleeding disorders (relative contra-indication), active gastric ulcer (relative contra-indication), gastro-oesophageal reflux with risk of aspiration (relative contra-indication), disseminated intra-abdominal malignancy, pregnancy (relative contra-indication).

CONCLUSIONS

No immediate procedure complications.

Antibiotics, either prophylaxis or concurrent, reduce the incidence of peristomal wound infection after PEG placement.

24% of patients had PEGs removed.

Mortality within 30 days of procedure was less than the national average of 10%.

90.5% of patients received antibiotic prophylaxis, either prior or at time of procedure.

Average patient's age on PEG insertion was 70 years.

More research needs to be undertaken to provide evidence so improved guidelines can be developed in this area.

Minor postoperative complications not requiring intervention occurred at 9.5% of patients, and major complications in 20 % of studied cases.

Infection was the most common post-operative complication.

No patient died as a direct result of the procedure.

Early PEG placement facilitates their transfer to a non-acute care environment.

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DISPLAZIA CLEIDOCRANIANĂ PIERRE MARIE-SAINTON

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PIERRE MARIE-SAINTON CLEIDOCRANIAL DYSPLASIA (Abstract): The paper presents a family with hereditary transmitted Marie-Sainton dysplasia affecting the father and his both children. This is a rare syndrome presenting an autosomal pattern of inheritance, characterized by a generalized defect in both membranous and endochondral bone formation resulting in clavicular aplasia, delayed ossification of the fontanelles and the sutures of the skull and prolonged retention of deciduous dentition with delayed eruption of the permanent teeth. The diagnosis is suggested by more or less complete clinical picture and confirmed by multiple radiological explorations (skull, thorax, spinal column, pelvis) and genetical examination. The genetic mutation for cleidocranial dysplasia (CCD) is found on chromosome six and is called CBFA1 (short for core biding factor a1 or RUNX2) and is the only gene known to be associated with CCD. The normal version of CBFA1 acts to induce osteoblasts which are the type of cells that lay down bone. Although associated psychosocial disorders can occur, the prognosis and life expectancy of this condition are favorable being conditioned however by the complexity of orthodontic procedures which are determinant for these patients life quality. **Key words:** CLEIDOCRANIAL DYSPLASIA, GENETICS, AUTOSOMAL DOMINANT, RADIOLOGY, ORTHODONTIC

Displazia (disostoza) cleidocraniană este o boală rară cu transmitere autosomal-dominantă, descoperită de obicei de la naştere în principal pe baza defectului de osificare a bolții craniene (1). Diagnosticul clinic este susținut de examenele radiologice (craniu, coloană, torace, pelvis, oase lungi, ortopantograma) şi confirmat prin efectuarea cariotipului care identifică mutații ale genei CBFA1 (1). Afecțiunea are un prognostic favorabil, intelectul nu este afectat, tulburările de creştere sunt moderate iar anomaliile claviculare nu sunt invalidante; supravegherea şi tratamentul stomatologic adecvat sunt însă esențiale (2). Prezentăm cazul unei familii cu doi copii, o fetiță de 3 ani şi un băiețel de 1 an şi 8 luni, diagnosticați în clinica noastră cu disostoză cleidocraniană Marie-Sainton. Cazurile celor doi pacienți însumează modificări osoase multiple, consecință a defectelor de osificare membranoasă cât şi cartilaginoasă, cu repercusiuni secundare asupra dezvoltării scheletice.

PREZENTAREA CAZULUI

Copiii M.A şi M.G se internează în clinică pentru investigații, suspicionându-se un rahitism carențial comun. Din AHC reiese că tatăl copiilor a prezentat fractură bilaterală de claviculă la naştere, închidere tardivă a fontanelei anterioare (la vârsta de 5 ani), deficit statural comparativ cu fratele său geamăn precum şi modificări importante ale dentiției definitive pentru care a urmat numeroase intervenții chirurgicale în adolescență. Alte AHC semnificative sunt cazul bunicului patern cu hipoplazie bilaterală de claviculă şi deficit statural cât şi cazul unei verişoare primare (de asemenea pe linie paternă) în vârstă de 13 ani cu spina bifida sacrată, scolioză dorsală, dinți supranumerari anormal implantați. APP relevă că ambii copii au prezentat fractură bilaterală de claviculă la naştere.

Din examenul clinic reținem că fetița prezintă la 3 ani: G=12kg (-1,6 DS), T=90 cm (-0,81 DS), PCr=49,8cm (+0,75 DS) iar băiatul, la 1 an şi 8 luni, G=8800g (-2,88DS), T=76,5cm (-2,07 DS) iar PCr= 48,2cm (+0,16 DS). Modificările de la nivelul craniului şi faciesului la ambii pacienți: discretă dismorfie facială cu hipertelorism, urechi mai jos inserate, fante palpebrale uşor antimongoloide (doar la băiat); fontanelele anterioare deschise, cu dimensiuni 3/3cm la fetiță respectiv 2/2cm la băiat. La tatăl copiilor am observat un prognatism pseudomandibular, consecință a dezvoltării insuficiente a maxilarului care este scurt în raport cu mandibula, urechi jos implantate precum şi proteza totală (fig. 1, 2, 3). Fetița prezintă dentiție temporară completă iar băiatul doar incisivii centrali şi laterali, atât superiori cât şi inferiori. La nivelul trunchiului se remarcă clavicule deformate bilateral, hipoplazice, umeri înguşti, stern înfundat şi torace asimetric evazat la baze (fig. 4)..

Fig. 1. M.G, 3 ani - umeri înguşti, claviculă deformată

Fig. 2. M.A, 1 an 8 luni - hipertelorism

Fig. 3.M.I,35 ani-prognatism pseudomandibular

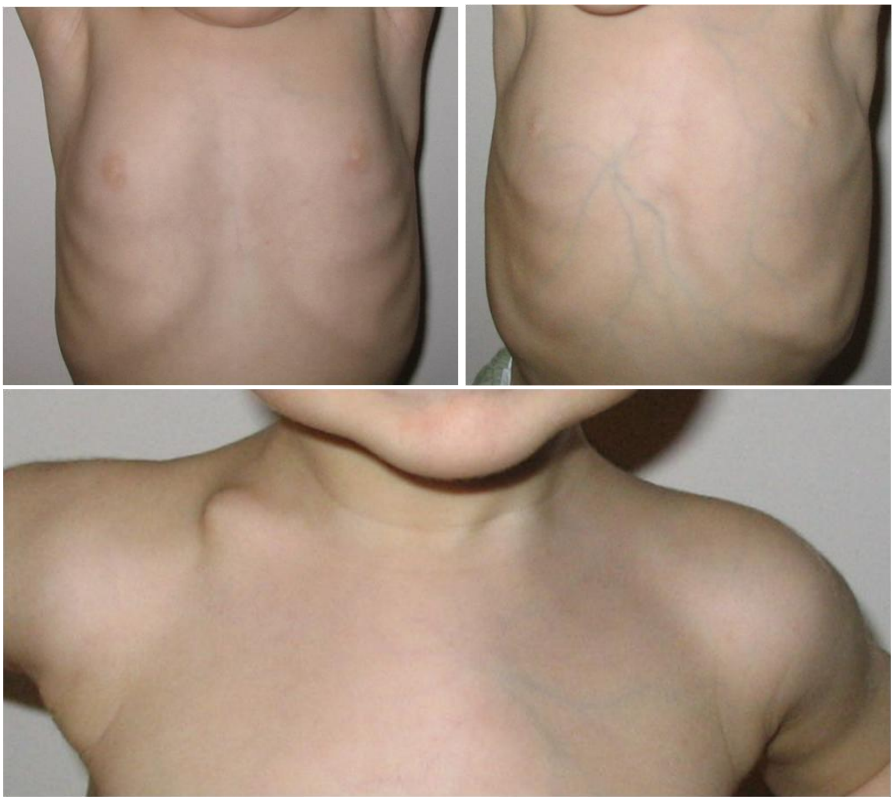

Fig. 4. M.G, 3 ani, M.A, 1 an 8 luni - stern înfundat, torace "în clopot", claviculă deformată

Testele de laborator au arătat doar uşoară anemie feriprivă la amândoi copiii (Hb= 10,2-10,9 mg/dl, Fe=48-56 µg/dl) şi valori la limita inferioară a normalului pentru calcemie şi fosfatemie (Ca=8-8,2 mg/dl, P=3,4-3,6 mg/dl); în acelaşi timp fosfatazele alcaline au fost în limite normale (F alc=369-543U/l). S-au exclus astfel un rahitism carențial comun precum şi o hipofosfatazemie congenitală de tip infantil.

Examinarea radiologică a inclus radiografii de craniu, coloană vertebrală, torace, pumn şi pelvis. Pe radiografiile de craniu (față şi profil) ale ambilor copii se remarcă proeminența boselor frontale, FA deschisă, prezența la nivelul osului occipital de numeroase fragmente osoase şi oasele masivului facial hipoplazice (fig. 5).

Radiografiile toracice relevă prezența a câte două fragmente de claviculă bilateral (fig. 6).

Pe radiografiile de pumn se observă întârziere în dezvoltarea nucleilor carpieni(fig. 7) iar pe cele de pelvis oasele iliace apar hipoplazice cu aripi etalate (fig. 8).

S-a efectuat examenul genetic (inclusiv cariotipul) la ambii pacienți si la tatăl acestora,care confirmă diagnosticul de displazie cleidocraniană Pierre Marie-Sainton pe baza elementelor clinice şi modificărilor radiologice sugestive. În prezent copiii sunt în evidența unei clinici de pediatrie din Spania, unde locuiesc împreună cu familia.

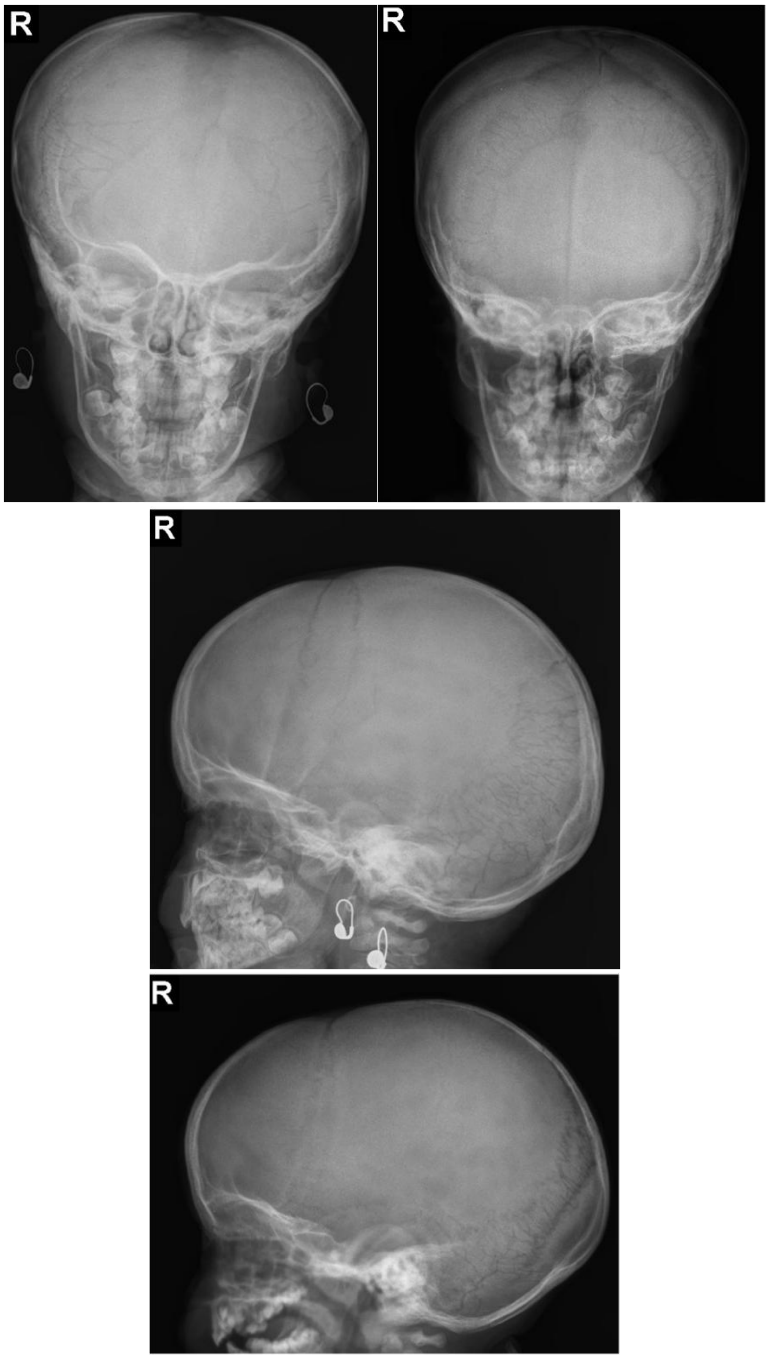

Fig. 5. Radiografii de craniu (față și profil) –
fontanela anterioară deschisă, bose frontale, hipoplazia masivului facial (M.G., M.A.)

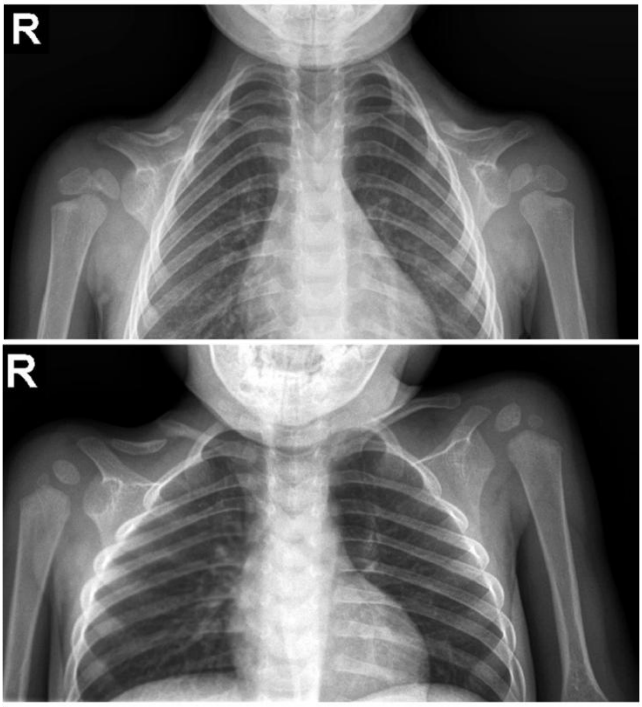

Fig. 6. Radiografii toracice - clavicule formate din două fragmente (M.G., M.A.)

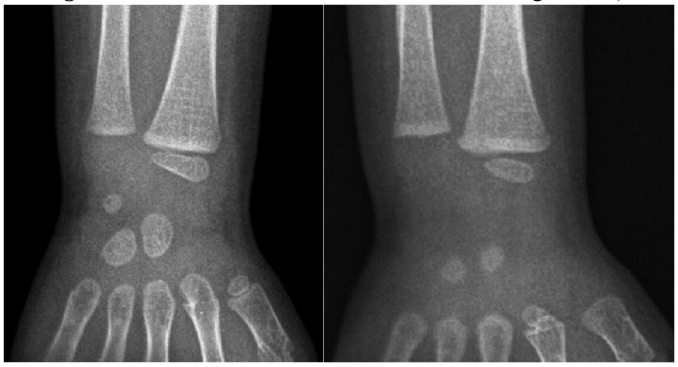

Fig. 7. Radiografii de pumn - nuclei carpieni hipoplazici

Fig. 8. Radiografii de pelvis - aripi iliace etalate

DISCUȚII

Disostoza cleidocraniană, posibil întâlnită şi la omul de Neanderthal a fost descrisă prima data sub acest nume în 1898 de Pierre Marie şi Pierre Sainton, dar observată încă din 1765 de Martin şi în 1871 de Scheuthauer. Soule şi Jackson au raportat alte cazuri în anul 1946 (3,4). Fiind vorba de o tulburare de creştere mai mult decât de o disostoză, este preferabil de a se denumi această maladie *displazie cleidocraniană* (Pierre Maroteaux) (4). Frecvența bolii a fost raportată ca fiind de 1/1000000, cu afectarea în egală măsură a ambelor sexe şi fără a exista o predispoziție etnică. Boala se transmite autosomal dominant în 2/3 din cazuri, în rest fiind vorba de mutații spontane. Gena mutantă implicată în producerea afecțiunii se găseşte pe cromosomul 6 şi se numeşte CBFA1 (prescurtarea pentru core binding factor a1 sau RUNX2); în mod normal aceasta stimulează producerea osteoblaştilor şi se pare că acționează nu numai în perioada embrionară dar este implicată şi în dezvoltarea fetală şi în cea postnatală (1). Aceasta afecțiune face parte din grupul osteocondrodisplaziilor care cuprinde anomalii de dezvoltare ale creşterii osului şi cartilajelor. Displazia cleidocraniană reprezintă prototipul tulburărilor în dezvoltarea oaselor craniului şi a claviculei. Diagnosticul clinic se bazează pe asocierea dintre hipoplazia claviculei, fontanela anterioară deschisă şi dinți supranumerari (1). Rareori clavicula poate fi absentă în totalitate; în general osul este redus la una sau două jumătăți rudimentare care se articulează cu sternul şi acromionul iar porțiunea sa mediană este deficientă. În formele grave umerii se apropie pe linia mediană. Frecvent modificările sunt asimetrice. Muşchii care se inseră pe claviculă pot avea o dezvoltare insuficientă. La nivelul craniului osificarea defectuoasă interesează fontanelele care sunt largi iar suturile dehiscente; la unii indivizi acestea pot să nu se închidă niciodată. Dezvoltarea excesivă a boselor frontale, parietale şi occipitale poate da craniului un aspect globulos (1,6). Frecvent se descriu şi oase vorniene. Anomaliile de dezvoltare la nivelul masivului facial (maxilarele, oasele nazale şi lacrimale) pot fi însoțite de absența sinusurilor (7). Mai pot apare micro/retrognația şi malocluzia. Bolta palatină este înaltă şi se descriu fante atât la nivelul palatului dur cât şi al celui moale (7). Copiii prezintă hipertelorism, deprimarea rădăcinii nasului şi glosoptoză. Modificările dentiției apar în până la 94% dintre cazuri. Formarea şi maturarea dentiției temporare sunt normale, existând însă un deficit de resorbție a laminei dentare ceea ce duce la persistența acesteia până în viața de adult, formarea de dinți supranumerari, întârziere în erupția dentiției definitive, hipodonție şi formarea de chisturi odontogene (8)**.** Extragerea dentiției temporare nu duce la erupția celei permanente (9). Dinții supranumerari apar cu o frecvență de 5% în regiunea molară şi de 22% în zona incisivilor maxilari superiori. Aceştia sunt impactați, ectopici şi prezintă anomalii ale coroanei şi rădăcinilor. Au existat cazuri la care s-au identificat până la 63 de asemenea dinți (7-9). Cutia toracică este deformată în pâlnie cu coaste verticalizate, modificări ce pot conduce la tulburări respiratorii iar la nivelul coloanei se descriu scolioza (uneori severă), malformații sacro-coccigiene şi spina bifida. Pot apare şi tulburări în osificarea oaselor ischio-pubiene şi iliace, uneori

simfiza pubiană este deschisă. Morfologia membrelor este puțin modificată dar deformările tip genu valgum sau platfus sunt frecvente; se mai întâlnesc degete scurtate și unghii rotunjite. Mâinile prezintă falanga distală scurtată, cu epifize masive și brahimezo-falanga degetului V (2,6). Modificări similare se observă și la plante. Talia este redusă, inclusiv în comparație cu cea a fraților/surorilor neafectați de boală (la băieți diferențe de 7,5 cm iar la fete de 15 cm) (1). Intelectul este normal. Ca probleme de patologie asociate se descriu sinuzitele recurente și alte IACRS, otite recidivante care pot conduce la pierderea auzului, retard motor mediu la copiii între 1 și 6 ani iar la fete distocii mecanice de bazin care impun, la vârsta maturității, nașterea prin cezariană (1). Diagnosticul este sugerat de anamneza familială pozitivă (se impune o anchetă amănunțită pentru ultimele trei generații) și de elementele clinice, fiind confirmat prin examene radiologice (radiografii de craniu, torace, coloană vertebrală, pelvis, oase lungi) și prin efectuarea cariotipului (totuși mutațiile genei RUNX2 sunt detectate doar în 60-70% dintre cazuri, de aceea un rezultat normal nu exclude diagnosticul) (1,5,6). Diagnosticul diferențial trebuie să excludă alte dezordini genetice dar cu transmitere autosomal recesivă și cu manifestari specifice ca sindroamele Crane Heise, Yunis Varon sau Maroteaux Lamy (picno-disostoza).

Managementul displaziei cleidocraniene include sfatul genetic în perspectiva unei viitoare sarcini pentru familiile în care cel puțin un membru este afectat, terapia susținută a sinuzitelor și otitelor recidivante (care poate include și montarea unor tuburi de timpanostomie), protecția cutiei craniene, suplimentare cu calciu și vitamina D în cazul în care densitatea osoasă este redusă și profilaxia precoce a osteoporozei.

Tratamentul stomatologic este esențial, se efectuează în echipă multidisciplinară pe parcursul mai multor ani și include extracția dinților supranumerari, expunerea dinților impactați sau încă neerupți pentru a forța erupția ortodontică, alinierea ortodontică în ocluzie, înlocuirea prin lucrări gnato-protetice a dinților absenți, măsuri de corecție estetică a anomaliilor de formă dentară și deseori chirurgie ortognatică a discrepanțelor scheletice maxilare (1,10, 11,12).

CONCLUZII

Cazul prezentat ilustrează atât modalitatea de transmitere a displaziei cleido-craniene cât și modificările clinico-radiologice tipice întâlnite în această afecțiune.

Diagnosticul tardiv în cazul tatălui a avut drept consecință modificări invalidante ale dentiției și necesitatea protezării totale; în același timp sfatul genetic ar fi putut preveni afectarea ambilor frați de către această maladie.

În cazul copiilor diagnosticul precoce va permite inițierea în timp util a tratamentului stomatologic complex.

Considerăm că închiderea tardivă a fontanelei anterioare este un semn clinic valoros, care poate orienta către diagnosticul de displazie cleidocraniană, mai ales în prezența unui tablou biologic normal; în astfel de cazuri se va prefera o evaluare radiologică minuțioasă și orientarea către un serviciu de genetică clinică.

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NOUTĂȚI

MODULAREA EXPRESIEI GENELOR DE VIRULENTA ALE *STAPHYLOCOCCUS AUREUS* DE CATRE ANTIBIOTICE BETA-LACTAMICE UTILIZATE IN CONCENTRATII SUBINHIBITORII

Stimularea expresiei genelor de virulență de către concentrații subinhibitorii ale anumitor antibiotice poate modifica evoluția infecției şi poate face ca terapia antimicrobiană să devină ineficientă. Investigarea efectelor concentrațiilor subinhibitorii ale antibioticelor care inhibă sinteza peretelui bacterian asupra expresiei genelor de virulență şi asupra producerii biofilmului la *Staphylococcus aureus* a fost realizată folosind metode difuzimetrice şi prin real time PCR cantitativ. Expresia genelor *spa* (*staphylococcal protein* A) şi *luk*E a fost stimulată de concentrații subinhibitorii de penicilină şi cefalotin, în timp ce expresia *agr* RNAIII (*accessory gene regulator*) nu a fost afectată. De asemenea, s-a observat producerea unui biofilm mai dens în prezența unor concentrații subinhibitorii de cefalotin (Subrt N, Mesak LR, Davies J. Modulation of virulence gene expression by cell wall active antibiotics in *Staphylococcus aureus. J Antimicrob Chemother*. 2011; 66: 979-84).

Teodora Vremera

POSSIBLE RELEVANCE OF PHYSICAL EXERCISING IN ALZHEIMER'S DISEASE AND OTHER DEMENTIAS

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POSSIBLE RELEVANCE OF PHYSICAL EXERCISING IN ALZHEIMER'S DISEASE AND OTHER DEMENTIAS (Abstract): The benefits of consistent exercising, either cardiovascular, strength training or a combination of both, have been recently shown in neuropsychiatric patients such as Alzheimer's disease and other dementia cases. In this way, an inverse relationship between cognitive impairment commonly observed in demented patients and cardiovascular fitness was demonstrated in a multitude of recent studies, which will be described and analyzed here. Moreover, several related features such as the relevance of oxidative stress, obesity and inflammation are discussed in this context. In addition, intensive muscle training was shown to be particularly effective leading to improved postural and motor response, and therefore reducing the motor impairments effects in dementia patients. Thus, the current trend in prevention is to support physical activity to delay dementia onset, knowing that physical exercising may be crucial in slowing or stopping the motor and general abilities decline in these patients. **Keywords:** ALZHEIMER'S DISEASE, MILD COGNITIVE IMPAIRMENT, MEMORY LOSS, EXERCISING, CARDIOVASCULAR TRAINING, STRENGTH TRAINING.

Alzheimer's disease (AD) is a form of dementia in which the memory decline is extremely severe causing distress and everyday cognitive performance impairment. Besides memory deficits, other cognitive impairments are exhibited by some individuals, but with no frequency or severity that would indicate a new specific feature caught in dementia diagnostic. In incipient stages, most of the AD patients develop very mild dementia or mild cognitive impairment (MCI) ("cognitively impaired no dementia") (1, 2).

AD is the most common form of dementia among the elders. This condition is characterized by the progressive cognitive function decline and is receiving an increased attention in the current literature and social aspects of life (3-5). Due to the intense psychological and physiological symptomatology, a detrimental global impact has been observed. Presently, an estimation counts more that 36 million patients worldwide and a prediction for the year

2015 even triples the number of cases (6).

In addition, AD is a complex, multifactorial, heterogeneous mental illness, which is characterized by an age-dependent loss of memory and an impairment of multiple cognitive functions. Molecularly, AD is associated with the presence of intracellular neurofibrillary tangles and extracellular beta-amyloid plaques which leads to neuronal subpopulations loss, mitochondrial oxidative damage, some specific genetic deficiencies, synaptic loss and the proliferation of reactive astrocytes and microglia (6).

As mentioned, recent studies support physical activity as an important preventive factor against AD onset. Furthermore, they are stating that physical exercising could be crucial for the maintenance or for slowing down the decline of optimal functional ability levels in these patients. However, small evidence points to a personalized exercise scheduled tasks which would help dementia patients.

In this way, as we will show in this mini-review, aerobic exercise is encouraged by therapists considering its role in angiogenesis neurotrophic factors release (increases in cerebral blood flow and cerebral plasticity).

Thus, the purpose of this investigation was to review the benefits of consistent exercising, either cardiovascular training, strength training or combinations of both in AD. Considering that an inverse relationship between cognitive impairment and cardiovascular fitness was demonstrated in several encouraging studies, and also that strength training was shown to be particularly effective for improving postural and motor functioning leading to reduced risk of motor failure in AD subjects, we will further discuss the possible implications and several presumed mechanisms of physical exercise on cognitive performance.

Cardiovascular exercises and Alzheimer's disease

Currently, studies regarding general fitness and cognitive impairments have focused on the effects of cardiorespiratory exercise on symptoms associated with these conditions (7). This may be due to the inverse relationship between cognitive impairment and cardiovascular fitness proven in some studies (7, 8).

For example, Burns *et al.* demonstrated that subjects who had higher levels of cardiorespiratory fitness (VO_2 peak) displayed lower levels of brain atrophy (8).

In addition, some randomized control trials have evaluated if cardiorespiratory exercise has a beneficial effect on cognitive response in older patients with AD. In this way, Lautenschlager *et al.* (9) conducted an intervention in elderly patients for a period of 6 months followed by 18 months follow-up period, but no significant difference in cognitive responses was found. We also must mention that Alzheimer Disease Assessment Scale-Cognitive subscale (ADAS-Cog) and ADL - was used for cognitive response assessment and compared between the exercise group and the control group.

On the contrary, Baker *et al.* (10) found that improved cardiovascular fitness was accompanied by an increased efficiency in activities, such as executive control processing, multitasking, information processing performances and selective attention when they evaluated the effect of a 6-month cardiorespiratory exercise intervention. However, when the results were adjusted for sex, a significant difference occurred only in women with increased oxygen consumption (VO_2 peak) and im-

provements in cognitive activities.

The effects of general fitness on cognitive performances may be also discussed in terms of the relationship between body mass index (BMI), age and oxidative stress (11). In this way, convincing evidence demonstrated that oxidative stress is a prominent feature in AD linking oxidative stress to the development of neuronal death and neural dysfunction, which suggests a key pathogenic role for oxidative stress in AD (12).

In addition, the evidence for the role of total adiposity, usually measured clinically as body mass index, and central adiposity, in AD is constantly increasing (11). This topic is of enormous public health importance given the global epidemic of obesity and its consequences.

Even more accumulating evidence is also suggesting that changes in lifestyle factors such as increasing physical activity will decrease the risk of developing dementia in later life (13). Other studies also showed reduced rate of age-related cognitive decline, decreased risk of incident dementia or AD in individuals who exercise regularly (14).

In this way, there are some possible explanations for the inequality between men and women regarding cognitive improvement in association with exercise. For example, it is speculated that estrogens may have a neuroprotective effect because many women after menopause are on hormone replacement therapy (15).

Therefore, regardless of biological gender, the conclusion is that cardiorespiratory training may be critical for a patient with AD, especially in the initial phase of the disease (16).

In addition, recently Middleton *et al.* (17) found that women which reported being physically active at any point over their life course had a lower likelihood of cognitive impairment in later life.

Molecular aspects for the association between physical exercise and ad care

As already mentioned, oxidative stress is a major factor associated with the development and progression of AD and other forms of dementia (2). A large part of data suggests that free radical oxidative damage-particularly of neuronal lipids, nucleic acids, and proteins are extensively implicated in the brain pathology of AD patients (12).

Increased oxidative stress is thought to result in the generation of free radicals and ROS, which is reported to be released by microglia activated by amyloid beta (18). When compared to other organs, the brain has been found to be more vulnerable to oxidative stress due to its high lipid content, its relatively high oxygen metabolism, and its low level of antioxidant defenses (19).

Also, some cytokines are potent stimulators to produce reactive oxygen and nitrogen by macrophages and monocytes and therefore a rise in the concentration of cytokines could be responsible for increased oxidative stress.

Even more, obesity increases the mechanical load and myocardial metabolism and therefore oxygen consumption is increased. Of course, one negative consequence of increased oxygen consumption is the production of ROS as superoxide, hydroxyl radical, and hydrogen peroxide derived from the increase in mitochondrial respiration and from the loss of electrons produced in the electron transport chain, resulting in the formation of superoxide radical (20).

Lately our group also demonstrated that oxidative stress is importantly related with

exercising (21-23) and that increased oxidative stress status may be characteristic to AD and MCI patients (2, 12).

Moreover, as one of the main factors that lead to obesity is the lack of sustained physical effort alongside some harmful alimentary habits (24), there is an increased awareness also on the relationship between obesity, oxidative stress and cognition.

In this way, the association between obesity and oxidative stress appears to be due to the presence of excessive adipose tissue itself, because adipocytes and preadipocytes have been identified as a source of proinflammatory cytokines, including TNF-α, IL-1, and IL-6, as sometimes obesity is considered a state of chronic inflammation. In fact, concerning the cardiorespiratory exercises there are many contradictory results regarding the connections that might exist between exercising and oxidative stress. A possible explanation for these different results could be differences that might appear between trained and untrained subjects. This theory was proven so far on rats. Senturk *et al*. demonstrated that exercise-induced oxidative stress affects erythrocytes in sedentary rats, but not in exercise-trained rats (25).

In this context, we could mention here the connection between inflammation and disorders such as rheumatoid myositis or psoriatic arthritis (26, 27), which were previously described as having some degree of correlation with AD, as demonstrated even by some previous Nature group publications (28-31).

Thus, concluding, it seems that over the years cardiorespiratory fitness development has received extensive research interest. Common methods for developing cardiorespiratory fitness are running, cycling, swimming, or brisk walking.

Strength training and AD management

For many years strength training potential positive effect on cognitive impairment was disregarded. However, since AD is associated with low muscle mass and strength (32), muscle strength and power training could in fact be crucial for AD patients.

In addition, balance training has been shown to improve the postural abilities of subjects which were moderately-to-severely affected by AD, and so reduce the risk of falling at a later stage of the disease (33).

In this way, a complete exercise training (including balance training, aerobic exercise and strength training) was shown to be particularly effective for improving postural and motor functioning and reducing the risk of falling in AD subjects (34).

Another randomized controlled trial, which was performed in elders' residential institutions in France, investigated the effect of an exercise program on the ability of AD patients to perform activities of daily living, physical performance, behavioral disturbances and depression. In this study, Rolland *et al.* (35) conducted a complete exercise program for 12 months with 1 hour of exercise twice a week, training program that included walking exercise, muscle strength, balance and flexibility tasks, while the control group received only traditional health care. Thus, at the end of the study the intervention group performed significantly better in the 6 m walking speed test. However, no significant effect was found for cognitive responses.

In another research conducted in Brazil (36), patients suffering from AD underwent a varied exercise program for 6 months that included various exercises, circuits, sport

dance sequences, recreational activities, stretching activities, weight training and relaxation. The results clearly indicated that cognitive functions (measured using the MMSE) were preserved during the interventional period, while the control group, whose members maintained their routine activities (no exercise), experienced a significant decline in MMSE score.

As for studies which particularly examined the effect of strength and strength training has on AD patients, in a cross-sectional study, Petrella *et al*. (37) evaluated the relationship between quadriceps muscle strength and cognitive impairment (CI) among frail elderly patients and physically independent patients. In this way, an inverse correlation was found between muscle power and CI in the physically independent individuals - that is, as results from the power tests decreased for the quadriceps muscle, signs of CI increased.

Similarly, Scherder *et al*. (38) found that in older women quadriceps muscle strength was associated with an improvement in completing function activities, such as attention and working memory.

Lastly, the increase in muscle strength was associated with a lower rate of decline in global cognitive function. However, despite this association between muscular fitness and cognitive function, relatively few studies have examined independently the effect of strength training on cognitive responses. Cassilhas *et al*. (39) studied the effect of 6 months of strength training on cognitive function in the elderly. In this study the intervention compared different intensities of strength training and the sample was distributed between moderate intensity (50% one-maximum repetition), high intensity (80% one-maximum repetition), and a control untrained group. In terms of muscle strength, both experimental groups performed better than the control group, but no differences were noted between them. Also, in both experimental groups (moderate and high intensity), positive changes were noted in cognitive function.

Furthermore, Liu-Ambrose *et al*. (40) investigated the effect of different frequencies of strength training (once a week compared to twice a week) or twice-weekly balance training on changes in cognitive function in older women. Their results demonstrated that12 months of once- or twice-weekly strength training significantly improved Stroop test performance in cognitively healthy women aged 65-75 years compared to the other two intervention groups.

Likewise, Nagamatsu *et al*. (41) demonstrated that 6 months of twice-weekly strength training improved selective attention/conflict resolution, associative memory and regional patterns of functional brain plasticity, as compared with twice-weekly balance exercises, providing novel evidence that strength training can benefit multiple domains in those at risk for dementia.

Furthermore, another study conducted on senior women with subjective memory complaints, six months of twice-weekly resistance training improved selective attention/conflict resolution, associative memory and regional patterns of functional brain plasticity, when compared with twice-weekly balance and tone exercises. In contrast, six months of twice-weekly aerobic training improved only physical function (41).

Also, some studies analyzed the impact that strength training has on specific age groups of patients with AD. As already

mentioned, Liu-Ambrose *et al*. demonstrated that 12 months of twice-weekly resistance training significantly improved Stroop Test performance in cognitively-healthy women aged 65-75 years old (40), while the same research group found later an improvement after only six months in women aged 70-80 years old with probable mild cognitive impairment. Thus, the benefits of resistance training on selective attention/conflict resolution may be more potent among those at greater risk for dementia.

In addition, loss of muscle strength is common and associated with a variety of adverse health outcomes in old age, but few studies have examined the association of muscle strength with the risk of AD or MCI.

Thus, Boyle *et al*. performed an analysis of 3.6 years on 138 persons who developed AD. The results showed in a proportional hazards model adjusted for age, sex, and education, each 1 unit increase in muscle strength at baseline was associated with about a 43% decrease in the risk of AD. The association of muscle strength with AD persisted even after adjustment for several covariates, including body mass index, physical activity, pulmonary function, vascular risk factors, vascular diseases and apolipoprotein E4 status. Furthermore, in a mixed-effects model adjusted for age, sex, education and baseline level of global cognition, increased muscle strength was associated with a slower rate of decline in global cognitive function. Finally, muscle strength was associated with a decreased risk of MCI, the precursor to AD (42).

Considering all these aspects, we can state that future research might assess the cost-effectiveness of the exercise interventions for the reduction of CI in AD, considering that for example in 2000 the cost of AD and CI treatment in the USA was estimated to be 100 billion US$. This could also have an increased relevance considering that there are a lot of issues that can be tackled by this alternative approach, including for example the self-neglect (43) aspects in these disorders discussed here, as well as various other aspects such as the overmedication (44) and other psychological, social or awareness issues on this matter (45, 46).

Still, although there is accumulating evidence for the effectiveness of exercise in combating cognitive decline, it is believed that there is still a gap in the literature on the indirect and economic costs of physical exercise interventions for CI and AD (47).

CONCLUSIONS

Thus, the current knowledge supports physical activity as an important preventive factor against the onset of Alzheimer's disease and that physical exercising could be crucial for the maintenance or for slowing down the decline of optimal functional ability levels in these patients. However, there is currently insufficient information to enable a precise definition of the best exercise regimen for patients with AD. Also, aerobic exercise is a necessary part of the treatment for AD, but aerobic exercise alone is probably not the best activity. Strength/power training, balance/coordination, and other types of physical exercises also contribute to reconditioning, maintaining, and improving the cognitive and motor functions of AD subjects. Therefore, it is advisable to combine aerobic exercises with other activities that are beneficial for the neuromuscular system (e. g., strength/power training and stretching), balance function, and the performance of motor coordination.

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NEWS

THE FUTURE IS NEAR: PARKINSON'S DISEASE – DIAGNOSIS

Parkinson's disease is a neurodegenerative disorder often confused with other atypical parkinsonian syndromes because of its difficult differential diagnosis. Due to recent technological progress in magnetic resonance imaging, the diagnosis of Parkinson's disease has considerably improved. A group of scientists from Innsbruck Medical University decided to write a review that focuses on the value of MRI techniques, including advanced sequences at high and ultra-high-field MRI. Imaging markers for neurodegeneration refers to loss of tissue volume, signal changes and increased iron deposition. Both structural and quantitative imaging techniques are analyzed, the latter including modern techniques such as diffusion imaging, magnetization transfer imaging, iron-sensitive sequences and sequences based on T1, functional imaging techniques including proton magnetic resonance spectroscopy, arterial spin labeling and rs-fMRI. In conclusion, advanced MRI techniques are nowadays used to make a correct differential diagnosis as well as for the early detection of Parkinson's disease. *(*Heim B, Krismer F, De Marzi R, Seppi K. Magnetic resonance imaging for the diagnosis of Parkinson's disease. *J Neural Transm* 2017; 124: 915-964)

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STUDIUL UNOR FACTORI CE POT INFLUENŢA DEFENSIVA ENZIMATICĂ ANTIOXIDANTĂ LA PACIENŢII OBEZI

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STUDY ON SEVERAL FACTORS INFLUENCING ANTIOXIDANT ENZYME DEFENSE IN OVERWEIGHT PATIENTS (Abstract): The notion of "oxidative stress" was introduced by Sies, in 1985, and designates all the oxidative damages caused by the free radicals of oxygen. Oxidative stress represents the imbalance of pro-oxidant and antioxidant ratio, in favor of the former, following the excessive action of the "aggressor" and/or the decrease of the "defender's" capacity to oppose. **Material and method**: In the current study we had 82 overweight patients (42 patients less than 65 years old and 40 patients over 65). The criteria for inclusion was weight over 80 Kg and BMI > 30. Patients were monitored and assessed every three, six, nine months and each year. **Results**: Obesity together with dyslipidemia, smoking, diabetes and high blood pressure intensifies oxidative stress. The improvement of these factors determines the growth of antioxidant enzyme level (SOD and GPX) and the reduction of malondialdehyde level. **Key words**: OXIDATIVE STRESS, OBESITY, ANTIOXIDANT ENZYME

Stresul oxidativ (SO) reprezintă o importantă verigă fiziopatologică pro-ateroscleroticăîn HTA, dar cu impact semnificativ crescut la persoanele vârstnice.

Stresul oxidativ pare a juca un rol vital în debutul şi progresia arterosclerozei. Efectele dăunătoare cauzate de speciile reactive de oxigen apar ca urmare a unui dezechilibru între formarea şi inactivarea radicalilor liberi de oxigen.

Inactivarea şi eliminarea speciilor reactive de oxigen (SRO) depinde de reacţii care implică sisteme de apărare antioxidante, dintre care cele mai importante sunt reprezentate de superoxid dismutază (SOD) şi glutation peroxidază (GPX) (1,2).

Radicalii liberi pot avea şi unele efecte benefice asupra organismului – de exemplu colaborează cu sistemul imunitar pentru a distruge bacteriile şi alţi invadatori ai organismului sau reglează tonusul muşchilor netezi vasculari contribuind astfel la controlul circulaţiei. Dar acumularea unei cantităţi crescute de radicali liberi ar fi pusă în legătură directă cu fenomenele de îmbătrânire şi de instalare a unor boli cronice (3).

Superoxidismutaza (SOD) reprezintă prima linie de apărare a organismului în faţa stresului oxidativ şi principalul „scavenger" al superoxidului în toate celulele cu metabolism predominant aerob.

Enzima catalizează dismutarea a doi radicali superoxid care se unesc şi formează peroxidul de hidrogen controlând astfel nivelul anionilor superoxid şi împiedicând iniţierea reacţiilor de formare a nocivilor radicali hidroxil şi peroxinitrit.

Pot exista variaţii interindividuale în ceea ce priveşte capacitatea de apărare antioxidantă, ceea ce afectează sensibilitatea indi-

TABELUL I
Corelaţii dintre stresul oxidativ, obezitate, fumat

		OBEZITATE	FUMAT
GPX iniţial	Pearson Correlation	-,689	-,401
	Sig. (2-tailed)	,000	,000
	N	82	82
SOD iniţial	Pearson Correlation	-,112	-,148
	Sig. (2-tailed)	,003	,002
	N	82	82
MDA iniţial	Pearson Correlation	,222	-,901
	Sig. (2-tailed)	,001	,001
	N	82	82
OBEZITATE	Pearson Correlation	1,000	,234
	Sig. (2-tailed)		,098
	N	82,000	82
FUMAT	Pearson Correlation	1,000	1,000
	Sig. (2-tailed)		
	N	82,000	82,000

vidual㠠împotriva efectului dăunător al reacţiilor oxidative (4,5).

MATERIAL ŞI METODĂ

În studiu au fost incluşi 82 de pacienţi obezi (42 de pacienţi cu vârste până în 65 de ani şi 40 pacienţi de peste 65 ani).

Criteriile de includere au fost greutatea peste 80 kg şi un indice de masă corporală peste 30.

Pacienţii au fost monitorizaţi şi evaluaţi la trei luni, şase luni, nouă luni şi un an. S-a considerat astfel că o evaluare la intervale fixe de timp reprezintă un factor predictiv şi de urmărire mai important al patologiei cardiovasculare şi/sau dismetabolice în cadrul loturilor studiate.

Pentru studierea existenţei unei relaţii între obezitate şi stresul oxidativ s-a realizat analiza de corelaţie între valorile GPX, SOD şi MDA şi prezenţa sau absenţa obezităţii.

REZULTATE ŞI DISCUŢII

Din tabelul I se poate observa că prezenţa obezităţii se corelează cu un nivel scăzut al enzimelor antioxidante (rGPX=-0,68 ; pGPX =0,00 ; rSOD=-0,112, pSOD=0,003) şi unul crescut al MDA (rMDA=0,22 ; pMDA =0,001).

Pentru studierea existenţei unei relaţii între fumat şi stresul oxidativ s-a realizat analiza de corelaţie între valorile GPX, SOD şi MDA şi prezenţa sau absenţa fumatului.

De asemenea, din tabelul I se poate observa că prezenţa fumatului se corelează cu un nivel scăzut al enzimelor antioxidante (rGPX= -0,401 ; pGPX=0,00 ; rSOD= -0,148, pSOD=0,002) şi unul crescut al MDA (rMDA=0,901 ; pMDA=0,001).

Pentru demonstrarea unei relaţii între dislipidemie şi stresul oxidativ s-a realizat analiza de corelaţie între valorile GPX, SOD şi MDA şi prezenţa sau absenţa dislipidemiei.

Din tabelul II se distinge faptul că prezenţa dislipidemiei se corelează cu un nivel scăzut al enzimelor antioxidante (rGPX= -0,114 ; pGPX=0,00 ; rSOD= -0,487, pSOD =0,00) şi unul crescut al MDA (rMDA= 0,237 ; pMDA=0,00).

Corelaţia dintre diabetul zaharat şi stresul oxidativ s-a remarcat prin efectuarea analizei de corelaţie între valorile GPX, SOD şi MDA şi prezenţa sau absenţa diabetului

TABELUL II
Corelaţii dintre stresul oxidativ, dislipidemie, diabet zaharat

		DISLIPIDEMIE	DIABET ZAHARAT
GPX iniţial	Pearson Correlation	-,114	,439
	Sig. (2-tailed)	,000	,003
	N	82	82
SOD iniţial	Pearson Correlation	-,487	-,168
	Sig. (2-tailed)	,000	,000
	N	82	82
MDA iniţial	Pearson Correlation	,237	,991
	Sig. (2-tailed)	,000	,001
	N	82	82
DISLIPIDEMIE	Pearson Correlation	1,000	,995
	Sig. (2-tailed)		,098
	N	82,000	82
DIABET ZAHARAT	Pearson Correlation	,995	1,000
	Sig. (2-tailed)	,098	
	N	82	82

TABELUL III
Corelaţii dintre stresul oxidativ şi hipertensiunea arterială

		AHC - HTA
GPX iniţial	Pearson Correlation	-,651
	Sig. (2-tailed)	,001
	N	82
SOD iniţial	Pearson Correlation	-,192
	Sig. (2-tailed)	,003
	N	82
MDA iniţial	Pearson Correlation	,123
	Sig. (2-tailed)	,000
	N	82
HTA	Pearson Correlation	1,000
	Sig. (2-tailed)	
	N	82,000

în antecedentele heredocolaterale.

În cadrul tabelului II este prezentat faptul că prezenţa diabetului se corelează cu un nivel scăzut al enzimelor antioxidante (rGPX = -0,43 ; pGPX=0,003 ; rSOD= -0,168, pSOD=0,000) şi unul crescut al MDA (rMDA=0,99 ; pMDA=0,001)

Realizarea analizei de corelaţie între valorile GPX, SOD şi MDA şi prezenţa sau absenţa hipertensiunii arteriale în antecedentele heredocolaterale, prezentată în tabelul III demonstrează că prezenţa HTA se corelează cu un nivel scăzut al enzimelor antioxidante (rGPX= -0,65 ; pGPX=0,00 ;

rSOD= -0,192, pSOD=0,003) şi unul crescut al MDA (rMDA=0,123 ; pMDA=0,000).

Bolile cardiovasculare se corelează cu intensificarea stresului oxidativ, demonstrate de scăderea nivelului enzimelor antioxidante (SOD şi GPX).

CONCLUZII

Obezitatea alături de fumat, dislipidemie, diabet şi hipertensiune creşte stresul oxidativ. Ameliorarea acestor factori duce la creşterea nivelului enzimelor antioxidante (SOD şi GPX) şi scăderea nivelului malondialdehidei.

Bolile cardiovasculare se corelează cu intensificarea stresului oxidativ, demonstrate de scăderea nivelului enzimelor antioxidante (SOD şi GPX).

Posibilele surse ale stresului oxidativ din diabetul zaharat includ creşterea producţiei de specii de radicali de oxigen, în special din procesele de glicare sau lipoxidare şi scăderea sistemului de apărare anti-oxidare enzimatic sau non- enzimatic.

Ateroscleroza este o stare în care nivelul stresului oxidativ este ridicat, caracterizată prin oxidarea lipidelor şi proteinelor în peretele vascular.

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FIVE-YEAR CLINICAL EXPERIENCE WITH MENINGOCOCCAL INFECTIONS AT "SF. PARASCHEVA" INFECTIOUS DISEASES HOSPITAL DURING 2014 – 2018

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FIVE-YEAR CLINICAL EXPERIENCE WITH MENINGOCOCCAL INFECTIONS IN INFECTIOUS DISEASES HOSPITAL "SF. PARASCHEVA" IAŞI DURING 2014-2018 (Abstract**): Aim:** To evaluate the epidemiological, clinical-biological and therapeutic data and to assess the influence of standard pretreatment prognosis score on the evolution on meningococcal infection. **Material and methods:** Retrospective analysis of the hospital-based medical records of patients with acute meningococcal infections admitted to the Iaşi Clinic of Infectious Diseases between 2014 and 2018. Selection of patients with classic clinical symptoms of meningitis was based on Gram staining, culture and latex agglutination. The risk factors for complications or death were assessed. **Results:** A total of 370 meningitis patients hospitalized between 2014 and 2018 were reviewed. Meningococcal infections were recorded in 8.05% cases of bacterial meningitis. CSF cultures were positive for only 60% of the study patients. Co-agglutination was clinically useful for confirming the diagnosis in 38% cases. Mortality rate was 14.3% and was related to meningococcemia. Platelet count was the most important predictor in fatal cases. The results showed a poor correlation between length of hospital stay and standard pretreatment prognostic score (r=0.201). **Conclusions:** Meningococcal infection remains a significant health problem because of its serious complications and high mortality rate. The results of this study highlight the importance of implementing the meningococcal immunization programs in our country. **Keywords**: MENINGOCOCCEMIA, MENINGITIS, MENB VACCINE.

Invasive meningococcal disease (IMD) remains a life-threatening disease and an important public health problem worldwide. Of the at least 12 identified serogroups of *Neisseria meningitidis*, six (namely A, B, C, Y and W) are responsible for the majority of cases, and serogroups B and C are the most frequent in Europe (1). *Neisseria meningitides* causes two serious diseases: meningococcal meningitis and fulminant meningococcemia which often occur together.

IMD was recorded as sporadic cases, focal outbreaks, or large epidemics (2). The incidence of IMD varies by geographic region, but also by socio-economic status,

with the highest records in African countries, 10 to 1,000 cases per 100,000 population (3). IMD is rare in Europe. In 2012, 0.68 cases per 100,000 population were reported. According to the most recent report of the European Center for Disease Prevention and Control a total of 3,121 cases of infectious meningococcal diseases were reported (with a notification rate of 0.61 confirmed cases/100.000 population) (4). Serogroups C, B, W and Y are responsible for IMD in Europe. Currently, serogroup B is the most common cause of outbreak associated disease after the significantly decrease in incidence of serogroup C-related meningococcal disease after the introduction of the specific vaccine (5). In 2015, almost 55% cases of IMD were associated with serogroup type B (4). The infants less than 1 year of age are at the highest risk of infection, followed by the 15 to 24-year age group. Patients with complement system deficiencies are also at increased risk for infection (mainly meningococcal meningitis) (6).

Even the event rate (like complications or mortality) is lower in case of meningococcal meningitis compared to other types of meningitis, the overall case fatality rate for IMD approximating 10% in developing countries. In addition, the neurological complications are significant in case of IMDs (4).

In this context, the aim of the present paper was to evaluate the epidemiological, clinical and biological features and therapeutic outcomes of IMDs based on clinical practice in the Iași Clinic of Infectious Diseases during a five-year interval.

MATERIAL AND METHODS

Study patients This was a retrospective analysis of hospital-based medical records of patients with infectious meningococcal diseases (meningococcal meningitis and/or meningococcemia) admitted to the Iași Clinic of Infectious Diseases between 2014 and 2018. The inclusion criteria were based on: clinical signs (e.g. fever, headache, neck stiffness, petechiae or purpura), blood tests results, at least one cerebrospinal fluid (CSF) test result: Gram stain, culture or latex agglutination. Patients with tuberculosis meningitis, nosocomial meningitis, other confirmed bacterial meningitis or meningoencephalitis were excluded.

Data collection. The following data were collected during the study: demographic data, onset of symptoms, physical examination on admission, blood tests results, CSF examination (appearance, cytology, Gram stain, culture, latex agglutination), computer tomography (CT) scan (if available), medication administered (name and duration of treatment) and outcome.

Prognostic score at admission was calculated using the updated Stiehm and Damrosch criteria (1966), namely: presence of petechiae for less than 12 hours prior to admission; cardiogenic shock/hypotension (systolic blood pressure 70 mmHg or below); absence of meningitis (less than 20 white blood cells/mm^3 in CSF); blood leukocyte count normal or low (less than10,000 white blood cells /mm^3); normal or low erythrocyte sedimentation rate (less than 10 mm/hour); thrombocytopenia, hyperpyrexia (7). In addition, fatality rate was calculated.

Statistical analysis Results were analyzed with descriptive statistics. The relationship between prognostic score at admission and disease course was evaluated using Pearson correlation test. Statistical analysis was performed using XLSTAT

version 2018 software.

Ethical approval This retrospective study was approved by the Ethics Committee of the Iasi Clinic of Infectious Diseases.

RESULTS

Patient characteristics Between 2014 and 2018, 370 cases of meningitis were admitted to our clinic. Meningococcal infections were recorded in only 8.57% cases of bacterial meningitis. The mean age of patients was 8.5 years (range 3 months – 69 years), but most patients were aged 1-5 years (fig. 1).

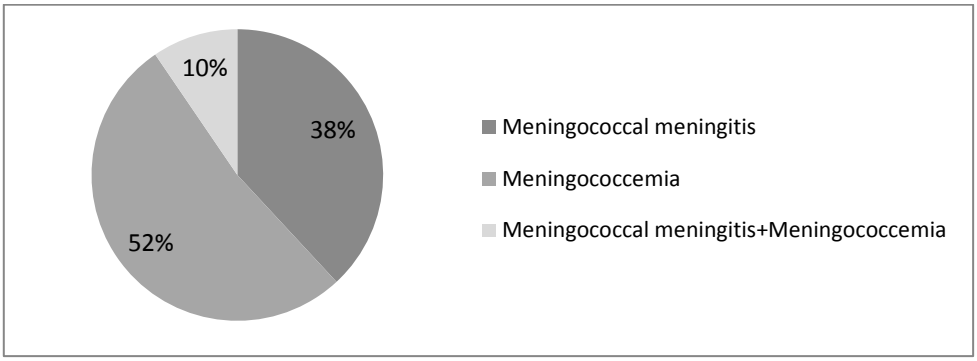

Fig. 1. Age distribution of study patients

The male: female ratio was almost 1:1. Meningococcal meningitis and meningococcemia were diagnosed together in most of the cases (fig. 2).

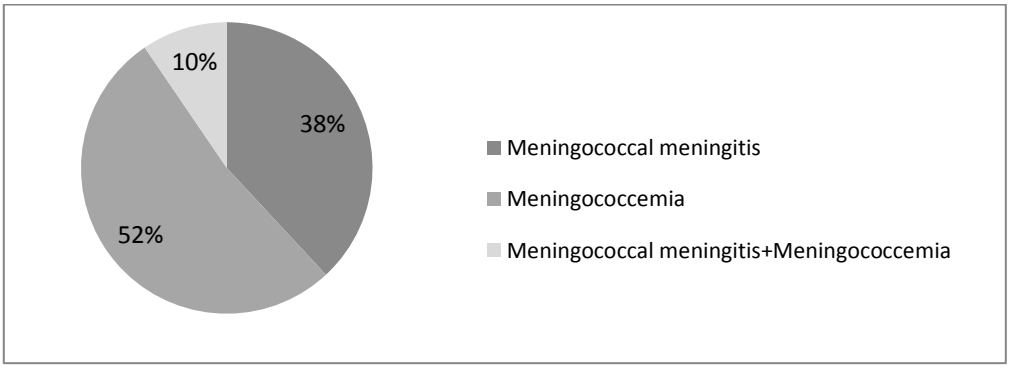

Fig2. Distribution of patients according to their diagnosis

Neisseria meningitides serogroup C was found only in two patients and serogroup B was identified in the remaining cases.

Clinical results. The clinical triad fever, neck stiffness, petechiae was identified in all pediatric patients. Symptom onset was less than 12 hours in81% cases. Level of consciousness was one of the most important criteria of severity considered. Changes in mental status from somnolence to loss of consciousness and coma were recorded in all cases. In the most severe cases, signs of coma (Glasgow 3), encephalopathy and convulsions were noted.

Biological results Mean leukocyte count was 18,358/mm^3 with neutrophils (%) between 29.5 and 93.2%. Leukocytosis was found in 80.95% cases.

Mean platelet count was 22,872/mm^3 and the lowest levels (16,000/mm^3) were identified only in patients with meningococcemia.

CSF findings Lumbar puncture and CSF collection were performed in all cases except two cases of meningococcemia where the significantly low level of thrombocytes contraindicated the procedure (tab. I). CSF analysis revealed pleocytosis in 52.4% cases, elevated protein levels in 62% cases and low glucose levels in only8.6% cases.

Overall, Gram stain test was positive in 52% cases. Positive cultures for *N. meningitides* were obtained in 43% cases. Latex agglutination test confirmed the diagnosis in 38% cases (Table II).

Treatment and outcomes. The prognostic factor was calculated for each patient before initiation of therapy. The values ranged between 1 and 4 but were not directly correlated with disease course (fig. 3).

TABLE I

CSF analysis

CSF parameter	Median (range)
Cell count (cells/mm^3)	1,266 (8 – 11,000)
Albumin (g/L)	1.75 (0.17 – 7)
Glucose (mg/dl)	57.0 (6 – 162)

TABLE II

CSF susceptibility tests

Positive susceptibility Tests (% cases)	Gram stain	CSF culture	Latex co-agglutination
Meningococcal meningitis	66.6	66.6	16.6
Meningococcal meningitis + Meningococcemia	63.64	45.4	54.5
Meningococcemia	0	0	25

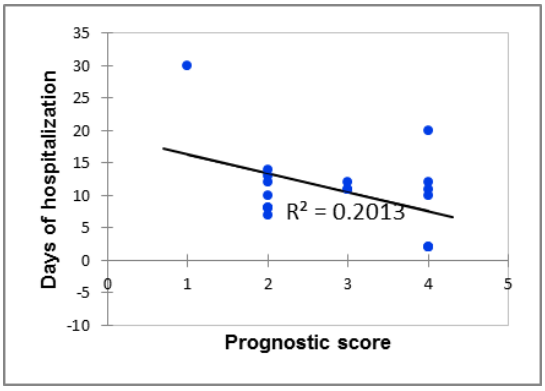

Fig 3. Correlation between days of hospital stay and prognostic score

The fatality rate was 14.3%. Three cases of fulminant meningococcemia had a rapidly progressive course, death occurring within less than 24 hours. Severe thrombocytopenia was the most important predictor of fatal outcome.

The remaining cases responded favorably to treatment with third generation semisynthetic cephalosporin (ceftriaxone), with a mean duration of treatment of 14 days.

DISCUSSION

We conducted a small sample size retrospective study that included 21 patients with meningococcal meningitis, meningococcemia or both. The small number of cases identified in the hospital database was expected since Romania is one of the European Union (EU) countries with a low incidence of IMDs. The notification rate for *N. meningitides* serogroup B recorded by the European Centre for Disease Prevention and Control (ECDC) in 2015 was 0.09 per 100,000 (8).

A previous multicenter study (9) that included our hospital among the participating hospitals reported an incidence of meningococcal diseases of 40 and 16 per 100,000 per year for children < 1year and 1 to 5 years, respectively. In our study, the majority of patients were aged under 5 years (75% cases), of which 33.3% cases were infants < 1-year-old.

Diagnosis was based on medical history, clinical examination, including signs of meningitis, blood tests and CSF analysis. The typical clinical (fever, neck stiffness, petechiae) and laboratory features (CSF analysis) of IMDs were present in all patients. However, the standard criteria to be met for positive diagnosis were very variable, as also noted in other surveillance studies (10).

Neisseria meningitidis was identified based on Gram stain method in less than 70% cases. This result was low compared to other reports, but a high variability of this test was reported in the literature (30 – 89%). A positive latex agglutination test identified the specific bacterial antigen in 50% cases. This result was expected since this method is characterized by a variable sensitivity (22-93%) (11, 12). A PCR analysis, the most sensitive and specific test in the diagnosis of IMDs, since at least one Gram stain, culture and/or latex agglutination tests were positive (13). *N. meningitidis* is some bacteria which rarely develops resistance to penicillin. In a trial performed on 506 *N. meningitides* strains, only 13% cases were identified as intermediate resistant and none of them were resistant to penicillin (14). By contrast, in our study we recorded penicillin resistance in 9% of the cases and intermediate resistance to penicillin in 20%, but the significance of these results could be affected by the small sample size. According to antimicrobial susceptibility tests and current guidelines, Ceftriaxone was selected for IMD treatment. It is well known that early diagnosis and initiation of antibacterial treatment are keys to avoid complications and death in IMDs (2, 15). Procedures like lumbar puncture or CT scan are often responsible for delayed treatment initiation (16). Specific IMD prognostic scores (e.g. Glasgow meningococcal septicemia prognostic score, Stiehm and Damrosch score, Niklasson score) were developed to quantify disease severity. In our study, the Stiehm and Damrosch score was calculated at admission. This factor score could not be accurately correlated with disease course. Of the factors found to be involved in the un favorable progression of IMDs (such as hypotension, tachycardia, low CSF protein level, high CSF/blood glucose, etc.) (16), we have identified thrombocytopenia as a major negative prognostic factor associated with disease-related mortality.

It was reported that almost 500 million people are carriers of *N. meningitidis*. Even though not all of these people develop acute disease, its epidemiological features and life-threatening risk classify the IMDs as a health problem (17).

In this context, prevention becomes one of the most important objectives in the management of IMDs. Chemoprophylaxis (quinolones, rifampin, ceftriaxone) and immunoprophylaxis (mainly meningococcal ACWY oligosaccharide conjugate vaccine) have been demonstrated to be effective in reduction of bacterial carriage of *N. meningitis* and prevention of meningitis-related complications and death (18). More recently, the first meningococcal B vaccine (menB) was authorized in EU for children with age over 2 months and in 2017 was licensed the second menB vaccine for children over 10 years. Also in 2017, ECDC reported the evaluation performed by most of the EU countries regarding the necessity of implementing the immunization program, based on: epidemiology of IMDs, efficacy, safety and immunogenicity of vaccine; cost- effectiveness studies. The European countries have expressed different opinions on this issue. For instance, countries like UK, Ireland, and Italy have already developed and implemented national immunization programs. By contrast, other countries (e.g. France, Belgium) do not recommend vaccination for different reasons. Romania, among other countries (e.g. Denmark, Netherlands, Bulgaria) has not made any decision (19). Romanian public health authorities should evaluate the effectiveness of mass immunoprophylaxis in case of suspected outbreak of meningitis and to define the target population to be vaccinated based on benefit-risk assessment.

CONCLUSIONS

In our retrospective study, typical clinical and laboratory presentations were common, but the positive criteria for IMDs were variable. The fatality rate was low. The prognostic score calculated at admission was not directly correlated with disease course, but low platelet count was one of the most significant predictors of meningococcemia-related mortality.

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ASPECTE CLINICO-PARACLINICE LA DIABETICII COLECISTECTOMIZAŢI PENTRU LITIAZĂ BILIARĂ VEZICULARĂ

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CLINICAL AND PARACLINICAL FEATURES IN DIABETIC PATIENTS CHOLECYSTECTOMIZED FOR GALLSTONES (Abstract) : Gallstones and diabetes mellitus represent a major economic burden especially when surgical treatment is involved. **Aim** : The purpose of our study is to identify particular laboratory, imaging and pathological aspects that correlate with gallstones in diabetic patients that undergo cholecystectomy. **Material and method** : This retrospective study enrolled 234 subjects diagnosed and treated through cholecystectomy for gallstones during 01.01-31.12.2008 at the 1st Surgical Clinic, Iaşi "St. Spiridon" Hospital. The subjects were divided into 2 groups comprising 62 patients diagnosed with diabetes mellitus and gallstones (group I), respectively 172 patients diagnosed only with gallstones (group II). **Results** : There is a statistically significant difference between the two groups regarding ASA class ($p=0.002$), body mass index, leukocyte count, serum amylase ($p=0.001$) and alanine--aminotransferase levels. Ultrasound examination revealed thickness of the gallbladder wall as an edematous aspect at 29.03% of the diabetic patients vs 13.95% nondiabetic patients and cholesterolotic appearance at 6.45% diabetics vs 2.91% nondiabetics. Pathological examination identified chronic cholecystitis at 88.96% of the diabetic and 70.97% of the nondiabetic patients ; there was no significant difference regarding the gallbladder pathological aspects. **Conclusion** : Diabetes mellitus does not appear to modify substantially the pathological course in patients with gallstones. There is a greater surgical risk for diabetic patients, possibly due to higher body weight and older age in this group and an increased risk for hepatopancreatic lesions. **Key words** : DIABETES MELLITUS, GALLSTONES, CHOLECYSTECTOMY

Litiaza biliară veziculară şi diabetul zaharat (DZ) sunt afecţiuni multifactoriale, caracterizate printr-o incidenţă crescută în populaţia generală şi reprezintă o încărcătură economică majoră pentru sistemul de sănătate, în special atunci când este implicată conduita chirurgicală a colelitiazei - colecistectomia. Asocierea celor două condiţii a fost sugerată de studiile epidemiologice, care au constatat prevalenţa de 2-3 ori mai mare a litiazei biliare la diabetici faţă de populaţia generală, relaţia fiind controversată datorită suprapunerii unor factori de risc pentru ambele boli : vârsta înaintată, obezitatea, dislipidemia, antecedentele familiale (1,2,3). La pacientul diabetic, cei mai importanţi factori litogenici incriminaţi sunt suprasaturarea în colesterol a bilei, hiperinsulinismul şi afectarea motilităţii colecistului, datorată neuropatiei autonome şi răspunsului diminuat al musculaturii netede veziculare la colecistokinină (4,5,6,7). Hiperinsulinemia pare să joace un rol mult mai important faţă de hiperglicemie în geneza calculilor, ceea ce determină o asociere mai puternică între litiaza biliară şi diabetul zaharat de

tip 2 (7). În ceea ce priveşte componenta chirurgicală a tratamentului, pacientul diabetic este, cel puţin teoretic, mai predispus la complicaţii perioperatorii, datorită afecţiunilor coexistente, cardiovasculare şi renale, precum şi riscului septic crescut.

Scopul prezentei lucrări este de a identifica şi de a demonstra câteva aspecte particulare ale tabloului biochimic, imagistic şi morfopatologic al pacienţilor diabetici colecistectomizaţi pentru litiază biliară veziculară, comparativ cu nondiabeticii supuşi aceleiaşi intervenţii curative.

MATERIAL ŞI METODĂ

S-a realizat un studiu de cohortă pe un număr total de 234 de pacienţi internaţi şi colecistectomizaţi în Clinica I Chirurgie a Spitalului "Sf. Spiridon" din Iaşi, în perioada 1.01.2008 – 31.12.2008. Pacienţii au fost împărţiţi în 2 loturi de studiu: lotul I, format din 62 de pacienţi cu diabet zaharat de tip 1, tip 2 sau tip 2 insulinotratat şi lotul II, format din 172 de pacienţi nondiabetici.

Bolnavii au fost incluşi într-unul dintre cele două loturi pe baza următoarelor criterii: diagnostic clinic şi imagistic de litiază biliară veziculară, rezolvată chirurgical prin colecistectomie (laparoscopică, clasică sau convertită), antecedente personale de DZ pentru includerea în lotul I sau absenţa acestui diagnostic (includerea în lotul II), absenţa evidenţelor imagistice sau intraoperatorii de litiază coledociană. Criteriile de excludere din studiu au fost reprezentate de litiaza coledociană, colecistectomie la o spitalizare anterioară, antecedentele personale de hepatită toxică, autoimună sau virală B şi C, bolile hepatice de stocaj, cirozele hepatice şi polipii veziculari.

Parametrii analizaţi au fost preluaţi din foile de observaţie clinică, protocolul operator aferent şi datele prezente în buletinele de examinare imagistică şi morfopatologică a specimenelor recoltate intraoperator. Informaţiile obţinute au fost grupate în date demografice şi clinice, parametri biochimici funcţionali hepatopancreatici (transaminaze, amilaze serice, fosfatază alcalină, bilirubină), număr total de leucocite, date ecografice (grosimea peretelui colecistic, numărul şi dimensiunea calculilor) şi aspecte microscopice ale piesei de rezecţie (veziculă biliară).

Datele au fost analizate şi exprimate în termeni de valori medii, exprimări procentuale şi deviaţie standard. Diferenţele semnificative dintre valorile medii au fost investigate cu ajutorul testelor *t-Student* şi *ANOVA*. Prelucrarea statistică a fost realizată prin intermediul programului *Statistica 8.0*.

REZULTATE

Parametrii demografici şi clinici: vârsta medie a pacienţilor incluşi în studiu a fost de $62,09 \pm 12,5$ ani în cazul pacienţilor diabetici şi de $55,19 \pm 13,08$ ani în cazul pacienţilor nondiabetici. În ambele loturi s-a înregistrat o prevalenţă crescută a pacienţilor de sex feminin, cu un raport dublu în favoarea acestora în cadrul lotului de nondiabetici (4, 2 : 1) faţă de lotul cu DZ (2,1 :

TABELUL I

Parametrii demografici şi clinici ai pacienţilor incluşi în studiu

Parametri	Lotul I (62 pacienţi)	Lotul II (172 pacienţi)	Semnificaţie statistică
Vârsta medie	62,09±12,5	55,19±13,08	NS
Sex (F/M)	42/20(2,1:1)	139/33(4,2:1)	NS
IMC	30,43±4,16	27,84±4,62	0,014
Scor ASA* I	0%	35,58%	0,002
Scor ASA II	85,48%	60,35%	
Scor ASA III	14,52%	4,07%	

*ASA - American Society of Anesthesiologists

TABELUL II
Valorile parametrilor de laborator în cazul pacienţilor studiaţi

Parametri de laborator	Lotul I	Lotul II	Semnificaţie statistică
Număr leucocite	10458,03±3788,12	7786,17±3454,69	0,032
Amilaza serică	273,2±858,03	91,23±628,88	0,001
Bilirubina totală	0,98±0,96	1,15±3,15	NS
Bilirubina directă	0,31±0,38	0,47±1,99	NS
Fosfataza alcalină	122,49±114,69	105,02±122,83	NS
*AST**	44,95±61,39	41,37±204,22	NS
*ALT***	53,58±58,7	55,89±211,86	0,025
ALT/AST	1,31±0,68	1,31±0,59	NS

*AST – aspartat-aminotransferaza **ALT – alanin-aminotransferaza

1). În ceea ce priveşte indicele de masă corporală (IMC), valorile medii ale acestuia au fost semnificativ mai mari la pacienţii diabetici, situându-se în intervalul corespunzător supraponderii şi obezităţii (tab. I).

Prin utilizarea clasificării ASA (*American Society of Anesthesiologists*), care evaluează riscul operator ţinând cont de statusul fizic al pacienţilor (8) şi analiza antecedentelor patologice personale, a tabloului clinic, bolnavii au fost incluşi într-una dintre următoarele categorii: clasa I (stare de sănătate bună), clasa II (patologie sistemică uşoară, fără afectare funcţională) şi clasa III (patologie sistemică severă, care limitează activitatea dar nu este incapacitantă); afecţiunile sistemice asociate cel mai frecvent au fost diabetul zaharat (100% în lotul I) şi hipertensiunea arterială (ambele loturi). Analiza comparativă a celor două loturi a relevat că pacienţii diabetici au prezentat un risc operator semnificativ crescut comparativ cu pacienţii non-diabetici (14,52% dintre pacienţii din lotul I au înregistrat scorul III pe scala ASA, spre deosebire de 4,07% dintre pacienţii lotului II).

Parametrii biochimici ai funcţiei hepatopancreatice au înregistrat diferenţe semnificative între cele două loturi studiate pentru valorile alanin-aminotransferazei (ALT) şi amilazemiei ($p=0,001$); markerul **inflamator**, reprezentat de numărul total de leucocite, a fost mai bine reprezentat în lotul diabeticilor, atingând nivelul de semnificaţie statistică ($p=0,032$) (tab. II).

Examenul ecografic a comparat grosimea pereţilor colecistului, dimensiunea şi numărul calculilor. În cazul ambelor loturi s-a evidenţiat îngroşarea marcată a pereţilor veziculei biliare, având ca substrat principal edemul (29,03% vs 13,95%) şi colesteroloza (6,45% *vs.* 2,91%); pereţii veziculei

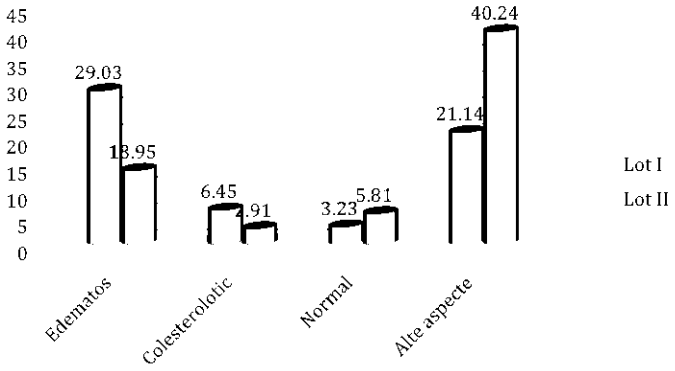

Fig. 1. Aspectul ecografic al pereţilor veziculei biliare

TABELUL III

Analiza comparativă a parametrilor ecografici în cazul pacienţilor studiaţi

Parametri ecografici	Lotul I	Lotul II	Semnificaţie statistică
Dimensiuni pereţi VB*	7,03±1,79	6,71±2,79	NS
Dimensiune minimă calculi	9,97±8,52	9,24±8,46	NS
Dimensiune maximă calculi	14,94±9,47	13,23±8,12	NS
Calcul unic	30,65%	25%	NS
Calculi multipli	69,35%	75%	NS

* VB – vezicula biliară

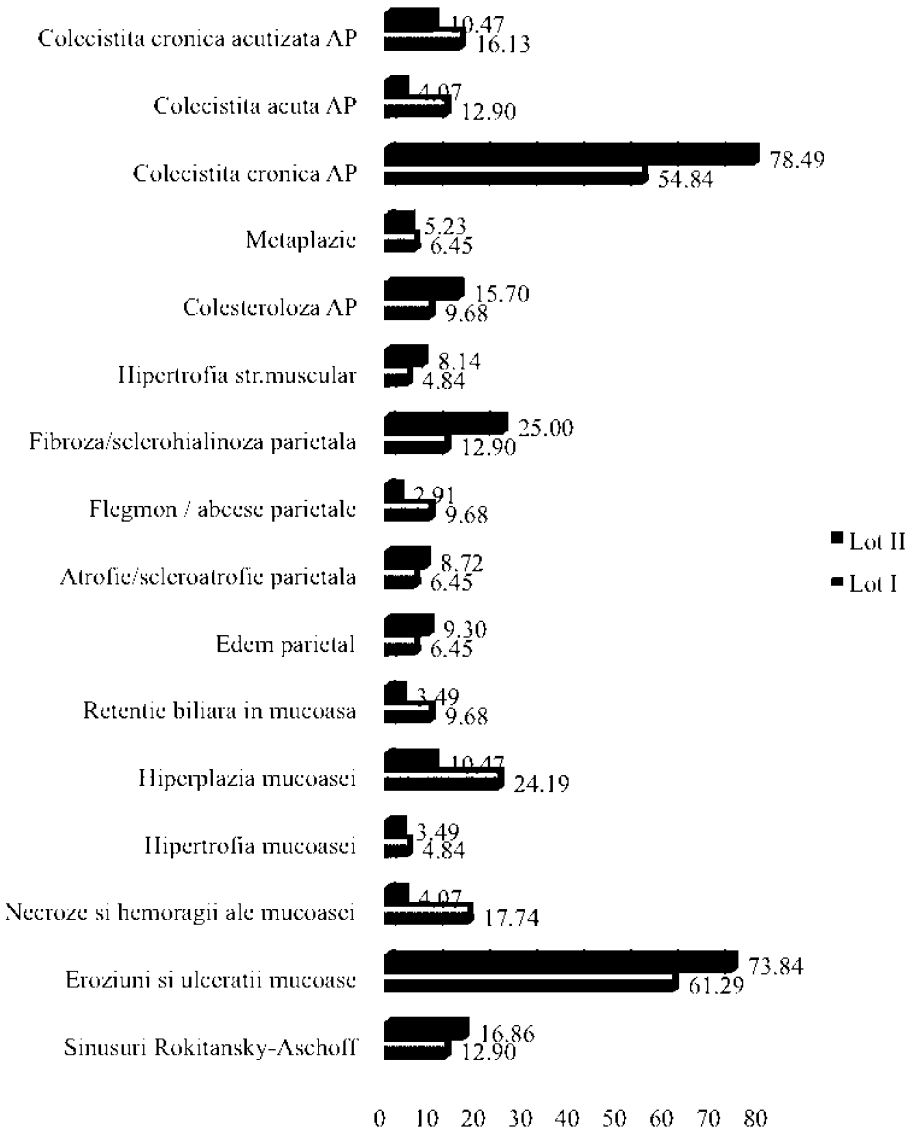

Fig. 2. Aspecte morfopatologice ale veziculei biliare la pacienţii colecistectomizaţi

biliare au avut un aspect normal ecografic la numai 3,23% dintre diabetici şi la 5,81% dintre pacienţii fără DZ (fig.1).

Calculii au avut dimensiuni extrem de variabile, de la microcalculi la dimensiuni relativ mari (20-25 mm); la 69,35% dintre pacienţii diabetici şi la 75% dintre pacienţii celui de-al doilea lot, calculii au fost multipli, fără a se înregistra diferenţe cu relevanţă statistică (tab. III).

Examenul morfopatologic al colecistului a obiectivat modificări la nivelul peretelui şi al mucoasei, majoritatea corespunzând tabloului colecistitei cronice calculoase sau unui puseu acut suprapus pe inflamaţia cronică, aceste aspecte, însumate, fiind mai frecvente la diabetici (88,96% *vs.* 70,97%); la nondiabetici au fost mai frecvente modificările de colecistită acută, abcesele parietale, necrozele şi hemoragiile mucoasei, metaplazia, fără semnificaţie statistică (fig. 2).

DISCUŢII

Analiza demografică a loturilor studiate a evidenţiat necesitatea colecistectomiei începând de la vârste medii şi predominanţa pacienţilor de sex feminin, concordant cu datele cunoscute de epidemiologie a litiazei biliare. În studiul nostru, se remarcă intervenţia chirurgicală survenită la vârste mai înaintate în cazul diabeticilor şi o tendinţă accentuată de scădere a raportului între sexe la diabetici (2,1 : 1) versus nondiabetici, la care sexul feminin predomină net (4,2 : 1); această constatare conduce la ipoteza unei echilibrări a riscului de litiază la diabeticii de ambele sexe, fie prin dezvoltarea unor factori de risc suplimentari pentru litiază, la bărbaţii diabetici, fie prin prezumţia că diabetul zaharat *per se* este un factor de risc mai puternic pentru colelitiază decât sexul feminin. Deloc surprinzător, pacienţii diabetici au avut un IMC semnificativ mai mare decât subiecţii din lotul II de studiu, obezitatea fiind puternic asociată cu colelitiaza, independent de prezenţa diabetului (2,6).

În ceea ce priveşte statusul fizic al pacientului pre-colecistectomie, diabeticii s-au încadrat majoritar în clasa ASA II (85,48%) şi 14,52% s-au încadrat în clasa ASA III, având un risc operator semnificativ crescut ($p=0,002$) faţă de nondiabetici, la care cea mai frecventă condiţie sistemică a fost hipertensiunea arterială, asociată cu un risc moderat. Riscul operator potenţial mai mare se poate explica datorită complicaţiilor sistemice multiple ale diabetului, riscului septic crescut precum şi complicaţiilor dezvoltate perioperator. În studiul nostru, vârsta mai înaintată şi obezitatea prezente la pacienţii diabetici ar putea fi factorii responsabili de diferenţa semnificativă de risc între cele două loturi, într-o măsură mai mare decât simpla evaluare a clasei ASA.

Datele din literatură privind chirurgia tractului biliar relevă un risc aproape similar la diabetici versus nondiabetici, diferenţele notabile fiind reprezentate de o rată mai mare de conversie a colecistectomiei laparoscopice la o intervenţie deschisă, de un risc mai mare de perforaţie a veziculei biliare şi de o incidenţă crescută a colecistitei gangrenoase şi emfizematoase (9,10), date neconfirmate în studiul nostru, posibil şi datorită numărului prea mic de diabetici raportat la cazurile de nondiabetici.

Parametrii biochimici determinaţi la internare au sugerat o injurie hepatocitară, exprimată prin creşterea alanin-aminotransferazei, semnificativ mai mare în lotul I (diabetici) faţă de pacienţii din lotul II. Această citoliză poate fi explicată în contextul inflamaţiei cronice veziculare de vecinătate, cu incidenţă, durată şi severitate mai mare la diabetic, în contextul unui stres oxidativ mai accentuat şi al preexistenţei steatozei hepatice metabolice, condiţie frecventă la diabetic (11,12). În acelaşi context, s-ar putea explica şi afectarea pancreatică mai pronunţată, exprimată prin creşterea amilazemiei, studii recente sugerând o incidenţă mai mare a pancreatitei acute la diabeticii

tip 2, fără a se nominaliza clar factorii cauzali ai acestei creşteri a riscului (13); în absenţa litiazei de coledoc, nu s-au înregistrat modificări semnificative ale parametrilor de colestază (bilirubina totală şi directă, fosfataza alcalină) la pacienţii studiaţi. O relevanţă mai mare decât injuria acută a ficatului ar putea-o avea determinarea seriată a acestor parametri, pentru urmărirea dinamicii lor postoperatorii; un studiu al lui Chang *et al*. a găsit această afectare ca fiind tranzitorie, pericolecistectomie, la pacienţii fără litiază de coledoc (14).

În studiul nostru, parametrii ecografici luaţi în calcul nu s-au dovedit discriminativi între cele două loturi, grosimea pereţilor colecistului, mărimea şi numărul calculilor dovedindu-se similare la cele două grupuri.

Modificările morfopatologice ale veziculei biliare se regăsesc mai ales la nivelul mucoasei, fiind prezente, cel mai frecvent, eroziuni şi ulceraţii, sinusuri Rokitansky-Aschoff, ca expresie a inflamaţiei cronice şi a obstrucţiei fluxului biliar cauzate de calculi, retenţie biliară în mucoasă, colesteroloză, hiperplazia mucoasei. În procente reduse s-au înregistrat aspecte cu grad înalt de severitate: necroze şi hemoragii, abcese sau flegmoane parietale, metaplazia mucoasei, fără ca aceste aspecte să fie mai frecvente la diabetici (15).

Absenţa unor diferenţe semnificative statistic între aspectele morfopatologice la cele două loturi dovedeşte faptul că prezenţa calculilor reprezintă o spină iritativă locală cu agresivitate similară la toţi pacienţii, diferenţele între bolnavi rezultând mai degrabă din complicaţiile sistemice ale diabetului.

Având în vedere aceste date, pare de înţeles de ce colecistectomia profilactică la toţi pacienţii diabetici, mai ales la cei asimptomatici, nu s-a demonstrat a creşte semnificativ calitatea vieţii la diabetic, ba chiar dimpotrivă (9,16).

CONCLUZII

Diabetul zaharat nu modifică fundamental tabloul clinico-paraclinic şi morfopatologic al pacienţilor cu boală litiazică veziculară. Cunoaşterea tabloului paraclinic şi a alterărilor acestuia în contextul asocierii diabetului zaharat cu litiază biliară precum şi evaluarea preoperatorie a complicaţiilor sistemice scade semnificativ riscul perioperator.

La pacienţii studiaţi, singurele elemente biochimice de alarmă au fost creşterea amilazemiei, ca răsunet al afectării pancreatice şi hepatocitoliză mai severă în cazul pacientului diabetic, ambele necesitând determinări în dinamică, pentru evaluarea reversibilităţii modificărilor.

Datele obţinute în studiul actual, coroborate cu informaţiile din literatură, sugerează că diabetul zaharat nu modifică semnificativ evoluţia clinică şi morfopatologică a litiazei biliare dar poate fi indicatorul unui risc crescut de afectare hepatopancreatică perioperator.

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NOUTĂŢI

Dovezi pentru şi împotriva profilaxiei în refluxul vezico-ureteral

Rolul profilaxiei antimicrobiene în refluxul vezico-ureteral (RVU) a devenit o cercetare în ascensiune prin folosirea celor mai bune metode analitice, prin dobândirea de cunostinţe în ceea ce priveşte RVU şi cicatricile renale diagnosticate fară a fi precedate de infecţie urinară (ITU), precum şi datorită celor mai bune metode de diagnosticare a cicatricilor renale. O meta-analiză a celor mai recente 5 studii randomizate cu un total de 809 pacienţi cu RVU diagnosticaţi după ITU relevă un risc relative a recurenţei ITU de 0,82 ş95% interval de confidenţă (CI) 0,62 – 1,08, $p=0,16$ţ cu profilaxie. O meta-analiza a 4 studii cu un total de 662 pacienţi cu ITU cu şi fără RVU evaluaţi pentru cicatrici renale a relevat un risc de 1,04 ş95% CI : 0,84- 1,30 ; $p =$ 0,69ţ cu profilaxie. Totuşi, aceste observaţii, necesita a fi interpretate cu precauţie datorită limitării acestor studii şi a eterogenităţii meta-analizelor, în particular pentru cicatricile renale. Sunt necesare mai multe cercetări pentru a valida rolul profilaxiei în RVU diagnosticat după ITU, mai multe cercetări putând garanta răspunsul la întrebarea privind profilaxia antibacteriană în legatură cu spectrul RVU în diferite circumstanţe clinice (Mattoo TK. Evidence for and against urinary prophylaxis in vesicoureteral reflux. *Ped Nephrol* 2010 ; 25 : 2379-2382).

Mihaela Munteanu

GUT MICROBIOTA - THE CINDERELLA OF NEUROLOGICAL DISORDERS

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GUT MICROBIOTA: THE CINDERELLA OF NEUROLOGICAL DISORDERS (Abstract): Gut microbiota (GM) modulates local and systemic immune mediated responses therefore its imbalance might be implicated in the pathogenesis of several human disorders. The pallet is wide including gastrointestinal diseases such as inflammatory bowel disease, irritable bowel syndrome, metabolic diseases such as metabolic syndrome, types 1 and 2 diabetes, allergic disorders and certain tumors. Regarding neurological disorders, dysbiosis is implicated in the pathogenesis of neurodegenerative conditions like Alzheimer's disease, Parkinson disease and amyotrophic lateral sclerosis. New data associate GM alterations with ischemic stroke and epilepsy. Also, a possible link between dysbiosis and myasthenia gravis is discussed. The aim was to synthetize the interaction between GM and the autoimmune system and the possible implications of this interaction in the pathogenesis of several neurological diseases, both autoimmune and non-autoimmune. These cause-effect relations open opportunities regarding potential new treatment measures. **Keywords**: GUT MICROBIOTA DYSBIOSIS, PATHOGENESIS, NEUROLOGICAL DISORDERS, MYASTHENIA GRAVIS, ISCHEMIC STROKE.

In a present time when several exogenous culprits such as antibiotics, preservatives, genetically modified food bombard the human organism through the gastro intestinal tract, gut microbiota (GM) becomes the center of attention as it modulates local and also systemic immune mediated responses. It is known that microbial colonization of the gut begins during the period of birth. The gut microbiota is distinctive of each individual, being influenced severely by numerous factors such as lifestyle, diet, environmental hygiene and metabolism, immune system of the host (1).

In the last two decades, medical publications focused on the alterations in GM, generally called dysbiosis, not only in terms of causes but also in terms of local and systemic repercussions. Many studies suggest that dysbiosis is implicated in the pathogenesis of gastrointestinal diseases such as inflammatory bowel disease, irritable bowel syndrome and *Clostridium difficile* infection (2). This is an important finding due to the fact that these diseases are frequent in the modern era. For exam-

ple, it is widely known that in the last two decades *Clostridium difficile* infection has become a prominent pathology especially among elderly hospitalized patients receiving antibiotics, with an increase in incidence and symptom severity (3, 4, 5).

GM and its alterations seem to play an important role in various diseases outside the gastrointestinal tract. Intestinal microbiota alterations appear to contribute to metabolic diseases such as metabolic syndrome, types 1 and 2 diabetes (6, 7), to allergic disorders and atopy (8) and to tumors both colorectal and extracolonic (9, 10). Regarding neurological disorders, several studies implicate dysbiosis in the pathogenesis of neurodegenerative conditions like Alzheimer's disease, Parkinson disease and amyotrophic lateral sclerosis (11).

Taking into consideration GM`s central immune functions in the gastrointestinal tract it is deducible that these functions can influence the immune system outside the gut. Actually, an increasing number of publications conclude that GM can be involved in the pathogenesis of immune-mediated, extraintestinal diseases such as thrombotic thrombocytopenic purpura and Hashimoto thyroiditis (12). Multiple sclerosis is a relevant example given the fact that both animal and clinical studies have shown its association with the intestinal microbiota (13). Following the same deductive pattern, dysbiosis may be involved in various types of autoimmune encephalitis including the one with N-methyl-D-aspartate receptor antibodies. According to Boangher, these particular antibodies can produce an individual type of autoimmune encephalitis or can be associated with *Herpes Virus* encephalitis (14).

Given the fact that Myasthenia Gravis is an autoimmune disorder caused by autoantibodies produced by autoreactive B lymphocytes and GM seems to influences immune mediated responses, one can extrapolate and assume that GM can be a key etiological factor in this intense studied yet incomplete understood disease of the neuromuscular junction.

The aim of this present article is to synthetize the interaction between GM and the autoimmune system and the possible implications of this interaction in the pathogeny of several neurological diseases, both autoimmune and non-autoimmune.

Gut microbiota –
an organ within an organ

The number of microbial entities in the GM being close to 10^{14} is almost ten times higher than the number of human cells (15). Also, its microbiome outnumbers by one hundred-fold the genes in the human genome (16). This is the picture present in the gastrointestinal tract of an adult and it is the result of diversification and multiplication that start at birth.

Information regarding GM`s composition have been emerging thanks to newer and more advanced molecular techniques. GM is practically an immense ecosystem comprised of over 1000 species of mainly anaerobic bacteria associated also with viruses, protozoa, archaea and fungi (17, 18). From the four phyla that dominate human intestinal microbiota, *Bacteroidetes*, *Actinobacteria*, and *Firmicutes* largely define GM (19).

The composition of the GM varies longitudinally and also transversely. Bacteria density increases downward the gastrointestinal tract with a peak in the colon. Transversely, certain species involved

especially in immune interaction adheres to the mucosal surface while others specialized in metabolic interactions with food are present in the lumen of the gastrointestinal tract (20, 21). Overall, GM composition varies from one individual to another.

Despite individual variations, GM is at an equilibrium which brings benefits to its host. These benefits regard metabolic, protective and trophic functions. GM influences the metabolism of not only cholesterol, polysaccharides and amino acids but also of certain medication, modulating their bioavailability (22, 23). For instance, GM contributes to fermentation of non-digestible polysaccharides into short-chain fatty acids which in turn increase in satiety (24). Also, GM produces certain vitamins as K and from the B group (25).

Pathways of interaction between gut microbiota and the central nervous system

It is well known that the gastrointestinal tract has a well-developed vegetative nervous system named the enteric nervous system which indirectly influences GM by coordinating gut motility and homeostasis (20). On the other hand, GM influences the enteric nervous system by producing many biologically active substances, including neuromodulators and neurotransmitters (26). The enteric nervous system is connected to the central nervous system via the vagus nerve. Therefore, vagus nerve can become a convenient pathway through which GM interacts with the central nervous system.

GM influences the enteric nervous system in several ways among which production of catecholamines, histamine and tryptophan is well known (25). An experimental study performed on mice concluded that normal GM influences cerebral metabolites through the microbiota-gut-brain axis and therefore it can contribute to normal brain development and to superior cortical functions such as memory and learning (27).

Another means is genetical interferences suggested by the fact that bacterial RNA, DNA, and proteins are detected in the human brain (28, 29). Also, short-chain fatty acids can produce epigenetic changes in the central nervous system by translocating in the brain and inhibiting deacetylases (25).

Overall, GM appears to influence nociceptive responses, depression, stress responsiveness, anxiety and social interaction (25). As proof of this theory, there are many publications which suggest that GM dysbiosis can be a cause of neuropsychiatric disorders such as schizophrenia, depression and autism and therefore propose as adjuvant treatment therapies which target the GM (16, 18).

Also, influence of GM on the central nervous system can partially explain Braak's theory in the development of Parkinson's disease. According to this theory, Parkinson disease neuropathology may occur first in the peripheral autonomic pathways and extend to the central autonomic pathways (30).

The influence of gut microbiota on the immune system

One of the modern definitions of human evolution is adapting changes in the human genome. The human genome is constantly interacting with the genome of commensal microorganisms, through epigenetic mechanisms. From the point of view of this "old friend hypothesis", human evolution depends on commensal flora, including GM (31).

In the last decades is has become a general belief that GM can influence the immune system of the host through its interactions with human intestinal cells and the diffuse gut associated lymphoid tissue. In physiological conditions, in the absence of a "leaky gut", GM interacts with human intestinal cells and plasma cells through Toll like receptors (32). Evidence of this immune modulation can be found in the variable IgA repertoire secreted by the intestinal plasma cells. Studies have shown that each individual harbors a private polyclonal and highly diverse IgA repertoire (33). Given the fact that intestinal IgA interacts with microbiota through several specific epitopes and regulates GM homeostasis, the IgA repertoire of a host can be considered a mirror of the actual GM composition (34).

The link between GM and the human immune system is also evident in the fact that some enteral commensal bacteria can influence some types of T lymphocytes. For example, certain species of *Clostridium* and *Bacteroides* can induce production of anti-inflammatory interleukin-10 and activation of regulatory T cells by the Toll-like-receptor pathway activation (35). In this manner, *Bacteroides fragilis*, a major GM bacterium can prevent inflammatory bowel disease. Also, *Firmicutes* can induce regulatory T cells (36). Colonic regulatory T cells can be induced also by short-chain fatty acids which are a product of GM fermentation (20). Also, GM are potent activators of innate lymphoid cells (25).

Gut microbiota and non-autoimmune neurological disorders

Several studies point that dysbiosis is a potential culprit in several neurological disorders. In Parkinson disease, although the mechanism is still unknown, it is proven that patients show a reduced population of *Prevotellaceae* in their fecal matter compared with normal patients. The overpopulation of other families of gut bacteria such as Enterobacteriaceae has been discovered in patients with postural and gait difficulty (36). Also, hypokinesia can be significantly diminished by eradicating *Helicobacter pylori* infection in parkinsonian patients, according to a randomized controlled trial (37).

In the past decade several reports suggest a link between GM composition and stroke outcome (38). The cause-effect relation seems to be mediated through two pathways. On one hand, GM modulates microglial maturation, activation and neuroinflammation through microbiota-derived short chain fatty acids (39). On the other hand, a recent theory points microbiota-dependently activated T-cells as an important pathogenic factor as these cells interact with other immunocompetent cells of the brain after an extensive ischemic lesion (38). A novel idea is a bidirectional relationship between brain and GM-immune axis. According to this, middle cerebral artery occlusions induce a specific change in the composition of GM with a decrease in diversity and an increase in Bacteroidetes phylum. In turn, gut dysbiosis increases brain lesion volume through immune-mediated mechanisms (40). The specific mechanism through which an ischemic brain lesion induces GM alterations is not completely elucidated. However, it has been proven that post ischemic gut dysbiosis are associated with reduced gastrointestinal motility and paralytic ileus, two features of autonomic disfunction (40,

41). This theory is consistent with the well-known fact that large brain ischemic lesions can induce autonomic disfunctions such as different hart rate responses depending on the side of the lesion: right hemisphere infarcts have an enhanced sympathetic control on the heart rate while left hemispheric ones have a dominant parasympathetic control (42, 43).

Epilepsy, one of the most frequent and stigmatizing neurological disorders has recently been linked to autoimmune dysregulations (44, 45). Due to the fact that GM significantly influences responses of the human immune system, both innate and adaptive it is a logical deduction that dysbiosis might be involved in the pathogenesis of idiopathic seizure disorders. According to some experts, a particular high count of *segmented filamentous bacteria* in the GM causes systemic proliferation of T helper 17 lymphocytes which in turn increases the level of interleukin 17-A in the brain. Apparently, high concentration of this cytokine in the cerebral spinal fluid or peripheral blood is correlated with increased seizure frequency and severity (46). Due to the fact that current antiepileptic drugs are quite limited in number and have numerous side effects, one can easily deduce the importance of new therapeutic alternatives, including recolonizing GM (47, 48).

Does gut microbiota contribute to development of neurological autoimmune disorders?

The complex link between GM and human immune system is incompletely understood. So is the etiology of many autoimmune disorders. However, there is evidence of the involvement of GM dysbiosis in the pathogeny of systemic autoimmune disorders such as thrombotic thrombocytopenic purpura, arthritis, systemic lupus erythematosus, Sjogren's syndrome and Hashimoto thyroiditis (12, 49).

The present tendency is to extrapolate the role of GM dysbiosis in the pathogenesis of neurological autoimmune disorders like multiple sclerosis. From an immunopathogenic point of view, multiple sclerosis is considered to be mediated by T lymphocytes and dependent on B lymphocytes: autoreactive T helper cells that have escaped thymic selection infiltrate the central nervous system where they produce inflammatory reactions and activate B lymphocytes (50). These cells along with autoreactive T cytotoxic cells appear to be responsible of the characteristic lesions in the central nervous system. In addition, pro-inflammatory mediators produced by astrocytes and microglia sustain continuous CNS inflammation which promotes gradual neurodegeneration (25). Given the fact that GM influences blood–brain barrier integrity and microglia maturation which are key factors in multiple sclerosis, it is only logically to consider that GM can be involved in this autoimmune neurological disease (51, 52).

Another well-known autoimmune neurological disorder is myasthenia gravis which is mediated by B lymphocyte and dependent on T lymphocyte (53). Myasthenia gravis is an extremely heterogenic disorder which can be classified using both clinical and serological criteria. Myasthenia gravis with antibodies against acetylcholine receptor is the most frequent given the fact that these antibodies can be detected in 85% of patients with generalized clinical form and in 40% of patients with the ocular form (54). This serological subtype of the

disease is caused by synthesis of autoreactive T helper cells within a thymus that lacks normal negative selection. This abnormal T lymphocytes cross- react with acetylcholine receptors situated on the postsynaptic component of the neuromuscular junction. Also there seems to be an alteration in the functionality of T suppressor lymphocytes which will fail to eliminate the autoreactive T effector cells (55).

Although its pathogenesis is well known, the etiology of myasthenia gravis remains partially obscure. Overall it is a multifactorial disease in which various exogenous factors bombard a human organism genetically predisposed to autoimmunity. The genetic predisposition is suggested by the fact myasthenia occurs in 35% of monozygotic twins whereas in heterozygous twins occurs only in 4-5% (22). Also, associations between certain myasthenic subtypes and different Human Leukocyte Antigens complex alleles, with significant differences between Caucasians and Asians indicates genetic implications (56).

Even though there is important evidence of genetic involvement, genetic mechanisms alone do not explain the complexity of myasthenia gravis. Taking into consideration on one side its immunopathogenesis and on the other side, the influence of GM on several T cell types and innate lymphoid cells, one can deduce that there might be a link between GM and myasthenia gravis.

Fecal microbiota transplantation and its role in neurological disorders

Some recent studies describe an important role in restoration of gut microbiota after fecal microbiota transplantation (FMT) and an improvement in some neurological and psychiatric disorders (57). The evidence is based on the modulation process of the gut-brain axis (58).

Recent report in literature relate to FMT as an important role in a widely neurologic and psychiatric disorders such as Parkinson's disease, fatigue, multiple sclerosis and autism (59).

FMT was used in patients with dysbiosis and Clostridium difficile refractory colitis, the technique consists in infusion of feces from a healthy donor in patients gastrointestinal tract (60). Now FMT is used for treatment in multiple sclerosis and autism.

Taking all into consideration, despite the fact that GM has been intensively studied throughout the years there still remains unknown information regarding its influence upon other organs outside the gastrointestinal tract. As knew information regarding its functions emerge, knew pathogenic hypothesis are tested. We are witnessing a tendency to involve GM and its dysbiosis in many autoimmune diseases including neurological ones as multiple sclerosis. Regarding myasthenia gravis and GM there is much uncharted territory, but immunomodulation seems to be the key.

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NEWS

DIGITAL BREAST TOMOSYNTHESIS: A THREE-DIMENSIONAL PICTURE OF THE BREAST

Digital breast tomosynthesis (DBT) creates a 3-dimensional picture of the breast using X-rays.. Digital tomosynthesis of the breast is different from a standard mammogram in the same way a CT scan of the chest is different from a standard chest X-ray. Mammography usually takes two views of each breast: cranio-caudal (CC) and oblique (MLO) view. Classic mammography records the pictures on film and digital mammography records the pictures in the computer. Mammograms are very good, but they have a significant limitation: the breast tissue and the pathology are overlapped, so breast cancer can be hidden in the overlapping breast tissue. Digital tomosynthesis overcomes this issue by taking multiple slices of each breast in CC and MLO projections; in DBT the X-ray tube makes an arc, during which a series of images is acquired; the acquisition time is 10-20 sec, slice thickness is 1mm and the images can be displayed in different ways (single slice, slab, cine loop. There is a great variability in the number of the acquired slices (between 13 and 25) and the acquisition angle (between 15 ° and 50 °).Tomosynthesis does not provide direct projection images, but reconstructed images of the individual layers through several available algorithms, more or less efficient, each aimed to remove from reconstructed slice the upper and lower layers. Because is relatively new and its availability is low, this method of examination is not yet used for breast cancer screening , but researchers believe that this new breast imaging technique will make breast cancers easier to see in dense breasts.

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RISCUL CARDIOVASCULAR AL HALOPERIDOLULUI VERSUS ANTIPSIHOTICE ATIPICE ÎN TERAPIA SCHIZOFRENIEI

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CARDIOVASCULAR RISK OF HALOPERIDOL VS. ATYPICAL ANTI-PSYCHOTIC DRUGS IN SCHIZOPHRENIA TREATMENT (Abstract): The present study examined the safety of the atypical antipsychotic drugs sertindol, olanzapine and quetiapine used in the treatment of schizophrenia. Haloperidol, a typical antipsychotic drug, was used for comparison. These data may account for the different therapeutic effects and side-effect profiles (cardiovascular risk) of typical and atypical antipsychotic drugs in schizophrenia. **Key words**: SERTINDOL, OLANZAPINE, QUETIAPINE, QTC

Medicaţia antipsihotică a reprezentat mult timp principalul remediu terapeutic în schizofrenie, odată cu introducerea clorpromazinei şi descoperirea ulterioară a diferitelor substanţe antipsihotice (1). În prezent există un număr impresionant de antipsihotice convenţionale al căror mecanism de acţiune constă în blocarea postsinaptică a receptorilor dopaminici D2, blocarea în diferite grade a receptorilor noradrenergici, colinergici şi histaminici (2). Prin această acţiune se realizează efectele terapeutice în faza acută şi în faza cronică, dar şi o serie de efecte secundare, care limitează eficienţa terapeutică. Noua generaţie de antipsihotice a fost completată prin lansarea olanzapinei, quetipinei, risperidonei, sertindole, zotipine (3,4). Aceste noi antipsihotice acţionează prin blocarea receptorilor D2 şi a receptorilor serotoninici de tip 2A la nivel frontal şi în sistemul striat, fapt care conduce la scăderea efectelor secundare extrapiramidale.

OBIECTIVE

Obiectivul acestui studiu este de a compara profilul de siguranţă al haloperidolului cu olanzapina, sertindolul şi quetiapina, în ceea ce priveşte efectul aritmogen al neurolepticelor prin creşterea intervalului QTc (5).

MATERIAL ŞI METODĂ

Studiul a constat în înrolarea, evaluarea, urmărirea clinico-terapeutică a 60 de pacienţi internaţi în cadrul Spitalului Clinic de Psihiatrie "Socola" între anii 2008-2009.

Pacienţii au fost împărţiţi în 4 subloturi egale în funcţie de medicaţia primită:

- LOT 1 - Haloperidol;
- LOT 2 - Olanzapină;
- LOT 3 - Sertindol;
- LOT 4 - Quetiapină

Criteriile de includere pentru acest studiu au fost:

- bărbaţi sau femei cu vârsta cuprinsă între 18 şi 65 de ani;
- diagnosticaţi cu schizofrenie (orice subtip) conform ICD-10 de cel puţin 1 an;
- să aibă un scor PANSS >70;
- CGI-S $=4$;
- traseu ECG (12 derivaţii) cu aspect mor-

fologic normal ;
- au semnat consimţământul informat după ce li s-a explicat scopul şi procedurile cerute de protocol ;
- capacitate de înţelegere.

Criteriile de excludere sunt enumerate mai jos :
- dacă erau diagnosticaţi cu sindrom de dependenţă la alcool sau la alte substanţe ;
- internaţi împotriva voinţei lor ;
- femeie însărcinată sau care alăptează ;
- istoric de alergie la haloperidol, olanzapina, sertindol şi quetiapina sau la excipienţii acestora ;
- istoric de rezultate pozitive la testele serologice pentru virusul hepatitei B, C şi HIV;
- istoric de sindrom neuroleptic malign ;
- istoric de glaucom cu unghi închis ;
- orice afecţiune somatică neechilibrată ;
- leucopenie ;
- orice probleme cardiovasculare, respiratorii, neurologice, renale, hepatice, endocrine, hematologice sau imunologice netratate ;
- istoric de malignitate în ultimii 5 ani ;
- diskinezie moderată sau severă în timpul înrolării ;
- ideaţie suicidară sau tendinţe violente în perioada de titrare a medicaţiei ;
- lipsa eficacităţii uneia dintre substanţele cercetate în antecedente.

Design-ul studiului

Design-ul studiului a fost structurat astfel : vizite săptămânale în prima lună, apoi vizitele au fost lunare până la 1 an. Retragerea pe parcursul studiului s-a făcut în urma :
- deciziei medicului în cazul unor evenimente adverse severe sau modificări clinic semnificative a unor parametri de laborator ;
- deciziei pacientului ;
- modificării sau întreruperii medicaţiei de studiu, indiferent de motiv (absenţa eficacităţii, solicitarea pacientului, sarcină etc.).

Perioada de studiu I : screening-ul

Vizita 1 (V1) (durata : 2 zile) Rezultatele testelor screening trebuie obţinute şi interpretate înainte de cea de a doua vizită (V2).

Pentru ca subiectul să poată continua participarea în studiu, a fost necesar ca toate criteriile de înrolare să fie îndeplinite la V2. Nu a fost obligatorie finalizarea testelor într-o singură zi. Pentru completarea tuturor procedurilor pot fi alocate două zile.

După V1 se va întrerupe orice medicaţie cu efect psihotrop ; invitarea pacientului de a lua parte la acest studiu şi semnarea consimţământului informat ; consemnarea datelor de identificare ; diagnosticul ; antecedentele personale patologice ; antecedentele heredo-colaterale ; anamneza socio-profesională ; verificarea parametrilor vitali (tensiune arterială, puls, temperatură) ; măsurarea taliei şi a greutăţii ; înregistrarea ECG ; test de sarcină (pentru femei) ; examene de laborator (ionogramă, glicemie, hemogramă, TGP, TGO, colesterol, uree, creatinină).

Perioada de studiu II

Pe parcursul acestei faze pacienţii au primit haloperidol 5 - 10 mg/zi, sertindol 12- 16 mg/zi, olanzapină 5 - 20 mg/zi, quetiapină 600-800 mg/zi. Doza zilnică eficace a fost stabilită prin titrare. Înregistrarea ECG a fost realizată la fiecare vizită.

Analiza statistică

Deoarece numărul de subiecţi este redus, eşantioanele sunt mici, repartiţia caracteristicilor studiate este normală, iar de la o vizită la alta scade numărul pacienţilor din eşantion, am aplicat testul *t-Student*. Testul reprezintă pragul ce separă descriptivul de începutul cercetării analitice.

Datele au fost introduse şi prelucrate cu ajutorul funcţiilor statistice din *Microsoft Excel* (Microsoft Corp.) şi pachetul de analiză statistică *SPSS 14.0* (SPSS Inc.).

Rezultatele sunt exprimate ca media plus sau minus deviaţia standard (DS). S-a considerat pragul de semnificaţie statistică p(Sig.) $< 0,05$ (fig. 1).

Din anii ’60, când au fost raportate primele cazuri de morţi subite la pacienţi aflaţi

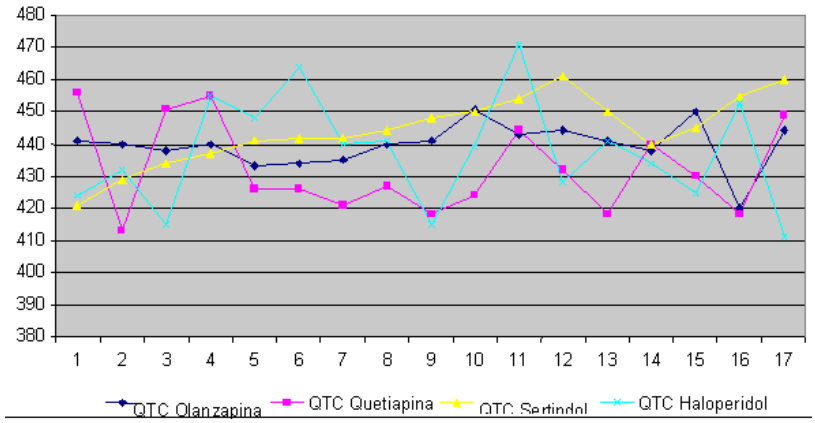

Fig. 1. Monitorizarea ECG pe parcursul studiului (variaţia intervalului QTc la cele patru antipsihotice)

în tratament cu antipsihotice, a existat o preocupare crescândă a comunităţii psihiatrice în legătură cu efectele adverse cardiovasculare ale antipsihoticelor. În anii ’90 a devenit clar că mesoridazina, un antipsihotic convenţional, poate provoca torsada vârfurilor (6).

Torsada vârfurilor reprezintă un tip specific de tahicardie ventriculară, caracterizată printr-un aspect polimorf al complexelor QRS, care variază în amplitudine şi în lungimea ciclului, creând aspectul unor oscilaţii în jurul liniei de bază. Acest ritm este precedat de o prelungire marcată a intervalului QT, adesea >600 ms. Intervalul QT, măsurabil pe ECG, include atât durata depolarizării, cât şi a repolarizării ventriculare şi variază invers proporţional cu frecvenţa cardiacă. Intervalul QT corectat în funcţie de frecvenţa cardiacă se calculează conform formulei QTc = QT/RR 0,5. Torsada vârfurilor poate progresa către fibrilaţie ventriculară şi moarte subită. Prelungirea intervalului QTc >450 ms sau o creştere cu >10% a intervalului QTc faţă de momentul iniţierii terapiei cu antipsihotice sunt privite ca indicatori ai unui risc crescut de apariţie a torsadei vârfurilor (7,8).

Din acest motiv a fost realizată monitorizarea ECG pe parcursul studiului, asigurând un profil de siguranţă din punct de vedere cardiovascular pentru pacienţii înrolaţi. Se observă că cea mai mare creştere a intervalului QTc a fost înregistrată în cazul haloperidolului la vizita 11 (471 ms). Sertindolul determină o creştere a intervalului QTc până la vizita 12, apoi urmează o scădere a acestor valori până la finalul protocolului unde înregistrăm din nou o creştere a valorilor. În ceea ce priveşte quetiapina, s-au raportat cazuri rare de prelungire a intervalului QTc, în timpul desfăşurării protocolului. Acestea au fost înregistrate la doi pacienţi din acest lot care şi-au administrat doze mai mari decât cele prevăzute în protocol.

În concluzie, toate antipsihoticele atipice au fost asociate cu o uşoară prelungire a intervalului QTc. Această prelungire nu s-a constituit într-un risc veritabil pentru pacient, din punct de vedere vital, ceea ce ne îndreptăţeşte să utilizăm cu încredere medicaţia antipsihotică. Global, beneficiile sunt semnificativ superioare, pacientul funcţionează, este reintegrat, motiv pentru care riscul cardiogen îl considerăm poate exagerat.

Prudenţa este oportună în prescrierea medicaţiei antipsihotice. Excesul de prudenţă defavorizează gestionarea cazului. Nu trebuie să uităm că tratamentul în schizofrenie este cronic şi obligatoriu va fi indicat un antipsihotic. Toate ghidurile recomandă

în prima linie un antipsihotic atipic, iar cele utilizate în studiul nostru fac parte din protocolul obişnuit de tratament. De asemenea, trebuie să reţinem că nici un antipsihotic folosit nu a fost asociat cu torsada vârfurilor.

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NOUTĂŢI

ANOMALII CITOGENETICE DECELATE LA PACIENŢII CU AZOOSPERMIE NON-OBSTRUCTIVĂ ŞI OLIGOSPERMIE SEVERĂ

Koşar şi Ozcelik au analizat 115 bărbaţi cu sterilitate (92 cu azoospermie şi 23 cu oligospermie severă) care au fost investigaţi citogenetic - înaintea utilizării unei tehnici de reproducere asistată – la Spitalul din Isparta, Turcia. A fost utilizat şi un grup martor constituit din 60 de bărbaţi cu ferilitate normală. Analiza cromozomială, efectuată la toţi pacienţii, s-a bazat pe culturi de limfocite din sângele periferic. De asemenea, la toţi pacienţii, au fost efectuate teste hormonale (LH, FSH, testosteron şi prolactina) şi ecografie testiculară. Prevalenţa anomaliilor cromozomiale decelate a fost de 4,3% (5/115) şi a inclus 4 cazuri cu sindrom Klinefelter şi 2 cazuri cu disgenezie testiculară, 46,XX. Toate anomaliile au fost decelate la pacienţi cu azoospermie (5,4% - 5/92). Bărbaţii cu oligospermie severă şi cei din lotul martor nu au prezentat anomalii cromozomiale. A existat o diferenţă semnificativă între valorile serice ale FSH, LH şi ale volumului mediu testicular la pacienţii cu azoospermie şi oligospermie severă, comparativ cu lotul martor ($p < 0,05$), precum şi între pacienţii cu azoospermie şi cei cu oligospermie ($p < 0,05$). Frecvenţa crescută a anomaliilor cromozomiale decelate la bărbaţii cu sterilitate, sugerează necesitatea efectuării de rutină a analizei cromozomiale şi a acordării sfatului genetic, în cazul acestor pacienţi, înaintea aplicării unei tehnici de reproducere asistată (Koşar PA, Ozçelik N, Koşar A. Cytogenetic abnormalities detected in patients with nonsobstructive azoospermia and severe oligozoospermia. *J Assist Reprod Genet* 2010 ; 27 (1) : 17-21).

Lăcrămioara Butnariu

LONG-TERM SURVIVAL IN HER2-POSITIVE BREAST CANCER WITH BRAIN METASTASES. CASE REPORT

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LONG-TERM SURVIVAL IN HER2-POSITIVE BREAST CANCER WITH BRAIN METASTASES. CASE REPORT (Abstract): We report the case of a 41-year-old woman diagnosed in October 2014 with bone-only metastatic HER2-positive breast cancer, who developed brain metastases (BM) 15 months after the initial cancer diagnosis. The patient initially received palliative chemotherapy with Paclitaxel, discontinued due to allergic reactions, and targeted therapy with Herceptin. BM were treated with antalgic radiotherapy and bisphosphonates. After the occurrence of secondary brain lesions, the patient underwent palliative whole-brain radiotherapy (WBRT) and systemic treatment with Capecitabine and Lapatinib from February 2016. In February 2018, Lapatinib was replaced with Trastuzumab emtansine, after the approval of this drug in Romania. Due to the deterioration of patient's clinical state, in October 2018 the administration of Trastuzumab emtansine was postponed, the patient received symptomatic treatment until she died 49 months after the diagnosis and 34 months after the occurrence of BM, respectively. The authors report this case in order to illustrate the importance of the multidisciplinary management of BM that had substantially improved the neurological symptoms and survival in this patient. Early recognition and appropriate therapy of breast cancer is imperative as it may be crucial for the outcome. **Keywords**: BREAST CANCER, BRAIN METASTASES, RADIOTHERAPY, TARGETED THERAPY.

Brain metastases (BM) are a significant cause of mortality in cancer patients, 20% to 40% of cancer patients will develop BM during their illness (1). The primary tumors that usually cause BM are lung cancer in 50% of cases, followed by breast cancer in 15-25% and melanoma in 5-20% (2).

Currently, about 20%-45% of patients with Her2-positive metastatic breast cancer will develop parenchymal BM (3). Moreover, in the case of Her2-positive cancers the extracerebral disease is controlled in 50% of cases at the diagnosis of BM.

Treatment for BM includes surgical resection, followed by stereotactic radiosurgery (SRS) or WBRT in a limited number of lesions. However, the optimal treatment remains controversial (4).

CASE REPORT

On October 2014, a 41-year-old obese, nulliparous woman presented at the Regional Institute of Oncology Iasi with a mass in her right breast, without palpable axillary nodes.

She had no significant past medical or family history and was not taking any medication. She admitted to drinking occasionally at social events but denied having ever smoked.

Mammography of her right breast (fig. 1) revealed a 1.8/1cm mass with associated microcalcifications.

A core-needle biopsy was taken from the lesion and highlighted an invasive ductal carcinoma overexpressing HER-2 (immunohistochemical score 3+) with low positive estrogen and progesterone receptors. Furthermore, in this case the Ki-67 index was extremely high, 60%.

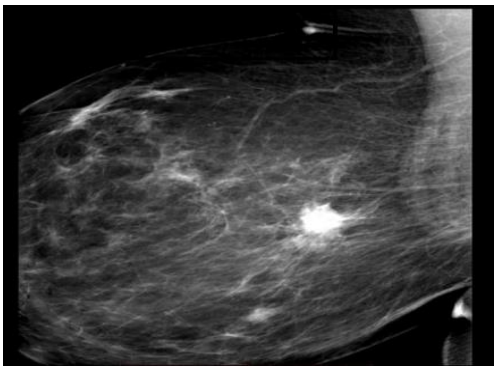

Fig. 1. Right mediolateral oblique view on screening mammogram revealed a focal asymmetry in the right lower outer quadrant with microcalcifications.

The initial chest-abdomen-pelvis computed tomography (CT) examination revealed an abnormal grow thin the lower outer quadrant of the right breast with extension to the aponeurosis of the pectoral muscle (T2), bone metastases in different sites (sternum, lumbar spine L2-L6, sacrum and bilateral femoral), multiple metastatic adenopathies (right axillary lymph nodes, ipsilateral internal mammary nodes, mediastinal and bilateral hilar lymphadenopathy) (N2). Clinical disease stage was assessed as cT2N2M1, stage IV.

Lactate dehydrogenase and alkaline phosphatase levels were elevated.

Serum study showed a normal level of cancer antigen 15-3 (15 U/mL).

Following the oncology consultation, palliative chemotherapy was started. Treatment with Paclitaxel (175 mg/m^2 every three weeks), discontinued in January 2015 due to third degree peripheral neuropathy, and targeted therapy with Trastuzumab (8 mg/kg iv loading dose, followed by 6 mg/kg every three weeks), from January 2015 to June 2017 were initiated.

For the bone metastases the patient received antalgic radiotherapy and treatment with 4 mg zoledronic acid every 3 weeks.

In January 2016 the patient was admitted for progressively worsening headaches and vomiting. A brain scan revealed multiple secondary parenchymal brain and cerebellar lesions (fig. 2).

WBRT (20 Gy / 5 fractions / 4 Gy / fraction) was performed. The neurological symptoms subsided very quickly. Otherwise, staging exams showed that all bone lesions were stable, and the primary tumor had not increased in size.

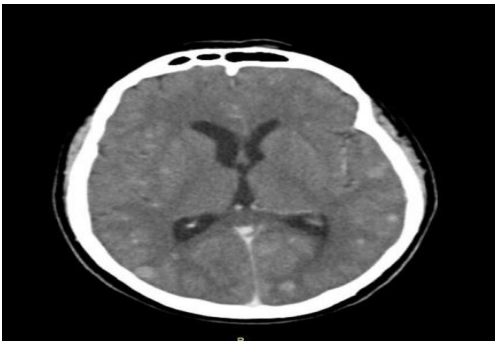

Fig. 2. CT scan of the brain showed multiple brain metastases.

Treatment with capecitabine 2,000 mg/m^2/ day (days 1-14) and lapatinib 1,250 mg/day was then initiated in February 2016. In December 2017, the patient underwent a whole-body CT scan that revealed the progression of brain metastases.

In February 2018, Lapatinib was replaced with Trastuzumab emtansine (3.6 mg/kg, every three weeks) following its approval in Romania. The therapy was well

tolerated without serious adverse events.

The patient's cardiac function was evaluated from the beginning of treatment by echocardiography once every 3 months. The first measurement of the Left ventricular (LVEF) was 65% and has been maintained during the treatment.

In October 2018 the patient presented a rapid and significant decline in general health status despite intensive symptomatic therapy. Unfortunately, our patient did not recover from her moribund neurological state and died several weeks later.

DISCUSSION

In this case, the objective of the multidisciplinary team in the management of BM was twofold: survival and quality of life.

HER2-positive tumors occur in 25% of all breast cancers (5). The human epidermal growth factor receptor 2 (HER2/ErbB2) is a member of the ErbB family of tyrosine kinase receptors which regulates normal cell growth. Dysregulated HER-2 activation supports tumorigenesis via cell proliferation, migration, survival, and angiogenesis (6). The risk of developing BM is higher among patients with overexpression of HER2-positive (7).

Median time from breast cancer diagnosis to BM is about 3 years with the longest reported interval of 97 months (8). Our patient developed BM 15 months after the initial diagnosis of breast cancer.

Bones are the most common secondary site of metastatic breast cancer, followed by the lungs, brain and liver (9, 10). Our patient was initially diagnosed with bone-only metastatic breast cancer that had later spread to the brain. Curiously, the patient did not develop other metastatic sites and the already present bone metastases remained stable, showing no progression.

In case of BM, the blood-brain barrier undergoes alterations around the metastatic sites. Radiotherapy can be used to increase the permeability of the blood-brain barrier and to favor the passage of various chemotherapeutic agents.

Ogawa *et al.* (11) reported that in 45 patients with breast cancer and BM, improvements in neurological symptoms were obtained in 35 patients (77.8%) using palliative radiotherapy without systemic chemotherapy. Therefore, the therapeutic strategy focused on BM allows the patient to regain a good quality of life.

Lapatinib, a dual inhibitor of the intracellular tyrosine kinase domain of HER1 and HER2, is expected to cross the blood brain barrier due to its very low molecular weight. Its efficacy has been investigated in combination with capecitabine in patients with previously untreated brain metastases from HER2-positive metastatic breast cancer (Landscape study) (12).

In the EMILIA study (13), Trastuzumab emtansine was compared with lapatinib plus capecitabine in advanced HER2- positive metastatic breast cancer pre-treated with Trastuzumab. Trastuzumab emtansine prolongs overall survival in patients with pre-existing BM (26.8 *vs.* 12.9 months, p=0.008).

Performance status (PS) is a strong independent prognostic factor for survival in breast cancer patients with BM (14). Throughout the disease course, our patient had a relatively good quality of life, the ECOG score being 1-2. Quality of life has been one of our objectives.

CONCLUSIONS

In this case, despite the unfavorable prognosis of breast cancer with BM, the multidisciplinary management had substantially improved the neurological symptoms and survival of the patient.

Thus, the patient died 49 months after

the initial diagnosis and 34 months after the occurrence of BM, respectively. Early detection of breast cancer remains the primary defense to prevent the development of this life-threatening disease.

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CORRELATIONS BETWEEN IADL SCALE AND CLINICAL PARAMETERS IN SEVERE-TO-VERY SEVERE COPD PATIENTS

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CORRELATIONS BETWEEN IADL SCALE AND CLINICAL PARAMETERS IN SEVERE-TO-VERY SEVERE COPD PATIENTS (Abstract): **Aim**: To identify the clinical parameters correlated with the Lawton Instrumental Activities of Daily Living (IADL) scale in severe-to-very severe COPD patients, admitted for an inpatient pulmonary rehabilitation program (PRP). **Material and methods**: We conducted a retrospective analytical study in 84 patients with GOLD 3 and GOLD 4 stage COPD, consecutively admitted to Iasi Pulmonary Rehabilitation Clinic, from January 2016 to December 2017, for an inpatient PRP. In addition to spirometry, the following functional evaluations were carried out: Lawton Instrumental Activities of Daily Living (IADL) scale, dyspnea assessment (on Modified Medical Research Council - mMRC and Borg scale), and COPD Assessment Test (CAT). **Results**: The study included 84 COPD patients, mean age 66.98±10.06 years and mean FEV_1 37.04±9.03% of predicted value. Mean values of FEV_1, dyspnea score on mMRC ($p=0.01$), and Borg scale ($p<0.001$) were significantly lower in GOLD 4 stage COPD. Median values of CAT and IADL scores were comparable, irrespective of gender or severity of COPD. There were negative correlations between IADL score and age ($r = -0.25$; $p=0.02$), mMRC dyspnea score ($r = -0.46$; $p<0.001$), Borg dyspnea score ($r = -0.412$; $p<0.001$), and CAT score ($r = -0.427$; $p<0.001$). In multivariate analysis, only age and the mMRC score remained significantly associated with IADL score. **Conclusions**: Pulmonary rehabilitation should be tailored for COPD patients with cognitive impairment, according to the severity of the disease and its assessment should be incorporated as a clinical outcome in the management of this chronic condition. **Keywords**: CHRONIC OBSTRUCTIVE PULMONARY DISEASE, PULMONARY REHABILITATION, IADL.

Chronic obstructive pulmonary disease (COPD) is a multi-systemic disease and a leading cause of morbidity and mortality worldwide. Self-management of this chronic condition as well as enrollment in pulmonary comprehensive rehabilitation programs are fundamental to coping with this invalidating disease.

Cognitive impairment and psychiatric disorders are well-recognized co-morbidities of COPD, with a high prevalence of 32% (1, 2). Neurotoxicity and multiple cerebral insults related to smoking, occupational exposure, hypoxia, and

inflammation lead to deficits in cognitive domains of memory, attention, motor and executive functions (3, 4, 5).

Current therapeutic strategy in COPD, according to the guidelines, requires the active involvement of the patients in smoking cessation, physical exercise, and pulmonary rehabilitation (6-10). Evaluation of cognitive abilities and disabilities may predict the amplitude of behavioral changes.

Lawton's Instrumental Activities of Daily Living (IADL) Scale was developed to assess the activities used for functioning in community settings, named "instrumental activities of daily living" (IADLs), unlike basic activities, like bathing, eating, toileting, named "activities of daily living" (ADLs) (11). The capacity to handle these more complex activities is lost before basic activities, so it allows identification of functional decline or signals the need for further assessment in older adults or other individuals, like those with chronic invalidating diseases, as COPD (11, 12).

The aim of our study was to identify the clinical parameters correlated with the Lawton Instrumental Activities of Daily Living (IADL) scale in severe-to-very severe COPD patients, admitted for an inpatient pulmonary rehabilitation program.

MATERIAL AND METHODS

We conducted a retrospective analytical study, in 84 patients with severe-to-very severe COPD, consecutively admitted to Iasi Pulmonary Rehabilitation Clinic, from January 2016 to December 2017, for an inpatient pulmonary rehabilitation program. Data were extracted from the medical records, ensuring security and confidentiality, in accordance with current regulations.

The inclusion criteria were the diagnosis of COPD, meeting the GOLD criteria for diagnosis and classification of severity, corresponding to stage 3 or 4 of the disease: suggestive symptoms (dyspnea, chronic cough, sputum production, history of exposure to risk factors for the disease), the presence of a post-bronchodilator $FEV_1 < 0.70$, and a $30\% \leq FEV_1 < 50\%$ predicted for GOLD 3 stage and $FEV_1 < 30\%$ predicted for GOLD 4 stage (13). Exclusion criteria were the diagnosis of mild-to-moderate COPD (GOLD 1 and GOLD 2 stage) and patients with missing values that might affect statistical results.

In addition to spirometry, the following functional evaluations were carried out: Lawton Instrumental Activities of Daily Living (IADL) scale, dyspnea assessment (on Modified Medical Research Council - mMRC and Borg scale), and COPD Assessment Test (CAT) (14).

The Lawton IADL scale (11) contains 8 items, measuring eight domains, rated from 0 (low functioning) to 8 (high functioning) and was administrated by a written questionnaire; scale took approximately 10 to 15 minutes to provide the answers, in the presence of a nurse, if additional explanations were required.

All subjects completed the CAT, consisting of 8 items, graded from 0 to 5 and designed to measure the global impact of COPD on a patient's life, with the range of CAT score from 0-40 (10, 14). The severity of dyspnea was assessed on mMRC and Borg scale of perceived exertion, which runs from 0-5, and 0-10, respectively.

Statistical analysis. The data were processed using *SPSS 16.0* software (SPSS Inc., Chicago, IL) and *Microsoft Excel* 2016 for *Windows*. The parameters and their frequencies were analyzed and the statistical significance of differences between groups (according to gender and

GOLD stage of COPD) was assessed by Student *t-test* for parametric variables and Kruskal-Wallis H or Sign-test for ordinal and nominal variables. Spearman's rank correlation coefficient was used for investigating the relationship between variables. The significance was set to a p value of 0.05 or less.

RESULTS

The study included 84 COPD patients, mean age 66.98±10.06 years, with the predominance of male gender (68 male patients - 81%), and mean FEV_1 37.04±9.03% of predicted value. Distribution according to the GOLD stage and gender shows no significant difference according to GOLD stage (45 patients stage 3 *vs.* 39 patients stage 4; p=NS) (tab. I).

TABLE I

Distribution of cases according to the GOLD stage of COPD and gender

Gender	GOLD 3	GOLD 4
Female (no pts.)	12	4
Male (no pts.)	33	35
TOTAL	45 patients	39 patients

Assessment of dyspnea on revised Borg scale allowed patients to subjectively rate their level of exertion and revealed aggregation of cases (55 patients, 65.47%) in the high severity area of perceived exertion, from 7 (very severe) to 10 (maximal) (fig. 1).

mMRC Dyspnea Scale assesses baseline functional impairment of individuals with COPD and 73 patients (86.9%) had 4 and 5 degrees of severity (fig. 2). There were significant differences between the two severity groups regarding dyspnea score on mMRC (p=0.01) and Borg scale (p<0.001), favoring stage GOLD 3.

Calculation of IADL score and the distribution of cases according to the results has shown surprising results, more than half of the cases (50 patients, 59.52%) having scores ranging from 6 to 8 (tab. II).

Mean values of FEV1 were significantly lower in GOLD 4 stage COPD as well as dyspnea score on mMRC (p=0.01) and Borg scale (p<0.001) but comparable between diseased men and women. Median values of CAT score and IADL score were comparable, irrespective of gender or severity of COPD (tab. III).

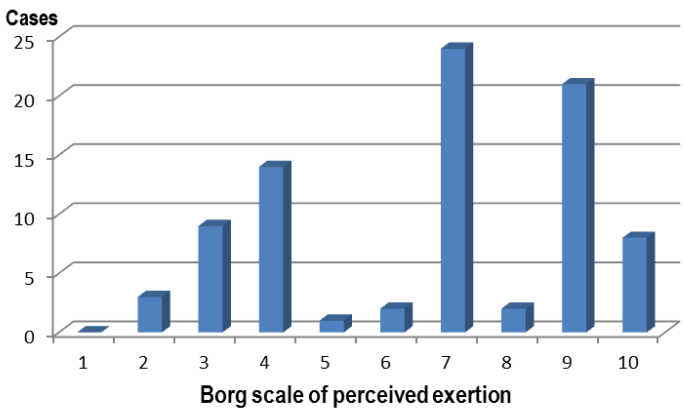

Fig. 1. Distribution of cases according to the severity of dyspnea on Borg scale

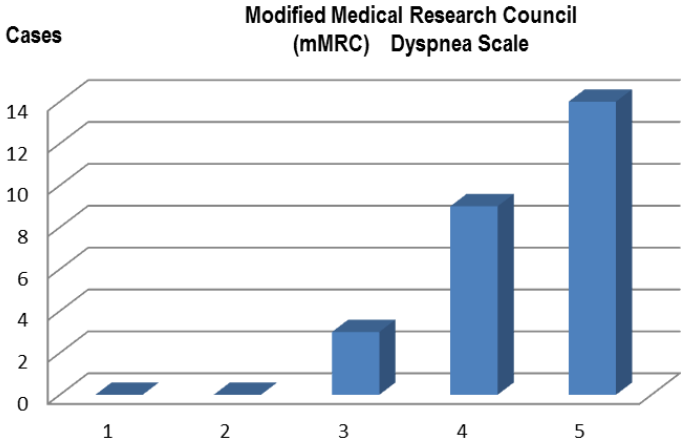

Fig. 2. Severity of dyspnea on mMRC scale

TABLE II

Distribution of cases according to total IADL score and GOLD stage of COPD

	IADL score (total)								
GOLD stage (no pts.)	0	1	2	3	4	5	6	7	8
GOLD 3	0	0	2	2	6	4	15	4	12
GOLD 4	2	3	3	4	3	5	9	5	5
Total patients	2	3	5	6	9	9	24	9	17

TABLE III

Mean values of FEV_1 according to gender and GOLD stage of COPD

		FEV_1 (% predicted)	p	CAT score (median value)	p	IADL score (median value)	p
Gender	Female	38.18±9.86%	NS	26 (18-37)	NS*	6 (0-8)	NS*
	Male	36.77±8.88%		28 (9-39)		6 (0-8)	
GOLD stage	GOLD 3	42.17±5.91%	<0.001	26 (18-37)	0.471* (NS)	6 (2-8)	0.457* (NS)
	GOLD 4	31.12±8.40%		28 (15-39)		5 (0-8)	

*Non-parametric tests

There is a significant correlation between IADL score and age (r = -0.25; P=0.02), mMRC dyspnea score (r = -0.46; P<0.001), Borg dyspnea score (r = -0.412; P<0.001), and CAT score (r = -0.427; P<0.001), for the studied group but in a negative or inverse relationship; that means the higher scores on the dyspnea scale, corresponding to a more severe dyspnea, are correlated with lower IADL scores, signifying a more prominent disability of the patient. In multivariate analysis, using the parameters significantly associated with IADL score in univariate analysis: age,

mMRC score, CAT 2 item - sputum production, CAT 3 item - chest tightness, CAT 6 item - self-confidence regarding living home, and CAT total, only age, and the mMRC score remained significantly associated with IADL score.

DISCUSSION

Multiple studies (3, 15-17) demonstrated a significant relationship between cognitive impairment and disability in COPD patients; disability is present in all disease stages, but it is related to disease severity. Braido *et al.* (15) showed that in COPD patients disability can be identified in all stages of the disease, the most relevant complaint being dyspnea, quantified on scales like MRC scale (15, 18), consistent with the final outcome of our research.

Our study revealed the aggregation of most of the cases in the more severe area of perceived breathlessness, both through estimation on Borg scale and mMRC scale, with this capital symptom being significantly worse in GOLD 4 stage of COPD, as expected.

When assessing the study group through CAT questionnaire, the median value of total CAT score was the same across the two GOLD stages, confirming that the burden of COPD on major symptoms and daily activities is not related in a linear manner to the severity of the disease, but is influenced by individual and environmental factors.

The distribution of cases according to total IADL score was somewhat surprising, 59.52% of the patients having scores ranging from 6 to 8 that means a high degree of functionality and independence among the COPD individuals addressed to the pulmonary rehabilitation program, despite the severity of the disease. A possible explanation would be the relatively low mean age (66.98±10.06 years) of the studied group and the involuntary selection of the most independent patients as candidates for pulmonary rehabilitation programs, the older and dependent patients being neglected in this regard both by themselves, relatives or family doctor. Still, the inverse or negative relationship between IADL score, age, and severity of dyspnea on mMRC and Borg scale means that the older the patient and the more severe the shortness of breath the lower the IADL score, meaning a more severe functional impairment.

Regular physical activity has proven positive cognitive outcomes, along with improved exercise tolerance and reduced symptoms like fatigue and dyspnea but requires ongoing sustained participation in regular exercise program (3, 19, 20) so that it is very important to first assess then develop support systems that will enable enrollment of COPD patients in pulmonary rehabilitation programs.

Our results showed a significant correlation between both IADL and CAT scores, but in multivariate analysis only age and shortness of breath remained significant contributors, stressing the necessity of tailoring the rehabilitation program to the individual's capacity and the degree of cognitive impairment and functioning.

CONCLUSIONS

Our study emphasizes the importance of assessing the functional capacity as well as the cognitive impairment in severe-to-very severe COPD, which seems to be related to age and dyspnea severity, regardless of the stage of disease, in such patients. Consequently, given the relevance of IADLs to the life of patients with COPD, pulmonary

rehabilitation should be adapted for COPD patients with cognitive impairment, according to the severity and its assessment should be incorporated as a clinical outcome in the management of this chronic disease.

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CHALLENGES IN THE MORPHO-FUNCTIONAL CHARACTERIZATION OF DILATED CARDIOMYOPATHY

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CHALLENGES IN THE MORPHO-FUNCTIONAL CHARACTERIZATION OFDILATED CARDIOMYOPATHY (Abstract): **Aim** refining the diagnosis of dilated cardiomyopathy (DCM) and improving diagnostic criteria. **Material and methods**: The study group consisted of 655 patients with heart failure (HF), NYHA class I-IV, of which 358 patients with DCM, 87 patients with inflammatory cardiomyopathy (ICM) and 210 patients with ischemic cardiopathy/ischemic cardiomyopathy (ICP/ICMP).The control group consisted of 120 healthy individuals. The diagnostics of DCM, ICM and ICP/ICMP comply with the recommendations of the current guidelines. **Results:** The radiopaque ventriculography showed increased ventricular volume, diffuse decrease of myocardial contractility and low ejection fraction of the left ventricle (LV). Perfusion abnormalities recorded in DCM patients (36.2%) did not differ significantly from those recorded in ICM (34.8%) and ICP/ICMP patients (40.3%). Holter monitoring revealed electrical instability of the myocardium in DCM and ICM patients, without statistically significant differences. Mitochondrial respiration study in DCM patients revealed a lower mean content of creatinekinase-15 and a greater content of creatinkinase MB-27 compared to healthy control group, and a marked impairment in acceptor control and creatine-stimulated mitochondrial respiration. The levels of myosin light chain kinase and related protein kinase in DCM and ICM were increased by 70% and 52%, respectively. We also have noticed a significant increase in annexin V and annexin VI levels in the myocardium of DCM patients compared to healthy control group: 122 ± 5% ($p < 0.05$) and 119 ± 5% ($p < 0.05$), respectively. **Conclusions:** We have conducted a complex cardio-vascular evaluation, using modern, high sensitivity and specificity techniques, with the purpose of identifying functional and structural elements, which will allow us to refine the DCM diagnostic. The results of our study grant a better understanding of the electrical and mechanical phenomena that underlie the functional and structural changes encountered in DCM. We thoroughly evaluated a series of morphological, morphometric and bioenergetic parameters of the myocardium, we determined intracardiac hemodynamic parameters and quantified the contractile status of the heart. Although we have identified significant morpho functional abnormalities, we have concluded that they have no specificity for DCM. These changes may rather be considered the expression of advanced disease status. **Keywords:** CARDIOMIOPATHY, HEART FAILURE ANNEXINS, ENERGY METABOLISM.

It has been more than six decades since Goodwin JF, based on his research on the peculiarities of morphological changes in the myocardium, proposed the definition

and the first classification of cardiomyopathies (1), which was adopted, without any change in principle, by the World Health Organization (WHO) and by the International Society and Federation of Cardiology. Over the years, due to their impact on medical practice, cardiomyopathies have been the subject of a tremendous number of clinical and experimental investigations, focused on various aspects of the problem (2, 3, 4, 5). But a question still remained: is dilated cardiomyopathy (DCM) a distinct nosological entity, which univocally implies a known etiology, or is it a syndrome, the result of various pathological processes, whose causes are not established or are not yet conclusively demonstrated?

Finding an answer to this question would make possible to clarify the definition either of DCM as a nosological form or of the probable DCM subtypes, conditioned by already established causal factors. Hence, it will become possible to characterize more accurately the syndrome's subtypes, to develop diagnostic criteria and construct a differential diagnosis (not only with other diseases, but also between DCM subtypes), as well as to find targeted, pathogenic and etiotropic treatments.

The aim of the study was to find answers to at least some of these problems, based on our experience accumulated over 20 years of studying the different aspects of DCM. To achieve this goal, we performed a thorough analysis and corroborated the multiple data obtained from our clinical and experimental studies.

MATERIAL AND METHODS

We included in our study 655 patients with heart failure (HF), NYHA class I-IV. The study group consisted of 358 patients with DCM [38.3 ± 0.6 years, 313 (87.4%) males and 45 (12.6%) females], 87 patients with inflammatory cardiomyopathy (ICM) [34.6 ± 1.3 years, 70 (80.5%) men, 17 (19.5%) women], and 210 patients with ischemic cardiopathy/ischemic cardiomyopathy (ICP/ICMP) [52.5 ± 1.2 years, 110 (91.7%) men, 10 (8.3%) women] (Tab. I). As inflammatory cardiomyopathy (myocarditis) is defined as inflammation of the heart muscle associated with impaired function of the myocardium regardless of its etiology, we enrolled in this group patients with infectious myocarditis (mainly of viral etiology) and also patients with myocarditis accompanying autoimmune disease (noninfectious myocarditis).

TABLE I

Distribution of patients according to study group and NYHA class

	DCM		ICM		IC/ICMP	
NYHA class	No.	%	No.	%	No.	%
I	56	15.7	24	27.5	42	20.0
II	122	34.1	28	32.2	88	41.9
III	162	45.3	28	32.2	80	38.1
IV	18	5.0	7	8.0	0	0

Clinical diagnosis of DCM and ICM was made in accordance with WHO expert group recommendations (1984, 1995, 2010). Positive and differential diagnostic were based

on coronary angiography, morphological and morphometric analysis of subendomycocardial biopsy samples, and in 79 (22.1%) cases based on autopsy reports. Overall, the clinical diagnosis of DCM and ICM was confirmed in 248 (74.9%) patients, respectively 60 (68.9%) patients, so we had a good anatomical-clinical correlation. We also selected a control group, consisting of 120 healthy individuals [mean age: 30.2 ± 1.4 years, of which 110 (91.7%) men and 10 (8.3%) women].

RESULTS

For all 79 HF patients autopsied we performed the macroscopic analysis of the hearts and the microscopic analysis of the myocardial tissue taken from the right ventricle (RV) free wall and from each wall of the left ventricle (LV). *Macroscopic analysis* revealed in all DCM patients the typical enlargement of all heart chambers, especially the left sided ones. Overall, heart mass exceeded normal values. Individual values ranged from 450 to 1300 grams. LV, RV and IVS masses, weighted separately, were also substantially increased, demonstrating total myocardial hypertrophy in all DCM patients. Microscopic examination revealed myocardial sclerosis. *Sclerotic changes* of the myocardium occurred most frequently in the LV (92.3%), mainly in the LV free walls (86.5%), and rarely in RV free wall (69.2%). In general, we identified diffuse, microfocal and diffuse microfocal sclerosis. Macrofocal sclerosis was mainly detected in LV (21% cases) and very rare in RV and IVS.

Invasive hemodynamic data were acquired in 104 patients with severe HF (NYHA class III-IV). *Central hemodynamic data* in patients with DCM revealed increased cardiac chamber volumes and pressures, with decreased ventricular ejection fraction. We also performed a comparative analysis of the mean values of the hemodynamic parameters in DCM, ICM and ICP/ICMP patients.

RAP - right atrial pressure; RVEDP - right ventricular end-diastolic pressure; PASP - pulmonary artery systolic pressure; PADP - pulmonary artery diastolic pressure; PAP - pulmonary artery pressure; LVEDP - left ventricular end-diastolic pressure; LVESV - left ventricular end-systolic volume; LVEDV - left ventricular end-diastolic volume; LVIB - left ventricular index-beat; LVEF - left ventricular ejection fraction; LVFSV - left ventricular fiber shortening velocity; RVESVI - right ventricular end-systolic volume index; RVEDVI - right ventricular end-diastolic volume index; RVIB - right ventricular index-beat; RVEF -right ventricular ejection fraction; NS - non significant.

During left cardiac catheterization, we assessed not only hemodynamic parameters, but also LV wall motion and contractility. Segments visible in the angiographic right anterior oblique view are five: anterobasal, anteromedial, apical, inferomedial and posterobasal. The study of *regional LV myocardial contractility* in all 5segments revealed similar changes in patients with DCM and ICM. Based on the percentage reduction of the surface in all segments, we concluded that the contractility was diffused decreased. The magnitude of the decrease has varied between 22 ± 2% to 31 ± 2% in DCM patients and 24 ± 3% to 32 ± 2% in ICM patients compared to controls. Regional LV contractility analyzed by radionuclide ventriculography did not differ between DCM and ICM patients.

The incidence and the type of arrhythmia were also evaluated. No significant

differences were observed between patients with DCM and ICM. When we adjusted by HF severity, the type and the incidence of heart rhythm and conduction abnormalities were higher in DCM patients than in ICM patients. However, the statistical significance was obtained only for ventricular ectopic beats ($p < 0.01$).

TABLE II

Central hemodynamic indices in patients with DCM, ICM and ICP/ICMP according to radiopaque ventriculography data

	DCM (n = 57)	ICM (n = 23)	ICP/ICMP (n = 24)	Statistical significance (p)
Mean RAP (mmHg)	5.9 ± 0.5	5.3 ± 1.1	8.0 ± 1.0	NS
RVEDP (mmHg)	7.2 ± 0.8	6.5 ± 1.5	7.3 ± 1.5	NS
PASP (mmHg)	45.1±2.9	32.2 ± 2.4	54.8 ± 3.4	1-2 < 0.01 1-3<0.05 2-3<0.001
PADP (mmHg)	19.3±1.7	12.1 ± 1.8	29.3 ± 2.6	1-2 < 0.01 1-3<0.01 2-3< 0.001
Mean PAP(mm Hg)	mean PAP (mm Hg)	19.2 ± 1.6	37.8 ± 3.2	1-2 < 0.01 1-3<0.02 2-3<0.001
LVEDP (mmHg)	16.2±1.2	14.3 ± 1.6	24.6 ± 1.8	1-2 – NS 1-3<0.001 2-3<0.001
LVESV (mL/m^2)	80.3±5.5	70.6 ± 6.1	99.0 ± 8.0	NS
LVEDV (mL/m^2)	122.7±5.8	113.4±7.0	141.0±9.0	NS
LVIB (mL/m^2)	42.0 ± 1.3	40.0 ± 1.2	42.0 ± 1.3	NS
LVEF (%)	34.0 ± 2.0	39.0 ± 3.0	32.0 ± 1.8	NS
LVFSV (sec^{-1})	0.49±0.04	0.62±0.09	0.46±0.03	NS
RVESVI (mL/m^2)	77.6 ± 4.6	82.1 ± 6.5	65.0 ± 7.0	NS
RVEDVI (mL/m^2)	118.7±5.1	126.2±7.1	103.0± 6.0	1-3<0.05 2-3<0.02 1-2 – NS
RVIB (mL/m^2)	42.2 ± 1.3	44.1 ± 1.4	38.0 ± 1.5	1-3< 0.05 2-3<0.01 1-2 – NS
RVEF (%)	33.0 ± 1.0	34.0 ± 1.9	37.0 ± 2.7	NS

Coronarography was performed on all patients catheterized on the left heart. In patients with DCM, sinuous coronary arteries were found. When coronary dilators were administered, increased resistance and decreased coronary artery dilation were noted. The dilation index in DCM was lower than in the control group, 8.0 ± 1.2 and 21.0 ± 1.2, respectively ($p<0.001$). No statistically significant differences in indices were

noted between DCM and ICM patients.

TI-201 myocardial scintigraphy at rest showed that the highest level of myocardium radiotracer extraction was in patients with ICM (4.5 ± 0.5%), and the lowest in patients with ICP (3.2 ± 0.4); in DCM patients this index had an intermediate value (3.6 ± 0.1%).

Intravital study of myocardial bioenergy showed an increase in cardiomyocyte mitochondrial respiration rate (Δ%) in both patients with DCM and ICM (fig. 1). The amplitude of variation in DCM patients with HF and NYHA class III-IV was obviously lower than that obtained in HF patients and NYHA class I-II.

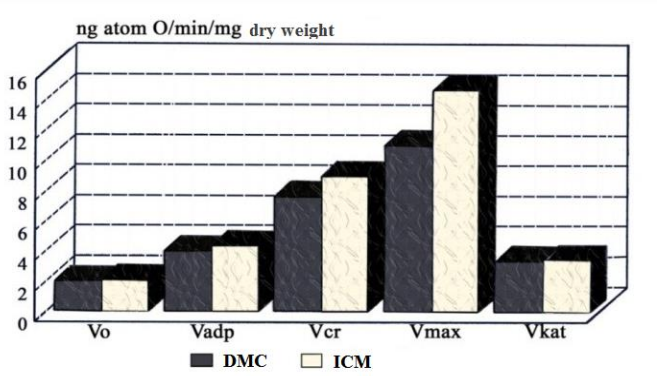

Fig. 1. Indices of cardiomyocyte mitochondrial respiration in patients with DCM and ICM

Optical microscopy identified cells with vesicular content, characteristic for apoptosis. Apoptotic corpuscles, along with other damaged structures, were detected in the sarcoplasm of isolated cardiomyocytes.

Throughout the study group, by *electron microscopy* were detected destroyed cardiomyocytes with large areas of abnormal orientation and myofibrillar lysis, mitochondrial destruction, sarcoplasmic reticulum vacuolization, sometimes disruption of intercalated disc-like cell contacts. In all cases, cardiomyocytes undergoing apoptosis were found.

TUNEL method (terminal deoxynucleotidyl transferase dUTP nick end labeling) revealed the presence of immunoreactive nuclei in all studied cases. The apoptotic index (AI) ranged from 1% to 12.86 ± 1.62%. In patients with DCM and ICM, IA was identical, namely 2.19 ± 0.82% and 2.17 ± 0.82%, respectively.

Using *immunoblot method* we assessed the levels of annexin V and VI in LV cardiomyocyte membrane. A significant increase in annexin levels was obtained in DCM patients compared to control group: 122 ± 5% ($p < 0.05$) and 119 ± 5% ($p < 0.05$) for annexin V and VI, respectively.

Expression of myosin light chain kinase (MLCK) and related protein kinase (KRP) was increased by 70% in DCM and by 52% in ICM.

DISCUSSION

The macroscopic analysis of the hearts performed during the autopsy revealed a significant increase, of up to 5 times, in heart mass in patients with DCM. This obvious hypertrophy of the myocardium is

one of the specific morphological characteristics of this disease. It should be noted that ventricular free walls and IVS thickness at different levels was within normal range, and no relationship could be established between walls thickness, ventricular volumes and heart mass. Based on this finding, we consider that ventricular walls thickness, which in daily practice is determined by transthoracic echocardiography, should not be considered a criterion for the presence or absence of myocardial hypertrophy in DCM patients. Relevant in DCM seems to be the biochemical aspects of the cardiomyocyte hypertrophy, namely the changes in cells protein composition. Thus, in the myocardium of the investigated patients we found hypertrophy of a significant part of cardiomyocytes, with partial return to the fetal program of protein expression. The increased MLCK and KRP expression may partially be responsible for the non-adaptive nature of cardiomyocyte hypertrophy. Despite the increase in size of functional fibers, the efficacy of contraction proves to be low (6).

In patients with DCM, morphological data reveal diffuse, microfocal or diffuse microfocal sclerosis. In more than 25% cases were detected foci of replacement myocardial sclerosis with intramural vessel sclerosis phenomena, which can prove that the onset of the disease was with myocarditis accompanied by proliferative and necrotizing vasculitis. The above presented data show not only the lack of specificity, but also the essential heterogeneity of myocardial changes in patients with DCM. Most likely, they indicate different stages of the pathological process and/or the primary influence of various etiological factors on the heart (7).

Central hemodynamic data showed moderate pulmonary hypertension in patients with DCM and ICM, which can be explained by the simultaneous involvement of both left and right cardiac cavities. As a result, the LVEF is lowered to a greater extent. Severe pulmonary hypertension may be determined by both severe LV systolic dysfunction and moderate RV changes, as well as by the development of sclerotic changes in small pulmonary vessels, as a result of long-term disease progression. In patients with DCM and ICM, which usually have a relatively short duration of disease, pulmonary hypertension fails to develop.

The segmental anomalies of LV contractility are most probably due to focal sclerosis and/or to conduction abnormalities in bundle of His, particularly at the level of the ventricular myocardium. Heart rhythm and conduction abnormalities were equally frequent in patients with DCM and ICM. In both groups, supraventricular and ventricular arrhythmias, as well as atrioventricular and intraventricular conduction abnormalities occurred as frequently. The need for an in-depth knowledge of the proarrhythmic substrate lies in the necessity of optimal control of the most common rhythm disorder associated with DCM, namely atrial fibrillation. The structural and functional changes that it determines in the atria (8) trigger a cascade of maladaptive events at the ventricular level, with negative consequences on the telediastolic pressure, parietal tension and LV ejection fraction. Long-term persisting arrhythmia and the treatment associated adverse effects have major impact on the patient's prognosis and quality of life (9).

The coronarography data clearly demonstrated the morphological and functional changes in the myocardial vasculari-

zation of DCM patients, thus confirming the presence of chronic myocardial hypoxia. It must be taken into account that 70% of the energy required from the myocardial cell is the result of aerobic oxidation of fatty acids, and prolonged ischemia results in reduction of this process, intensification of anaerobic glycolysis and accumulation of no oxidized fatty acids (10).There is increasing evidence that cardiac lipid accumulation worsens heart function and leads to structural myocardial damage, including cardiac fibrosis, myocyte apoptosis, and reduced contractility (11), which is often linked to mitochondrial dysfunction. This persistent deficiency of cardiomyocyte oxygenation and its crucial involvement in the primary and/or secondary injury of the cardiomyocytes is strongly supported by our intravital study of myocardial bioenergy. For this highly specialized evaluation, we used the permeabilized fiber method, a high-fidelity technique, of great utility in clinical trials in patients with different pathological conditions (12). Our data revealed serious abnormalities in the biosynthesis chains of various isoenzymes in DCM patients, but similar to those recorded in ICM patients.

A major breakthrough in understanding the complex mechanisms that leads to DCM was achieved by assessing apoptosis in myocardial cells. By combining electron and optical microscopy with the TUNEL test, a highly specialized immunohistochemical method, we proved the presence of immunoreactive nuclei in all myocardial biopsies from DCM patients. Based on this undeniable evidence, we affirm without hesitation that myocardial wall thinning, as well as the reduction in cardiomyocyte count occurred in the absence of collagen accumulation in the myocardium. This phenomenon indicates a low coefficient of necrotic cell death and allows us to confirm previous studies (13), who states that apoptosis is the primary factor responsible for the pronounced dilation of cardiac cavities in patients with DCM.

Immunoblotting method allowed us to highlight the increased levels and the particular disposition of annexin V and VI in myocardium of DCM patients. In healthy myocardium, at subcellular level, annexin V and VI are present in the sarcolemma, intercalated discs and T-tubules, connected to the terminal cisternae of the sarcoplasmic reticulum where excitation-contraction coupling takes place. In DCM patients, we found a particular localization of annexin V in the myocardium, especially in the interstice, between myocyte clusters, at sites corresponding to fibrous tissue. This abnormality, together with annexin V ability to bind at the extracellular matrix proteins (14), are likely to contribute to the development of “reactive” interstitial fibrosis and to the structural reorganization of ventricular myocardium. These are the clearest features of heart failure.

Although the changes we found are not specific for DCM and rather confirm the common characteristics of advanced stages of the disease, they elucidate the incidence and the pathophysiological substrate of heart rhythm disorders in patients with DCM and ICM. All the morphological, morphometric and bioenergetic abnormalities that we documented, along with the impairment in the regional myocardial contractility and changes in the intracardiac hemodynamic parameters strongly support the fact that DCM has multiple and incompletely elucidated mechanisms, that lead to a unique clinical aspect, regardless of etiology.

CONCLUSIONS

The results of our research states that the identified changes are not related to the etiopathogenic characteristics of DCM. However, they reflect a clearly contoured syndrome, characterized by morphological, morphometric and bioenergetic myocardic indices, intracardiac hemodynamic parameters and, regional and global myocardial contractile dysfunction. These data confirm the common character of structural and functional changes of the myocardium in advanced stages of the disease. The concept is supported by the similarity of incidence and pattern of heart rhythm disorders and also by abnormalities of myofibrillar proteins in patients with DCM, ICM and ICMP. Certainly, more studies are necessary to correlate accurately the parietal kinetic parameters with intracellular metabolic processes.

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TOXICITATEA ACUTĂ A INHIBITORILOR ENZIMEI DE CONVERSIE A ANGIOTENSINEI. EXPERIENŢA CLINICII DE MEDICINĂ INTERNĂ ŞI TOXICOLOGIE, SPITALUL CLINIC DE URGENŢE "SF. IOAN" IAŞI

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ACUTE TOXICITY OF ANGIOTENSIN CONVERTING ENZYME. INTERNAL MEDICINE AND TOXICOLOGY CLINIC OF IASI EMERGENY HOSPITAL EXPERIENCE (Abstract): Regarding angiotensin-converting enzyme inhibitors (ACEI) poisoning, only few data are available in the last decade literature. In the previous couple of years especially isolated case reports were published. **Material and method**: We analyzed retrospectively all the patients with acute ACEI poisoning admitted in Iaşi Internal Medicine and Toxicology Clinic between 2004 and 2009. **Results**: 17 cases of poisoning were recorded (enalaprilum-9 cases, captoprilum-3 cases, perindoprilum-3 cases, lisinoprilum-2 cases). All the poisonings were intentional. A favorable outcome was consistently observed, and were recorded no sequelae or death in this study. The main complain was hypotension, required fluid administration, only one case with 500 mg enalaprilum and severe hypotension required injection of vasopressive amines. No abnormal renal function and no angioedema were noted. **Key words**: ANGIOTENSIN-CONVERTING ENZYME INHIBITORS TOXICITY, ANGIOTENSIN-CONVERTING ENZYME INHIBITORS POISONING, HYPOTENSION

LUNG CANCER IN PATIENTS WITH PULMONARY TUBERCULOSIS: EPIDEMIOLOGICAL AND CLINICAL FEATURES

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LUNG CANCER IN PATIENTS WITH PULMONARY TUBERCULOSIS: EPIDEMIOLOGICAL AND CLINICAL FEATURES (Abstract) **Aim**: To describe the clinical and epidemiological features of patients who developed simultaneously lung cancer (LC) and pulmonary tuberculosis (PTB). **Material and methods**: Retrospective study of the medical records of patients diagnosed with LC and PTB at the Constanta Pneumophtysiology Hospital between January 1, 2014 and December 31, 2018. Demographic data (age, gender, occupation), risk factors, clinical status, histopathological type of LC, stage at diagnosis, LC therapy, and laboratory results (sputum smear or bronchoalveolar lavage microscopy, culture, susceptibility testing for *Mycobacterium tuberculosis*) were collected. All hospitalized patients were evaluated. **Results**: Of the 559 LC patients, we identified 54 patients that had active TB at the time of LC diagnosis, mean age 57.27 ± 11.26 years, 37 (67.27%) men and 18 (32.73%) women. Most patients were smokers or former smokers (90.4%), diagnosed at an advanced stage (90.7% stage III and IV), and adenocarcinoma was the most frequent histologic type (40.7%). Positive smear microscopy was recorded in 24 (44.4%) patients, and 7 patients reported a history of TB contact. Thirty-eight (70.3%) of patients were new TB cases, 16 (29.6%) were relapses, and one patient was diagnosed with multidrug resistant (MDR) TB. **Conclusions**: LC patients with concurrent pulmonary TB were mostly males, smokers, with advanced LC, adenocarcinoma being the most common histologic type, with almost half of the cases, with positive smear microscopy.
Keywords: LUNG CANCER, PULMONARY TUBERCULOSIS, COMORBIDITY.

At the end of the 20th century, lung cancer (LC) became one of the leading causes of death worldwide. LC is a tumor of extreme severity, less than 1/3 of cases being diagnosed at a localized stage (1). Smoking is the most common risk factors not only for LC (2), but also for other pulmonary or extrapulmonary diseases (3-5). Involuntary inhalation of cigarette smoke and the use of electronic cigarettes are as harmful as active smoking (6, 7). In addition to smoking, there are many other risk factors associated with LC, such as environmental exposure to noxious particles or gases, predisposing conditions (pulmonary fibrosis, TB scars) or a family or personal history of cancer (1, 8).

PTB is a major health problem globally, Romania reporting the highest incidence

(72/100,000 population) in the European Union (9). In Romania, every year, more than 1,000 people die of and other 16,000 are newly diagnosed with PTB. The most common risk factors for PTB are various immunosuppressive conditions, malnutrition, indoor air pollution, smoking and alcohol use, and low socioeconomic status (10, 11).

Coexistence of LC and PTB remains a clinical problem that presents a real challenge for the management of both diseases. There are patients diagnosed with LC who develop active TB disease, mainly by reactivation of a latent PTB due to prolonged immunosuppression caused by chemotherapy, radiation therapy or their overall poor health status and malnutrition. On the other hand, chronic inflammation, the major pathogenic process in TB, appears to be the basis for the future development of LC (12).

The aim of the study was to describe the clinical and epidemiological characteristics of patients who developed simultaneously LC and PTB.

MATERIAL AND METHODS

A retrospective study of the medical records of patients diagnosed with LC and PTB at the Constanta Pneumophtysiology Hospital between January 1st , 2014 and December 31st, 2018, was conducted. There were assessed all LC patient files and demographic data (age, gender, occupation), risk factors, clinical status, histopathological type of LC, stage at diagnosis, LC therapy, and laboratory results (sputum smear or bronchoalveolar lavage microscopy, culture, susceptibility testing of *Mycobacterium tuberculosis*) were collected. The diagnosis of PTB was based on the national consensus criteria (13): positive Ziehl-Neelsen staining for acid fast bacilli (AFB); positive culture for *Mycobacterium*; or epidemiological, clinical, and radiological features consistent with PTB, associated with a favorable response to anti-tuberculosis treatment. LC diagnosis was based on cytology and/or anatomopathological findings.

This study was approved by the Ethics Committee of the Constanta Pneumophtysiology Hospital. Patient informed consent was not needed because the study was retrospective.

Statistical analysis was performed using the *Statistical Package for the Social Sciences version 18.0* (SPSS Inc., Chicago, IL, USA). A descriptive analysis of the study variables was carried out. Qualitative data are expressed as n (%), and quantitative data as mean ± SD.

RESULTS

There were assessed 559 files of patients diagnosed with LC during the study period, of which 54 (9.6%) patients with active TB disease were included in the study. Yearly and gender distribution of the cases diagnosed and hospitalized for LC and concomitant PTB during the study period is presented in figure 1.

Patients' data including age, sex, smoking and clinical status, histopathological type of LC, stage at diagnosis, and LC therapy are shown in first table. The study group consisted of 54 patients diagnosed with LC and PTB, of which 37 (68.51%) men and 17 (31.38%) women. Mean age was 57.27 ± 11.26 years (55.86 ± 12.43 years in men, and 60.65 ± 11.34 years in women). Most of patients (n=49/54; 90.74%) were smokers or ex-smokers, and more than half of them (n=29; 53.7%) were living in urban areas. Six (11.11%) patients had a history of respiratory toxicant exposures. The most common histopathological type was adenocarcinoma (n=22; 40.74%), followed by squamous cell carcinoma (n=19; 35.18%), while

small cell carcinoma was diagnosed in only 5 (9.25%) patients.

Most patients (n=49; 90.73%) were diagnosed in advanced stages (III B, C and IV). Of the 5 patients diagnosed in operable stages (I, II, IIIA), only 3 underwent surgery, the other two presenting serious cardiovascular comorbidities that prevented the surgical procedures. 36 patients underwent chemotherapy, of which 8 patients had concurrent or sequential radiation therapy. One patient benefited from all 3 therapeutic possibilities, while 16 patients received only symptomatic treatments, either because of severe comorbidities or because of refusal of therapy.

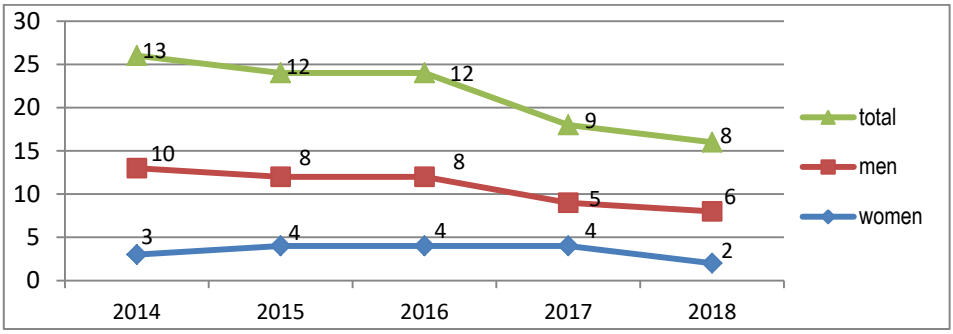

Fig. 1. Number of LC and PTB patients diagnosed during the study period

TABLE I

Characteristics of the patients diagnosed with LC and PTB

Variables	Number	Percent (%)
Age, years, mean ± SD	57.27±11.26	
Male gender	37	68.51 %
Smoker or former smoker	49	90.74 %
Urban residence	29	53.70 %
History of respiratory toxicant exposure	6	11.11 %
Performance status ECOG ≤ 1 ECOG ≥ 2	 31 23	 57.4 % 42.59 %
Pathological type Adenocarcinoma Squamous cell carcinoma NSCLC, undetermined Small cell carcinoma	 22 19 8 5	 40.74 % 35.18 % 14.81 % 9.25 %
Stage I and II III A III B, C IV	 1 4 16 33	 1.8 % 7.4 % 29.62 % 61.11 %
Treatment Chemotherapy Radiotherapy Surgery	 36 8 3	 66.66 % 14.81 % 5.55 %

The PTB diagnosis was made using the following methods: sputum smear microscopy in 24 (44.44%) patients; sputum culture in 44 (81.48%); bronchoalveolar lavage (BAL) microscopy in 2 (3.7%); BAL culture in 4 (7.4%); PCR positivity for M. tuberculosis in BAL samples in 6 (11.11%); and clinical and radiological findings consistent with tuberculosis in 8 (14.81%) patients (tab. II). Thirty-eight patients were new TB cases, while 16 were relapses. One patient was diagnosed with multidrug resistant TB. The treatment of PTP was completed by 40 patients, 5 patients died and for 9 patients the data regarding treatment outcome were unavailable.

TABLE II
Characteristics of PTB

PTB characteristics	Number	Percent (%)
History of TB contact	7	12.96
Positive sputum smear microscopy	24	44.44
Positive BAL microscopy	2	3.7
Positive PCR for *M. tuberculosis* in BAL samples	6	11.11
Positive smear culture	44	81.48
Positive BAL culture	4	7.4
Clinical and radiological findings consistent with tuberculosis	8	14.81
New cases	38	70.37
Relapses	16	29.62
MDR TB	1	1.85

DISCUSSION

The present study describes the characteristics of 54 patients simultaneously diagnosed with PTB and LC over a 5-year period. This period was characterized by a decrease in the annual number of patients (13 patients in 2014 versus 8 patients in 2018). This descending trend can be explained by the decrease in TB incidence at country level (9).

The association between PTB and LC has been studied for years (14-20). These two diseases can mimic the clinical and radiological features of each other, therefore the diagnosis may be delayed. There are various hypotheses regarding a plausible biological mechanism explaining the association between LC and PTB. One of these would be that chronic inflammation associated with prolonged infections can contribute to carcinogenesis (15). Activated neutrophils produce reactive oxygen or nitrogen species that can bind to the DNA, inducing genetic damage and neoplastic transformation (16). In patients with chronic infections, such as PTB, it has been demonstrated that histidine triad gene alterations may be involved in lung carcinogenesis, along with increased cell proliferation and angiogenesis during tissue repair (15, 16).

In conditions of impaired local immunity due to lung carcinoma or various administered therapies, primary mycobacterial infection, reactivation of a latent TB, or new exogenous infection may cause TB disease (17).

In the present study, of the 559 patients diagnosed with LC, 54 (9.6%) were found to have simultaneously PTB. In a cross-

sectional study (18) involving 24 patients diagnosed with tuberculosis and LC, the diagnoses of PTB and LC occurred simultaneously in 10 patients, whereas PTB was diagnosed prior to LC in 14. The high number of patients diagnosed concomitantly with LC and PTB in our study can be explained by the fact that TB is highly endemic in our country, Constanta county being one of the highest endemic areas.

In this study the most common histopathological type of LC was adenocarcinoma (40.74% of cases), similar to those reported in previous studies (18,19). A meta-analysis (20) of 37 case-control studies and 4 cohort studies showed a significant association between tuberculosis and adenocarcinoma (RR = 1.6; 95% CI = 1.2-2.1), but not with squamous cell carcinoma or small cell carcinoma.

Almost 91% of patients included in this study were smokers or former smokers, fact that can lead to the idea that these two diseases share the same risk factor, tobacco smoking. Despite the well-known effect of tobacco as a carcinogen, the association between PTB and LC persists even in never smoking individuals (RR=1.8, 95% CI=1.4-2.2) (20). Similar with previously reported data, approximately one of 10 patients were exposed to respiratory toxicants, thus demonstrating the negative impact of occupational exposure on respiratory system (21).

Approximately 60% of our patients presented distant metastases at the time of LC diagnosis and almost half of them with positive sputum smear or BAL microscopy showing an advanced stage of both diseases (22). This can be explained by the fact that these two diseases have common symptoms that can cause a delay in their diagnosis. Prevention measures, early-stage disease diagnosis with the use of "red-flags" during anamnesis in the general practicians' offices and more often updates of the protocols are needed (23).

CONCLUSIONS

The present study showed that patients with lung cancer and concurrent pulmonary TB were mostly males, smokers, with advanced stage of LC. Adenocarcinoma was the most common histologic type, almost a half of the cases had positive smear microscopy.

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THE PROGNOSTIC VALUE OF THE NEUTROPHIL-TO-LYMPHOCYTE RATIO IN PATIENTS WITH ADVANCED NON-SMALL-CELL LUNG CANCER AND PULMONARY COMORBIDITIES

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THE PROGNOSTIC IMPACT OF THE NEUTROPHIL-TO-LYMPHOCYTE RATIO IN PATIENTS WITH ADVANCED NON-SMALL-CELL LUNG CANCER WITH PULMONARY COMORBIDITIES (Abstract): **Aim:** This study assesses the prognostic role of the neutrophil-to-lymphocyte ratio (NLR) and other clinical parameters in patients with advanced non-small-cell lung cancer (NSCLC) who also suffer from pulmonary comorbidities. **Material and methods:** This single-center retrospective research was conducted during January 2015 – December 2018 on patients with the above-mentioned diagnosis. The statistical analysis was carried out with SPSS version 25.0 and focused on evaluating the independent effects of NLR and other parameters by means of univariate and multivariate Cox regression analyses. **Results:** Regarding progression-free survival (PFS), the results of the multivariate analysis did not indicate NLR as a prognostic factor (HR: 1.34; 95% CI: 0.85-2.11; p=0.2), unlike the ECOG performance status (HR: 1.71; 95% CI: 1.06-2.77; p=0,02). In terms of overall survival (OS), NLR was an independent negative prognostic factor (HR: 1.8; 95% CI: 1.21-2.69; p=0.004), and so was smoking (HR 1.97; 95% CI: 1.23-3.15; p=0.005). **Conclusions:** While we did not find NLR to be an independent prognostic factor for PFS in patients with NSCLC and pulmonary comorbidities, it proved to be a negative prognostic factor for OS in these cases. **Keywords:** ADVANCED NSCLC, PULMONARY COMORBIDITIES, NLR, PROGNOSIS.

Worldwide, lung cancer is currently both the most common type of neoplasm as well as the deadliest (1). Up to 80% of cases feature non-small-cell lung cancers (NSCLC). Over the course of the last ten years, survival rates have improved for patients in all stages of lung cancer, with particularly good outcomes in early stages significantly alleviated by multimodal therapies (2, 3). TNM staging has proven useful in classifying lung cancer cases based on severity and one of the most important practical implications is in predicting outcome. However, it is limited in that it does not meet all the necessary criteria for being a complete prognostic tool. Nevertheless, most studies employ TNM in order to stratify and assess patient outcomes (2). The current situation raise an unmet need for more cost-effective biomarkers capable of providing reliable prognostic information which may then guide therapeutic decision-

making (4). Numerous studies have so far demonstrated the prognostic role of various clinical, biological and genetic factors in advanced and metastatic lung cancer, but none that we could identify has yet researched this in a population of Romanian patients and specifically on a cohort with pulmonary comorbidities. Our study aims to fill this gap and assess the prognostic role of NLR and other variables in patients with non-small-cell lung cancer and pulmonary comorbidities

MATERIAL AND METHODS

Patient selection

Upon approval by the Ethics Committee of the Regional Institute of Oncology Iași (RIO) and the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy Iași, we proceeded to conduct a retrospective study on patients previously diagnosed with locally-advanced NSCLC ineligible for surgical/radiotherapy treatment or already in the metastatic stage. The patients to be taken into consideration had undergone chemotherapy and targeted molecular therapies at RIO in Iași over the course of 3 years (2015-2018). The data was retrospectively collected using the Hippocrates information system with which RIO manages all the patient data. The selection process resulted in a database including clinical, biological, histopathological and molecular parameters for each case. Information regarding investigations, therapeutic decisions, and patient outcomes were also considered. The studied variables were: age, gender, ECOG performance status, smoking and number of packages per year, comorbidities, pathological diagnosis, oncogenic mutations of the epidermal growth factor receptor (EGFR) gene, TNM staging, location of the primary tumor, location of the metastases, undergone treatment, first-line chemotherapy, neutrophil-to-lymphocyte ratio (NLR), first-line targeted molecular therapies, toxic reactions to treatment, treatment outcomes, progression-free survival (PFS) and overall survival (OS).

The inclusion criteria were:

- age > 18 years old;
- histology of squamous cell carcinoma or adenocarcinoma;
- locally advanced either ineligible for surgery/radiotherapy or in metastatic stage;
- no prior oncologic treatment for lung cancer;
- treatment carried out at RIO Iași;
- diagnosis of pulmonary comorbidities.

The exclusion criteria were:

- other histologies than typical of squamous cell carcinoma and adenocarcinoma;
- history of cancer;
- severe cardiovascular comorbidities – recent myocardial infarction, severe angina pectoris, stroke and severe peripheral arterial disease.

The tumor staging was established using the 7th edition of the staging system developed by the American Joint Committee on Cancer.

Statistical analysis

The Chi-square test was used to compare the clinical characteristics of the groups with elevated and low NLR. Overall survival was defined as the length of time between the lung cancer diagnosis date and the time of death due to cancer, and the Kaplan-Meier method was used to estimate it. The univariate analysis was performed using the log-rank test, also helpful in establishing the prognostic differences between the groups. Cox regression was used to develop multivariate models. Hazard ratios and 95% confidence intervals were computed, and the default threshold for statistical significance was set at 0.5. All the patient data collected over a peri-

od of 3 years from the time of diagnosis (or less in case of death) was considered as explained. The characteristics of the patients are summarized in first table.

RESULTS

Patient characteristics

During January 2015 – December 2018, a total of 488 new cases of NSCLC were recorded as being treated at RIO. Of them, 109 patients met the criteria for inclusion in this study and did not feature any of the exclusion criteria. These patients underwent first-line chemotherapy or targeted molecular therapies if manifesting oncogenic mutations of the EGFR.

TABLE I

Clinical characteristics of the patients

No. of cases		109 (100%)
Median age (years)		63
Gender	Male	88 (80.7%)
	Female	21 (19.3%)
ECOG	0	2 (1.8%)
	1	67 (61.5%)
	2	40 (36.7%)
Smoking status	Smokers	83 (76.1%)
	Non-smokers	26 (23.9%)
Histology	Adenocarcinoma	58 (53.2 %)
	Squamous cell carcinoma	51 (46.8 %)
Stage	IIIA	8 (7.3 %)
	IIIB	18 (16.5 %)
	IIIC	9 (8.3 %)
	IVA	39 (35.7 %)
	IVB	35 (32.1 %)
Metastases	Absent	33 (30.2 %)
	Pulmonary	56 (51.3 %)
	Hepatic	9 (8.2 %)
	Osseous	13 (11.9 %)
	Cerebral	2 (1.8 %)
	Adrenal	10 (9.1 %)
	Other locations	0 (0 %)
1st line chemotherapy	Cisplatin Gemcitabine	48 (44%)
	Carboplatin Gemcitabine	30 (27.5%)
	Carboplatin Paclitaxel	12 (10.1%)
	Other protocols	19 (17.5%)
Pulmonary comorbidities	COPD	73 (69.6%)
	Pulmonary fibrosis	6 (5.5%)
	Asthma	6 (5.5%)
	TB history	2 (1.8%)
	Other comorbidities	19 (17.6)

The clinical characteristics of this patient population are summarized in Table I. In all, data on 109 patients treated during January 2015 – December 2018 were eligible for analysis. The median age of the patients was 63. Regarding gender, 88 patients were men (80.7%) and 21 were women (19.3%). A majority of 83 patients were smokers (76.1%). Adenocarcinoma-type histology was present in 53.2% of the cases, and squamous cell carcinoma in the other 46.8%. There were 35 cases diagnosed as stage III (32.1%) and 74 as stage IV (67.8%). The most common secondary lesions were pulmonary (51.3%). The most frequently associated chronic pulmonary pathology was chronic obstructive pulmonary disease (COPD), in 66.9% of cases), while a prior history of pulmonary fibrosis was rare (only 5.5%). The most commonly employed first-line chemotherapy protocol Cisplatin Gemcitabine (44%) and Carboplatin Gemcitabine (27.5%). Considering that EGFR mutations were also present in some of the patients, first-line Erlotinib was used in 3 cases and Afatinib in one other. For the studied population, the median PFS was of 7.7 months and the median OS was of 12.6 months.

Inflammation markers based on biological sampling

The median values of the levels of neutrophils and lymphocytes were 8.7 x 10^9/l and 2.9 x 10^9/l, respectively. The NLR was defined as the number of neutrophils divided by the number of lymphocytes, thus resulting in a median NLR value of 3.32 for the entire cohort (with individual values ranging from 0.2 to 16.3). The cases were further grouped in two: patients with elevated NLR (≥ 3) and those with low NLR (< 3).

NLR-related parameters

The clinical and pathological characteristics correlated with NLR values are summarized in second table. There were no significant associations found between NLR and age, gender, smoking status, the histopathological type of the TNM clinical staging. There were significantly more ECOG 1 and ECOG 2 patients in the group with NLR ≥ 3 compared to the NLR<3 group (p=0.02).

TABLE II

Correlations between clinical characteristics and NLR

	NLR		
	<3 n=46, (42.2%)	**>=3 n=63, (57.8%)**	**p value**
Age			0.78
<65	24 (52.2)	34 (54.8)	
>=65	22 (47.8)	29 (45.2)	
Gender			0.94
Men	37 (80.4)	51 (81)	
Women	9 (19.6)	12 (19)	
ECOG			**0.02**
0	2 (4.3)	0 (0)	
1	33 (71.7)	34 (54)	
2	11 (23.9)	29 (46)	

	NLR		
	<3 n=46, (42.2%)	**>=3 n=63, (57.8%)**	**p value**
Smoking status			0.9
Non-smoker	11 (23.9)	15 (23.8)	
Smoker	35 (76.1)	48 (76.2)	
Histopathological type			0.3
Adenocarcinoma	22 (37.9)	36 (57.1)	
Squamous cell carcinoma	24 (52.2)	27 (42.9)	
Clinical staging			0.6
III	16 (34.8)	19 (30.2)	
IV	30 (65.2)	44 (69.8)	
Location of the primary tumor			0.59
Right lung	25 (54.3)	31 (49.2)	
Left lung	21 (45.7)	32 (50.8)	
EGFR mutation			0.45
Absent	41 (89.1)	53 (84.1)	
Present	5 (10.9)	10 (15.9)	

NLR and PFS

The median PFS was of 7.8 months (ranging between 1 and 47.5 months). At 1-year PFS was 16% of the NSCLC patients with pulmonary comorbidities and NLR ≥ 3, as well as in 32.5% of the patients with NLR < 3. The univariate analysis was conducted in order to establish any existing correlations between PFS and various parameters. Age (p=0.89), gender (p=0.85), smoking (p=0.3), the histopathological type (p=0.2), the clinical staging (p=0.9), the location of the primary tumor (p=0.9) and the presence of EGFR mutation (p=0.58) did not significantly associate with PFS, unlike the ECOG performance status (p=0.005) and NLR (p=0.03), as summarized in Table III. Also, the patients from the elevated NLR group had shorter PFS than those with low NLR (6.1 months versus 9.5 months – see fig. 1). However, the multivariate analysis did not highlight a significant association between NLR and PFS (HR: 1.34; 95% CI: 0.85-2.11; p=0.2). The ECOG performance status proved to be an independent prognostic factor for PFS (HR: 1.71; 95% CI: 1.06-2.77; p=0.02) (tab. III). The median PFS of the patients with ECOG 0 was 12 months, while for patients with ECOG 1 it was 8.5 months and for those with ECOG 2, it was only 4.6 months (fig. 2).

TABLE III

Univariate and multivariate analysis of PFS for patients with advanced NSCLC and pulmonary comorbidities

	Univariate analysis			Multivariate analysis		
	HR	**95% CI**	**P value**	**HR**	**95% CI**	**P value**
Age	0.97	0.63-1.48	0.89			
<65						
>=65						

	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Gender Men Women	0.95	0.55-1.62	0.85			
ECOG 0 1 2	**1.91**	**1.21-3**	**0.005**	**1.71**	**1.06-2.77**	**0.02**
Smoking status Non-smoker Smoker	1.31	0.78-2.22	0.3			
Histopathological type Adenocarcinoma Squamous cell carcinoma	0.76	0.5-1.16	0.2			
TNM clinical staging III IV	1.02	0.6-1.59	0.9			
Location of primary tumor Right lung Left lung Bilateral	1.02	0.67-1.56	0.9			
EGFR mutation Absent Present	0.83	0.42-1.61	0.58			
NLR <3 ≥3	**1.56**	**1.02-2.4**	**0.03**	**1.34**	**0.85-2.11**	**0.2**

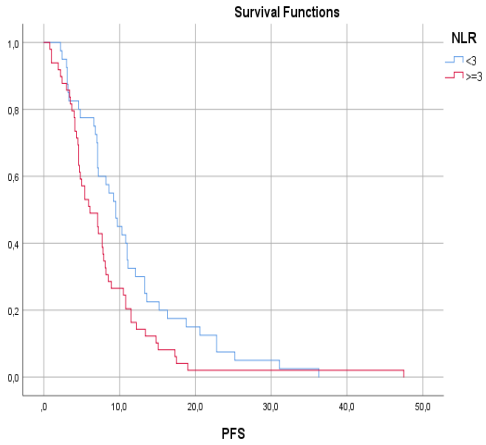

Fig. 1. PFS and NLR for patients with advanced NSCLC and pulmonary comorbidities

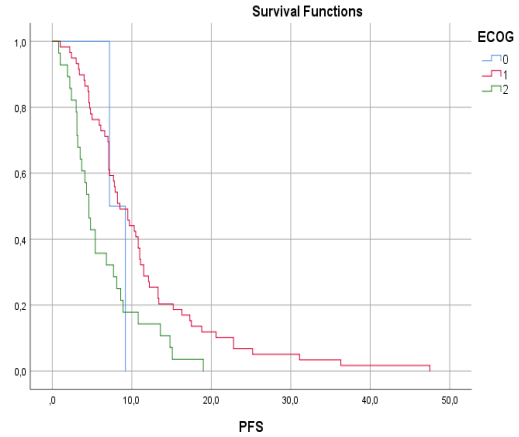

Fig. 2. PFS and ECOG for patients with advanced NSCLC and pulmonary comorbidities

NLR and OS

The median overall survival was of 15.4 months (ranging between 10.1 and 21.8 months). The 1-year OS rate was significantly lower in patients with elevated NLR compared to the group with low NLR (40% vs 70%). According to the univariate analysis, the variables correlated with poor OS were smoking (p=0.01) and elevated NLR (p=0.01). The multivariate analysis pointed elevated NLR as an independent prognostic factor for short OS (HR: 1.8; 95% CI: 1.21-2.69; p=0.004). Also, smoking status was a negative prognostic factor (HR 1.97; 95% CI: 1.23-3.15; p=0.005) (tab. IV). The median OS values were lower in the case of patients with advanced NSCLC and pulmonary comorbidities who also had elevated NLR than those with low NLR (10.5 months vs 15.8 months, p=0.01) (fig. 3).

TABLE IV

Univariate and multivariate analysis of OS for patients with advanced NSCLC and pulmonary comorbidities

	Univariate analysis			Multivariate analysis		
	HR	**95% CI**	**P value**	**HR**	**95% CI**	**P value**
Age <65 >=65	1.16	0.79-1.72	0.43			
Gender Men Women	0.64	0.38-1.05	0.08			
ECOG 0 1 2	1.35	0.93-1.96	0.1			
Smoking status Non-smoker Smoker	**1.81**	**1.14-2.88**	**0.01**	**1.97**	**1.23-3.15**	**0.005**
Histopathological type Adenocarcinoma Squamous cell carcinoma	1.19	0.81-1.75	0.37			
TNM clinical staging III IV	1.06	0.7-1.06	0.75			
Location of primary tumor Right lung Left lung Bilateral	0.98	0.67-1.43	0.93			
EGFR mutation Absent Present	0.68	0.38-1.2	0.18			
NLR <3 ≥3	**1.67**	**1.13-2.48**	**0.01**	**1.8**	**1.21-2.69**	**0.004**

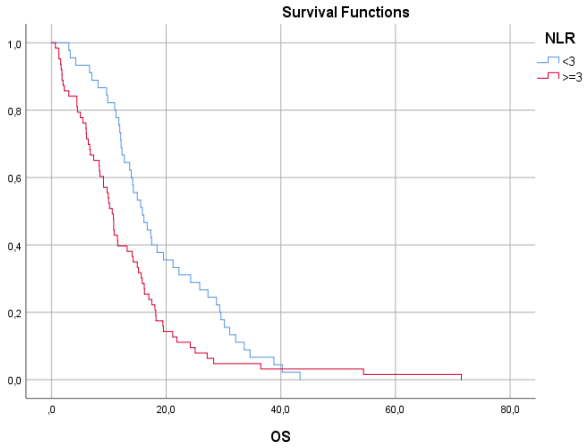

Fig. 3. OS and NLR in patients with advanced NSCLC and pulmonary comorbidities

DISCUSSION

NLR is the ratio between neutrophils and lymphocytes. This ratio influences the prognosis for cancer patients, but the underlying mechanism is unclear. Several hypotheses have been raised. For one, neutrophils seem to play several protumoral roles. Those from the tumor stroma exert protumor genic effects by inhibiting apoptosis, promoting angiogenesis and stimulating the formation of metastases, creating an inflammatory microenvironment conductive to malignant growth (5). Also, lymphocytes are essential in immunosurveillance and tumor suppression (6). CD41 helper T cells and CD81 cytotoxic T cells take part in immunosurveillance. Increased densities of CD41 helper T cells and CD81 cytotoxic T cells in tumor stroma are considered favorable independent prognostic factors in patients with NSCLC (7, 8). Moreover, lymphocytes engage in antitumoral activities which may cause cellular death and may inhibit the migration as well as the growth of tumor cells. In fact, they are the main inflammatory agents that inhibit the proliferation of cancer cells. Since an elevated NLR points to a large number of neutrophils and/or a small number of lymphocytes, this may explain why it is typically associated with an unfavorable prognosis in cancer patients (4).

According to our research, the NLR before starting the treatment of the studied patients represented a negative prognostic factor for OS (HR: 1.8; 95% CI: 1.21-2.69; p=0.004), but not for PFS (HR: 1.34; 95% CI: 0.85-2.11; p=0.2). In patients with pulmonary comorbidities, the median OS was 5.3 months shorter in patients with NLR < 3 compared to those with NLR ≥ 3.

In other clinical trials, elevated NLR has been linked to poor survival in patients with gastrointestinal cancers, pancreatic cancer, breast cancer, bladder cancer and other types of cancers. A recent meta-analysis of studies conducted on a total of 40 599 patients with solid tumors also showed that NLR > 4 was associated with a substantial increase in mortality risk. However, the cutoff value and the prognostic effect of NLR remain controversial (9, 10). Liu *et al.* assessed 934 patients with advanced lung cancer and demonstrated that

NLR is correlated independently with a favorable prognosis (11). In another study coordinated by Guo, low values of NLR were associated with increased OS in patients with advanced NSCLC who were treated with first-line platinum-based chemotherapy. Thus, NLR was demonstrated as an independent predictor for survival (12). Yu *et al.* conducted a meta-analysis on data from 7,219 patients and showed that elevated NLR predicted reduced OS (HR: 1.46l 95% CI: 1.30-1.64) as well as reduced PFS (HR: 1.42; 95% CI: 1.15-1.75) (13). Scilla's research confirmed the hypothesis that an elevated baseline NLR was associated with reduced OS in patients with advanced NSCLC who underwent chemotherapy (14). Building on the latest progress in the field of immunotherapy for lung cancer, a recent study found that elevated NLR can be linked to poorer prognosis ($p < 0.001$) in patients with metastatic NSCLC undergoing treatment with nivolumab. Accessibility to inflammation biomarkers could, in the future, make this a reliable predictive biomarker for immunotherapy in NSCLC (15).

The research hereby presented features several limitations. Mainly, this is a retrospective study conducted in a single center and on a relatively small number of patients. Also, despite our best attempts to compile the database using strictly managed inclusion and exclusion criteria, the results may still be influenced by uncontrolled selection biases.

CONCLUSIONS

Given the multitude of preclinical and clinical data reported in the literature, we believe there are multiple studies in support of NLR as a prognostic factor in stage III and stage IV NSCLC. Our study does more than corroborate by further enhancing the understanding that elevated NLR works as a negative prognostic factor in patients with pulmonary comorbidities. Also, we completed the existent literature with a population from Romania offering data about clinical, pathological and prognostic in patients with advanced NSCLC and pulmonary comorbidities.

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NEWS

PEDIATRIC AND ADOLESCENT ONCOFERTILITY - S.O.S .- FUTURE IS HERE!

Oncofertility is the multidisciplinary approach of oncologists and reproductive fertility specialists working together to achieve the goals of patients with cancer. The treatments for cancer disease like radiotherapy, chemotherapy and surgery or maybe combined therapy with the above methods may lead to premature ovarian insufficiency (POI) or substantial male germ cell loss. Therefore, reproductive medicine experts strongly suggest that all young patients diagnosed with a malignant tumor should immediately undergo a consultation with suggestions for fertility and endocrine function protection and preservation- methods such as sparing surgery, cryopreservation and so on. In this review authors discuss about the background knowledge, methods, indications, pros and cons, and experimental and clinical applications of fertility preservation, and new strategies for future fertility conservation to help physicians, especially oncologist, pediatrician, hematologist, and surgeons-"multidisciplinary team", become aware of the concepts, methods, and importance of fertility and endocrine function protection. Although there are many reasons for fertility preservation in children and adolescents, cancer is currently the main indication for fertility preservation, and interest in this group of patients has led to development of the oncofertility focus (Zhao H, Jin L, Yufeng L, *et al.* Oncofertility: What can we do from bench to bedside? *Cancer Lett* 2019; 442: 148-160. doi: 10.1016/j.canlet.2018.10.023).

THE ANALYSIS OF THE RELATIONSHIP BETWEEN INFLAMMATION, DEPRESSION AND SUICIDAL BEHAVIOR

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THE ANALYSIS OF THE RELATIONSHIP BETWEEN INFLAMMATION, DEPRESSION AND SUICIDAL BEHAVIOR (Abstract): Major Depressive Disorder is in direct connection with serotonin, noradrenaline, and dopamine but the exploration of the role that inflammation has in depression has drawn to new conclusions that come in completion of the etiopathogenesis of depression. **Material and methods:** We have made a retrospective study consisting of a statistical comparative analysis that targets the patients committed to the Vth Clinic of the "Socola" Institute of Psychiatry from Iasi, between 2010-2017, diagnosed with major depressive disorder. There have been analyzed: demographic data, the intensity of the depression measured on the Hamilton scale, VSH values, and the suicidal associated risk. **Results:** There is a cumulation of factors that can determine the bidirectional relationship between depression and inflammation. The mechanisms that are at the base of it are not fully known but for women with associated suicidal risk from 2010, the elevated values of HAMD were associated with the higher values of VSH, that means at least for women, that depression is associated with a higher degree of inflammation. **Conclusions:** From the statistical analysis of the studied groups, the average value of HAMD was relatively constant as well in men and women, without considerable variations over the seven years. The difference consists in a stronger association between the women with severe depression and a higher VSH, over men, whereas we can observe lower VSH values, but with tendencies towards the superior limit, that supports an association between depression with the inflammation status, especially in patients with suicidal risk. **Keywords:** DEPRESSION, INFLAMMATION, SUICIDAL BEHAVIOR, IMMUNE SYSTEM.

A major depressive disorder is one of the more frequent conditions in psychiatric pathology and one of the main reasons for mortality and morbidity at a global level. Among the main criteria of this diagnostic are sad mood, anhedonia, fatigability, low vitality levels, symptoms that need to persist for at least two weeks. From the physiological point of view, the patient is presented with sleep disorders manifested as insomnia, low appetite, waking mood inconsistency, low libido. Also, the patient may experience feelings of futility, low energy levels and low focusing throughout the day, low self-esteem, predominant obsessive ideas of an autolytic nature the affects the overall ability of relationship functioning in society and family (1, 2).

The role of the inflammation in the etiopathogenic of depression is of very high

interest withing the research upon this field. Kafka, in 1990, in a letter to Miena, described his experience with tuberculosis *"The mind is the sick one and the lung condition is nothing else but the externalization of the psyche"* (3).

Depression consists of a rich heterogeneity both etiopathogenic and clinical. From the etiopathogenic point of view - depression can be classified as exogenous, endogenic, primary, and secondary, seasonal, or not, reactive, neurotic or psychotic, serotonergic, nor-adrenalin, or of dopaminergic nature.

Serotonin (5-HT) is a neuro-mediator with a modulator purpose within the neuronal circuit. Serotonin is created from the amino-acid tryptophan and is at the base of forming melatonin. It does not break the hematoencephalic barrier and it is secreted also at the central and peripheral levels. Some of the serotonin functions are the adjustment of the sleep and awake states, temperature adjustment, metabolism, sexuality, affectivity, motivation, pain, and adjustment of the blood pressure (4). From the limbic system point of view, dopamine has an uninhibited role and stimulates impulsivity. The inhibiting effect of the serotonin on the dopaminergic receptors dials down impulsivity, in such a way that when a deficit of serotonin occurs, therefore an imbalance between dopamine and serotonin levels, the excess of dopamine produces an uninhibited and impulsive behavior. Depression with high suicidal risk and bulimia are associated with increased impulsivity, and in some cases, the impulsivity may increase the risk of suicidal attempts (5).

Noradrenaline (NA) is a neuro mediator that affects multiple circuits that originate in the ocus coeruleus, which represents the nucleus with the most connections from the central nervous system that responds to new stimuli to manage the individual attention. Hypoactivity of these types of neurons generates hypoprosexia and hyperactivity produces an excessive fluctuation in attention that can be found as a symptom in maniacal behavior and ADHD (6).

Dopamine (DA), is the primary neuro mediators of the central nervous system and is a pre-component of nor-adrenaline. Dopamine is produced by the middle hypothalamic nucleus and has a role in motility control and muscle movement. It also has an inhibitory effect on prolactin. It reduces the levels of insulin production by the pancreas. Dopamine also reduces the activation of the lymphocytes within the immune system and from the renal point of view it modules the elimination of sodium by increasing the volume of excreted urine. DA also affects the pineal gland, having a very important role in the adjustment of the circadian/nictemeral rhythm. The deficit of dopamine can lead to certain neurological disorders such as Parkinson's Disease. The excess of dopamine may cause psychotic disorders and schizophrenia (7).

The connection between the immune system and the brain was not established until 1985 when a group of scientists has brought scientific proof regarding the suppression of the immune response in depression. The study had as primary objective exploring the role of inflammation in depression and the connected inflammatory mechanisms involved. The study leads to 3 main conclusions: High levels of inflammatory markers are present in patients with major depressive disorder, in absence of somatic conditions; the coexistence of depression with inflammation, and high risk of depression in the treatment with a cytokine (8). Numerous inflammatory bi-

omarkers have been identified with high values within the patients with depression (9). It is a well-known fact that major depressive disorder involves immune suppression that is proved by a reduction if cytotoxicity of the natural killer cells and a lymphocyte proliferation as well as an activation of the immune response indicated by an increase of the inflammatory mediator levels (10).

The effects of the inflammation on the psychic state and behavior could be partially mediated by the metabolites of the kynurenine pathways by modulating the neuroinflammation and neurotransmission of the glutamate (11). At the same time, in patients with suicidal tendencies, the factors that contribute to the inflammatory modifications can be attributed to mechanisms such as autoimmune responses, neurotropic pathogens, stress, and traumatic cerebral lesions (12, 13). Exploring the inflammatory systems from the perspective of its contributions to the suicidal behavior could offer new therapeutical approaches such as potential biomarkers for identifying patients with a high risk. To achieve the objective of improvement of the mechanisms of identifying and treating suicidal patients, the origin of the mechanisms of the effects of the suicidal behavior must be carefully studied (14, 15).

The therapeutical alliance between doctor, patient, and family represents the first step in initializing the depression treatment and ensuring better compliance of the treated patient. This aspect is essential both with patients treated ambulatory and with hospitalized patients (16). For many depressive patients, the association between psychotropic medication and psychotherapy seems to be the best option. Because of the high frequency of this particular pathology within the psychiatric practice, new directions in research of optimization of treatment need to be implemented and we are talking here of the hypothesis of the inflammatory mechanisms in depression (17, 18).

MATERIAL AND METHODS

To evaluate the correlation between inflammation, the intensity of depression, somatic and psychiatric comorbidities associated, we have made a retrospective study consisting of a statistical comparative analysis that targets patients committed to the V clinic of the "Socola" Institute of Psychiatry from Iasi, between 2010-2017 diagnosed with major depressive disorder (tab. I). The following parameters have been analyzed: demographic data, the intensity of the depression measured on the Hamilton scale, VSH values, and the suicidal associated risk.

The groups of patients were selected by accessing the electronic archive of the "Socola" Institute of Psychiatry from Iasi, and by consulting their observation files. The patients have expressed their consent when they were initially committed and agreed with the use of their data for scientific purposes.

Data analysis

The statistic calculations were made in *MS Excel* and pursued the correlations between the intensity of the depression using the HAMD score and VSH value. At the same time, the linear regression function was used and the statistical analyses *ANOVA* to underline the presence of a significant p, the significative threshold is 0.05.

RESULTS

The analysis of the values, graphics, and percent followed the VSH increasing values, according to the age group standards: groups of women and men ages between 20-40 years old and 41-60 years old

with VSH < 20 and women and men > 61 years old with VSH < 30. The age groups represent the second classification criteria. For each patient, it has been registered the level of depression (according to the Hamilton Depression Rating Scale / HAMD) and the anxiety level (according to the Hamilton Anxiety Scale / HAMA), and the interval and average values have been presented for each age group because, in the moment of commitment, the depression level (HAMD) was considered severe.

TABLE I

The report for men/women committed between the years 2010 and 2017

Year	Total of patients	MEN	WOMEN
2010	198	105	93
2017	156	79	67

By analyzing the graphic from above, we can conclude that there were no major differences between the number of women and men committed in the year 2010, and also 2017, the percentage is 53% men over 47% women and 51% men over 49% women. The statistic differences are very small even though in 2010 the differences were not far superior, it can be noted a tendency of equalization between the number of cases in men and women altogether.

If we are considering the environment whereas the patients were from, in the years 2010 and 2017, the stats are very similar with 54% from the urban environment over 46% from the rural environment. The environment does not represent a precise variable so it can influence this actual study, considering the population migration from rural to urban, most of them being probably classified in the electronic archive as coming from a rural environment.

Regarding the distribution on age groups, it has been noted that in the year 2010 as well as in 2017 the fact that the majority of patients are part of the age group 41-60 years old, with a significant decrease of the number of depression cases in the younger age group from 20-40 years old (from 10% in 2010 to 3% in 2017) and a slight increase in the number of patients with an age over 61 years old (from 22% in 2010 to 31% in 2017).

The main psychiatric comorbidity associated with the patients included in the study group was anxiety, measured through the Hamilton anxiety scale (tab. II).

TABLE II

Type of depression and the associated anxiety level

	2010	2017
Anxiety		
Mild Anxiety	16	20
Moderate anxiety	20	18
Severe anxiety	30	35
Depression		
Mild depression	0	0
Moderate depression	0	0
Severe depression	198	146

The Hamilton scale application for depression for the patients in the study group received a score of at least 27 points and the patients who did not get at least 27 points were not included in the present study. The average of the depression scores in 2017 for men was 32.25±2.19, and for anxiety of 23.62 ±5.83, and the average

value of VSH was 13.35±10.34. In regard to the women patients, in 2017 was registered a HAMD average of 32.04±2.23, and in the case of HAMA 26.3±1.33 with an average VSH value of 19.17±8.17. Following the same parameters, in 2010 the average of scores for depression calculated with the HAMD scale in the case of men was 32.41 ±2.52, with average anxiety of 25.6 ±4.36 and an average VSH of 21.11 ±7.27. In the women's case study, in 2010 the average HAMD value was 31.14 ±2.56, for HAMA 25.51 ±1.46 with an average value of VSH of 22.25 ±6.76.

Between the psychiatric comorbidities, the most frequently associated with depression for the patients included in the study, were the following: emotionally unstable personality disorder, addictive personality disorder, panic disorder, autolytic tendency, autolytic ideation, and dementia, according to the table below. In the following we will consider the autolytic ideation and autolytic tendencies, as a small sub-group with suicidal tendencies, to follow the associated particularities (tab. III).

TABLE III

Associated psychiatric comorbidities

Associated psychiatric comorbidities	2010	2017
TP emotional instability	7	12
TP addictive	15	18
Panic disorder	10	32
Autolytic tendencies	25	27
Autolytic ideation	12	18
Dementia	5	2

By analyzing the distribution between the age groups of the patients with suicidal risk, there noted significant differences between the young patients between 20-40 years, whereas a constant increase has been noted starting from only 4 patients in 2010 to 11 patients in 2017. The age group with the biggest number of patients with suicidal risk remains the over 60 years group for 2010, with 20 patients, as well as for 2017 with 22 patients, and for patients over 61 years old, a relatively constant number remained from 11 to 12.

We analyzed the women's age group with suicidal risk identified in the year 2017.

For the 14 patients, we identified with an average value for the HAMD score of 33 ± standard deviations 2.44, an average value of VSH of 24.9 ± standard deviations of 7.57, and a HAMA average value of 26.37 ± standard deviations of 1.3.

To identify and study the relationship between VSH and HAMD in the study group the coefficient *Pearson* was calculated at r=0.04, a value that suggests a moderate correlation between the 2 variables. The positive sign suggests that the low scored withing the first variable, correlates with the low scores in the second variable, average scores from the first variable associated with average scores in the second variable, and high scores in the first variable associated with high scores in the second variable. To establish the value of p the linear regression function from Excel was used, the significand threshold being of <0.05. It has been noted that the statistical analysis

ANOVA between the values of HAMD and VSH F(1.12)=0.5, at a p 0.1.

As the indicated value from the graphic above, in the case of women with suicidal risk selected in 2017, the higher the depression score HAMD the higher the value of VSH - that suggests that the inflammation status tends to go up.

Similarly, we analyzed the group of women with suicidal risk from 2010 and we identified an average value of HAMD of 32.181 ± 3.33 standard deviation, with an average value of VSH of 25.090 ± 6.33, standard deviation and a HAMA average value of 25.545 ± 1.47 standard deviation.

The Pearson coefficient was calculated, with an r=0.61, which suggests a powerful correlation between HAMD and the VSH value. The statistical analysis continues to evidentiate a p=0.05, statistically significant.

We concluded that in the cases of women with associated suicidal risk from 2010, the elevated values of HAMD are associated with the higher values of VSH, and we can conclude, at least for women, that depression is associated with a higher degree of inflammation.

In the men cases with associated suicidal risk from 2017, we identified an average HAMD score of 32.56 ± 2.15 standard deviation, with an average VSH of 16.75 ± 11.9 standard deviations and a HAMA value of 26.5 ± 1.54 standard deviation. We can observe an average value a lot lower than the VSH associated, compared with the women study group.

The calculation of the Pearson coefficient evidentiate a r=0.26, suggest a powerful correlation between HAMD and VSH. The linear regression function and statistical analysis ANOVA have identified a p=0.04 significantly statistic.

The graphic representation sustains the hypothesis according to which the highest HAMD values are associated with the over the average that tends to go towards the superior limit of VSH.

The men group from 2010 with a suicidal risk have the following particularities: average HAMD value of 32.41 ± 2.95, standard deviation, average VSH=20.67 ± 8.05 standard deviation and HAMA average =23.64 ± 4.22 standard deviation.

DISCUSSION

For women, the decreased state of anxiety in the last years is related to other factors. Therefore, we consider that the increased understanding of the factors that maintain the anxiety problems were acquired through efficient strategies of relaxation, with changes in the way of thinking that predisposes to anxiety and the correction of the behaviors that maintain anxiety.

Also, there have been acquired some strategies of developing positive emotions, and the positive side of life, strategies for prevention of the relapses with reduction of the symptoms of depression that come with anxiety.

Once the cytokines reach the cerebral level, they influence the ax involved in the psychopathology of depression by metabolically altering the neurotransmitter 5-HT, DA, and NA. Tryptophan is a component of serotonin and melatonin. Within the immune response of the T cells helper 1 (TH1) microglia and macrophages produce interleukin 1 (IL-1), interferon ɣ (IFN ɣ) and the necrosis tumor factor α that induce the production of indolamine 2,3-dyoxigenase (IDO) favoring the conversion of tryptophan in quinolinic acid, an agonist of the N-methyl-D-aspartic (NMDA) acid and kynurenic acid, the antagonist of NMDA (19).

One of the most debated associations in this field is the connection with the bilateral potential between depression and pain affecting the population at a global level. We are talking about the co-existence of almost 80% (20), contributing often to an increased mortality rate (21).

According to a report from the World Health Organisation, every year occur 800,000 deaths by suicide (22). The cultural taboos, as well as the fact that death by suicide is considered a criminal act in some countries can influence the statistics (23). In the present day, it is estimated that the suicide attempts are from 10 to 20 times more frequent than the actual number of deaths by suicide. The number of deaths by suicide as well as the attempted suicides is the result of a major severe psychiatric sufferance. The carried-out attempts of suicide carry with them an emotional burden that affects both the individual and the family for a long period (24).

Even though the pharmaceutical treatment and addressability for depressive patients with suicidal behavior are continuously rising, the frequency of suicides and suicide attempts does not register considerably lower rates. It is estimated that approximately 1.53 million deaths by suicide will be registered in 2020, an estimation that is at an alarming rate, and changes in the field are a must (25). Being able to assess suicidal behavior is a constant challenge for psychiatrists, consider the fact that most patients do not present the actual gravity of the symptoms, and diminish them. In some cases, the medical system cannot precisely detect the gravity of the phenomenon even though it is well known that 50% of these patients get in contact with the suppliers of medical services, several months before the first suicidal attempt (26).

In consequence, it is required an improvement within the methods of diagnosing the risk of suicide, access to more efficient options of treatment, and counseling towards suicidal patients (27, 28).

Here in our results, we showed that there is a cumulation of factors that can determine the bidirectional relationship between depression and inflammation. The mechanisms that are at the base of it are not fully known.

The inflammation hypothesis was under discussion more and more often lately, and the biological abnormalities in major depressive disorder were highlighted in numerous studies.

There is a cumulation of factors that can determine the bidirectional relationship between depression and inflammation, but the mechanisms that are at the base of it are not fully known.

CONCLUSIONS

The current data do not allow us to draw definitive and clear conclusions over the hypothesis about the inflammation in depression, but we can speculate a tendency and a repetitive pattern in this way.

From the statistical analysis of the groups studied, we understood that the average value of HAMD was relatively constant as well in men and women, without considerable variations over 7 years. The difference consists in a stronger association between the women with severe depression and a higher VSH, over men, whereas we can observe lower VSH values, but with tendencies towards the superior limit, a fact that supports the hypothesis of an association between depression with the inflammation status, especially in patients with suicidal risk.

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NEWS

COMMON ALLERGY DRUG MAKES RESISTANT BACTERIA VULNERABLE TO ANTIBIOTICS

The common antihistamine loratadine-the active ingredient in over-the-counter medications such as Claritin, Alerclear, and Alavert-makes some species of antibiotic-resistant bacteria susceptible to antibiotics in lab experiments. To cope with the rise of these stubborn bacterial strains, clinicians often combine different antibiotics or prescribe them along with drugs that help prevent bacterial enzymes from degrading antibiotics. But for now, there are no drugs that increase bacterial susceptibility to existing antibiotics. “It’s totally unexpected to see an antihistamine compound have this effect” says chemist Steven D. Townsend of Vanderbilt University, who was not involved in the study. He adds that if the findings hold up in clinical studies, loratadine’s antibacterial activities “could be really powerful”. The researchers cultured varieties of *S. aureus* and *S. epidermidis*, including standard laboratory strains and ones isolated from hospital-acquired or community-acquired infections. They treated the bacteria with a common antibiotic, oxacillin, in the presence of loratadine and desloratadine, a bioactive metabolite of the drug. When treated with loratadine, *S. aureus* strains were 8 to 500 times as sensitive to the antibiotic. Desloratadine did not affect *S. aureus* susceptibility, and neither compound had any effect on *S. epidermidis*. Antibiotic resistance and biofilm formation are regulated by similar genetic mechanisms. Biofilms enhance drug resistance and are the main cause of catheter-associated infections. When the researchers tested the effects of applying the antihistamines to bacteria in culture, they found that loratadine blocked biofilm formation and broke up preformed biofilms of all *S. aureus* strains and of some *S. epidermidis* strains tested. The team then sought loratadine’s targets. Both microbes carry a gene named *stk* whose protein triggers the synthesis of antibiotic-blocking enzymes. Mutant *S. aureus* strains that lack *stk* were not made more sensitive to oxacillin by loratadine, suggesting the gene’s product could be loratadine’s target. Further studies in animal models will be needed to see if loratadine is effective against infections. Moreover, the authors add that loratadine—taken as a pill for allergies-is converted into desloratadine in the body, and the latter was ineffective in their experiments. “Loratadine could have applications as a topical ointment on burns or wounds” where it would not be metabolized, Blackledge says. (Jyoti Madhusoodanan, special to *C&EN , Chemical and Engineering News*, June 12, 2019,Volume 97, Issue 24 about the article *From Antihistamine to Anti-infective: Loratadine Inhibition of Regulatory PASTA Kinases in Staphylococci Reduces Biofilm Formation and Potentiates β-Lactam Antibiotics and Vancomycin in Resistant Strains of Staphylococcus aureus*. Cutrona N, Gillard K, Ulrich R, Seemann M, Miller HB, Blackledge MS, *ACS Infect Dis* 2019; doi: 10.1021/acsinfecdis.9b00096).

ANALYSIS OF THE NEWBORNS FROM HIV POSITIVE-MOTHERS IN A PERIOD OF THREE YEARS FROM THE REGIONAL HIV-AIDS CENTER IASI, ROMANIA

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ANALYSIS OF THE NEWBORNS FROM HIV POSITIVE-MOTHERS IN A PERIOD OF THREE YEARS FROM THE REGIONAL HIV-AIDS CENTER IASI, ROMANIA (Abstract) **Aim:** With proper medication and regular monitoring, pregnant women with human immunodeficiency virus (HIV) can give birth to a healthy child. With the implementation of programs to prevent vertical transmission, the number of HIV-positive newborns has decreased significantly. The aim of this paper is to evaluate the HIV status of newborns perinatally exposed through the infection. **Material and methods:** We conduct a retrospective study on the HIV positive women registered at the "Sf. Parascheva" Infectious Disease Hospital from Iasi, which became mothers between 2017-2019. **Results**: During the three years mentioned period, 101 newborns from HIV positive mothers were included in the study, four being from untreated mothers. All the children received prophylactic antiretroviral compounds. At the end of the monitored period, two children had detectable viremia. **Conclusions**: Evaluation of HIV-positive mothers and prophylaxis of newborns during the evaluated period, which was conducted according to the protocol, lead to a low percentage of HIV-positive children. **Keywords:** MATERNAL-FETAL TRANSMISSION, ANTIRETROVIRAL THERAPY, PROFYLAXIS, HIV.

Human immunodeficiency virus (HIV) / acquired immune deficiency syndrome (AIDS) is more than a public health priority. It is a complex problem that affects all components of the society (1).

Although HIV infection is perceived as one of the most feared conditions, most infected couples (both partners being HIV positive) or serodiscordant couples want at least one child. From a medical point of view, due to the evolution of medicine, the risks of transmitting HIV infection from mother to fetus is much lower (2).

The detection of HIV-positive pregnant women is essential to prevent the transmission of the infection (3). The vertical transmission of the infection may occur during pregnancy, labor or birth delivery, or breastfeeding. Without treatment, about 15-30% of children born from HIV-positive mothers will become HIV infected during pregnancy or childbirth. Other 5-20% will

be infected by breastfeeding (4).

Maternal-fetal transmission in Romania has decreased dramatically since the beginning of the 1990s, from 45% to <3% at the beginning of 2018 (5). In the last years, two frequency peaks were registered, in 2010 (4.7%) and in 2013 (3%), so that in 2016, they registered the lowest value (0.7%). Among children (0-14 years), the vertical transmission path is on the 2nd place (6).

Epidemiological surveillance, antiretroviral treatment (ARV) and prevention of the maternal-fetal transmission of infection are performed according to national protocols, respecting the recommendations of the World Health Organization (WHO) (7). Programs to prevent vertical transmission started in the North-eastern part of Romania since 2004, due to the increasing number of cases of the virus transmission from mother to fetus (prenatal, perinatal, and postnatal). That is determined by the high incidence of HIV infection in young women with a low educational level, and also of the increasing number of injecting drug users (8).

A strategy consists in three components that are necessary to prevent the transmission of infection from mother to her child: preventing HIV infection among future parents, avoiding unwanted pregnancies among HIV-positive women, and preventing HIV transmission from HIV-positive mothers to their children during pregnancy, contractions, births, and breastfeeding (9).

MATERIAL AND METHODS

Studied patients

The monitoring sheets of HIV-infected pregnant women from the North-Eastern part of Romania were analyzed retrospectively, in order to highlight the status of the newborns, as well as to comply with the prophylaxis programs regarding the vertical transmission of the virus.

The study included females diagnosed with HIV who became pregnant and gave birth to live children during the study period. The exclusion criteria consisted of pregnancies that did not result in the birth of live children and the abortions.

Data analysis

The study summarized a period of three years, respectively from January 2017 to December 2019, and the data were obtained from the medical records of the patients from Iasi Regional HIV-AIDS Center.

Data were analyzed by descriptive statistics, where applicable. Statistical analysis was performed using *XLSTAT* version 2019 software.

RESULTS AND DISCUSSION

The Regional HIV-AIDS Center from Iasi, a center that is serving to the HIV-positive population from the North-Eastern region of Romania, is part of the St Parascheva Infectious Disease Hospital from Iasi.

Currently, in the records, there are more than 1440 patients, 46% of whom are female. Analyzing their monitoring sheets, we found that, during the aforementioned period, 101 patients became mothers. The largest group was represented by female between 26-35 years old (77.23%), and most of them were from urban areas (67.32%). If we look back, the average age of the mothers in the study group reminds us of the pediatric cohort from 1989-1992. Then over 13,000 children were infected with the HIV virus in Romanian hospitals. More than half of them still managed to survive, although no one gave them a chance (10). Today, they are between 28

and 31 years old and can live normally. Of the studied group, 52 (51.48%) females are part of that cohort.

Most women diagnosed with HIV are of childbearing age, which requires an increased need for choices about whether or not they want biological children. The opinions of the life partner, of the family members, the fear of stigmatization can significantly influence the decision. An additional factor is the fear of transmitting HIV to their child (11). The support of the attending physician associated with psychotherapy sessions can positively influence the decision of having a child (12).

The vulnerability of women living with HIV is higher than other population groups. This can be determined by the person's status, the social and economic context in which the person lives, and the stereotypes present in society (13). Also, the presence of comorbidities in people with HIV contributes to the increase of vulnerability and in the decisions about giving birth. For example, the association of infection with hepatitis B virus, an infection that can also be transmitted vertically from mother to fetus (14). The results of our study show that 6.93% (7 cases) associated HBV infection.

Once the decision to have a child has been made, careful monitoring is necessary to stop the transmission of the infection to the fetus. One of the components of the prophylaxis program concerns the administration of antiretroviral therapy to the mother (15). In our study, 97 (96.03%) of the pregnant women were monitored during pregnancy and received prophylactic treatment. Ninety of them throughout all pregnancy, 6 only in the last trimester of pregnancy, and in one case the monitoring began at birth, because the mother was tested for HIV at birth. The mother's ARV therapy is shown in first table.

TABLE I

Mother's ARV therapy

ARV therapy	No of patients (%)
KLT + CBV	58 (57,42%)
KLT + KIV	3 (2.97%)
KLT + RAL	2 (1.98%)
RAL + KIV	5 (4.95%)
RAL + CBV	2 (1.98%)
DRV/RTV + KIV	11 (10.89%)
DRV/RTV + ETV	2 (1.98%)
DRT/RTV + CBV	1 (0.99%)
DRV/RTV + 3TC	1 (0.99%)
FTC/TDF + RAL	3 (2.97%)
FTC/TDF + RIL	1 (0.99%)
CBV + KLT + RAL	1 (0.99%)
FTC/TDF + ATZ + RTV	1 (0.99%)
FTC/TNF + DRV/RTV	2 (1.98%)
DRV/RTV + 3TC + RAL	1 (0.99%)
DRV/RTV + ETV + RAL	1 (0.99%)
DRV/RTV + CBV + RAL	2 (1.98%)

KAL – Kaletra, CBV – Combivir, KIV – Kivexa, RAL – Raltegravir, DRV – Darunavir, RTV – Ritonavir, ETV – Etravirine, FTC – Emtricitabine,3TC – Lamivudine, RIL – Rilpirine, TDF – Tenofovir, AZT - Zidovudine

It consisted in a combination of Lopinavir – Ritonavir (Kaletra) with Zidovudine- Lamivudine (Combivir) throughout the pregnancy in 58 cases (57.42%), other antiretroviral regimens in 39 cases (38.61%) and in four cases (3.96%), no treatment regimen was administered, due to the mother's non-compliance or because the mother was tested for HIV at birth.

Thus, between 2017-2019, 101 children were born, 41 females (40.9%) and 60 males (59.40%). The distribution by years is shown in the second table.

TABLE II

Distribution of newborns from HIV-positive mothers by years

2017		2018		2019	
F (%)	M (%)	F (%)	M (%)	F (%)	M (%)
15(45.45%)	18(54.54%)	12(33.33%)	24(66.66%)	14(43.75%)	18(56.25%)

F – female, M – male

Another strategy to prevent maternal-fetal transmission is scheduled caesarean section (16). From the data obtained, we observed that the birth of the children in the study was by caesarean section in most cases, respectively 99 cases (98.01%), except for 2 children who were born through the natural way (1.98%), the mother was not previously diagnosed with HIV3.

In addition to maternal prophylaxis, child prophylaxis should be performed, with the risk of perinatal transmission being reduced to 0.4% in developed countries (17). Postexposure prophylaxis can offer protection against HIV infection even to babies of women who missed opportunities to be counselled and tested before or during pregnancy. The nevirapine and zidovudine regimen is safe and easy to implement (18). For all newborns from this study the prophylaxis was done with Zidovudine and Lamivudine in 98 cases, with Zidovudine only in 2 cases and in one case was associated Kaletra to Zidovudine and Lamivudine (tab. III). All babies received postexposure prophylaxis for a period of 6 weeks.

TABLE III

Distribution of newborns from HIV-mother by ARV prophylaxis

ARV prophylaxis	2017	2018	2019
AZT + 3TC	31	36	31
AZT	1	0	0
AZT + 3TC + KLT	1	0	1

ARV – antiretroviral therapy, AZT – Zidovudine, 3TC – Lamivudine, KLT – Kaletra

As soon as the fetus is born, they are under regular monitoring to evaluate the viral load and the number of CD4 lymphocytes, as well as serological testing. Monitoring takes place at birth, at one month, at 3, 6, 9, 12 and 18 months, respectively. The HIV status of a child is finally declared at 18 months, after a PCR- HIV test. A patient of 18 months who comes from an infected mother and who has positive serological tests is considered HIV infected (19,20).

By the time this article was written, the surveillance period for 78 children had ended, with 76 having undetectable viremia at the age of 1 and a half. Two children had

detectable viremia at birth, respectively they remained positive at 18 months. Two children remained positive at 18 months and the further therapy for them consisted of Zidovudine + Lamivudine + Nevirapine. For some newborns in 2019, the surveillance period is not over. Twenty-three children continue to be under clinical and para-clinical supervision. However, the data obtained so far show that none show detectable viremia, which indicates the good evolution of the cases and the non-transmission of the infection from mother to fetus.

CONCLUSIONS

The evaluation of HIV-positive mothers and prophylaxis of newborns during the evaluated period was conducted according to the protocol, which resulted in a low percentage of HIV-positive children (1.9%).

All pregnant women in vulnerable populations should have the same access to services during pregnancy to prevent the transmission of HIV from mother to fetus. Preventing the vertical transmission of HIV aimed to reduce the incidence of this pathology.

In this epidemiological context, the issue of transmitting the infection from mother to fetus remains current, which is why the monitoring of this phenomenon should be continued in the coming years.

With proper medication and regular monitoring, pregnant women infected with the human immunodeficiency virus (HIV) can give birth to a healthy child. With the implementation of programs to prevent vertical transmission, the number of HIV-positive newborns has significantly decreased.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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NEWS

HEARING LOSS IN RHEUMATOID ARTHRITIS

Although hearing loss is not officially recognized as an extra-articular manifestation of rheumatoid arthritis, it is the subject of many studies that investigate its association with this disease. A recent systematic review and meta-analysis explored 12 such observational studies which included a total number of 20,022 patients and 79,244 controls. The authors concluded that rheumatoid arthritis is significantly associated with increased odds (OR 3.42, 95% CI 2.50-4.69) compared to controls and with increased risk of sensorineural hearing loss (RR 2.28, 95% CI 1.88-2.76), while there is no association with conductive hearing loss or mixed hearing loss. This can be due to direct damage to the cochlea by autoantibodies, vasculitis affecting the cochlea or the cochlear nerve blood supply, or an iatrogenic mechanism. Therefore, an early screening of rheumatoid arthritis patients with pure tone audiometry could be of value. (Chaitidis N, Theocharis P, Festas C, Aritzi I. Association of rheumatoid arthritis with hearing loss: a systematic review and meta-analysis. *Rheumatol Int* 2020; 40: 1771-1779).

THE ROLE OF COMPREHENSIVE GERIATRIC ASSESSMENT IN THE COMPLEX MANAGEMENT OF SENIOR PATIENTS WITH HIP FRACTURE

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THE ROLE OF COMPREHENSIVE GERIATRIC ASSESSMENT IN THE COMPLEX MANAGEMENT OF SENIOR PATIENTS WITH HIP FRACTURE. (Abstract): The increased incidence of hip fracture in the frail elderly patients requires the development of differentiated care, adapted to their physical, psychological and emotional profile. Pre- and postoperative comprehensive geriatric assessment (CGA) is the most accurate tool for developing a personalized therapeutic and care plan. **Aim:** To investigate the consequences of the surgical treatment of hip fractures on senior patients using CGA preoperatively and in the immediate postoperative period. **Material and methods:** The prospective study, from which we present the preliminary data, was conducted on a group of 95 patients admitted in the Trauma Clinic with hip fracture. Of these, only 59 underwent CGA preoperatively and 48 hours postoperatively. Particular attention was given to frailty - evaluated with Groningen Frailty Index (GFI), neurocognitive status - evaluated with Mini-Mental State Examination (MMSE), and psycho-emotional status - evaluated with Geriatric Depression Scale (GDS). **Results:** Analyzing the GFI scores ($p = 0.014$) it was found that of the preoperatively robust patients 71.4% became frail postoperatively. No statistically significant differences in cognitive and psycho-emotional status were found in the immediate postoperative compared to the initial preoperative assessment, probably due to the small sample size. **Conclusions:** The results of this study confirm the importance of evaluating the degree of frailty for the prediction of the postoperative evolution of hip fractures in senior patients. **Keywords:** COMPREHENSIVE GERIATRIC EVALUATION, HIP FRACTURE, SENIOR PATIENTS, SURGERY.

Falls represent a frequent cause of morbidity, mortality and decreased QoL in senior population, as they are associated with severe physical and psychological trauma. Statistics report that 30% of the elderly fall annually, and more than half of this group fall several times a year (1-4). Hip fractures are among the most frequent types of traumas in aged people, fractures caused by backward falls being increasingly common (5, 6).

In this vulnerable population, a traumat-

ic event is associated with the aggravation of functional decline (frailty, sarcopenia), social withdraw, anxiety, depression, prolonged bed immobilization, fear of future recurrent falls, and increased use of medical services (7). These are the main reasons why these patients should be hospitalized immediately in a Trauma Department - preferably an integrated orthogeriatric clinic - in order to identify the best therapeutic approach. The surgical treatment is always preferred over immobilization in a cast (8-11). The decision of whether or not to perform surgery in these patients should be made according to the results of the geriatric assessment, preferably the CGA (12-14). The postoperative recovery of these patients relies on their degree of frailty, resilience, and family support, important factors that will help them to fulfill the physical and psychological program designed by the geriatric team. The prognosis of recovery in these patients is strongly related to the geriatric evaluation (15-17).

MATERIAL AND METHODS

We present a prospective study carried out between June 2019 and March 2020 on a group of senior patients admitted with hip fracture in the Orthopedics-Traumatology Clinic of the Iasi County "St. Spiridon" Emergency Hospital. The CGA was performed by a geriatric team in the preoperative period and 48 hours postoperatively. Special attention was paid to assessing the degree of frailty using GFI, neurocognitive status using MMSE, and psycho-affective status using GDS. Of the 95 patients, only 59 (62.1%) patients were assessed postoperatively, due to the epidemiological context that restricted multidisciplinary access and the inability of patients to collaborate postoperatively (mainly due to significant pain).

The informed consent was obtained from all patients prior to the completion of the questionnaires by the same trained medical team (18).

Data were analyzed using *SPSS 18.0* software. ANOVA test, F test (ANOVA), multivariate analysis, ROC curve, χ^2 and *t-Student's* test, Kruskall-Wallis and Pearson correlation were used for the quantitative interpretation of the data. Statistical significance was set at $p < 0.05$, (95% confidence interval).

RESULTS

The analyze of the demographic data showed that the age of the study patients ranged from 61 to 96 years, with a mean of 77.58 (± 9.02) years, close to the median (78 years), suggesting that age-range was homogeneous, so tests of statistical significance could be run.

Of all study patients, 50.8% were older than the median, 64.4% were male, and 61% were not married (tab. I).

TABLE I

Descriptive demographic data

Demographic characteristics	n = 59
Mean age ± SD, years (median/min-max)	77.58 ± 9.02 (78/61-96)
≥ 78 years, n (%)	30 (50.8%)
Male, n(%)	38 (64.4%)
Unmarried / divorced / widowed, no (%)	36 (61.0%)

Preoperative assessment – statistical indicators

Preoperatively, the GFI score ranged from 0 to 12; the mean 5.20 ± 3.11 was close to the median value (score = 4), and a value < 2 for skewness suggested that the

range of GFI scores was homogeneous (tab. II).

The score for cognitive assessment (MMSE) ranged from 5 to 30; the mean 22.20 ± 5.73 was close to the median value (score = 23), and a value of >-2 for skewness suggested that the preoperatively recorded range of MMSE scores was homogeneous (tab. II).

The score for emotional assessment (GDS) was in the range 0-14; the mean 6.34 ± 3.58 was close to the median value (score = 6), and the value of >-2 for skewness suggested that the preoperatively recorded range of GDS scores were homogeneous (tab. II).

TABLE II

Statistical indicators of preoperatively monitored scores

Score		MMSE	GDS	GFI
Evaluations		59	95	59
Mean		22.20	6.34	5.20
Median		23	6	4
Standard Deviation		5.73	3.58	3.11
Variance		25.81	12.85	9.68
Skewness Test		-0.609	-0.047	0.376
Std. Error of Skewness		0.311	0.311	0.311
Minimum		5	0	0
Maximum		30	14	12
Percentile	**25**	17	4	3
	50	23	6	4
	75	27	9	8

Postoperative assessment – statistical indicators

Postoperatively, the GFI score ranged from 1-13; the mean 7.39 ± 3.20 was close to the median value (score = 7), and the value of > -2 for skewness suggested that the range of GFI scores was homogeneous (tab. III).

The postoperative MMSE score ranged from 8 to 30; the mean 21.58 ± 5.71 was close to the median value (score = 22), and the value of > -2 for skewness suggested that the range of MMSE scores was homogeneous (tab. III).

The postoperative GDS score ranged from 1 to 14; the mean 6.76 ± 3.32 was close to the median (score = 7), and the value of >-2 for skewness suggested that the range of GDS scores was homogeneous (tab. III).

The homogeneity of preoperative and postoperative MMSE, GDS and GFI scores allowed us to use significance tests for continuous variables.

The percentage distribution of cases with cognitive (p = 0.052) or emotional (p = 0.157) impairment had slightly increased postoperatively (tab. IV).

The percentage of cases with frailty increased significantly postoperatively, from 64.4% preoperatively to 86.4% postoperatively (p = 0.002) (tab. IV).

TABLE III

Statistical indicators of postoperatively monitored scores

Score		MMSE	GDS	GFI
Evaluation		59	95	59
Mean		21.58	6.76	7.39
Median		22	7	7
Standard Deviation		5.71	3.32	3.20
Variance		32.59	11.01	10.21
Skewness Test		-0.499	-0.022	-0.010
Std. Error of Skewness		0.311	0.311	0.311
Minimum		8	1	1
Maximum		30	14	13
Percentile	**25**	18	4	5
	50	22	7	7
	75	26	9	10

TABLE IV

Postoperative cognitive, emotional, and frailty status

Score	Preoperative	Postoperative	Significance test	p
MMSE Cognitive impairment	22.20±5.73	21.58±5.71	Paired Samples T test	0.151
severe moderate mild normal	1 (1.7%) 17 (28.8%) 20 (33.9%) 21 (35.6%)	3 (5.1%) 16 (27.1%) 23 (39.0%) 17 (28.8%)	Wilcoxon Signed Ranks Test	0.052
GDS Emotional impairment	6.34±3.58	6.76±3.32	Paired Samples T test	0.123
very depressed mildly depressed normal	7 (11.9%) 31 (52.5%) 21 (35.6%)	8 (13.6%) 66 (55.9%) 18 (30.5%)	Wilcoxon Signed Ranks Test	0.157
GFI	5.20±3.11	7.39±3.20	Paired Samples T test	0.001
frail robust	38 (64.4%) 21 (35.6%)	51 (86.4%) 8 (13.6%)	Wilcoxon Signed Ranks Test	0.002

Analyzing the postoperative GFI scores, the following was found (p = 0.014) (fig.1): 71.4% of the preoperatively robust patients became frail postoperatively.

After plotting the ROC curve, it was found that the marital status (AUC = 0.632; 95% CI: 0.442-0.823; p = 0.232); emotional score (AUC = 0.735; 0.579-0.891; p = 0.034) and preoperative frailty (AUC = 0.767; 0.635-0.900; p = 0.016) are good predictors of postoperative frailty (fig. 2, tab. V).

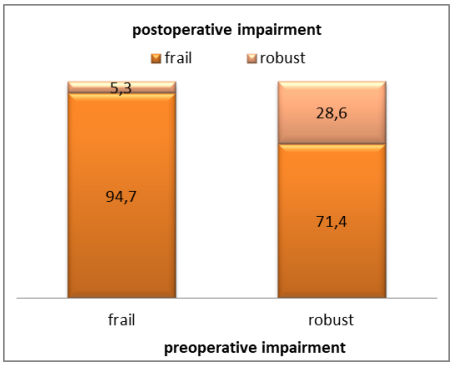

Fig. 1. Correlation of pre- and postoperative emotional impairment

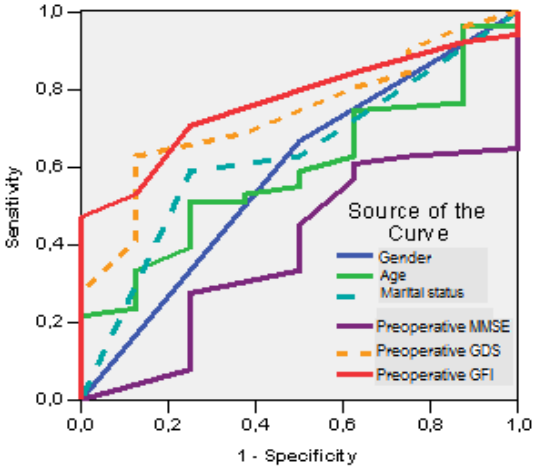

Fig. 2. ROC curve. Demographic characteristics, scores for cognitive and emotional Impairment, and preoperative frailty in the determinism of postoperative frailty

TABLE IV

Demographic characteristics, scores for cognitive and emotional impairment and preoperative frailty in the determinism of postoperative frailty

Parameters	AUC	Standard error	Statistical significance	Confidence interval 95%
Gender	0.583	0.111	0.452	0.365-0.802
Age	0.589	0.092	0.419	0.408-0.770
Marital status	**0.632**	0.097	0.232	0.442-0.823
Preoperative MMSE	0.386	0.096	0.303	0.197-0.575
Preoperative GDS	**0.735**	0.080	0.034	0.579-0.891
Preoperative GFI	**0.767**	0.068	0.016	0.635-0.900

DISCUSSION

In the context of demographic aging, the incidence of hip fractures will increase directly proportional to life expectancy, risk of falling, and tendency to osteoporosis. On average, one in three patients dies within the first year after suffering this type of fracture, and in over half of these patients mobility is still limited one year after trauma. Age-related issues, such as psycho-emotional status, frailty, sarcopenia, and others, indicate that the appropriate treatment is a complex one, and patient assessment in the pre- and postoperative period must be a holistic one. The risks for patients to develop severe complications and loss of functionality are considerable and often associated with high treatment costs (19-22).

The assessment of the personal status of the injured patient pre and postoperatively using a most reliable geriatric tool (CGA) is an important predictor of the physical and psychological resilience of the patient, an essential component of the recovery period (23, 24). Of the many aspects of the CGA our study focused on frailty, cognition impairment and depression as predictors of postoperatively evolution.

Most studies report frailty as a major component in the occurrence of postoperative adverse outcomes in senior patients (25-28). Our results confirm these data, alarming increase of frailty being recorded postoperatively in the preoperatively robust patients. Thus, the need for complex and sustained treatment of frailty through early rehabilitation: physical therapy, prevention of protein-calorie malnutrition and sarcopenia, and psychological support.

Congruent results suggest that cognitive impairment, was found to be correlated with limited functional gains and poor postoperative rehabilitation outcomes. Therefore, cognitive impairment has traditionally been regarded as an obstacle in geriatric rehabilitation. Specific studies in senior patients with hip fractures have led to the assumption that they should not be considered suitable candidates for active rehabilitation programs. It seems obvious that cognitive status plays a major role in the success of rehabilitation. A significant number of elderly patients undergoing rehabilitation programs associate cognitive deficits, therefore it is important to measure the success of rehabilitation in the senior patients with cognitive impairments who have suffered a hip fracture (29-32).

Our results do not confirm these data, the cognitive function of our patients being preserved independently of the impact of the fracture and/or surgical treatment. We consider that our data are influenced by the small sample size, and we intend to continue the study as soon as the epidemiological situation allows it.

Regarding the psycho-emotional status, some data suggest that in the senior patients who underwent hip fracture surgery, depression symptoms were associated with the underlying Alzheimer's disease and postoperative delirium. According to these results, the initially mild depression symptoms were the strongest predictor of postoperative delirium, representing a prodrome of dementia (33). Our results are not statistically significant for the psycho-emotional component. Thus, emotional status does not undergo the deconditioning inherent in trauma or surgery. In this respect, the literature indicates that fracture type or the degree of post-traumatic disability are not as important predictors of

psycho-emotional impairment in the post-operative period as the state of apathy. The only direct relationship is between depression and fall risk. The same studies highlighted a correlation between hip fracture, depression and 5-HTTLPR genotype, indicating a genetic predisposition for the development of an emotional impairment (34, 35).

CONCLUSIONS

Integrated orthogeriatric treatment should be the standard approach to the multidimensional needs of senior patients with hip fracture. Patient characteristics play a decisive role in recovery outcomes. For clinicians, it is critical to gain a clear perspective of the underlying factors influencing the poor outcome after a hip fracture, such as the degree of frailty and psycho-emotional impairment.

Preoperative assessment should accurately estimate the degree of frailty, cognitive and psycho-emotional status, as any degree of recorded dysfunction is a negative predictor for all components of post-operative recovery. Frailty assessed as GFI score > 4 is decisively correlated with the traumatic and surgical impact, independent of patient preoperative status. In this respect, cultivating the resilience-enhancement interventions is the primary goal of orthogeriatric treatment. The holistic, multidisciplinary, and at the same time individualized approach of the senior patients who suffered a hip fracture prevents the negative prognostic value of any cognitive-emotional prototype, but also of any frailty phenotype.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

REGIONAL DIFFERENCES IN THE IMPACT OF DIABETES ON POPULATION HEALTH

The study carried out in the USA evaluated regional disparities in the influence of diabetes on population health and examined life expectancies at age 50 between population with diabetes and healthy population and life quality among the population with diabetes among native-born Americans by birth region and current residence. Using data on a cohort of 17,686 native-born individuals from the Health and Retirement Survey (1998-2014), the authors applied a Bayesian multistate life table method to estimate life expectancies at age 50 between population with diabetes and healthy population by each birth/current region combination. They estimated the proportion of life remaining without either chronic conditions or disabilities as a quality-of-life measure and the probabilities that one region is worse than the other in terms of different health outcomes. As results, the authors showed that, at age 50, persons with diabetes were expected to live on average 5.8-10.8 years less than their healthy equivalents across regions. Diabetes had the greatest influence on life expectancy for older adults who lived in the South at the time of interviews. Persons with diabetes born in the South were more likely to have developed chronic conditions or disabilities and spent greater proportions of life with these two issues compared to other regions. (Zang E, Lynch SM, West J. Regional differences in the impact of diabetes on population health in the USA. *J Epidemiol Community Health* 2021; 75: 56-61)

ACCURACY OF AST TO PLATELET RATIO FOR PREDICTING LIVER FIBROSIS IN PATIENTS WITH CHRONIC HCV INFECTION AFTER VIRAL ERADICATION

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ACCURACY OF AST TO PLATELET RATIO FOR PREDICTING LIVER FIBROSIS IN PATIENTS WITH CHRONIC HCV INFECTION AFTER VIRAL ERADICATION (Abstract): Determining the stage of fibrosis is an essential element of chronic hepatitis C (HCV) management. This study aims to evaluate aminotransferase to platelet ratio (APRI) score in comparison with Vibration-Controlled Transient Elastography (VCTE) for assessing the severity of liver disease in HCV-infected patients. **Material and methods:** We retrospectively enrolled 293 HCV-infected patients with different stages of fibrosis who achieved sustained virological response (SVR) after direct-acting antivirals (DAAs) therapy, which have been evaluated by VCTE from 1st September 2020 to 30th July 2021. **Results:** Two hundred and ninety-three patients (72.7% females, mean age of 61.75 ± 10.87 years, and mean BMI of 27.85 ± 4.63 kg/m^2) were evaluated. As estimated by VCTE, 44 (15%) of patients were without liver fibrosis (F0), 80 (27.3%) with F1 (mild), 39 (13.3%) with F2 (significant), 43 (14.7%) with F3 (advanced) liver fibrosis, and 87 (29.7%) with F4 (cirrhosis), with a mean value of liver stiffness measurements (LSM) of 10.17 ± 7.42 kPa. The APRI score has a positive correlation with the LSM score (p=0.047), predicting severe liver disease adequately. The optimal receiver operator curve (ROC) for predicting advanced fibrosis was > 1.15 (area under the curve [AUC] 0.79, 95% CI 0.71-0.83; p < 0.001) with a sensitivity (Ss) of 82.5%, specificity (Sp) 28%, a positive predictive value (PPV) of 72%, and negative predictive value (NPV) of 81%. **Conclusions:** APRI score can predict well the advanced liver fibrosis and cirrhosis and can be a useful non-invasive biochemical marker for resource-limited management of HCV-infected patients.
Keywords: CHRONIC HEPATITIS C, VIBRATION-CONTROLLED TRANSIENT ELASTOGRAPHY, AMINO-TRANSFERASE TO PLATELET RATIO.

Chronic hepatitis C (HCV) is one of the most common causes of liver cirrhosis and hepatocellular carcinoma and a growing global public health problem, affecting approximately 71.1 million of people worldwide (1). The new therapy with direct-acting antivirals (DAAs) had revolutionized the treatment of HCV, with more

than 95% of the patients achieving sustained virological response and a very good safety profile (2).

According to current guidelines, an evaluation of liver fibrosis with non-invasive methods in HCV-infected patients is required prior to therapy (3). This recommendation is based on the residual risk of hepatocellular carcinoma and further decompensation of liver cirrhosis in those with advanced/severe fibrosis, even after viral clearance (4).

Up to this point, liver biopsy (LB) has been considered traditionally the "imperfect" gold standard method for the staging of liver fibrosis. However, LB is an invasive procedure, with several limitations (high cost, sampling errors, intra-, and inter-observer variability, variable accessibility, and a low but real risk of morbidity and mortality), which led to the development of non-invasive methods such as Vibration-Controlled Transient Elastography (VCTE) with a high accuracy, currently being the most frequent imaging method for the evaluation of liver fibrosis in United States of America (5, 6). However, due to its high cost and limited availability in low- and middle-income countries, the use of VCTE could be replaced with surrogate markers such as the APRI or FIB-4 score (7, 8). The main advantages of these biochemical markers are the low costs and wide availability (9). In a recent study, Wong *et al.* found that the cut off value of 0.5 for APRI score predicted the existence of advanced fibrosis in 100% of the cases, thus highlighting the efficacy of this score (10). Moreover, APRI score is recommended by World Health Organization (WHO) for the evaluation of liver fibrosis in limited resources-areas (7-Sripongpun). However, data regarding the correlation of APRI score and VCTE are scarce and the need for the validation of APRI score in different patient populations is still required (11).

Hence, the aim of this study was to evaluate the diagnostic accuracy of APRI score compared with VCTE in staging the liver fibrosis in Romanian HCV-infected patients. As a secondary outcome, we aimed to determine the best cut-off values for APRI score to predict advanced fibrosis or cirrhosis.

MATERIAL AND METHODS

This is a retrospective study from a tertiary referral hospital in North-East of Romania that included 293 consecutive patients with chronic HCV infection and different stages of fibrosis who achieved SVR after DAA therapy, between 1st September 2020 and 30th July 2021. DAA regimens were either Paritaprevir/Ritonavir, Ombitasvir and Dasabuvir (PrOD), Ledipasvir/Sofosbuvir (LED/SOF) or Elbasvir/Grazoprevir (ELB/GRZ).

Demographic data, anthropometric measurements, blood tests and FibroScan evaluations were recorded for all patients. Reliable VCTE data was defined by following criteria: at least 10 valid measurements, and an interquartile range $\leq$ 30%. LSM results were expressed in kilopascals (kPa) ranging from 1.5 to 75 kPa, with cut-off values for fibrosis as follows: F0 (without), F1 (mild), F2 (significant), F3 (advanced) fibrosis, and F4 (cirrhosis); $\leq$ 5.5, 5.6-7.1, 7.2-9.4, 9.5-12.4, $\geq$ 12.5 kPa, respectively. APRI score was calculated using the proposed formula: APRI = [(AST level/ULN)/platelet count (109 /L)] × 100.

Patients with non-HCV related liver disease, coinfection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV), HCC or other malignant pathologies, decompensated liver cirrhosis, acute

hepatitis, pregnancy, women in nursing period, incomplete or invalid FibroScan assessments, were excluded.

All patients signed the informed consent. Our study was conducted according to the criteria established by the Declaration of Helsinki.

Statistical analysis

All data were analyzed using *SPSS software version 22.0* (IBM SPSS Inc, Chicago, IL, USA). Continuous variables are expressed as median and interquartile/median range (IQR) or as mean ± standard deviation (SD). For comparing group's variables were used Student t-test for continuous variables, or *Chi-squared test* for comparing qualitative data as appropriate. One-way *ANOVA* was considered appropriate to assess the difference in APRI score values according to the fibrosis stages, while for checking the association between two variables *Pearson* (r) coefficient of correlation was used. Diagnostic performance of APRI score compared to TE was analyzed by constructing the receiver operator characteristic (ROC) curve, obtaining sensitivity (Ss), specificity (Sp), positive predicting value (PPV), and negative predicting value (NPV). A p value < 0.005 (two-tailed) was considered to be significant.

RESULTS

This study included 293 consecutive patients with chronic HCV infection and different stages of fibrosis, mostly females (72.7%), mean age 61.75 ± 10.87 years with mean BMI 27.85 ± 4.63 kg/m2. All patients were treated with DAAs and obtained SVR. One hundred thirty (44.3%) patients received PrOD, 120 (40.9%) LED/SOF and 43 (14.8%) ELB/GRZ. The mean LSM value was 10.17 ± 7.42 kPa, and the mean APRI score was 1.14 ± 3.91 (tab. I).

TABLE I

Baseline characteristics of the study population

Characteristics	Value
Gender (female), n (%)	213 (72.7)
Age, years	61.75 ± 10.87
BMI (kg/m^2)	27.85 ± 4.63
Platelet count (x10^3/cm^3)	177 ± 103
Fasting plasma glucose (mg/dL)	107.8 ± 35
ALT (U/L)	27.94 ± 23.89
AST (U/L)	28.34 ± 18.93
GGT (U/L)	37.29 ± 31.73
ALP (U/L)	95 ± 53.74
Total bilirubin (mg/dL)	1.14 ± 0.83
Total cholesterol (mg/dL)	186.62 ± 44.37
Triglycerides (mg/dL)	119 ± 57.91
Albumin (g/dL)	4.24 ± 0.51
LSM (kPa), median (IQR)	10.17 ± 7.42
APRI score	1.14 ± 3.91

BMI, body mass index; INR, international normalized ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; LSM, liver stiffness measurement; APRI, aspartate aminotransferase to platelet ratio

According to LSM, 44 (15%) patients had no liver fibrosis (F0), 80 (27.3%) patients had F1 (mild), 39 (13.3%) had F2 (significant), 43 (14.7%) patients had F3 (advanced), and 87 (29.7%) patients had F4 (cirrhosis).

TABLE II
APRI score in different stages of liver fibrosis

Liver fibrosis stages based on LSM		APRI	
Stage	**n (%)**	**Mean ± SD**	**p-value**
F0	44 (15)	0.34 ± 0.29	0.002
F1	80 (27.3)	0.41 ± 0.42	
F2	39 (13.3)	0.35 ± 0.16	
F3	43 (14.7)	1.32 ± 3.47	
F4	87 (29.7)	2.48 ± 6.55	

TABLE III
Comparison between APRI score and different stages of liver fibrosis

Liver fibrosis stages	p-value
F0 *vs.* F1	0.348
F0 *vs.* F2	0.801
F0 *vs.* F3	0.066
F0 *vs.* F4	**0.003**
F1 *vs.* F2	0.444
F1 *vs.* F3	**0.022**
F1 *vs.* F4	**0.004**
F2 *vs.* F3	0.087
F2 *vs.* F4	**0.045**
F3 *vs.* F4	0.190

We found significant statistical differences of APRI score between different fibrosis stages based on LSM (p<0.001) (tab. II). The differences remained highly significant when the mean APRI score value was compared between F0 *vs.* F4 (p=0.003), F1. *vs.* F3 (p=0.022), F1 *vs.* F4 (p=0.004), and F2 *vs.* F4 (p=0.045) (tab. III). Moreover, the APRI score had a positive correlation with AST (p = <0.001), ALT (p = 0.001), and LSM score (p=0.047), and a negative significant correlation with total cholesterol (p = <0.001) and albumin (p=0.022).

The optimal receiver operator curve (ROC) of the APRI score for diagnosing advanced fibrosis was calculated as > 1.15 (area under the curve [AUC]=0.79, 95% CI 0.71-0.83; p < 0.001) with a sensitivity (Ss) of 82.5%, specificity (Sp) 28%, a predictive positive value (PPV) 72%, and negative predicting value (NPV) of 81% (fig. 1).

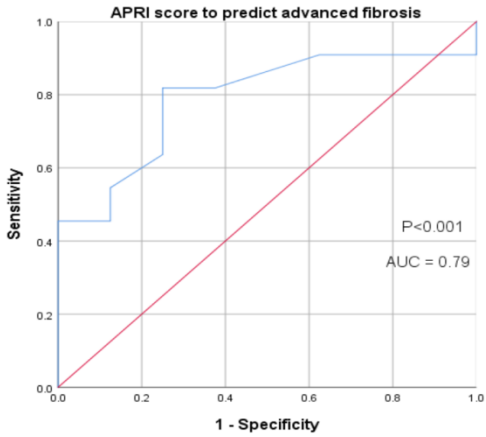

Fig. 1. Performance of APRI score in the prediction of advanced liver fibrosis (F3/F4) according to LSM

DISSCUSION

The outcome of chronic HCV infection varies significantly from patient to patient, depending on the level of fibrosis and viral status at the time of diagnosis. Recent years brought an extraordinary progress in the management of HCV infection, by introducing the new DAAs in the eradication strategies (12). Still, even after viral clearance, the severity of liver fibrosis needs to be acknowledged in order to evaluate the prognosis of the liver disease. There are several non-invasive methods to determine the severity of fibrosis which allow bypassing LB, previously known as the gold standard, nowadays leaving behind due to numerous shortcomings. In this regard, we chose to analyze the APRI score based on

consistent data showing its usefulness in predicting liver fibrosis. According to recent data, the APRI score has the best accuracy of all serum markers for the identification of advanced fibrosis and cirrhosis in patients with chronic hepatitis, demonstrating an excellent ability to discriminate between F3 and F4 (13). Moreover, a recent meta-analysis showed a significant association between increased APRI and the occurrence of HCC in patients with chronic hepatitis of various etiologies, demonstrating the usefulness of the APRI score in risk stratification in patients with chronic HCV infection (14).

In a large cohort study consisting of 3619 patients with HCV or HBV chronic infection, Wong et al demonstrated that APRI score has similar predictive value as LSM for advanced fibrosis (7). In line with these results, our study showed that APRI and LSM strongly correlated especially when comparing F0 *vs.* F4 and F1 *vs.* F4. Despite the similar findings between our study and the aforementioned, the fact that there are different cut-offs of TE in HBV *vs.* HCV, must be kept in mind (15). A more recent German study which analyzed a similar group as ours, consisting of 260 patients with HCV infection treated with DAAs, concluded that liver fibrosis regresses significantly after SVR (16). Although this was not initially an objective of the study, the authors found that APRI strongly correlated with TE, highlighting the efficacy

We found a good performance of APRI score in the prediction of advanced liver fibrosis (F3/F4) according to LSM. Considering a cut-off value of > 1.15, the PPV and NPV for APRI were 72% and 82%, respectively. In a prospective cross-sectional study, Mendes *et al.* showed that for advanced fibrosis, an APRI score less than 1 had a NPV of 81% (AUROC 0.80) whilst for a cut off above 2, the specificity for F ≥3 was 93% (17). Furthermore, the authors compared the performance of APRI *vs.* LB for predicting significant fibrosis and cirrhosis and found a slightly decreased accuracy of APRI (AUROC 0.78 and 0.82, respectively).

The main strength of our study is represented by the comparative analysis between LSM and APRI, which underlines the possibility of replacing the TE evaluation with this simple and widely available serum score. The limitations are represented by the lack of data regarding fibrosis stages assessed by both methods before the start of DAAs and also, we did not calculate the FIB-4 index, which could've been useful to compare with predictive power of APRI.

CONCLUSIONS

Considering the decreased availability of TE in some areas, APRI score is a good surrogate marker for liver fibrosis, with several advantages as low cost and simple to use in clinical practice. As the number of HCV-cured patients will dramatically increase and will require simpler ways to assess liver fibrosis.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to acquisition of data, or analysis, conception and design, and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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IMPLICATIONS OF LIPID AND CARBOHYDRATE METABOLISM IN PERIODONTAL DISEASE ASSOCIATED WITH DIABETES, CARDIOVASCULAR AND NONALCOHOLIC FATTY LIVER DISEASE

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IMPLICATIONS OF LIPID AND CARBOHYDRATE METABOLISM IN PERIODONTAL DISEASE ASSOCIATED WITH DIABETES, CARDIOVASCULAR AND NONALCOHOLIC FATTY LIVER DISEASE (Abstract): The systemic inflammatory response in periodontal disease is characterized by the presence of inflammatory mediators. The systemic inflammatory response positively influences the risk of developing diabetes and its complications, atherosclerotic diseases and other chronic conditions. Metabolic syndrome is a consequence of the modern lifestyle, an accumulation of metabolic abnormalities that increase the cardiovascular risk. Abnormal distribution of adipose tissue with obesity, insulin resistance, and dyslipidemia are some of these abnormalities but also increased blood pressure, atherogenic dyslipidemia, hypertension, and pro-thrombotic and pro-inflammatory status are other conditions. The study's main **objective** was to evaluate the hypothesis that inflammatory changes in gingival tissue are correlated to lipid and carbohydrate metabolism abnormalities in patients with diabetes mellitus (DM), cardiovascular disease (CVD), and nonalcoholic fatty liver disease (NAFLD). The medical records of 181 subjects were used in this retrospective study. The **results** suggested that periodontal disease can influence the systemic level of inflammatory mediators in patients with systemic diseases (DM, CVD, and NAFLD) by mechanisms that rely on oral microbial flora exposure and control. **Conclusions**: by influencing lipid and carbohydrate metabolism, NAFLD is closely correlated to cardiovascular disease or diabetes, and study into these factors in patients with periodontal disease helps to highlight the association between these diseases. **Keywords**: SYSTEMIC CONDITION, PERIODONTAL DISEASE, METABOLIC SYNDROME, DIABETES MELLITUS, CARDIOVASCULAR DISEASE, LIVER DISEASE.

The two-way relationship between periodontal tissue inflammation and a number of systemic conditions as risk factors, such as diabetes, cardiovascular disease, obesity, and liver disease, can be supported by studies that have evaluated metabolic disorders

in these patients (1).

Liver disease such as viral hepatitis or nonalcoholic fatty liver disease are often diagnosed in patients who have other comorbidities, such as cardiovascular disease. This association of these systemic diseases is frequently involved in the death of people who have been diagnosed with these conditions (2).

The endocrine system in the body achieves control in organs and tissues and thus establishes metabolism and weight (3, 4), Frequently associated dyslipidemia and obesity are risk factors for abdominal surgery due to the association with ischemic heart disease, hypertension, diabetes and respiratory failure (5).

Studies have shown that about 40-80% of variations in blood lipids are related to genetic factors (6, 7, 8). The increased incidence of type 2 diabetes is closely related to the presence of obesity but can also occur independently of this (9, 10).

There are many complications caused by diabetes with oral manifestations that require treatment appropriate to the patient's needs: oral infections, periodontitis or gingivitis. (11)

Glycosylated hemoglobin is a test that indicates a patient's glycemic control. Maintaining glycosylated hemoglobin < 7% can prevent cardiovascular complications, with a high risk of death for diagnosed patients (12, 13, 14).

Bleeding on probing is strongly associated with some systemic inflammatory mediators, in patients with coronary heart disease, liver diseases and periodontitis. This association reflects the potential of local periodontal inflammation for the development of systemic inflammation (15, 16, 17).

Liver disease such nonalcoholic fatty liver disease is associated with components of the metabolic syndrome: obesity, diabetes and insulin resistance, high blood pressure and dyslipidemia (18, 19).

As a major objective of the study, we will verify the study hypothesis inflammatory changes occurs within gingival tissue in correlation with disturbances related to lipid and carbohydrate metabolism in patients with diabetes (DM), cardiovascular diseases (CVD), nonalcoholic fatty liver disease (NAFLD).

The specific objectives are related to: analysis of demographic data of the group of patients, to highlight the role of sociodemographic factors related to gender, age, environment of origin in the evolution of periodontal disease and diabetes, cardiovascular disease, nonalcoholic fatty liver disease; evaluation of the clinical parameters of the periodontal status, in order to highlight the modification of the preexisting clinical picture of the periodontal diseases under the influence of the systemic pathology due to diabetes, cardiovascular diseases, nonalcoholic fatty liver disease.

MATERIAL AND METHODS

This retrospective study was conducted on a medical record of 181 subjects, selected from subjects who addressed the Periodontology Departments of the University of Medicine and Pharmacy of Craiova, Romania, between 2018 and 2020. The inclusion criteria for groups: the history of treatment for periodontitis, minimum age 25 years. Patients declared medically diagnosed by a specialist to have DM, CVD or NAFLD were included in the test groups. The age of the patients ranged from 28 to 68 years, the mean age being 47.33 years. The gender distribution of the patients consisted of 80 females (44.2%) and 101 males (55.8%), divided into four groups, for data analysis

and comparison, depending on systemic pathology (DM, CVD or NAFLD) in association with periodontal disease and another group of systemically healthy patients with periodontal disease. We will consider P group - patients with periodontal disease (35 patients), P+DM group - patients with diabetes and periodontal disease (64 patients), P+CVD group - patients with cardiovascular disease and periodontal disease (17 patients), P+NAFLD group - patients with liver disease (NAFLD) and periodontal disease (19 patients) and P+SYS group - patients with at least two of the systemic diseases (DM, CVD, NAFLD) in the presence of periodontal disease (46 patients).

For the study, we analyzed the data recorded after medical and dental history, examination of clinical aspects, periodontal assessment, radiographic and serological analyzes. We recorded information regarding demographic data (patient's age, environment, gender), working and living conditions, vicious habits, medical history, dental history, patient's hygiene, history of periodontal disease (duration of the disease, onset and evolution over time). The O'Leary Index for bacterial plaque control record has been used for oral hygiene status. The main reason for the last visit to dental services was gingival bleeding when brushing, spontaneous bleeding, calculus, swollen or red gingiva, halitosis, masticatory deficiency, esthetic and functional deficiencies in the missing tooth regions, dental caries, endodontic problems, prevention and treatment. The following data were related to periodontal disease, so the number of missing teeth was recorded, the number of teeth with periodontal pockets measured at different depths (between 4-5 mm, 6-7 mm, over 7 mm), the degree of bleeding, the level of attachment loss and the periodontal diagnosis of the patient.

Serological analyzes of each patient were consulted: blood sugar level, total cholesterol, hepatic enzymes - transaminases ALT and AST, triglycerides and cholesterol.

The data were analyzed and statistically processed using the *Microsoft Excel Data Analysis Tools Pack extension*.

RESULTS

The mean age of the patients included in the study was 47.33 years. The average age of the subjects groups was 43.33 in P group, 54.21 in P+DM group, 46.56 in P+CVD group, 44.88 in P+NAFLD group and higher, 59.55 in P+SYS group.

Human environments are complex and different, influencing the success rate of treatments depending on the availability and information accumulated. Of the 181 patients, 135subjects (74.58%) were from urban areas and the rest, 46 subjects (25.42%) from rural areas.

The main reason for the last visit to dental services is represented in the first table.

The data regarding the age of periodontal diagnosis for each subject group is presented in the first figure.

On average the O'Leary index for P group was 38.92%, for P+DM 29.45%, for P+CVD 22.42%, for P+NAFLD 33.21% and for P+SYS was 38.65%. Most patients had a poorly controlled bacterial biofilm with negative consequences on periodontal disease.

Patients with periodontal disease and diabetes or cardiovascular disease had 4-9 teeth lost. As well as patients with periodontal disease associated with cardiovascular disease and NAFLD, over 56% of them did not have 4-9 teeth on the dental arches (tab. II).

TABLE I

Specific issues that are presented at the medical visit

The main reason	Number	Percent	P	P+DM	P+CVD	P+NAFLD	P+SYS
Bleeding	45	24,86%	35,55%	26,66%	17,77%	6,66%	
Calculus	31	17,13%	29,03%	12,90%	25,80%	25,80%	6,
Red gingiva	27	14,91%	22,22%	25,93%	18,52%	14,81%	18
Halitosis	24	13,26%	12,50%	33,33%	16,66%	16,66%	20
Masticatory deficiency	15	8,29%	13,33%	33,33%	13,33%	13,33%	26
Esthetic and functional deficiency	20	11,05%	30,00%	30,00%	15,00%	10,00%	15
Dental caries	8	4,42%	12,50%	25,00%	25,00%	12,50%	25
Endodontic problems	6	3,31%	16,66%	16,66%	0%	33,33%	33
Prevention and treatment	5	2,77%	40,00%	20,00%	0%	20,00%	20

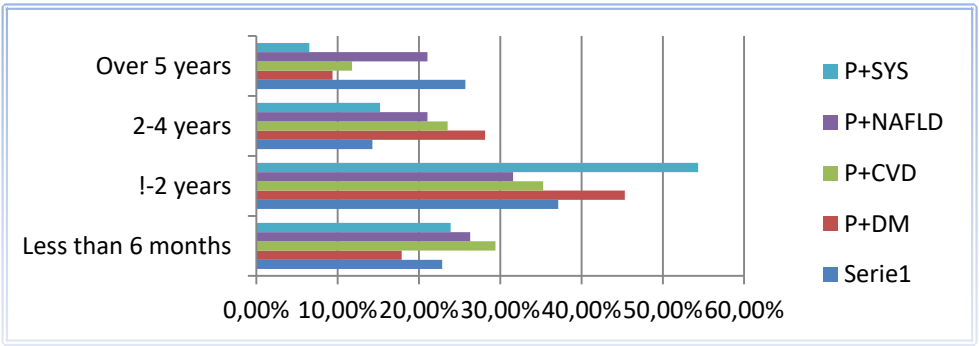

Fig. 1. The age of diagnosis for periodontal disease.

TABLE II

Average tooth loss for each group

Patients groups	0-3 lost teeth	4-9 lost teeth	>10 lost teeth
Group P	51.42%	22.85%	25.73%
Group P+DM	32.82%	37.50%	29.68%
Group P+CVD	23.54%	41.17%	35.29%
Group P+NAFLD	36.86%	31.57%	31.57%
Group P+ SYS	23.91%	56.53%	19.56%

The average number of periodontal pockets with a depth of 4-5 mm is approximately equal for all groups, being 2.43 for the P group, 2.48 for the P+DM, 2.44 for the P+CVD, 2.40 for the P+NAFLD and 2.55 for the P+SYS group. In the case of 6-7 mm periodontal pockets, the P group has an average of 3.11, the P+DM of 3.83, the P+CVD of 3.77, the P+NAFLD of 3.12 and the P+SYS group of 3.95.The average number of periodontal pockets with a depth of more than 7 mm is significantly higher for the P+DM group, being 3.13, compared to the other groups where there were variations of 1.67. The average level of attachment loss is 1.42 mm for the P group, 3.25 mm for the P+SYS, being higher than that of the P+CVD where we found 1.92 mm, for the P+NAFLD which was of 1.76 mm and compared to the P+DM which was

2.21 mm.

The average value of the bleeding index is 11% for the P group, being higher in the P+SYS (19%) compared to the P+DM, P+CVD and P+NAFLD groups, which is 16% (tab. III).

TABLE III

Average values of periodontal measurements

Patients group	The average depth of the periodontal pockets			The average level of attachment loss	The average value of the bleeding index
	4-5 mm	6-7 mm	>7 mm		
P	2.43	3.11	1.08	1.42 mm	11%
P+DM	2.48	3.83	3.13	2.21 mm	16%
P+CVD	2.44	3.77	0.98	1.92 mm	16%
P+NAFLD	2.40	3.12	1.78	1.76 mm	16%
P+SYS	2.55	3.95	2.84	3.25 mm	19%

Regarding the diagnosis of diabetes, most subjects, 169 patients (93.37%) were diagnosed with type 2 diabetes. We recorded in the table the blood glucose value and the glycosylated hemoglobin value, both for patients considered healthy but also for those who confirmed that they suffer from diabetic impairment, for a closer monitoring of the data.HbA1c levels obtained from the medical records for the previous 6 months were ≥ 6.5% (≥ 48 mmol/L).The average levels of glycosylated hemoglobin are 7.2 for subjects included in P+DM group. Blood glucose values in the studied group were between 99.3 mg / dL and 145.5 mg/dL with an average value of 123.5 mg/dL.

The diagnosis of cardiovascular disease in patients in the P+CVD group is hypertension, being found in 12 patients. History of stroke is present in 3 patients. Two of the patients have ischemic cardiomyopathy. The average age of diagnosis of cardiovascular disease is 3 years. The most common type of treatment for patients in the P+CVD group is diuretics in 9 cases, followed by β-blockers in 5 cases and anticoagulants in 3 cases.

Biochemically, patients diagnosed with NAFLD had increased levels of aspartate transaminase (AST) and alanine aminotransferase (ALT). When AST has values higher than ALT, it may indicate the presence of nonalcoholic steatohepatitis or hepatitis C. Patients included in our study had average AST level48.93U/L and ALT level38.21U/L.

Analysis of the mean value of triglycerides in the blood shows an increase in the group of patients diagnosed with NAFLD and higher values for those who also have CVD disease (triglycerides ≥1.70 mmol/L). Triglycerides reference values are normal = < 150 mg/dL (<1.7 mmol/L), mild hypertriglyceridemia = 150-199 mg/dL (1.7-2.3 mmol/L) moderate hypertriglyceridemia = 200-999 mg/dL (2.3-11.2 mmol/L), severe hypertriglyceridemia = 1,000-1,999 mg /dL (11.2-22.4 mmol/L), very severe hypertriglyceridemia = 2,000 mg/dL (22.4 mmol /L). Serum triglyceride levels for patients in the other study groups were normal. Total cholesterol levels lower than 200 mg/dL being the accepted limit for an optimal level, while values above 240 mg/dL are considered high. HDL together with

LDL and triglycerides represent total cholesterol whose values above the normal limit represent an imbalance (tab. IV).

TABLE IV
Triglyceride and cholesterol mean levels

Study group	Triglyceride (mg/dL)	Cholesterol (mg/dL)
Group P	—	—
Group P+DM	152	210
Group P+CVD	155	213
Group P+NAFLD	198	289
Group P+SYS	202	340

DISCUSSION

A two-way relationship, through the spread of oral microorganisms and pro-inflammatory mediators, can be highlighted between periodontal disease and the systemic disorders, such as cardiovascular disease, metabolic syndromes, including DM and NAFLD, cancer and respiratory diseases that could spread oral microorganisms and pro-inflammatory mediators (20, 21).

Fentoğlu *et al.* showed that the mean values of the clinical parameters: plaque index, depth of periodontal pockets, bleeding at probing and the level of clinical attachment were significantly higher in the hyperlipidemia group than in the control group (22).

The preponderance of the data seems to support the concept that periodontitis may contribute to systemic levels of inflammatory mediators and markers associated with an increased risk of cardiovascular disease. Among the most common cardiovascular diseases are ischemic heart disease, stroke, hypertension the main cause in over 95% of deaths (16).

Similar results were presented by Kumar and colleagues in a study aimed at determining the frequency and nature of oral manifestations in hypertensive patients, of whom 85.38% had probing gingival bleeding and marginal gingival erythema and 16.9% patients showed an increase in gingival volume characterized by nodular growth, observed on both the oral and vestibular surfaces of the gingival margin. Inflammatory changes in the gingival mucosa were mainly due to antihypertensive drugs. The frequency of potential oral manifestations in patients with hypertension was significantly high, thus showing an association of gingival and periodontal pathology in hypertensive patients (23).

The average level of attachment loss of 3.25 mm in the group of patients with liver and heart disease and 2.21 in P+DM group, significantly higher compared to patients diagnosed with periodontal disease without other systemic conditions. In the Pham and Tran study published in 2018, the mean value of the level of attachment loss was calculated to be 2.80 mm in their study group represented by diabetic patients (24).

Dhir *et al.* showed that the average value calculated for glycosylated hemoglobin was 7.4% (25).Our study reveals the value of 7.2 for glycosylated hemoglobin in the P+DM group. The blood glucose value during a fasting period, but also after the patients ate was published by Rajhans in 2011. For the group of patients with diabetes there is an average value of 122 mg/dL before meals and 212.36 mg/dL after eating and for the control group 76.24 mg/dL before and after 117.16 mg/dL (26). In our study average value indicates 123.5 mg/dL.

In a study conducted at the clinics of the University of Taubaté the results obtained are similar to those of the current study and showed that the average levels of choles-

terol (192.1 mg / dL ± 40.9) and triglycerides (153.5 mg / dL ± 105.6) in individuals with periodontitis were higher, not statistically different from those without periodontitis (186.1 mg / dL ± 35.4 and 117.5 mg / dL ± 68, respectively) (27).

High values for AST and ALT we concluded in patients with liver disease, in association or not with cardiovascular disease, all other patients presenting normal values on biological tests.

According to studies, high values of AST and ALT are present in patients with periodontal destruction, periodontal pockets and gingival bleeding, being experimentally proved that these biomarkers had high value in serum and saliva in the presence of other different conditions (28-30). According to a study published by Yoneda in 2012, periodontal pathogens were identified more frequently in patients diagnosed with NAFLD than in those in the control group and the initial periodontal treatment decreased AST and ALT levels, this being the reason why we did not include in our study patients previously periodontally treated (31).

CONCLUSIONS

The data suggest that periodontal disease can influence the systemic level of inflammatory mediators in patients with systemic disorders (DM, CVD, NAFLD) through mechanisms that depend on the exposure and control of oral microbial flora. NAFLD is frequently associated with cardiovascular disease or diabetes, by altering lipid and carbohydrate metabolism and the study of these aspects in patients suffering also from periodontal disease, helps to highlight the relationship between these conditions.

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CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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IMPACT OF APOE POLYMORPHISM IN A FAMILIAL HYPERCHOLESTEROLEMIA POPULATION

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IMPACT OF APOE POLYMORPHISM IN A FAMILIAL HYPERCHOLESTEROLEMIA POPULATION (Abstract): Familial hypercholesterolemia is a genetic disease caused by a mutation in one of the genes implied in the low-density lipoprotein (LDL) catabolism, leading to increased seric values of LDL and a very high cardiovascular risk. ApoE is a ligand for the LDL receptor and the polymorphisms at the level of ApoE will alter the lipid profile. **Material and methods:** A cross-sectional study was conducted in the Cardiovascular Rehabilitation Clinic from Iasi Clinical Rehabilitation Hospital between December 1st, 2020, and December 1st, 2021, including 52 patients with familial hypercholesterolemia in the study group and 17 patients in the control group. **Results:** In our research, the patients with ApoE4/E4 had the highest lipid profile concentrations. Statistical significance was observed between ApoE2/E3 and ApoE3/E3, ApoE3/E4 and Apo E4/E4 and between ApoE3/E3 and ApoE4/E4 regarding the creatinine clearance in the familial hypercholesterolemia group. In the same group, statistical significance correlations were observed between ApoE3/E3 and ApoE3/E4 for triglycerides concentration, between ApoE3/E3 and ApoE4/E4 for the glycosylated hemoglobin. **Conclusions:** For a better understanding of the genetic and environmental factors in patients with familial hypercholesterolemia, larger studies are needed, in order to increase their quality of life. **Keywords:** FAMILIAL HYPERCHOLESTEROLEMIA, APOE, LIPID PROFILE, CARDIO-VASCULAR DISEASE, CREATININE CLEARANCE.

Familial hypercholesterolemia is a genetic disease of the metabolism of lipoproteins, causing high levels of low-density lipoproteins (LDL). The loss of function mutations in LDLR, APOB, or PCSK9 genes can lead to homozygous or heterozygous familial hypercholesterolemia. Relatively recent research identified APOE as part of the gene network involved in familial hypercholesterolemia (1, 2). The elevated serum LDL-C induced by any of these mutations promotes atherosclerosis and cardiovascular disease, putting these individuals at a tenfold increased risk of coronary artery disease compared to those without FH. Clinical scoring systems for

the diagnosis of FH are widely used and approved, and numerous sets of criteria have been created, encompassing clinical, biochemical, and genetic factors. The most commonly used criteria are Simon Broome, MedPed and Dutch Lipid Clinic Network (DLCN), the last one being used predominantly in the European countries (3, 4).

ApoE is a component of chylomicrons, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), LDL, high-density lipoproteins (HDL), and lipoprotein a (LPa). ApoE regulates the clearance of these lipoproteins from the plasma and the homeostasis of tissue lipid content as a ligand for cell membrane lipoprotein receptors. ApoE is also involved in the catabolism of VLDL particles, inhibits lipoprotein lipase and the triglyceride-rich lipoprotein lipolysis, but may also increase the hepatic production of VLDL by activating the particle assembly cascade. All these data support the involvement of a dysfunctional apoE in the etiology of dyslipidemia (5).

The APOE gene is located on 19q13.2 and encodes for a precursor that follows peptide cleavage and glycosylation, resulting in mature apoE that is secreted as a 299 amino acid protein with a relative molecular mass of 34,200 kDa. According to current literature, one of the main factors affecting the lipid profile levels are ApoE polymorphisms. ApoE2, ApoE3 and ApoE4 are the protein isoforms that are encoded by the most frequent alleles (ε2, ε3, and ε4). Among the population, six phenotypes can be observed: E2/2, E3/2, E3/3, E4/2, E4/3, and E4/4, each of them with different prevalence. Up to date, it is known that the ε2 allele determines a reduction of the cholesterol absorption and also a decrease of the LDL cholesterol values compared to ε4 allele which correlates with high serum cholesterol levels (6, 7). Moreover, the association between dyslipidemia and chronic kidney disease, to be correlated with apoE isoforms, suggesting the importance of apoE polymorphisms study for an accurate prognosis and management plan (8).

MATERIAL AND METHODS

A cross-sectional study was conducted in the Cardiovascular Rehabilitation Clinic from Iasi Clinical Rehabilitation Hospital between December 1st, 2020, and December 1st, 2021. From the 1,499 patients that were admitted in this period, only 52 adults were included in the study. Inclusion selection consisted of patients diagnosed with familial hypercholesterolemia with a DLCN score over 8 that signed the informed consent. Exclusion criteria were considered secondary causes of hypercholesterolemia such as patients diagnosed with severe liver diseases, hypothyroidism, severe chronic kidney disease or nephrotic syndrome, uncontrolled diabetes mellitus or patients that eat meals with high concentrations of saturated and trans-unsaturated fatty acids. The 52 patients were compared to a control group that included 17 adult patients with normal lipid profile who signed the informed consent.

The participants in the study group had a clinical diagnosis of FH, as determined by a DLCN score greater than 8, and were included in the study. A score below 3 points is considered improbable, between 3 and 5 is possible, probable between 6 and 8 points, and more than 8 is considered as a definite clinical diagnosis for familial hypercholesterolemia. One of the criteria used in the diagnosis score is the LDL value, which was adjusted for the patients who

underwent in the last 6 months treatment with statins or ezetimibe. This correction factor was described by Haralambos *et al.* based on a review of 71 original papers gathered prior to the establishment of FH criteria.

A single investigator conducted the anamnesis and physical examination, recording the patients' medical history, body mass index, blood pressure, and heart rate as well as the drug history (including the lipid-lowering therapy and antiaggregant drugs). Regarding the laboratory tests, we included the lipid profile (total cholesterol, LDL, HDL, triglycerides), uric acid, urea, creatinine, blood glucose, and glycated hemoglobin.

DNA was prepared from peripheral blood cells using PureLink® Genomic DNA Kit (Thermo Fisher Scientific). We evaluated relevant mutations in PAI-1 (4G/5G), MTHFR (C677T, A1298C), ACE I/D, apolipoprotein B (R3500Q), and apolipoprotein E (Apo E) E2/E3/E4 by reverse-hybridization using CVD StripAssay kit (ViennaLab Diagnostics, Vienna, Austria).

After PCR amplification with biotinylated primers, PCR products were hybridized directly on the StripAssay® test strips, which contain allele-specific oligonucleotide probes immobilized as parallel lines. The presence of a wild type or/and mutant alleles was visualized by an enzymatic color reaction visible on the strip.

Positively stained lines were analyzed using the online tool StripAssay® Online Calculator (ViennaLab Diagnostics) and for each targeted gene were identified underlying genotypes.

The informed consent was collected from all the patients included in the study and the ethics committee approval was obtained from both "Grigore T. Popa" University of Medicine and Pharmacy Iasi (certificate of approval dated 15th June 2020) and Iasi Clinical Rehabilitation Hospital (certificate of approval dated 25th November 2020).

The data analysis was realized using SPSS 20.0 (Statistical Package for the Social Sciences, Chicago, Illinois). For continuous variables, the data were presented as median with interquartile range or as mean±standard deviation (SD). For categorical variables, the data were presented as the number of cases with percent frequency. Continuous normally distributed variables were compared by Independent Samples t-Test in case of two samples, or by One-Way *ANOVA*, in case of comparisons of more than two samples. Categorical comparisons were performed by Fisher's exact test or by Chi-square test. In the cases where the distribution of the continuous variables didn't satisfy the assumption of normality, we applied the *Mann-Whitney U* test. Spearman correlation coefficient was calculated when evaluating the correlations between continuous variables. A statistical significance was considered at a two-sided p-value < 0.05.

RESULTS

In this research were enrolled 52 patients that were clinically diagnosed with familial hypercholesterolemia and 17 healthy controls. More than half of the included patients were females (63.5%), with a mean age of 55.5±12.8. Regarding the males, they had a lower mean age of detection 38.88±11.72. Regarding education, most of the patients finished university studies (38.5% from the FH group and 70.5% from the control group) followed by patients that have a bachelor's degree (34.6% *vs.* 11.8%). The patients included

were predominantly married (78.8% from the FH group and 58.8% from the control group). When compared to the control group, the patients diagnosed with FH had more associated pathologies such as atherosclerosis, heart failure, metabolic syndrome, or chronic kidney disease. The first table summarize the general characteristics of the patients included in the study.

Apo E3/E3 was the most frequent genotype observed in our study in both groups (80.8% in the FH group and 64.7% in the control group), followed by Apo E3/E4 form (9.6% in the FH group and 0% in the control group). Apo E2/E3 had a lower frequency (5.8% in the FH group and 23.5% in the control group) and Apo E4/E4 had the lowest frequency (3.8% in the FH group and 11.8% in the control group).

TABLE I.

General characteristics of the patients included in the study

Patients characteristics	**FH**	**Controls**	**p value**
Gender, n (%) Females	33 (63.5)	11 (64.7)	0.927
Gender, n (%) Males	19 (36.5)	6 (35.3)	
Age, years (mean±SD)	55.5±12.80	38.88±11.72	0.000
BMI , kg/m^2 (mean±SD)	28.71±5.22	23.99±5.89	0.003
Lipidic profile, mg/dL (mean±SD)			
Total cholesterol	286.93±55.10	207.24±42.15	0.000
HDL cholesterol	58.52±17.51	46.34±11.12	0.009
Non-HDL cholesterol	228.40±57.52	160.90±43.39	0.000
LDL cholesterol	202.41±45.86	129.62±40.05	0.000
Corrected LDL cholesterol	312.73±91.67	129.62±40.05	0.000
Triglycerides	185.88±120.05	178.07±276.96	0.871
Uric acid, mg/dL (mean±SD)	4.44±1.57	4.45±1.37	0.971
Urea, mg/dL (mean±SD)	36.12±14.50	32.51±7.17	0.328
ATS, n (%)	25 (48.1)	3 (17.6)	0.045
HF, n (%)	10 (19.2)	2 (11.8)	0.024
CKD, n (%)			
Stage 1	12 (23.1)	8 (47.1)	0.009
Stage 2	25 (48.1)	9 (52.9)	
Stage 3A	13 (25.0)	0 (0)	
Stage 3B	2 (3.8)	0 (0)	

ATS – atherosclerosis; BMI – body mass index; CKD – chronic kidney disease; FH – familial hypercholesterolemia; HDL – high density lipoprotein; HF – heart failure; LDL – low density lipoprotein.

Regarding the ApoE genotype (tab. II), statistical significance correlations were observed between Apo E3/E3 and Apo E3/E4 for triglycerides concentration, between Apo E3/E3 and Apo E4/E4 for the glycosylated hemoglobin, urea and creatinine clearance value and between Apo E3/E4 and Apo E2/E3 for the creatinine clearance and also when comparing with the gender. In addition, a p value < 0.05 was observed for the creatinine concentration and for the clearance of creatinine between Apo E3/E3 and Apo E2/E3 and only for the creatinine clearance between Apo E2/E3 and Apo E4/E4.

TABLE II.

Correlations between ApoE genotypes variants in the FH group

	Apo E3/E3	Apo E3/E4	Apo E2/E3	Apo E4/E4	P_1	P2	P_3	P4	P5
Gender (Females)	26 (78.8%)	5 (15.2%)	1 (3%)	1 (3%)	0.150	0.555	0.035*	1.000	1.000
Gender (Males)	16 (84.2%)	0 (0%)	2 (10.5%)	1 (5.3%)					
TC	289.91±53.66	251.80±76.84	284.33±45.44	316.00±28.28	0.718	0.996	0.873	0.699	0.781
LDL	203.45±45.50	175.70±58.63	209.53±29.07	236.70±29.13	0.746	0.985	0.710	0.599	0.756
C-LDL	318.25±93.79	252.06±56.73	308.03±78.43	355.55±138.95	0.196	0.996	0.723	0.975	0.964
HDL	58.50±17.83	64.22 ±19.66	46.30±9.36	63.10±15.13	0.921	0.344	0.385	0.968	0.621
Non-HDL	231.40±57.81	187.58±62.98	238.03±52.35	252.90±13.15	0.510	0.996	0.641	0.449	0.960
TG	198.74±125.58	112.72±40.02	174.16±128.30	116.35±72.61	0.022*	0.986	0.849	0.602	0.912
Glucose	133.77±160.72	110.02±15.15	106.00±12.66	93.80 ±3.53	0.792	0.707	0.976	0.388	0.517
HbA1c	5.82±1.34	5.65±0.85	5.75±0.48	5.11±0.04	0.978	0.996	0.997	0.007*	0.333
IFG	20 (80%)	3 (12%)	2 (8%)	0 (0%)	0.666	0.608	1.000	0.493	0.400
BMI	29.07±5.14	26.11±5.04	29.30±8.16	26.60±4.47	0.632	1.000	0.922	0.870	0.960
MS	18 (85.7%)	1 (4.8%)	2 (9.5%)	0 (0%)	0.635	0.577	0.464	0.505	0.400
ATS	23 (92%)	2 (8%)	0 (0%)	0 (0%)	0.654	0.109	0.464	0.222	1.000
Uric acid	4.64±1.52	3.19±1.61	3.88±1.88	4.02±1.62	0.333	0.894	0.948	0.940	1.000
Urea	37.29±15.70	32.94±6.55	32.06±5.40	25.65±0.07	0.671	0.587	0.997	0.000*	0.391
Creatinine	1.03±0.25	0.95±0.10	0.87±0.04	0.96±0.16	0.569	0.009*	0.463	0.916	0.896
Creatinine clearance	70.61±17.86	72.00 ±10.79	93.66±3.21	80.50±2.12	0.994	0.000*	0.030*	0.030*	0.036*

ATS – atherosclerosis; BMI – body mass index; C- LDL – corrected LDL; HDL – high density lipoprotein; IFG – impaired fasting glucose; LDL – low density lipoprotein; MS – metabolic syndrome; TC – total cholesterol; TG – triglycerides; P1 – statistical significance between ApoE3/E3 and Apo E4/E4; P2 – statistical significance between ApoE3/E3 and ApoE2/E3; P3 – statistical significance between ApoE3/E4 and ApoE2/E3; P4 – statistical significance between ApoE3/E3 and ApoE4/E4; P5 – statistical significance between ApoE2/E3 and ApoE4/E4.

Regarding the patients with FH and Apo E3/E3 genotype, most patients (45.2%) were diagnosed with third-degree hypertension, 11.9% (5 patients) had second-degree hypertension, and only 4 patients (9.5%) presented first-degree hypertension. From the ones who presented Apo E3/E4 genotype, 3 (60%) had first degree hypertension and 1 (20%) had second degree hypertension. When referring to the patients with Apo E2/E3, there was one patient (33%) with second and third-degree of hypertension. A significant correlation between the hypertension and the ApoE genotype has been observed ($p = 0.014$).

In table III it can be observed the statistically significant correlation between Apo E3/E3 and Apo E4/E4 for the BMI value in the patients from the control group.

TABLE III.

Correlations between ApoE genotypes variants in the control group

	Apo E3/E3	Apo E2/E3	Apo E4/E4	P_1	P_2	P_3
Gender (F)	7 (63.6%)	2 (18.2%)	2 (18.2%)	1.000	1.000	0.467
Gender (M)	4 (66.7%)	2 (33.3%)	0 (0%)			
TC	205.03±41.71	222.70±55.61	188.50±6.36	0.838	0.457	0.520
LDL/C-LDL	139.71±32.68	107.42±62.28	118.50±1.97	0.625	0.131	0.934
HDL	47.90±10.84	40.42±14.08	49.65±4.45	0.632	0.923	0.517
Non-HDL	157.13±36.05	182.27±68.74	138.85±1.90	0.779	0.262	0.502
TG	111.82±40.95	398.37±564.67	101.80±0.56	0.619	0.705	0.601
Glucose	99.05±8.88	101.00±7.57	91.65±2.75	0.909	0.141	0.187
HbA1c	5.27±0.32	5.22±0.39	5.00±0.11	0.971	0.171	0.595
IFG	2 (66.7%)	1 (33.3%)	0 (0%)	1.000	-	-
BMI	24.26±5.82	26.14±6.49	18.17±1.59	0.817	0.049*	0.174
MS	1 (50%)	1 (50%)	0 (0%)	-	-	-
ATS	2 (66.7%)	1 (33.3%)	0 (0%)	1.000	-	-
Uric acid	4.30±1.00	5.01±2.37	4.15±1.22	0.838	0.985	0.835
Urea	33.37±6.57	28.10±9.34	36.65±3.04	0.593	0.563	0.326
Creatinine	0.92±0.13	0.95±0.11	0.94±0.02	0.905	0.870	0.991
Creatinine clearance	87.36±17.89	88.75±19.08	81.00±2.82	0.991	0.530	0.730

ATS – atherosclerosis; BMI – body mass index; C- LDL – corrected LDL; HDL – high density lipoprotein; IFG – impaired fasting glucose; LDL – low density lipoprotein; MS – metabolic syndrome; TC – total cholesterol; TG – triglycerides; P1 – statistical significance between ApoE3/E3 and ApoE2/E3; P2 - statistical significance between ApoE3/E3 and ApoE4/E4; P3 - statistical significance between ApoE2/E3 and ApoE4/E4.

In table IV are compared the lipid profile, and the Apo E genotypes in the FH group. We can observe that the lowest values of the total cholesterol, LDL, corrected LDL, triglycerides and non-HDL are in the patients with Apo E3/E4. Slightly increased values compared to this form can be seen in Apo E2/E3, followed by Apo E3/E3. The highest values of the lipid profile can be observed in the Apo E4/E4 form.

TABLE IV

Lipid profile and Apo E genotypes in the FH group

	Apo E3/E3	Apo E3/E4	Apo E2/E3	Apo E4/E4
Total cholesterol (mean±SD)	289.91±53.66	251.80±76.84	284.33±45.44	316.00±28.28
LDL (mean±SD)	203.45±45.50	175.70±58.63	209.53±29.07	236.70±29.13
Corrected LDL (mean±SD)	318.25±93.79	252.06±56.73	308.03±78.43	355.55±138.95
Triglycerides (mean±SD)	198.74±125.58	112.72±40.02	174.16±128.30	116.35±72.61
HDL (mean±SD)	58.50±17.83	64.22±19.66	46.30±9.36	63.10±15.13
Non-HDL (mean±SD)	231.40±57.81	187.58±62.98	238.03±52.35	252.90±13.15

DISCUSSION

Almigbal *et al.* recently published a study in which they evaluated the role of apolipoprotein E in patients with familial hypercholesterolemia. Regarding the frequency of the studied genotypes, our results are consistent with their study, the most frequent being Apo E3/3 and Apo E3/E4 (9).

Regarding the lipid profile and the Apo E genotypes, Liu *et al.* published in 2021 an article that described the fact that Apo E2/E3 had lower values of total cholesterol and LDL cholesterol compared to Apo E3/E3 and Apo E4/E4, results obtained also by us. The authors describe also that Apo E2/E3 has lower lipid profiles when compared to Apo E3/E4, these results being in contradiction to ours (10). Rodrigues *et al.* studied the relationship between Apo E genotypes and dyslipidemia in six main regions of Portugal. The study included 778 men and 1153 women between the ages of 15 and 74. The authors have shown that there is a relationship between E4 allele and high cholesterol blood levels (11).

In our study, we have observed a statistical significance between E3/E3 and E4/E4 for glycated hemoglobin in the patients diagnosed with familial hypercholesterolemia. Ukola *et al.* performed a study on 271 Caucasian patients diagnosed with diabetes to examine whether there was a link between Apo E polymorphisms and the onset of diabetic complications. The authors observed that the E4-bearing genotypes had a higher risk of developing micro and macrovascular complications in patients diagnosed with non-insulin-dependent diabetes mellitus, regardless the gender, when compared to the carriers of E2 genotype (12).

Our research described a statistical significance regarding the creatinine clearance between Apo E2/E3 and Apo E3/E3, Apo E3/E4 and between Apo E4/E4. Studies suggest that patients with Apo E2 genotype have a low glomerular filtration rate when compared to Apo E3 or Apo E4 carriers, also associating a high concentration in the serum creatinine (13). A recently published review illustrates the existence of Apo E2 homozygote glomerulopathy associated

with the mutations of the apolipoprotein E. A possible explanation for this can be the internalization of the lipoproteins by the macrophages into the cytoplasm, being therefore responsible for the accumulation in the glomerulus of lipoproteins (14). Thus, our results are in accordance with the ones already published.

When referring to the control group, a statistical significance regarding the BMI can be observed between Apo E3/E3 and Apo E4/E4. Tabatabaei-Malazy *et al.* described that the Apo E4 allele had a significant impact on the central obesity (15). According to the Atherosclerosis Risk in Communities (ARIC) study, the most common Apo E genotype that was related to BMI was the Apo E4 form (16). Another study found also that the E4-bearing genotypes (Apo E3/E4 and Apo E4/E4) were correlated with higher waist circumference and obesity in elderly women with a family history of diabetes (17).

CONCLUSIONS

Common ApoE variants are well known to be associated with different concentrations of the parameters included in the lipid profile. In our study, the patients with ApoE4/E4 had the highest lipid profile concentrations. In addition, we observed a low creatinine clearance in patients with Apo E2 genotype when compared to Apo E3 and Apo E4.

Despite the fact that these data are statistically significant, larger studies are needed in order to have a better understanding of the impact of the genetic factors in patients with familial hypercholesterolemia. These findings could improve the quality of life of these patients, with a decrease in morbidity and mortality.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding the scientific research.

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NEWS

PROENKEPHALIN CORRELATES WITH THE SEVERITY OF SEPSIS-ASSOCIATED ACUTE KIDNEY INJURY

In this study, 215 patients were enrolled. They were diagnosed as having sepsis (N=109, 50.7%) or septic shock (N=106, 49.3%) according to the Sepsis-3 criteria. The PENK levels were significantly higher in the septic shock group than in the sepsis group (118.7 pmol/L *vs.* 75.7 pmol/L, p=0.02). The PENK levels were also significantly higher in patients with vasopressor use than in those without vasopressor use (116.9 pmol/L *vs.* 72.7 pmol/L, p=0.007) and in the non-survivors than in the survivors (171.5 pmol/L *vs.* 79.8 pmol/L, p<0.001). PENK levels gradually increased according to the increased SOFA renal sub scores and CKD-EPI eGFR categories; this significance was constantly observed across each group (P<0.05, post-hoc test), except for eGFR categories G3a and G3b. Another noticeable finding in this study was the significant association between the PENK quartile and 30-day mortality rate. The present data also support the use of a clinical cut-off for PENK (80 pmol/L). In the groups with poor clinical status or outcomes, including septic shock, vasopressor use, and non-survivors, the median value of PENK levels was higher than the clinical cut-off. PENK could thus be an objective and reliable marker that has the potential to substitute for or augment the current role of the SOFA scoring system in critically ill septic patients. (Hanah K, *et al*. Proenkephalin Predicts Organ Failure, Renal Replacement Therapy, and Mortality in Patients With Sepsis. *Ann Lab Med* 2020; 40(6): 466-473).

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DIETARY PATTERNS IN YOUNG AND MIDDLE-AGED SUBJECTS WITH FAMILIAL HYPERCHOLESTEROLEMIA FROM NORTH-EASTERN ROMANIA

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DIETARY PATTERNS IN YOUNG AND MIDDLE-AGED SUBJECTS WITH FAMILIAL HYPERCHOLESTEROLEMIA FROM NORTH-EASTERN ROMANIA (Abstract): Familial hypercholesterolemia (FH) is a genetic autosomal dominant disease that is characterized by very high values of low-density lipoproteins. An unbalanced diet associated with FH will increase the cardiovascular risk of fatal and non-fatal events. **Material and methods:** 32 patients with FH were admitted in the Cardiovascular Rehabilitation Clinic from Iasi Clinical Rehabilitation Hospital between 1st December 2020, and 31st March 2022. Dutch Lipid Clinic Network score was the instrument used for the patient's diagnosis. Nutritional assessment was evaluated through a validated food frequency questionnaire. **Results:** In our study, we have observed that men diagnosed with FH eat more chicken meat, fried food, and fast food, while women have a more balanced diet, which includes vegetables and fruits. Regarding the blood tests, we noticed higher values of triglycerides and uric acid in males when compared to women. Moreover, the HDL cholesterol level was higher in women. Regardless of gender, the subjects with FH lack in eating dairy products. **Conclusions:** As far as we know, this is the first study that evaluates the gender-related eating habits of young and middle-aged patients with FH. The information obtained can form the framework for the implementation of active and comprehensive prevention measures in order to increase the awareness of the disease. **Keywords:** FAMILIAL HYPER-CHOLESTEROLEMIA, FOOD FREQUENCY QUESTIONNAIRE, LIPID PROFILE, DIET, CARDIOVASCULAR RISK.

Familial hypercholesterolemia (FH) is a genetic autosomal dominant disease that is characterized by very high values of low-density lipoproteins (LDL) (1). Due to its genetic component, the LDL values are high since childhood, thus increasing the deposits of cholesterol in the vessels intima and intensifying the atherosclerosis process (2). Recent research declares that without effective treatment, the cumulative risk of a coronary event by the age of 60 is more than 50 percent in men and over 30 percent

in women (3). Moreover, researchers affirm that FH is underdiagnosed and undertreated, thus the necessity to prevent the associated cardiovascular disease and make important lifestyle changes are mandatory for them (4).

In order to make a correct diagnosis, there are three clinical criteria that are used worldwide: Simon Broom criteria (used predominantly in the United Kingdom), MedPed (applied in the United States), and Dutch Lipid Clinic Network criteria (DLCN, applied worldwide and most frequently used). These criteria can put the clinical diagnosis of definite FH, but genetic tests for mutations in the LDL receptor gene, APOB gene, or PCSK9 gene are definite for this diagnosis (5).

The current trend is for an accelerated lifestyle, with late work hours, unbalanced meals, and a sedentary lifestyle. As far as we know, this is the first study that aims to evaluate the gender-related eating habits of young and middle-aged patients with FH. The information obtained can form the framework for the implementation of active and comprehensive prevention measures in order to reduce the risk of fatal and non-fatal future cardiovascular events.

MATERIAL AND METHODS

Study Design and Setting

Between 1st December 2020 and 31st March 2022, we conducted a cross-sectional study in the Cardiovascular Rehabilitation Clinic from Iasi Clinical Rehabilitation Hospital, on a group of patients with HF. For their classification, we used the Dutch Lipid Clinic Network (DLCN) score as it follows: if the score was above 8, they were classified as a certain FH case, probable case if the score was between 6-8, possible between 3 and 5 points, and unlikely to suffer from FH under 3 points. We decided to adjust the DLCN score using a correction factor validated in previous studies (6), because a great part of the patients had in their chronical prescription LLT. Consequently, if the patient has not been treated for high LDL for more than 6 months, was considered to be without conical treatment. For those who followed one LLT, the value was adjusted so that the impact of the treatment could be observed An important note is that the correction factor differs depending on the type of statin and dose, alone or in association with ezetimibe.

Study Participants

From a total number of 2005 patients admitted in the specified period of time, 32 patients were accepted in our research. The inclusion criteria were the age between 18-54 and more than 8 points in the Dutch Lipid Clinic Network score. We excluded patients diagnosed with severe hepatic or renal impairment, unbalanced diabetes, nephrotic syndrome, hypothyroidism, or those who ate a great amount of saturated and trans-unsaturated fatty acids.

Patients Evaluation

In order to avoid subjectiveness one investigator performed both the anamnesis and the clinical examination. Demographic data included: age, gender, BMI, degree of education (gymnasium, high school, university, and 10 classes or less), and employment status (working full time, part-time, retired or not working).

Some of the measurements performed from the blood samples were : Hb (mg/dL), uric acid (UA, mg/dL), proteins (g/dL), TC (mg/dL), triglycerides (mg/dL), HDL (mg/dL), LDL (mg/dL). These laboratory tests performed using the Erba XL 1000 Spectrophotometer® (Erba Diagnostics,

Mannheim, Germany).

Besides clinical and biological data, we applied a food frequency questionnaire that included information about eating habits, triggers for overeating, and feelings felt afterwards.

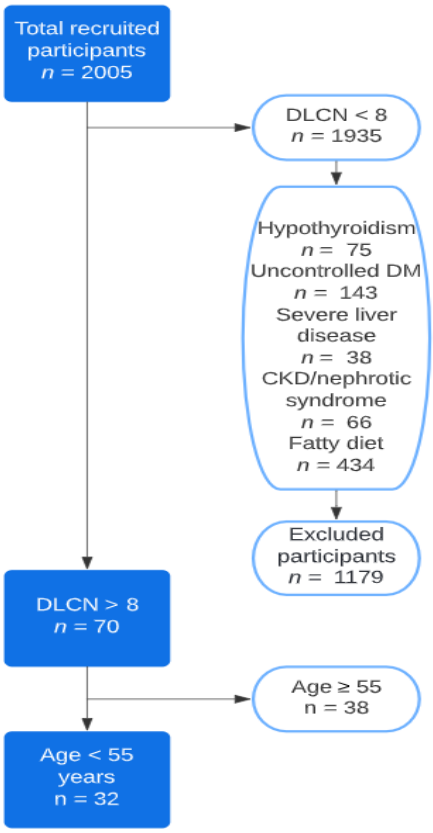

Fig. 1. Flow chart of the studied population

Ethical Approval

In order to be enrolled in the research, all of the patients had to complete a written informed consent form. Our study had received the approval from the Ethics Committee of both "Grigore T. Popa" University of Medicine and Pharmacy Iasi (certificate of approval dated 15th June 2020) and Iasi Clinical Rehabilitation Hospital (certificate of approval 25th November 2020).

Statistical Methods

Statistical analysis was performed using *SPSS 20.0* (Statistical Package for the Social Sciences, Chicago, Illinois). Data were expressed as mean ± standard deviation for continuous variables with normal distribution, as median with interquartile range for variables in which the normality of the distribution is not observed, and as the number of cases with frequency expressed as a percentage, in the case of categorical variables. In the case of continuous variables with normal distribution, the comparisons were made by applying the T-test for independent samples, or the ANOVA test,

for the comparison of more than two independent subgroups. The comparison of continuous variables that do not respect the normal distribution was performed using nonparametric tests: the Mann-Whitney's test, for the comparison of two subgroups, respectively the Kruskal-Wallis test, for the comparison of more than two subgroups. The comparison of the categorical variables was done using the Chi-square test. The correlation between different variables was evaluated by applying the Spearman correlation test. Statistical significance for all statistical tests performed was defined by a p-value <0.05.

RESULTS

Our study group included 32 patients, of whom 19 were women and 13 men. The average age was, respectively. We noticed that most of them came from urban areas (78.9% *versus* 84.6%), had higher education (63.2% *versus* 53.8%), and were employed full-time (84.2% *versus* 84.6%).

The evaluation of the body mass index revealed a higher average in the case of men, the difference being statistically significant (p=0.015).

TABLE I

Characteristics of the study group

	Female	Male	p-value
Age	43.78±9.41	44.15±9.04	0.869
Place of origin			
Rural	4 (21.1%)	2 (15.4%)	0.687
Urban	15 (78.9%)	11 (84.6%)	
Last form of graduated education			
Gymnasium	1 (5.3%)	1 (7.7%)	
10 classes/less	2 (10.5%)	1 (7.7%)	0.909
Highschool	4 (21.1%)	4 (30.8%)	
University	12 (63.2%)	7 (53.8%)	
Employment status			
Full time working	16 (84.2%)	11 (84.6%)	0.933
Retired	1 (5.3%)	1 (7.7%)	
Not working	2 (10.5%)	1 (7.7%)	
BMI	25.49±4.74	29.60±3.85	**0.015**
Blood samples			
Hemoglobin	12.41±1.08	14.88±0.79	**0.000**
Uric acid	3.07±1.14	4.67±0.85	**0.000**
Proteins	7.18±0.39	7.19±0.28	0.904
TC	260.21±56.93	283.20±78.66	0.344
TG	110.75±53.83	247.05±120.30	**0.002**
HDL	62.51±14.55	50.93±17.32	**0.050**
Non-HDL	197.69±54.36	232.26±78.24	0.150
LDL	177.84±48.41	203.10±66.89	0.224
LDLc	286.66±70.04	311.17±107.30	0.439

HDL – high-density lipoprotein; LDL – low-density lipoprotein; LDLc – corrected low-density lipoprotein; TC – total cholesterol; TG – triglycerides.

Regarding the laboratory tests, we notice significant differences in the value of hemoglobin, uric acid, and HDLc, but what stands out most is the difference between the average values of triglycerides, in men being much higher (247.05 ± 120.30 *versus* 110.75 ± 53.83, p = 0.002).

The diagnosis of familial hypercholesterolemia was made using the Dutch Lipid Clinic Network Score, criteria widely used in the European countries. Table II describes each criterion from the DLCN score and their frequency in the patients included in our study.

TABLE II

Dutch Lipid Clinic Network diagnostic criteria applied to the studied population

DLCN Criteria	FH
First-degree relative	
Premature CVD	16 (50%)
LDL > 95th percentile	11 (34.4%)
Tendinous xanthoma a/o arcus cornealis	7 (21.9%)
Child with LDL-C > 95th percentile for age and sex	14 (43.8%)
Personal history	
Premature CVA/PVD	4 (12.5%)
Coronary artery disease	10 (31.3%)
Physical examination	
Arcus cornealis < 45 years of age	18 (56.3%)
LDL	
155–189 mg/dL	0 (0%)
190–249 mg/dL	12 (37.5%)
250–329 mg/dL	10 (31.3%)
> 330 mg/dL	10 (31.3%)

FH: familial hypercholesterolemia; CVD: cardiovascular disease; LDL: low-density lipoprotein; CVA: cerebrovascular accident; PVD = peripheral vascular disease.

In table III the food questionnaire results are presented regarding how often certain specific food groups or foods are consumed.

Two-thirds of patients eat bread daily, regardless of gender. We notice that women tend to consume pasta more frequently, 94.7% prefer them a maximum of 3 times a week, unlike men. In their case, the percentage decreases by 10%.

Potatoes remain a staple food, being chosen on the menu almost daily, probably offset by the presence of other vegetables. We notice a food deficit when it comes to fruit, especially for men: only 23.1% have a portion of fruit every day, the percentage doubling for women (42.1%). Dairy products are also consumed with a low frequency, with less than a quarter of the participant consuming them daily.

When we evaluated the consumption of meat, we noticed that chicken is consumed 2-3 times a week by 76.9% of men and only 42.1% of women, who prefer pork/

beef. The vast majority of patients consume fish in less than 2 meals/week.

Fried food is chosen less than 3 times a week by 73.6% of women. But men eat more often, appearing even 4-6 times/week in the case of 46.2% of the subjects.

Soft juices and fast food have a low frequency in the weekly menu, regardless of gender.

TABLE III

Eating habits for female and male patients diagnosed with FH

	Female	*Male*
Bread		
<2x/week	1 (5.3%)	1 (7.7%)
2-3x/week	2 (10.5%)	2 (15.4%)
4-6x/week	3 (15.8%)	1 (7.7%)
Daily	13 (68.4%)	9 (69.2%)
Pasta		
<2x/week	9 (47.4%)	9 (69.2%)
2-3x/week	9 (47.4%)	2 (15.4%)
4-6x/week	0 (0%)	1 (7.7%)
Daily	1 (5.3%)	1 (7.7%)
Potatoes		
<2x/week	5 (26.3%)	3 (23.1%)
2-3x/week	11 (57.9%)	7 (53.8%)
4-6x/week	3 (15.8%)	2 (15.4%)
Daily	0 (0%)	1 (7.7%)
Green peas		
<2x/week	12 (63.2%)	8 (61.5%)
2-3x/week	6 (31.6%)	5 (38.5%)
4-6x/week	1 (5.3%)	0 (0%)
Daily	0 (0%)	0 (0%)
Other vegetables		
<2x/week	2 (10.5%)	2 (15.4%)
2-3x/week	4 (21.1%)	4 (30.8%)
4-6x/week	7 (36.8%)	4 (30.8%)
Daily	6 (31.6%)	3 (23.1%)
Fruits		
<2x/week	2 (10.5%)	2 (15.4%)
2-3x/week	3 (15.8%)	2 (15.4%)
4-6x/week	6 (31.6%)	6 (46.2%)
Daily	8 (42.1%)	3 (23.1%)
Dairy products		
<2x/week	3 (15.8%)	5 (38.5%)
2-3x/week	7 (36.8%)	3 (23.1%)
4-6x/week	5 (26.3%)	3 (23.1%)
Daily	4 (21.1%)	2 (15.4%)
Beef/pork		
<2x/week	6 (31.6%)	2 (15.4%)
2-3x/week	10 (52.6%)	5 (38.5%)
4-6x/week	3 (15.8%)	5 (38.5%)
Daily	0 (0%)	1 (7.7%)
Chicken		
<2x/week	2 (10.5%)	1 (7.7%)
2-3x/week	8 (42.1%)	10 (76.9%)
4-6x/week	5 (26.3%)	2 (15.4%)
Daily	4 (21.1%)	0 (0%)
Fish		
<2x/week	13 (68.4%)	8 (61.5%)
2-3x/week	3 (15.8%)	4 (30.8%)
4-6x/week	3 (15.8%)	1 (7.7%)
Daily	0 (0%)	0 (0%)
Fried food		
<2x/week	7 (36.8%)	1 (7.7%)
2-3x/week	7 (36.8%)	5 (38.5%)
4-6x/week	5 (26.3%)	6 (46.2%)
Daily	0 (0%)	1 (7.7%)
Sweets		
<2x/week	2 (10.5%)	3 (23.1%)
2-3x/week	9 (47.4%)	5 (38.5%)
4-6x/week	6 (31.6%)	3 (23.1%)
Daily	2 (10.5%)	2 (15.4%)
Soft juices		
<2x/week	16 (84.2%)	8 (61.5%)
2-3x/week	3 (15.8%)	2 (15.4%)
4-6x/week	0 (0%)	2 (15.4%)
Daily	0 (0%)	1 (7.7%)
Fast-food		
<2x/week	13 (68.4%)	10 (76.9%)
2-3x/week	5 (26.3%)	2 (15.4%)
4-6x/week	1 (5.3%)	0 (0%)
Daily	0 (0%)	1 (7.7%)

DISCUSSION

Food choices can be explained primarily by characteristics of the studied group. It consists mainly of young people with secondary-higher education, from urban areas, employed full time.

The average BMI is above normal for both women and men. However, we note that male subjects are closer grade 1 obesity, while women have a slightly higher value than normal. Similar results were also published by Liberopoulos *et al* . that observed a higher BMI in male patients with FH compared to women. Moreover, the researchers declared that men usually are diagnosed earlier with FH than women, due to their unhealthier lifestyle (7). When it comes to laboratory tests, we see high levels of uric acid and triglycerides in men, possibly explained by overeating, and sitting in front of a screen for long times, these being also evidence for the increased values of BMI discussed above. Regarding the triglycerides values, similar results were published recently by Borg *et al.* who evaluated the lipid profile in patients with FH from Faroe Islands. The authors observed higher median triglycerides values in men than women, values that were increased even since childhood and had a maximum around 45-50 years. On the other hand, women had almost constant values in all the age groups (8). Another study conducted by Iyen *et al* . that included 3553 patients with FH from 1st January 1980 to 20 December 2010, observed that women had statistically significant lower values of the triglycerides compared to men. Also, the BMI in men was higher when compared to women (9).

One aspect that should be mentioned is the increased value of HDL cholesterol in women, with an average of 62.51 ± 14.55, as opposed to men, who have 50.93 ± 17.32, a sign that women's food choices are more thoughtful. Moreover, we have included in our study young and middle-aged women. Thus, most of them are premenopausal and do not have a proatherogenic lipidic profile, having therefore high values of HDL cholesterol. These findings are supported also by Wang *et al* . (10) and by Iyen *et al* (9).

Given that the articles evaluating the nutrition of patients with HF are extremely few, we have initiated a series of studies on this topic. The first choice to evaluate nutrition was the frequency food questionnaire, a validated, easy-to-apply tool that gave us valuable, starting information about the eating habits of this category of patients.

The main observations are that there is a discrepancy between the diet of men and women. Men tend to eat more potatoes, chicken, fast-food, and fried food, and the last two categories appear even daily in the menu of a small percentage of men. A pertinent explanation is the daily work schedule that limits the culinary choices of men, who prefer to eat outside during breaks, so they choose accessible dishes. The same results were obtained also by Grzymisławska *et al.* that published in 2020 an article that mentioned the fact that men eat more fried and fatty meals or sweets in front of the television (11). Masella and Malorni agree also with the results mentioned above, observing the opposite types of meals between men and women: while men have a high fatty meal, women tend to eat more vegetables and whole food (12). No female consume fried food or fast food daily.

Even if women eat pasta more often the triglycerides value is significantly lower. A

possible explanation might be the larger portions for men, who probably have other sources of carbohydrates.

Women eat more fruits and vegetables but even so, the daily intake is reduced for both genders. Similar results were published by a group of researchers that evaluated more than 3,500 subjects from Lebanon where females have a higher intake of fibers and fruits compared to males (13).

About dairy products, about 25% of men and women eat them 4-6 times per week, while approximately 20% of women and only 15% of men eat them daily. Heuer *et al.* evaluated the eating habits of Germans and Regarding the dairy products, the males aged up to 50 years old ate more cheese, dairy products, and milk compared to women, while the ratio was inversely proportional when considering the age group 35-80 years old (14).

CONCLUSIONS

Further research is necessary in order to apply even more accurate nutritional assessments, thus including in the research team in addition to the cardiologist, internist, and psychologist, the nutritionist. Correct patient information is as important as the medical treatment, ensuring better compliance with lifestyle changes. Prevention should be initiated from the family doctor's office, adherence to treatment increasing with the confidence in the medical staff. The training of specialists in lipidology is imperative for a comprehensive assessment of the patient with FH.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding the scientific research.

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NEWS

CLINICAL FEATURES AND MANAGEMENT OF HUMAN MONKEYPOX: A RETROSPECTIVE OBSERVATIONAL STUDY IN THE UK

Human monkeypox is a zoonosis caused by monkeypox virus, an orthopoxvirus and close relative of variola virus (smallpox). It was first reported in central Africa in 1970 and has historically affected some of the poorest and most marginalized communities in the world. The clinical syndrome is characterized by fever, rash, and lymphadenopathy. Complications of monkeypox can include pneumonitis, encephalitis, sight-threatening keratitis, and secondary bacterial infections. Case fatality rates ranging from 1% to 10% have been reported in outbreaks in the Congo Basin and the virus clade circulating in this region appears to be associated with higher virulence. Human-to-human transmission of monkeypox is well described, including nosocomial and household transmission. However, human-to-human chains of transmission have historically been less well recognized. Two oral drugs, brincidofovir and tecovirimat, have been approved for treatment of smallpox and have demonstrated efficacy against monkeypox in animals. Our aim was to describe the longitudinal clinical course of monkeypox in a high-income setting, coupled with viral dynamics, and any adverse events related to novel antiviral therapies. We reviewed all cases since the inception of the HCID (airborne) network between Aug 15, 2018, and Sept 10, 2021, identifying seven patients. Of the seven patients, four were men and three were women. Three patients were treated with brincidofovir (200 mg once a week orally), all of whom developed elevated liver enzymes resulting in cessation of therapy. One patient was treated with tecovirimat (600 mg twice daily for 2 weeks orally), experienced no adverse effects, and had a shorter duration of viral shedding and illness (10 days hospitalization) compared with the other six patients. One patient experienced a mild relapse 6 weeks after hospital discharge. Human monkeypox poses unique challenges, even to well-resourced health-care systems with HCID networks. Prolonged upper respiratory tract viral DNA shedding after skin lesion resolution challenged current infection prevention and control guidance. There is an urgent need for prospective studies of antivirals for this disease (Adler H, Gould S, Hine P, *et al.* Clinical features and management of human monkeypox: a retrospective observational study in the UK; *Lancet Infect Dis* 2022 Published Online May 24, 2022 / https://doi.org/10.1016/ S1473-3099(22)00228-6).

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CLINICAL PROFILE ASSOCIATED WITH BLEEDING SMALL BOWEL TUMORS IN PATIENTS UNDERGOING CAPSULE ENDOSCOPY

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CLINICAL PROFILE ASSOCIATED WITH BLEEDING SMALL BOWEL TUMORS IN PATIENTS UNDERGOING CAPSULE ENDOSCOPY (Abstract): Since the development and implementation into clinical practice of the small bowel (SB) capsule endoscopy (CE), obscure gastrointestinal bleeding (OGIB) is no longer an answerless question. In many cases of SB pathology, a definite diagnosis is made by SBCE, while other cases, as SB tumors (SBT), require additional investigations. Our aim was to identify predictors for SBT as OGIB etiology. **Material and methods:** We retrospectively studied all patients with OGIB who underwent SBCE in a two-year period. Only patients with positive findings were included. Patients with SBTs were compared to patients with non-tumoral cause of OGIB, and analyses for possible associated clinical factors were performed. **Results:** 205 SBCE examinations were performed for OGIB. Positive findings were noted in 134 patients (male gender: 72, female gender: 62; age range 21-79): 25 cases of SBTs, and 109 cases with non-tumoral causes responsible for bleeding (angioectasia, Crohn's disease, NSAIDs enteritis, and other). SBT patients underwent further investigations, for confirmation and stadialization. Patient's age < 65 years, lack of anticoagulant treatment, and overt type of bleeding significantly associated with SBTs. Gender was not predictor for SBT. Surgery was the main therapeutic option for SBTs, significantly more frequently compared to the non-tumoral causes. **Conclusion:** Young age, overt bleeding and lack of anticoagulant treatment are factors associated with the final diagnosis of SBT. Because SBCE is only a visual technique and additional investigations are needed, predictors could anticipate the need for additional explorations and could help optimizing the investigational plan in terms of time and resources. **Keywords:** SMALL BOWEL TUMOR, OBSCURE GASTROINTESTINAL BLEEDING, IRON DEFICIENCY ANEMIA, SMALL BOWEL CAPSULE ENDOSCOPY.

Small bowel capsule endoscopy (SBCE) has revolutionized the approach of patients with suspected small bowel (SB) pathology. Since its launch and its implementation into the clinical practice more than two decades ago, SBCE has become an essential tool in the exploration of the gastrointestinal tract, allowing the diagnosis and warranting the choice of the appropriate management in many types of SB patholo-

gy (1). Actual guidelines from all over the world are now consensually sustaining the use of SBCE as first-line investigation whenever SB pathology is suspected unless absolute contraindication exists (2-4).

SBCE has made important contributions in the diagnostic work-up of obscure gastrointestinal bleeding, unexplained iron deficiency anemia (IDA), inflammatory bowel diseases - especially Crohn's disease and inflammatory disease type unclassified, celiac disease, and small bowel tumors (SBT).

Since the use of SBCE, and thanks to various technical progresses aiming to improve its performance, OGIB is no longer an answerless question. Suspected small bowel bleeding, recognized recently as separate entity called middle gastrointestinal bleeding, represents the main indication for SBCE (5). Diagnostic yield of SBCE in suspected SB bleeding has raised to almost 70%, and the most frequent lesions found to be responsible are angiodysplasia, Crohn's disease, various types of enteritis, and SBT (6, 7).

Traditionally, SBT are considered relatively rare, counting 5-8% from all gastrointestinal tract tumors (8). Small bowel is classically considered the most challenging part to be explored form the whole gastrointestinal tract, due to its "hidden" location, length and lack of ideal approach methods, both effective and safe. Not so long ago, data were showing that SBCE has doubled the rate of SBT diagnosis (9). According to recent analysis, SBCE has practically almost tripled it, the reason for this accomplishment being undoubtedly its capability to visualize in a relatively simple, non-invasive manner the entire small bowel (10). On the other hand, the most frequent circumstance for detecting SBT by SBCE is obscure (manifest or occult) gastrointestinal bleeding.

However, SBCE offers only a macroscopic view of the lesion. Therefore, the final and complete diagnosis of SBT is established after further investigations allowing histopathological analysis and, respectively, stadialization, or even only after surgical approach. Even if SBCE is the first line investigation for suspected SB pathology, any suspicion of SBT will require supplementary investigations. Thus, in terms of resources, anticipating the need of more explorations could optimize the work-up strategy.

Our aim was to identify predictors for SBT as cause of OGIB, so in the high probability of SBT, additional work-up should be timely previewed and consequently provided.

MATERIAL AND METHODS

Study design and patients

We retrospectively analyzed the clinical data and medical records of all patients with OGIB (overt or occult) who underwent SBCE in a two and a half-year period, from January 2019 to June 2021 in our tertiary referral care center. Only patients with positive findings, which were further concordant with the final diagnosis, were included in the final analysis.

This study was performed in accordance with the ethical principles of the Declaration of Helsinki and was approved by the institutional review board. All patients underwent SBCE examination after informed consent was signed. No additional consent was required for this study owing to its retrospective nature.

SBCE procedure

The third-generation CE system (PillCam SB3) was used, in combination with

RAPID reading software. All the procedure were performed after negative upper and lower gastrointestinal endoscopy. Patients were informed about the procedure and signed the informed consent. SBCE examination was performed after contraindications were excluded. They were prepared according to current guidelines, with 2 liters of polyethylene-glycol electrolyte lavage solution the day before the examination. Saurin's classification was used for potential small bowel bleeding lesions (11).

Data collection

Patient-related variables, including age, gender, type of OGIB, and current anticoagulant treatment, were reviewed. The patients were classified into two groups according to the final diagnosis: patients with SBT and patients with non-tumoral cause for OGIB, respectively. The two groups were compared in order to assess clinical parameters associated with the diagnosis of SBT.

Statistical analysis

Categorical variables were reported as frequencies and percentages. Continuous variables are presented as mean ± standard deviation values, and differences between groups were statistically assessed using Fisher's exact test. A two-sided P value of < 0.05 was considered statistically significant Statistical analyses were performed using *SPSS version 22.0* software (IBM SPSS Inc, Chicago, IL, USA).

RESULTS

Baseline characteristics of patients and diagnostic yield of SBCE in OGIB

In total, 205 patients underwent SBCE for OGIB in the above-mentioned period. SBCE found the responsible lesion in 134 patients, corresponding to a diagnostic yield of 65%.

The clinical characteristics of the 134 patients entering our study are presented in the Table I. We have noted a slight predominance for male gender among all patients. The majority of patients belonged to the young age group (age < 65 years), and the most frequent type of bleeding was the occult one. Approximatively one fifth of the examined patients were taking anticoagulants, for associated cardio-vascular or neurological diseases.

TABLE I.

Clinical characteristics of patients, findings at SBCE and type of intervention

	Total number of patients = 134
Age, years, mean ± SD Range, years	64 ± 12.5 21-79
Gender ratio Women/Men, n (%)	 62/72 (46.3/53.7)
Type of OGIB, n (%) Overt Occult	 50 (37.3) 84 (62.7)
Patients with anticoagulant treatment, n	26
Further investigations, n	66
Type of lesion responsible for OGIB, n **SBT** GIST Adenocarcinoma	 **25** 13 6

	Total number of patients = 134
Neuroendocrine tumor	2
Lymphoma	2
Lipoma	1
Metastatic melanoma	1
Non-tumoral	**109**
Angioectasia	65
Crohn's disease	30
NSAIDs enteropathy	4
Enteritis	6
Meckel's diverticulum	2
Parasitosis	1
Intestinal tuberculosis	1
Type of intervention, n	
Medical treatment	82
Endoscopic treatment	27
Surgery	25

Findings at SBCE

The interpretation of the lesions discovered by SBCE was made according to Saurin's classification. Following SBCE, SBTs as etiology for OGIB were noted for 25 patients (18.6%), while for the remaining 109 patients (81.4%), non-tumoral causes were recorded. After complete work-up, consisting in further imaging or enteroscopic investigations and/or surgical approach, a final histological diagnosis was established. The most frequent types of tumors were GIST and adenocarcinoma. Cases of neuroendocrine tumor and lymphoma were also recorded, as well as a case of jejunal ulcerated lipoma. Amid non-tumoral causes, the most frequent were represented by angioectasia, Crohn's disease, NSAIDs enteropathy, and enteritis; other lesions were also described (diverticulum, parasitosis, intestinal tuberculosis).

The final management of the cases, consisted in medical treatment for the majority of cases, endoscopic or surgical treatment, according to the positive diagnosis. SBCE procedure-related complications, such as retention, did not occur in any of the cases.

Correlations between clinical characteristics of patients and the etiology of OGIB

The results of the analysis regarding patients' age, type of bleeding, concomitant anticoagulant treatment according to the etiology of bleeding (tumoral lesion vs. non-tumoral cause, respectively) are showed in the Table II. Age group under 65, overt type of OGIB and lack of anticoagulant treatment significantly associated with SBT as cause of OGIB ($p<0.05$).

The comparative analysis regarding the need for further investigations and the type of therapeutic intervention according to the same two groups is shown in the Table III. With one exception, all the rest of the patients with SBT (24 out of 25) required additional investigations, significantly more compared to the patients with non-tumoral cause of OGIB (25 out of 109, respectively; $p<0.001$). In the same time, significantly more patients with tumoral bleeding needed surgery as therapeutic solution, while the most patients with non-tumoral bleeding were treated by medical or endoscopic interventions ($p<0.0001$).

TABLE II.

Clinical characteristics according to the etiologic diagnosis

	SBT, n Total = 25	Non-tumoral, n Total = 109	
Women/men, n (%)	9/16 (36/64)	53/56 (48.7/51.3)	p=0.27
Age group <65/≥65, n	21/4	67/42	p=0.03*
Type of OGIB Overt/occult	18/7	32/77	p<0.0001*
Anticoagulant treatment	1	25	p=0.04*

*Significant $p<0.05$

TABLE III.

Diagnostic and therapeutic characteristics according to the etiological diagnosis

	SBT, n Total = 25	Non-tumoral, n Total = 109	
Further imaging studies	24	42	$p<0.0001$*
Surgical treatment	23	2	$p<0.0001$*

*Significant $p<0.05$

DISCUSSION

SBT are rare, and their diagnosis is difficult. Clinical presentation of SBT is often non-specific. Even when the clinical index suspicion is high, choosing and applying the right methods of exploration is difficult. These are main the reasons for why, generally, the approach of the small bowel is considered a challenge.

After having proved along the years the usefulness, together with its non-invasive character, SBCE became the first line investigation method recommended when small bowel pathology is suspected.

However, a positive diagnosis at SBCE examination is not always the final diagnosis. The identification and the right description of a typical, benign lesion at SBCE usually represents the last step before applying the right treatment, but the diagnosis of SBT in patients undergoing SBCE is usually an intermediate cornerstone across a long investigational path. Finding a tumoral mass at SBCE is followed, as appropriate, by anterograde or retrograde enteroscopy with biopsy, and further entero-CT or entero-IRM in order to establishing the extension.

At the same time, another challenge in the diagnosis of SBT are represented by the cases of false SBT, which require, especially in the case of submucosal lesions, thorough differential diagnosis with the bulges having similar appearance. Several criteria and scores have been proposed along the years, as predictive methods to differentiate between the true masses and the innocent bulges (12, 13).

Thus, knowing the factors associated with the SBT could help anticipate the diagnosis and plan in a timely manner the diagnostic sequence and the therapeutic decision.

Epidemiological studies showed that, according to gender, men are more prone to develop malignant SBT (14). In our analysis, there was a higher predominance of male patients in the SBT group, but male gender was not predictive for the diagnosis of SBT.

In our study, the main indication for OGIB was represented by the occult bleed-

ing, with IDA. A significant lower proportion of patients with occult OGIB had tumoral lesions as cause for their anemia (8%), while more than one third of patients with overt OGIB had SBT at SBCE (36%, $p<0.05$). Consequently, overt OGIB associates with higher frequency of SBT diagnosis. Lower diagnostic yield of SBCE in IDA, compared to overt bleeding, has been stated by many studies so far (15,16). Additionally, anemia itself has many and often combined causes and mechanisms and may become relatively common in the elderly population. Thus, when SBCE is performed for IDA, there are usually less chances to have a diagnosis. This is also how we could further explain the higher proportion of patients aged under 65 in the SBT group, compared to that in the non-tumoral group (84% *versus* 61%, respectively, $p = 0.27$).

Anticoagulant treatment was associated in our analysis with the non-tumoral cause of OGIB. Minor lesions bleeding may be triggered by the anticoagulant medication. In the lack of anticoagulant drugs, they would have probably remained silent. So, minor lesions are prone to manifest and be discovered more frequently when the patient follows this type of treatment, for various, especially cardio-vascular, comorbidities. SBT present a bleeding potential by themselves, so they do not need an extra trigger factor for hemorrhagic exteriorization.

As discussed before, identifying a tumoral mass at SBCE implies further work-up. In our study, in order to complete the diagnosis, all patients with SBTs underwent additional investigations (enteroscopy with biopsy, entero-CT, and/or entero-IRM), while only 38% of patients with non-tumoral lesions at SBCE needed supplementary exploration before the therapeutic decision ($p<0.05$).

Depending on the type, dimension and extension, SBTs have usually surgical indication. In our study, one single SBT was benign, with the final histological diagnosis established after surgery - jejunal ulcerated lipoma. The bleeding potential, and the lack of a histological diagnosis after enteroscopy with biopsy, justified the surgical approach. Metastatic SBTs are rare, but they exist, and they may complicate with bleeding; a particular case of metastatic melanoma was diagnosed in our analysis with metastatic melanoma, managed by conservative treatment, with no surgery involved. The other case in our study which did not require surgery was a case of SB lymphoma, which was referred to the hemato-oncologist.

Capsule retention is a complication with 1-2% overall occurrence rate, with various frequencies reported depending on the indication of the examination, the highest in known CD (17, 18).

For OGIB, retention rate was 1.3% in a large European single-center 10-year experience (19). Nor retention or other complication occurred in our analysis, probably due to the prior work-up which excluded patients with high obstruction risk from the SBCE examination.

CONCLUSIONS

Age under 65, overt bleeding and lack of anticoagulant treatment sketch the profile of the patients with SBT; these patients will also be those who will require additional investigation methods and, finally, will have surgery as therapeutic option.

A complete early characterization of the clinical profile of the patient is of upmost importance, before deciding the diagnostic work-up. Some clinical characteristics may associate, as we showed, with a higher probability of having a tumoral lesion responsi-

ble for their bleeding. Being prepared of advance in front of such patients may assure a temporal gain and a more fluent investigation algorithm, finally reflected into the right therapeutic option choice.

CONFLICT OF INTEREST AND FUNDING

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BEYOND RESPIRATORY DAMAGE – NEUROLOGICAL INVOLVEMENT IN SARS-CoV-2 INFECTION

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BEYOND RESPIRATORY DAMAGE-NEUROLOGICAL INVOLVEMENT IN SARS-Cov-2 INFECTION (Abstract): Although the respiratory damage associated with SARS-CoV-2 infection is well known, clinical and experimental studies have shown that the *Coronaviridae* family has a certain tropism for both the central and peripheral nervous system. Most patients with this pathology also associate neurological manifestations, often of a central type, with varying degrees of difficulty. The aim of this study was to evaluate the newly developed neurological manifestations in SARS-CoV-2 infected hospitalized patients. Material and method: We retrospectively analyzed medical records of patients with SARS-CoV-2 infection admitted in the Clinical Hospital of Infectious Diseases "Sf. Parascheva" Iasi between May 2020 – May 2022. Clinical features, frequency and correlation with risk factors (for example age, gender, comorbidities) and also severity of infection were evaluated. **Results:** We have identified 133 patients hospitalized during analyzed period and the majority presented significant comorbidities (94.84% cases). Fatigability (51.88% cases), myalgia (32.33% cases), impaired consciousness (decreased consciousness or confusion to encephalopathy) (27.88% cases), headache (28.57% cases) were the most frequent neurological features identified. A changing pattern in neurological manifestations was found during the pandemics' waves, with an increase of incidence of fatigability and myalgia during the fifth wave of pandemic. **Conclusions:** Neurological symptoms were a relatively constant part of the clinical picture of SARS-CoV-2 infection. They had polymorphic and various features during the pandemic waves. The incidence of headache, myalgia and fatigability was highly increased during the last wave of pandemics. Impairment of consciousness was significantly correlated with age, neurological and cardiovascular comorbidities. **Keywords**: SARS-CoV-2 INFECTION, NEUROLOGICAL, HEADACHE, CONFUSION.

Declared in March 2020, the COVID-19 pandemic was associated with over 650 million illnesses or relapses and over 6 million deaths (1). The pathophysiology of the illness, a description of the clinical manifestations, the discovery of an efficient and secure etiological treatment, and last but not least, the identification of suitable particular prophylaxis measures were all necessary for the face of an emergent infection. These factors required special consideration and quick understanding. Under the pressure of the unceasing evolution of viral transmission in parallel with the high number of illnesses,

the therapeutic approach was constantly followed and updated national and international protocols, aiming at the most effective treatment depending on the severity and risk factors of the infection. The SARS-CoV-2 infection affected people of all ages, and the risk factors for the severe infection, such as newborns, prematurity, elderly, or male gender, were found (2). Pre-existing pathologies such as obesity, diabetes, cardiovascular and renal diseases, chronic respiratory diseases, or immunosuppression were risk factors for all age groups throughout the pandemic (2,3).

Clinical manifestations of SARS-CoV-2 infection include:

- fever, chills, rigor.
- respiratory: cough, odynophagia, dyspnea, thoracic pain.
- digestive: nausea, vomiting, diarrhea.
- cutaneous: maculopapular/morbilliform, urticarial, and vesicular eruptions and transient livedo reticularis.
- neurological: headache, myalgia, anosmia, ageusia or dysgeusia, fatigability, confusion, dizziness. Rare cases of myositis, rhabdomyolysis, paresthesia, Guillain Barre syndrome and focal and multifocal neuropathies (e.g., facial nerve palsy, lower cranial neuropathy (vagus, accessory, and hypoglossal; Tapia syndrome or multiple cranial neuropathies) were also reported (4-6).

The SARS-CoV-2 virus produces neurological manifestations through various mechanisms, either by direct action secondary to the invasion of the CNS through the blood–brain barrier, via the hematogenous route attached to the ACE2 receptors or by infecting the nerve terminals of the vagus nerve located in the respiratory system and the gastrointestinal tract (7). The pathological changes secondary to hypoxemia, including acute hypoxic-ischemic damage and the proinflammatory state associated with the "*cytokine storm*" hypercoagulation, endotheliopathy, platelet activation, or cardio-embolism, are responsible for the neurological complications of SARS-CoV-2 infection (8). These complications are represented by encephalopathy, meningitis, encephalitis, acute necrotizing encephalopathy, or cerebrovascular disorders, such as stroke (6,8). The incidence of neurological manifestations and complications varied during the waves of the COVID-19 pandemic (9).

In this context, we performed a retrospective analysis of the clinical-biological, therapeutic and evolutive data of patients with SARS-CoV-2 infection who presented neurological manifestations and were admitted to the Clinical Hospital of Infectious Diseases "Sf. Parascheva" Iasi, between May 2020 and May 2022.

MATERIAL AND METHODS

This was a retrospective analysis of the hospital–based medical records of the patients with SARS-CoV-2 infection in patients with neurological manifestations hospitalized during the mentioned period. The study period was stratified based on the month of test screening and diagnosis to identify temporal trends in cases. Population data were divided into three phases:

- Phase I: May 2020–February 2021 (first three waves)
- Phase II: August–December 2021 (fourth wave)
- Phase III: January–May 2022 (fifth wave).

The clinical and paraclinical data were collected from observation files and reports. The following data were collected during the research: demographic data, medical and medicine history, the onset,

and severity of symptoms at admission (neurological, respiratory and other manifestations), treatment administered (name and duration of administration), and outcome (complications or death).

The severity of infection was graded as follows (5): *mild disease* - mild symptoms and normal or non-pneumonia findings on radiographic examination; *moderate disease* - respiratory symptoms and fever, with evidence of pneumonia on radiographic examination; *severe disease*: dyspnea, respiratory frequency ≥ 30/minute, blood oxygen saturation ≤ 93%, PaO_2/FiO_2 ratio < 300, and/or lung infiltrates > 50% of the lung field within 24–48 hours and c*ritically ill*: respiratory failure and mechanical ventilation required septic shock, and/or multiple organ dysfunction/failure and requires ICU monitoring and treatment.

Continuous data are expressed as means ± standard deviation. Pearson correlation coefficients were used for correlation between different variables. A p-value ≤ 0.05 was considered statistically significant. Statistical analysis was performed using XLSTAT software version 2022.

The Ethics Committee of the Infectious Diseases Clinic "Sf. Parascheva" Iaşi approved the study.

RESULTS

In the current analysis, we have included 133 patients corresponding to each phase, with a mean age of 65.5 years (range 25 – 92 years), mainly males (60%). In most of the cases were recorded metabolic (obesity or diabetes) and cardiovascular (e.g., hypertension), and only 5,26% of cases had no associated disorders (tab. I).

TABLE I.
Patients' characteristics

Statistic Variable	Comorbidities			Provenience Urban (U)/ Rural (R)	Gender Male(M)/ Female(F)	Statistic Variable	Age (years)	Weight (kg)	Height (m)
	Metabolic	Cardiov-ascular	Neurologic						
No. of observations	133	133	133	133	133	No. of observations	133	133	133
Rel. frequency per category (%)	42	52	9.77	71/29	59/41	Median	69	81.5	1.68
Lower bound on frequencies (95%)	32	41	0	64/21	50/33	Mean	65.49	86.36	1.7
Upper bound on frequencies(95%)	53	62	7	79/36	67/50	Standard deviation (n)	13.96	18.86	0.10
Proportion per category	0	1	0	1/0	1/0	Variation coefficient (n)	0.21	0.22	0.06
Lower bound on proportions (95%)	0	0	0	1/0	1/0	Lower bound on mean (95%)	63.08	78.91	1.66
Upper bound on proportions (95%)	1	1	0	1/0	1/0	Upper bound on mean (95%)	67.89	93.80	1.74

The most frequent neurological symptoms extracted from the medical records were experienced from the onset of infection to discharge, as follows: fatigability (51,88% cases), myalgia (32,33% cases), impaired consciousness (decreased consciousness or confusion to encephalopathy) (27,88% cases), headache (28,57% cases) and less frequent loss of smell (anosmia) or taste (ageusia) (fig. 1).

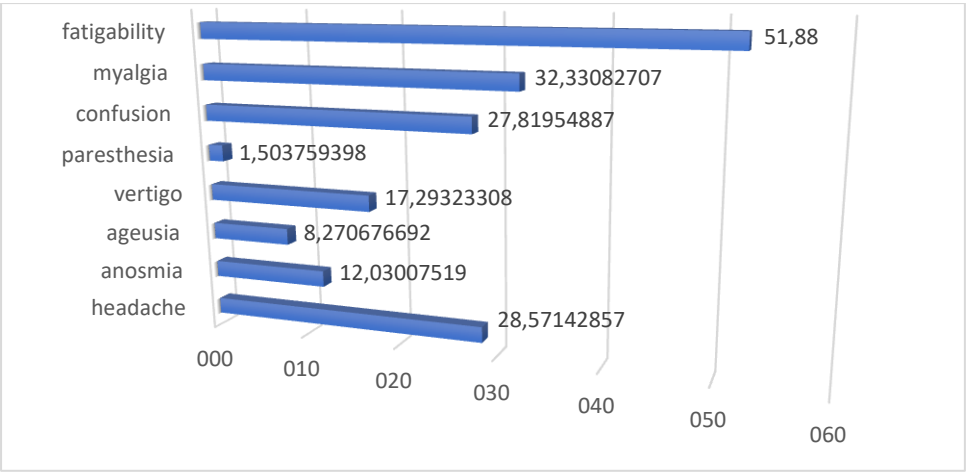

Fig. 1. Neurological manifestations

In our study, alterations in consciousness status prevailed during the fourth wave of pandemics (delta variant). At the same time, myalgia and fatigability were noted more often during the fifth wave, which was the omicron variant wave. Interestingly, the incidence of cases of headache decreased up to the last wave of pandemics (fig. 2).

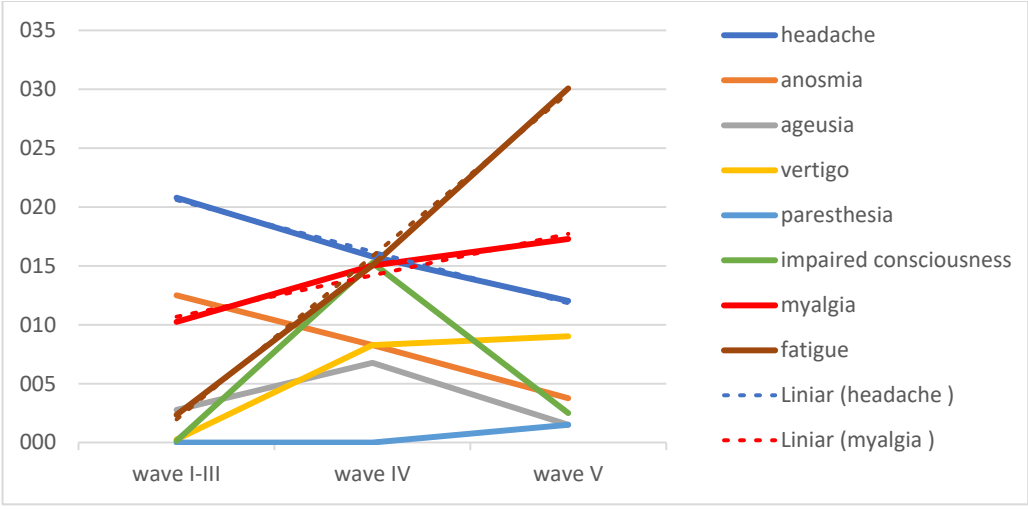

Fig. 2. Trends of neurological symptoms by pandemic wave (percentage of cases)

The Pearson correlation test did not find any link between neurological symptoms and risk factors like gender or comorbidities. Nevertheless, a weak but significant correlation was found between age, neurological and cardiovascular illnesses, and the different levels of altered consciousness (from confusion, disorientation, and somnolence to coma or encephalopathy) (tab. II).

TABLE II.
Pearson correlation test

Pearson coefficient	Comorbidities				SARS-CoV-2 infection		Age	Gender
	Metabolic	Cardiovascular	Neurologic	Oncologic	Severity	Evolution		
Fatigability	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Headache	0.00	0.00	0.01	0.02	0.01	0.00	0.01	0.00
Anosmia	0.00	0.00	0.02	0.02	0.00	0.03	0.01	0.02
Ageusia	0.00	0.00	0.01	0.01	0.00	0.03 **P=0.04**	0.01	0.02
Vertigo	0.00	0.01	0.02	0.02	0.04 **P=0.03**	0.00	0.01	0.00
Paresthesia	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
Impaired consciousness	0.00	0.04 **P=0.04**	0.11 **P=0.00**	0.00	0.00	0.00	0.10 **P=0.00**	0.00
Myalgia	0.01	0.00	0.05	0.00	0.00	0.00	0.03	0.00

DISCUSSIONS

Declared in March 2020, the evolution in "waves" of the COVID-19 pandemic was the result of the very large number of mutations, especially at the level of the viral Spike protein. Emerging strains of the SARS-CoV-2 virus, with increased transmission capacity and high degree of infectivity, have been called "*variants of concern"* (VOC). The World Health Organization has established 5 VOCs of the SARS-CoV-2 virus, namely: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529) with different characteristics (tab. III) (10).

The SARS-CoV-2 infection is mainly characterized by pneumonia, hypoxemic respiratory failure, cardiac and kidney dysfunction, and important morbidity and mortality. However, a varied range of manifestations with temporal trends depending on the wave and variant involved has been described in COVID-19 patients. Our findings are in line with previously published data (11-14). anosmia, ageusia/dysgeusia, headache, alterations of consciousness and myopathies were the most common neurological features identified in SARS-CoV-2-infected patients. Amin et al (2022) described the evolution of COVID-19 symptoms during the five waves based on the results from a population-based cross-sectional analysis. The incidence of neurological manifestations with peripheral involvement was increased during the third wave and prevailed during the last two waves. In our study, various levels of impairment of consciousness (from altered

mental status to encephalopathy) were identified mainly in the infection produced by the delta variant (9). These manifestations were expected mainly as a result of silent hypoxia described in patients with COVID-19 pneumonia (15). We have noted a very weak, but statistically significant correlation between these alterations and other negative prognosis factors in the evolution of SARS-CoV-2 infection, such as male gender, elderly, and other associated diseases (neuropsychiatric and cardiovascular diseases). The severity of these symptoms decreased towards the end of the pandemic, not only due to changes in the virulence of the virus but also due to the introduction of the anti-SARS-CoV-2 vaccination (16).

TABLE III.
Characteristics of main VOCs

WHO	Alpha	Delta	Omicron
Pango Lineage	B.1.1.7,Q	B.1.617.2, AY.4.2	B.1.1.529, BA
Transmission	↑ 43-82% transmissible	↑~↑↑ ≥alpha	↑↑↑ X3.19-4.2 more transmissible than delta
Severity	→/↑ X60% mortality	↑↑ X2 alpha Risk of hospitalization and death	↓ Severity and mortality low
Immunology	→	↑	↑↑
		high risk of reinfection	

The incidence of myopathies characterized by fatigability and myalgia significantly increased during the fifth wave, while other symptoms (e.g., loss of taste or smell) which were frequent at the beginning of the pandemic, were less often recorded in COVID-19 patients. The frequency of cases with headache was found decreased during the last two waves of pandemics, which is different from our clinical practice. This could be a result of the small sample size analyzed. Therefore, we will perform further analysis extending the number of patients.

The main limitation of our analysis is the small number of analyzed patients. The following studies will expand the study sample size and analyze in detail the correlations between the neurological manifestations, the severity of the disease and other known prognostic factors in SARS-CoV-2 infection.

CONCLUSIONS

Neurological symptoms were a relatively constant part of the clinical picture of SARS-CoV-2 infection. They had polymorphic and various features during the pandemic waves. The incidence of headache, myalgia and fatigability was highly increased during the last wave of pandemics. Impairment of consciousness was significantly correlated with age, but also with neurological and cardiovascular comorbidities.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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IN VITRO ASSESSMENT OF TGFβ3/TGFβ1 RATIO VARIATION PATTERN FOLLOWING ANTISEPTIC TREATMENT ON ADIPOSE-DERIVED STEM CELLS AND FIBROBLASTS IN 2D *VS.* 3D CULTURE SYSTEMS

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IN VITRO ASSESSMENT OF TGFβ3/TGFβ1 RATIO VARIATION PATTERN FOLLOWING ANTISEPTIC TREATEMNT ON ADIPOSE-DERIVED STEM CELLS AND FIBROBLASTS IN 2D *VS*. 3D CULTURE SYSTEMS (Abstract): Wound management, especially chronic wounds and burns, remain a challenge despite advances regarding topical antimicrobial agents and emerging stem-cell based strategies. Moreover, the current available data is controversial regarding antiseptics drawbacks on wound healing and minimal on the effects on stem cells, although it seems they modulate cytokines, including TGFβ. The aim of the study was to evaluate *in vitro* the effects of commonly used antiseptics on the TGFβ3/TGFβ1 ratio in cell cultures. **Material and methods:** Four cell constructs were developed with different cell ratios using adipose-derived stem cells and normal human dermal fibroblasts in both 2D and 3D mono and co-cultures. Following treatment with either 1% povidone-iodine, 0.05% chlorhexidine digluconate or silver-coated dressing, TGFβ1 and TGBβ3 levels were evaluated using ELISA method. Subsequently, TGFβ3/TGFβ1 ratio was calculated, and statistical analysis performed. **Results:** TGFβ3/TGFβ1 ratio for untreated stem cells and co-cultures increase pattern follows the stem cell ratio in both 2D and 3D culture systems. Following povidone-iodine treatment for all cell constructs in 2D systems, TGFβ3/TGFβ1 ratio increases significantly, whereas in 3D culture systems increased only in stem cell monoculture and co-culture with fibroblast as a majority cell fraction. Moreover, there are significant differences in TGFβ3/TGFβ1 ratio when 2D and 3D culture systems are compared, especially after chlorhexidine digluconate and silver-coated dressing exposure for fibroblast monocultures and co-cultures. **Conclusions:** TGFβ3/TGFβ1 ratio pattern modulation *in vitro* is dependent on antiseptic, cell type, cell ratio and culture system. **Keywords:** TGFβ3/TGFβ1 RATIO, WOUND HEALING, ANTISEPTICS, SPHEROIDS, 3D CULTURES.

Wound care improved significantly, although management of chronic wounds, burns or any other wound at high risk of infection remains a clinical challenge (1,2). Often to either control the bacterial burden or to treat an infected wound, topical antimicrobial is used (3, 4). Also, emerging stem cell-based strategies represent an important topic in wound management (5).

Antiseptics are chosen based on antimicrobial spectrum and advantages and possible drawbacks on wound healing. One pa-

rameter used to choose an antiseptic is the biocompatibility index, which should be above 1, meaning a broad activity with minimal cytotoxicity on fibroblasts and keratinocytes (1, 4). Current available data on antiseptics effects on wound healing are heterogeneous and conflictual, difficult to translate *in vitro* data in clinical setups, with an insufficient standardized testing and evaluation methods and without any global guidelines available (1, 2, 4).

Most commonly used topical antimicrobial agents are povidone-iodine, chlorhexidine digluconate and silver based. The first two agents are used especially for wound irrigation, while silver based, such as silver containing dressing are recommended for infected wounds and not prophylactic (2, 4, 6). Povidone-iodine (PVI) is frequently used, although it is not recommended for chronic wounds due to its cytotoxicity and contraindicated in new-borns, pregnancy, thyroid disorder and for no more than 7 days (1, 4). Some data suggest that PVI promote wound healing by increasing TGFβ levels, even in the absence of infection (7). Chlorhexidine (CHX) is used for wound cleaning/irrigation, predominantly for burns. There is low evidence regarding wound healing impairment, including when compared with PVI. Moreover, it has a lower antimicrobial spectrum with no effect on Proteus spp., Pseudomonas aeruginosa and fungi (4, 6). Silver-coated dressings (Ag) seem to have anti-inflammatory effects, by modulating cytokine levels, including TGFβ. Also, some studies suggest that Ag, apart from a good antimicrobial effect, promotes wound healing (3, 4, 8).

TGFβ superfamily plays an important role throughout the wound healing process. TGFβ1 it is usually associated with dermal fibrosis, while TGFβ3 is known to decrease scar tissue formation. Also, when TGFβ3 to TGFβ1 ratio is increased, wounds tend to heal with less scarring (9, 10).

As the stem cell-based strategies emerge, there is limited data regarding antiseptic effects on adipose-derived stem cells (5, 11). Most *in vitro* studies are on fibroblasts and keratinocytes and using varying cell species. For example, murine fibroblasts tolerate better PVI compared with human fibroblasts, making more difficult to translate *in vitro* results in clinical settings (12).

Herein, as part of a larger study, we present the effects of the three most commonly used topical antimicrobial agents on human adipose derived stem cells (ADSC) and normal human dermal fibroblasts (NHDF) in different culture systems regarding TGFβ3/TGFβ1 ratio variation patterns.

MATERIAL AND METHODS

Antiseptics

Most commonly used antiseptics were selected: PVI (PVP1-100G, Sigma-Aldrich), CHX (C9394-25ML, Sigma-Aldrich) and a silver-coated dressing (66000808, Smith& Nephew). PVI and CHX were diluted in PBS and 1% PVI and 0.05% CHX selected as working concentrations. The 5/5 cm silver-coated dressing (Ag) was cut in 5/5 mm pieces that contain approximate 0.21-0.33 mg silver per mm^2.

Cells and cell constructs

Adipose-derived stem cells (PT-5006, Lonza) and fibroblasts (C12302, PromoCell), cultured at 37^0C, 5% CO_2, and in humidified atmosphere were used for both 2D and 3D models. For both cell culture systems, four cell constructs were developed: ADSC and NHDF monocultures; and

co-cultures with 80% ADSC and 20% NHDF (A80N20) and inverted ratio with 20% ADSC and 80% NHDF (A20N80). A 1:1 media mix was used for co-culture conditions (FGM-2 Bullet Kit, CC-3132, Lonza and ADSC™ Growth Medium, PT-4505, Lonza).

For 2D culture constructs, $5x10^3$ cells per well (final number) were seeded in either 96-well flat bottom plates (3904, Costar) or 24-well plates (CLS3527-100EA; Sigma-Aldrich) and incubated 24h. All conditions were treated for 30 seconds with PVI and CHX and 24h with Ag, followed by 2 washes with PBS. After treatment, fresh media was added and incubated for 3 days prior to ELISA sample collection.

Spheroids were generated using 96 ultra-low adherence plates (650979, Greiner), 1% Matrigel (356237, Corning), as previously described by Ivascu and Kubbies (13). For each cell condition $5x10^3$ cells/well were used, followed by 3 days incubation to allow spheroid formation. The spheroids were treated with antiseptics for 30 minutes with PVI and CHX and 72h with Ag, washed two times with PBS and incubated in fresh media for 5 days prior to sample collection.

Enzyme-linked immunosorbent assay (ELISA)

TGFβ isoforms levels were assessed using sandwich ELISA kits (TGFβ1 - ab100647, Abcam; TGFβ3 - LS-F2825, LSBio). Assays were performed according to supplier protocols, in duplicates. Plate reader SpectraMax i3 (Molecular Devices) was used to obtain absorbance values.

Statistical analysis

Data collection was performed using Excel. Absorbance values were used to generate standard curve interpolation in GraphPad to calculate cytokine levels. The results were used to calculate TGFβ3 to TGFβ1 ratio. Graphs and statistical analysis were performed with GraphPad software and one-way ANOVA with multiple comparation analysis (Sidak's multiple comparation test).

RESULTS

TGFβ3/TGFβ1 ratio pattern varies for untreated conditions, with cell type, cell ratio and culture system. When compared the untreated cell conditions in 2D culture systems, TGFβ3/TGFβ1 ratio increases as stem cells are introduced in the cultures conditions, with a statistically significant difference ($p < 0.015$, R^2 0.908) between fibroblasts and stem cell monocultures (fig. 1A). In 3D culture systems there are ratio shifts, but with no statistical significance. The ratio is increased for fibroblasts condition compared with stem cell monoculture (fig. 1B). Moreover, when stem cells are present, the ratio pattern is similar with 2D culture systems, increasing with the stem cell ratio (20% < 80% < 100% stem cells).

In 2D cultures after PVI treatment, TGFβ3/TGFβ1 ratio increases significantly for all cell conditions ($p < 0.0001$, R^2 0.9900) and the pattern variations follows the stem cell ratio. For stem cell monocultures, TGFβ3/TGFβ1 ratio increases significantly ($p < 0.0001$, R^2 0.9900) following treatment with all three antiseptics. For A80N20 the ratio increases also following CHX and Ag treatment, but with no significant difference for Ag; compared with A20N20 when following both CHX and Ag treatment, TGFβ3/TGFβ1 ratio shifts are minimal. The ratio increases also in fibroblast monoculture after exposure to CHX ($p < 0.0001$, R^2 0.9900). Moreover, when the response to the three antiseptics are

compared between cell culture conditions, the ratio increase is significant between PVI and both CHX and Ag ($p < 0.0001$, R^2 0.9900) (fig. 2A).

In 3D cultures, TGFβ3/TGFβ1 ratio variations following antiseptic treatment are less compared with 2D conditions. TGFβ3/TGFβ1 ratio increases significantly following PVI treatment only for stem cell cultures and A20N80 condition; and following Ag exposure for co-culture conditions ($p < 0.0087$, R^2 0.7668). Moreover, there was no significant differences between antiseptics (PVI ~ CHX ~ Ag) (fig. 2B).

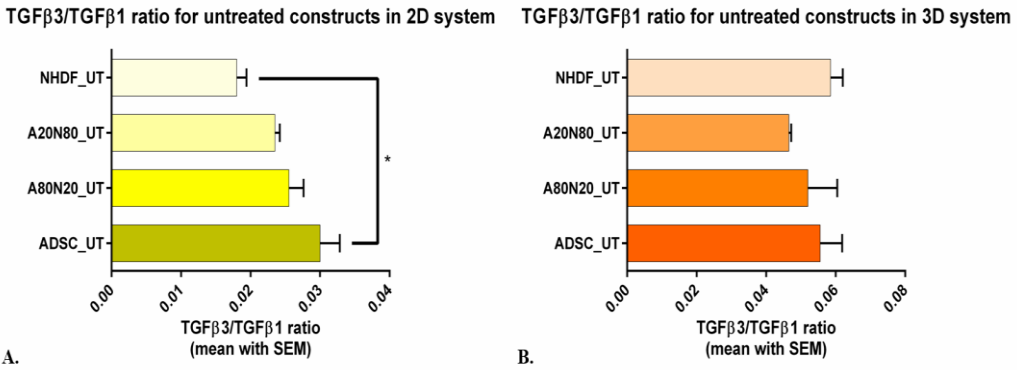

Fig. 1. TGFβ3/TGFβ1 ratio for untreated cell constructs: A. 2D system – Ratio increases significantly for ADSC monocultures compared with NHDF (*$p < 0.015$, R^2 0.908; CI 95%); B. 3D system – TGFβ3/TGFβ1 increasing pattern proportional with stem cell ratio.

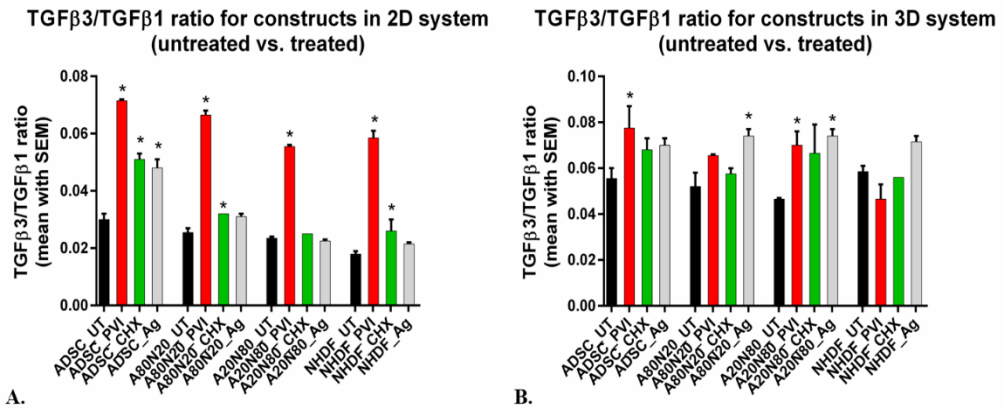

Fig. 2. TGFβ3/TGFβ1 ratio following treatment: A. Constructs in 2D system – The ratio increases significantly following PVI treatment for all cell condition and the variation pattern follows the stem cell ratio. TGFβ3/TGFβ1 ratio is significant following CHX treatment in monocultures and A80N20 condition (*$p < 0.0001$, R^2 0.9900; CI 95%); B. Constructs in 3D system – TGFβ3/TGFβ1 ratio shifts: significant differences following PVI for ADCS and A20N80; following Ag only for co-cultures. (*$p < 0.0001$, R^2 0.9900; CI 95%).

When TGFβ3/TGFβ1 ratio is compared for the same untreated cell condition in 2D culture system vs. 3D culture system, it is increased significantly in 3D cultures. For stem cell monocultures, although the ratio increases following treatment more in 3D cultures than in 2D cultures, it is significant only following Ag exposure ($p < 0.001$, R^2 0.914) (Fig. 3A). Following CHX and Ag treatment, TGFβ3/TGFβ1 ratio increases for fibroblasts ($p < 0.0001$, R^2 0.9731) and co-cultures ($p < 0.0001$, R^2 0.9771 for A80N20 and $p < 0.0002$, R^2 0.9429 for A20N80) more in 3D cultures (figs. 3C-D). Following PVI treatment, in all cell conditions the TGFβ3/TGFβ1 ratio shifts are minimal, with no statistical significance between the two culture systems.

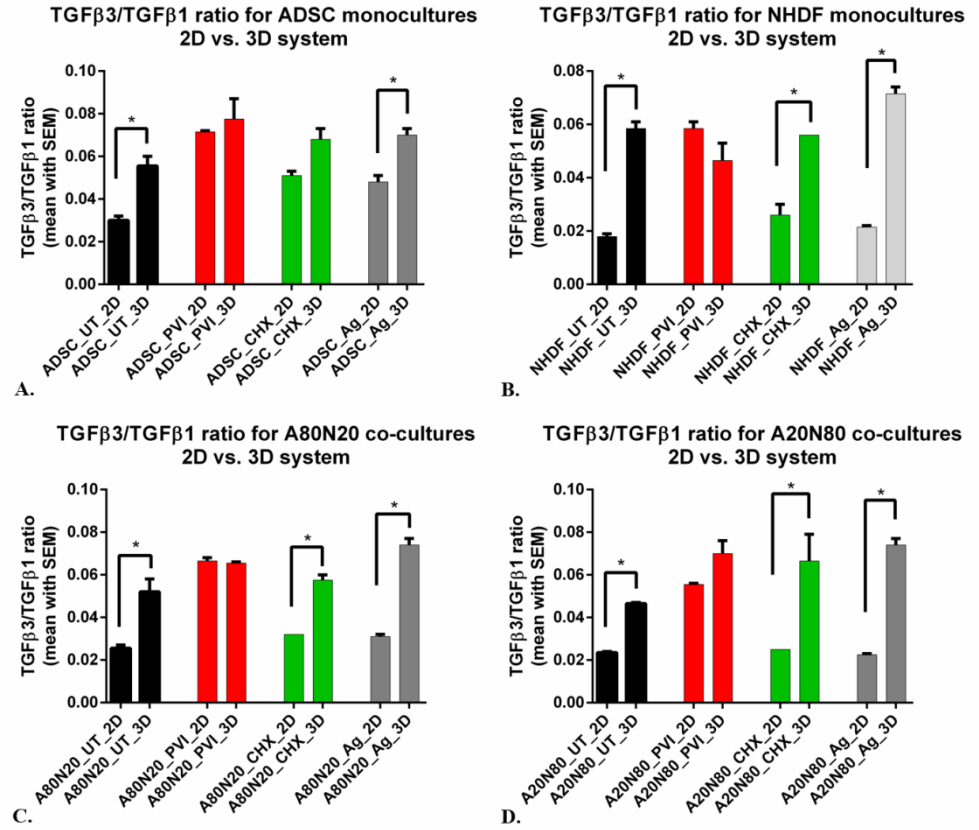

Fig. 3. TGFβ3/TGFβ1 ratio comparison between 2D and 3D culture systems: A. ADSC monocultures - Ratio is more increased for untreated and following Ag exposure in 3D culture system (*$p < 0.001$, R^2 0.914; CI 95%); B. A80N20 co-cultures – TGFβ3/TGFβ1 ratio increases significantly following CHX and Ag treatment for 3D culture conditions (*$p < 0.0001$, R^2 0.9771; CI 95%); C. A20N80 co-cultures – TGFβ3/TGFβ1 ratio has similar pattern as in A80N20 condition, with differences for CHX and Ag treatment in 3D cultures (*$p < 0.0002$, R^2 0.9429; CI 95%); D. NHDF monocultures – As in co-cultures, TGFβ3/TGFβ1 ratio is more increased for 3D culture systems following CHX and Ag treatment (*$p < 0.0001$, R^2 0.9731; CI 95%)

DISCUSSION

Currently there are no global guidelines in regards with antiseptics and wound care, although chronic wounds and burns can be clinically challenging (1, 6, 14). Critical colonization and wound infection impair the healing process, thus aside wound healing promotion, obtaining bioburden control is a critical point towards proper wound healing (3). An antiseptic should have an effective antimicrobial activity with a minimal cytotoxicity (1). Some studies suggest that that antiseptics promote healing by the sole control of bacterial load, although there is emerging data that they may promote wound healing by other mechanism as well, including cytokine modulation (4, 7, 15). Current available data regarding antiseptics cytotoxicity and possible drawbacks on wound healing is controversial and heterogeneous. Moreover, there is a lack of standards in evaluation, both regarding research and clinically, that makes it difficult to develop management guidelines (2, 4, 16).

Povidone-iodine is one most used antiseptics, as wound dressings, ointments, or irrigation/cleansing, although the data regarding efficiency and adverse effects is conflicting (1, 6, 15). Wang *et al*. reported that topical application of 0.5% PVI on post excisional full thickness wounds in rats promote αSMA and TGFβ expression and enhanced healing process (7). Murine wound healing is mainly through wound contraction, compared with granulation tissue formation and reepithelization (17). Moreover, it seems that murine fibroblasts tolerate better PVI compared with human fibroblasts (12). Fumal *et al*. compared topical PVI with CHX and silver sulfadiazine in chronic leg ulcers management and reported that PVI increased the healing rate and did not significantly affect fibroblast (14). Regarding PVI effects on ADSC, there are limited reports. Kim *et al*. evaluated PVI effects on freshly isolated ADSC and they reported that PVI decreased proliferation and differentiation rates, promote necrosis and down regulated stem cell markers (18). The results presented suggest that PVI may modulate cytokines expression, by increasing TGFβ3/TGFβ1 ratio in both 2D and 3D cell culture systems. Although, the data also reveals that this is dependent also on cell types and construct conditions.

Although, alcohol-based chlorhexidine is superior to PVI for preoperative cleansing, data regarding CHX effects on wound healing is limited. It is an antiseptic used for wound irrigation, mainly burns and oral cavity (6, 19, 20). Park *et al*. reported that CHX decreases viability of cultured stem cells isolated from buccal fat pad (21). Pilloni *et al*. evaluated CHX effects on human gingival tissue and concluded that it promotes a "scar wound healing response" by increasing αSMA expression (20). Herein, following CHX treatment TGFβ3/TGFβ1 ratio increased, although with statistical significance difference only in some cell conditions cultured in 2D systems.

Silver nanoparticles bring new and particular properties, including through anti-inflammatory effects, decreasing cytokine release and wound healing efficacy (4). Mainly used in chronic wound and burn management, the available data in regards with Ag is diverse (1, 2). Lin *et al*. obtained better healing with faster wound reduction when using Ag, compared with gauze (3). Tian *et al*. evaluated Ag effects on a thermal injury model in rats and reported that TGFβ1 had a peak at day 3 following injury, followed by a decreased and maintained low level in the wound area treated with silver nanoparticles. Moreover, they suggested a better cosmetic outcome (8). Herein, the effect of Ag treatment was

more pronounced on 3D culture conditions, increasing significantly TGFβ3/TGFβ1 ratio on co-cultures, suggesting that silver nanoparticles within the dressing modulate TGFβ levels. Nešporová *et al*. shown that wound dressings containing silver display toxicity on ex vivo skin by decreasing viability, inducing oxidative stress and DNA damage (22). Also, regarding Ag effects on stem cells, current data is limited. Hackenberg *et al*. concluded that silver nanoparticles display a cyto- and genotoxic on human mesenchymal stem cells in higher concentrations than levels needed for antimicrobial efficiency (11).

Ex utero healing process implies scar tissue formation; thus, efforts are made towards therapies that improve scars based on fetal healing. TGFβ cytokines are known as key players in wound healing process and as TGFβ1 is associated with scar tissue formation and fibrosis, TGFβ3 has an anti-scaring effect (9, 10). Tyrone *et al*. improved and accelerated fascial wound healing using TGFβ3 delivered in the wound using a methylcellulose vehicle (23). In the present study, although further research is required, the results suggest that antiseptics may have an effect including on scar tissue formation, by modulating the TGFβ3/TGFβ1 ratio.

Furthermore, although 2D monolayer cultures are widely used, cost-efficient and simple, 3D cultures provide a cell microenvironment closer to in vivo setting by adding the architectural cell arrangement. Thus, in 3D culture settings, cells display a phenotype closer to in vivo cellular responses (24). Herein, regarding TGFβ3/TGFβ1 ratio, there are differences on cytokine levels between 2D and 3D culture systems, for both treated and untreated conditions.

CONCLUSIONS

TGFβ3/TGFβ1 ratio pattern modulation *in vitro* is dependent on antiseptic, cell type, cell ratio and culture system. Although the data suggests that antiseptics may improve scar tissue formation, further research is required. Moreover, based on the differences displayed between cell types and cell culture conditions and diverse current published data, results are difficult to translate in clinical settings. Thus, standardized methods for antiseptic testing and evaluation should be developed to facilitate data extrapolation into clinical wound management guidelines.

CONFLICT OF INTEREST AND FUNDING

The authors declare there is no conflict of interest.

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PLASTIC SURGICAL TECHNIQUES FOR THE TREATMENT OF BURN ABNORMAL SCARS IN PEDIATRIC PATIENTS

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PLASTIC SURGICAL TECHNIQUES FOR THE TREATMENT OF BURN ABNORMAL SCARS IN PEDIATRIC PATIENTS (Abstract): Post-burn scars have a major impact on the quality of life of pediatric patients, both from a psychological and functional point of view. Pediatric patients with post-burn scars usually have difficulties integrating into the collective due to the low self-esteem determined by the poor aesthetic outcome of the deep or large burns. From a functional standpoint, burn contractures are a significant cause of disability in burn survivors worldwide, as they can affect the tendons and muscles or impair joint mobility. The statistical information that results from this study plays a role in improving the therapeutic protocol. The reconstruction methods of post-burn abnormal scars can be used in order to prevent or to treat the psychological consequences and the functional disability of the affected segment. **Material and methods:** The study includes a group of 147 patients aged between 1-24 years, male and female, admitted through the emergency or outpatient service in the Department of Plastic Surgery and Reconstructive Microsurgery in the "Sf. Maria" Emergency Children Hospital Iasi. **Results:** From the total number of pediatric patients with post-burn scarring sequelae (n=147), 32.65% (n=48) were treated by local triangular skin flaps plasty, 18.36% (n=27) by full-thickness skin graft plasty, 12.92% (n=19) by scar excision and suture, 11.56% (n=17) by tissue expander implant, 7.48% (n=11) by employing the Z-plasty technique, 5.44% (n=8) by associating a local triangular skin flap with a full-thickness skin graft, 5.44% (n=8) by combining Z-plasty with a full-thickness skin graft, 2.04% (n=3) benefited from plasty with synthetic dermis (Integra®), 1.36% (n=2) were treated by degranulation, 1.36% (n=2) by Triamcinolone injection, less than 1% (n=1) of patients received dermabrasion or hair transplantation. **Conclusions:** Skin flaps represent the most effective and frequently utilized method of treatment of post-burn scar sequelae. **Keywords:** BURNS, HYPERTROPHIC SCARS, KELOID SCARS, SKIN FLAPS, SKIN GRAFTS, EXPANDER, Z-PLASTY.

Burns represent one of the most frequent causes of accidental injuries and are a life-threatening and potentially disabling pathology with major psychological and social implications.

The essential therapeutic objectives are

the survival of the patient, prevention of complications of the acute phase, preservation of the functions of the affected segments, an acceptable cosmetic result, and limitation of psychological trauma (1). Approximately 90% of burns in pediatric patients occur at home, while in adolescents there is a higher rate of occurrence outside of the home (2). An accurate assessment of the extent of burns is critical because resuscitation efforts are based on the affected body surface area. Overestimating or underestimating the surface area of burns can lead to therapeutic failure. Computer applications such as the SAGE chart can provide more accurate assessments (3).

There are multiple methods used for treating burn injuries: skin grafting combined with negative pressure therapy, skin flaps, tissue expanders, artificial dermis, and stem cell therapies. Management practices regarding burn wound healing include initial wound assessment, debridement, dressing, and reconstruction with primary or secondary closure, skin grafts or flaps (4, 5). Skin grafts are divided into 4 categories: autograft, allograft, xenograft, and artificial skin (6). Skin flaps differ from skin grafts by their mobilization along with preserving the vascular source (5). A flap may comprise skin, skin and muscle, muscle and bone, or various combinations of tissues. Flap techniques are based on the local angiosome vascular anatomy (7). Tissue expander reconstruction was originally used for breast reconstruction, but later found utility in the reconstruction of excisional defects resulting from a variety of causes, including postburn surgery. The tissue expander is a silicone balloon with an injection port that allows the injection of a saline solution that will increase its volume and consequently expand the healthy adjacent tissue (8). Compared to other techniques, such as skin grafting or flap transfers, tissue expansion allows a better match of skin color and texture, in addition, to preserving hair follicles and sensory nerves (9, 10). In terms of artificial dermis utilized in post-burn sequelae treatment, there are several types such as Integra®, Matriderm®, or Pelnac® (11, 12, 13).

The aim of this study is to highlight the modern methods of treatment of post-burn sequelae and to improve therapeutic conduct to prevent psychological trauma and functional impotence of the affected segment.

MATERIAL AND METHODS

The study includes a group of 147 patients aged between 1-24 years, male and female, admitted through the emergency or ambulatory service in the Department of Plastic Surgery and Reconstructive Microsurgery in the "Sf. Maria" Emergency Children Hospital from Iași, between the years 2010-2020, most of which were admitted repeatedly. Data was collected from observation charts from the hospital's archives and the patient's attending physicians. The collected data comprised of date of birth, age, sex, living conditions, history of burns, and surgical therapeutic plan to which was added information from the observation protocols for patients during the hospital stay and periodical surgeries that were performed. Also, the study was carried out respecting ethical norms, by protecting the confidentiality of the information and the anonymization of the patients in the database. All patients benefited from paraclinical explorations consisting of collecting the usual analyses: complete blood count, inflammatory markers (ESR,

CRP), urea, creatinine, blood glucose, transaminases, exploration of hydro electrolytic and acid-base metabolism, urine summary, coagulation tests, blood group, Rh and imaging studies such as lung X-ray to identify the presence of disorders that may delay surgical intervention and their correction. Moreover, in the morning before surgery, an intensive care physician performed a full pre-anesthetic check-up to assess the anesthetic-surgical risk.

RESULTS

The incidence of hypertrophic scars at 86% (n=127) is six times higher compared to that of keloid scars at 14% (n=20) (fig. 1).

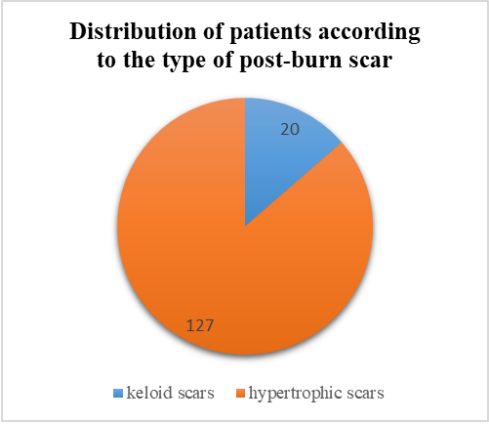

Fig. 1. Distribution of patients according to the type of post-burn scar

From the total number of pediatric patients with post-burn scarring sequelae (n=147), 32.65% (n=48) were treated by local triangular skin flaps plasty, 18.36% (n=27) by full-thickness skin graft plasty, 12.92% (n=19) by scar excision and suture, 11.56% (n=17) by tissue expander implant, 7.48% (n=11) by employing the Z-plasty technique, 5.44% (n=8) by associating a local triangular skin flap with a full-thickness skin graft, 5.44% (n=8) by combining Z-plasty with a full-thickness skin graft, 2.04% (n=3) benefited from plasty with synthetic dermis (Integra®), 1.36% (n=2) were treated by degranulation, 1.36% (n=2) by Triamcinolone injection, less than 1% (n=1) of patients received dermabrasion or hair transplantation (tab. I).

TABLE I

Distribution of patients according to the surgical intervention of postburn scar sequelae

Type of surgery	Cases
Dermabrasion	1
Hair transplant	1
Degranulation	2
Triamcinolone injection	2
Synthetic dermis	3
Combination of skin flap and skin graft	8
Combination of Z-plasty and skin graft	8
Z-plasty	11
Expander implantation	17
Scar excision	19
Skin graft	27
Triangular skin flap	48

In fig. 2, we note that 45% (n=9) of the patients diagnosed with keloid scars benefited from local triangular skin flap plasty. This indicates that this method of treatment for post-burn keloid scars is both commonly used and very effective. And even though the patient group is scant and poses low statistical significance, it still confirms the hypothesis that skin flaps are the most used method of reconstruction of post-burn scar sequelae. Also, 20% (n=4) of these patients were treated by scar excision and suture, 10% (n=2) by full-thickness skin graft plasty, another 10% (n=2) by tissue expander implantation, 5% (n=1) by combining Z-plasty with a full-thickness skin graft.

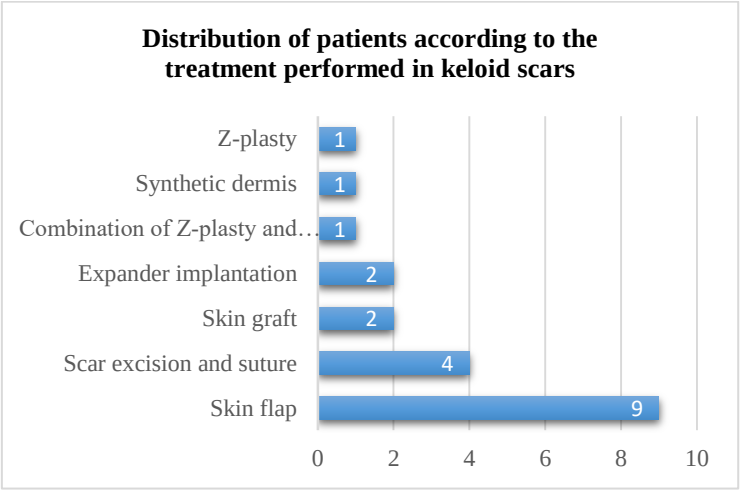

Fig. 2. Distribution of patients according to the treatment performed in keloid scars

The graphic representation in fig. 3, illustrates that 31.49% (n=40) of the patients diagnosed with hypertrophic scars benefited from a local crossed triangular skin flap plasty. This result confirms the previously presented idea: skin flaps are the most used method for the reconstruction of post-burn scar sequelae because they maintain their vascular source with mobilization, unlike skin grafts that are avascular and require a well-vascularized wound bed.

In figures 4 and 5, the pictures describes a case of post-burn syndactyly at 3/4 fingers and a case with retractable hypertrophic post-burned cervical scar, both treated with local flaps.

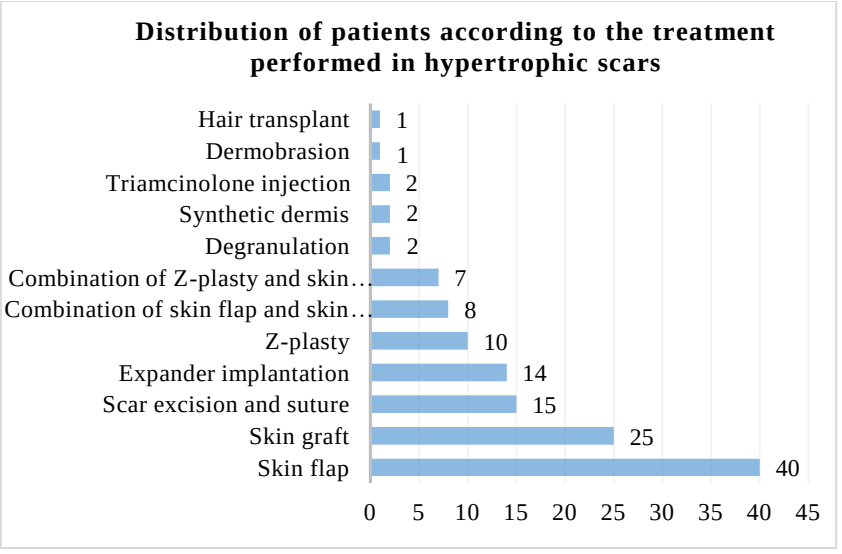

Fig. 3. Distribution of patients according to the treatment performed in hypertrophic scars

Fig. 4. Post-burn syndactyly at 3/4 fingers

Fig. 5. Retractable post-burn hypertrophic scar

Also, 19.68% (n=25) of the patients diagnosed with postburn hypertrophic scars benefited from full-thickness skin graft plasty, 11.81% (n=15) from scar excision and suture, 11.02% (n =14) of implantation with an expander, 7.87% (n=10) of Z-plasty, 6.29% (n=8) from a combination of skin flap with a skin graft, 5.51% (n=7) Z-plasty and skin graft association, under 2% of the patients benefited either from scar degranulation, artificial dermal substitute (Integra®), Triamcinolon injection, dermabrasion or hair transplantation.

It is observed that 45% of patients diagnosed with post-burn keloid scar sequelae benefited from treatment with crossed triangular skin flaps and 10% from skin grafting, as a result, the most common method of skin plasty is flap plasty with a flap:graft ratio of 4.5:1. This difference was found to be statistically significant (p=0.03). Also, 31.5% of patients diagnosed with hypertrophic scar benefited from plasty through crossed triangular skin flaps, as opposed to 19.7% of patients who benefited from plasty with skin graft, with a flap:graft ratio of 1.6:1. This difference was found to be statistically significant (p=0.03).

DISCUSSION

We observe in the results of our study that the most common post-burn scar sequela developed in pediatric patients is the hypertrophic scar with a percentage of 86% (n=127) of the total number of patients included in the group (n=147), followed by the keloid scar with a percentage of 14% (n=20), hypertrophic scar: keloid ratio of 6.35:1. This confirms the involvement of the genetic factor in the pathogenesis of keloid scars, according to the study by Gurtner *et al.* in 2018 in which he specifies that keloid scars occur more frequently in pigmented individuals and in African populations. These types of sequelae produce patients with functional impotence if the burn is located at or near a joint. Atrophic scars are not present in our group of patients because this type of scar occurs more frequently after a trauma, and less often due to post-burn (7).

Since the intention of this study is to discover the modern methods of treatment for post-burn scar sequelae in pediatric patients, the initial distribution of patients had to be made according to the treatment

method used. Later, they were divided into two diagnostic groups (post-burn keloid scar sequelae, respectively post-burn hypertrophic scar sequelae) to find which treatment method was more frequently used depending on the type of scar. Thus, we observed that the most used and effective treatment method in both types of scar is the use of the local triangular crossed skin flap, from which 32.65% of patients (n=48) benefited.

Our results are consistent with the systematic review of free tissue transfers in burns by Shehab Jabir *et al.* in 2014 which included 30 studies conducted between 1984-2012 stating that free flaps can play an essential role in primary reconstruction as well as secondary reconstruction in burn patients. When used in primary reconstruction, they can significantly reduce the length of hospital stay, and the number of operations to achieve wound closure and, in some cases, even save limbs that would otherwise have to be amputated. When used in secondary reconstruction, they may be the only way to provide the necessary amounts of tissue to achieve sufficient release of the contracture, close chronic wounds, and treat unstable scars. This translates into reduced morbidity and improved functionality and aesthetics for patients (14).

Also, in our group of patients, 18.36% (n=27) benefited from a full-thickness skin graft. This type of plasty is most frequently used in acute skin burns with defects spanning a large surface area. There is no evidence that full-thickness skin grafting is clearly superior to split skin grafting for covering palmar burns according to the meta-analysis by Theddeus *et al.* in 2015. The current trend is to use FTSG as a wound dressing for hand burns in children because it is believed to have a lower rate of secondary contracture and it could decrease the need for the secondary reconstructive procedure; however, FTSG has some limitations in its use. FTSG has limited donor sources, so it cannot be used to cover large burn areas. It also has a lower absorption rate compared to STSG due to edema and inflammation reactions. STSG also has some disadvantages, such as a higher rate of secondary contracture and a relatively lower aesthetic value. However, using STSG is advantageous in that it can cover a large area (15). It can also be combined with artificial dermis like Integra or Matriderm for a better functional result (16, 17, 18). The scientific literature states the existence of 3 methods of fixing the skin graft: stapler, cyanoacrylate adhesive, and tie-over dressing. The tie-over dressing is the traditional technique that no longer always provides satisfactory results in some areas of the body (19). Pediatric patients with post-burn cicatricial sequelae in which skin grafting was performed as a method of healing also benefited from VAC treatment because an improvement in the healing rate and a decrease in the wound infection rate was observed. This result is consistent with the randomized controlled trial conducted by Zheng XP *et al.* in 2019 in the Department of Burn Surgery of the First Affiliated Hospital of the University of Naval Medicine (20).

According to our results, 11.56% (n=17) of the patients benefited from tissue expansion with the help of an expander. Unlike other treatment techniques, such as skin grafting or the use of skin flaps, tissue expansion allows for a better match of skin color and texture (8, 21). The number of patients who have benefited from this method of skin plasty is reduced because following the presentation to the parents by

the attending physician of the available skin plasty methods, respectively their advantages and disadvantages, the parents were much more reluctant to choose the skin expander as a treatment method.

The conservative methods to prevent abnormal scarring, the long-term follow-up of the scars along with intense kinesitherapy are extremely important (22, 23). There is a possibility to treat the burn wounds in the acute phase and as well in the late reconstruction with skin substitutes, along with intermittent fat grafting of the scar (11, 24, 25).

CONCLUSIONS

Burns are a serious health problem, especially in low-income countries. They cause many deaths each year and are the leading cause of disability in many countries. Post-combustion contractures on extremities present a clinical challenge. Flexion contractures are more common than extension contractures. In mild contractures (defined simply as more than 50% of the range of motion of the joint is still possible), plastic surgical techniques, for example, the Z-plasty technique can be used to elongate the scar by transposition of the adjacent normal tissue. In more severe cases, there is not enough adjacent tissue to allow contracture elongation and a different surgical approach is required. The most used and effective method of treatment in both types of post-burn scar sequelae is the use of the skin flap. This method is followed by scar excision and suture in the case of keloid scars, respectively by full-thickness skin graft plasty used in the case of hypertrophic scars.

Compared to skin grafts, flaps have the ability to expand to allow full extension of the affected region. Flaps are essential reconstructive modalities frequently used by plastic surgeons. As techniques evolve, many varieties of tissue transfer appear. A thorough understanding of the underlying principles, the ability to apply them to specific situations, and careful assessment of patient needs are necessary for the successful use of this tool. Recent advances in burn reconstruction offer methods to reduce scar tissue and joint contractures.

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EPIDEMIOLOGY OF CHRONIC KIDNEY DISEASE AND COMORBID ILLNESS IN HOSPITALIZED GERIATRIC PATIENTS

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EPIDEMIOLOGY OF CHRONIC KIDNEY DISEASE AND COMORBID ILLNESS IN HOSPITALIZED GERIATRIC PATIENTS (Abstract): **Background and aims**: The number of older people in Europe is expanding. Although chronic kidney disease (CKD) becomes more common with increasing age, data concerning epidemiology of CKD in older people are scarce, especially in Eastern Europe countries. In the current analysis, we aimed to fill the gap on CKD epidemiology in older people from Romania, by describing a large population of hospitalized geriatric patients. **Material and methods:** In this retrospective cohort study we included all patients aged ≥ 65 years hospitalized in the Geriatric Department of "Dr. C. I. Parhon" Hospital from Iași, Romania, between 2012 – 2022. CKD was identified as decreased estimated glomerular filtration rate (<60 mL/min/1.73 m^2) according to CKD-EPI equation. Investigated comorbid conditions included diabetes mellitus, hypertension/hypertensive heart disease, coronary artery disease, congestive heart failure, atrial fibrillation, chronic respiratory failure and anemia. **Results**: 3,563 geriatric patients (59.2% females) were hospitalized between 2012-2022. 3116 patients (87.4% of the total population) had a serum creatinine assay. Among these patients, the prevalence of CKD using the CKD-EPI equation was 38%. We found an unequal annually distribution between the number of patients with a diagnosis of CKD according to electronic health records and the number of patients with estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2. 86,7% of the patients with eGFR < 60 mL/min/1.73 m^2 had at least 2 comorbidities. Only 2,4% of the patients with eGFR < 60 mL/min had no other chronic condition. **Conclusions:** In Romanian older hospitalized patients, the prevalence of individuals with eGFR <60 mL/min/1.73 m2 is higher than previous reports from our country. In geriatric adults, CKD is not an isolated entity, and the burden of cardiovascular comorbid illness is high. **Keywords**: EPIDEMIOLOGY, CHRONIC KIDNEY DISEASE, COMORBID ILLNESS, HOSPITALIZED GERIATRIC PATIENTS.

The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines define chronic kidney disease (CKD) as abnormalities of kidney structure or function, present for > 3 months, with implications for health (1). The classification of CKD requires the determination of the cause of CKD, glomerular filtration rate (GFR) category (G1-G5) and albuminuria category (A1-A3). By standardizing the definition and staging of CKD, data on CKD burden have grown. Globally, in 2017, there were 697.5 million

cases of all-stage CKD, for a global prevalence of 9.1 % (2). However, based on a meta-analysis of 100 studies (roughly 7 million patients), the global CKD prevalence could be as high as 13.4 %, with a prevalence of CKD stages G3 to G5 of 10.6 % (3). In Europe, estimates of CKD prevalence indicate a regional variation across 27 countries that ranges from 3.31% in Norway to 17.3 % in Germany (4). Regional disparities in CKD burden arise from differences in geographic area and ethnicity, issues related to GFR estimation (e.g., type of equation used) ore one-off testing assessments in large-scale reports.

Although the growing CKD burden parallels the expanding proportion of older individuals, data concerning epidemiology of CKD in older people are scarce, especially in Eastern Europe countries. These patients have multiple chronic conditions that need to be correctly quantified for guiding risk stratification and subsequent management. Previously, European Renal Best Practice (ERBP) guidelines shed some light on management of older patients with advanced CKD, by stimulating implementation of validated tools for predicting the most important risks, death and progression to end-stage renal disease (ESRD). When comparing the above - mentioned risks, it is important to note that in older people with CKD, death is a more likely event than progression to ESRD, even at severe reductions in GFR (5).

The starting point for an effective strategy of screening for CKD consists of increasing awareness of the true burden of the disease and utilization of simple laboratory tests (serum creatinine to estimate GFR and proteinuria) to identify CKD in people at risk. A systematic review suggested that screening for CKD is cost effective in people with diabetes and hypertension, the two most common causes of CKD worldwide (6). From a practical point of view, early identification of CKD in people at-risk is beneficial in the primary care settings if the programs are associated with treatment strategies. However, there are no current evidence-based recommendations regarding the frequency of screening in people at risk of CKD.

In the current analysis, we aimed to fill the gap on CKD epidemiology in older people from Romania, by describing a large population of hospitalized geriatric patients. We found only two other analysis (7, 8) published in that last twelve years that investigated the prevalence of CKD in the adult population of Romania, none with focus on geriatric patients.

MATERIAL AND METHODS

In this retrospective cohort study, we included all patients aged ≥ 65 years hospitalized in the Geriatric Department of "Dr. C. I. Parhon" Hospital from Iasi, Romania, between 2012 - 2022.

This Geriatric Unit covers the entire North-East region of the country, so patients are representative for a population of almost 4 million people. We selected for our analysis those in which serum creatinine was performed. In this population, we calculated the estimated glomerular filtration rate (eGFR) by using CKD-EPI equation. Based on the KDIGO definition and classification of CKD (1), we first divided our population into "no CKD" (defined as eGFR > 60 mL/min/1.73 m2) and "CKD" (eGFR < 60 mL/min/1.73 m^2). The CKD group was further classified based on GFR category in G3a (45-59 mL/min/1.73 m^2),

G3b (30-44 mL/min/1.73 m^2), G4 (15-29 mL/min/1.73 m2) and G5 (<15 mL/min/1.73 m^2). Investigated comorbid conditions included diabetes mellitus, hypertension/hypertensive heart disease, coronary artery disease, congestive heart failure, atrial fibrillation, chronic respiratory failure and anemia. We investigated the distribution of these comorbidities in the entire geriatric population and in the CKD group.

RESULTS

In the Geriatric Unit from "Dr. C. I. Parhon" Hospital from Iasi between 2012 and 2022, there were 3,563 patients hospitalized with an age greater than 65 years old at admission - 1,453 (40.8%) male, 2,110 (59.2%) female.

Out of the total patients > 65 years old (3,563), only 3116 patients had at least one serum creatinine value determined between 2012 and 2022 (a total of 7954 values determined). Taking into account the first creatinine value corresponding to an estimated GFR of < 60 mL/min/1.73 m^2 (CKD-EPI), there were 1,187 individual patients with an eGFR <60 mL/min/1.73 m^2, corresponding to a prevalence of 38%. Mean age of patients with eGFR < 60 mL/min/1.73 m^2 was 79 years and gender distribution was unequal (63.4% females).

We explored the distribution of the primary or secondary diagnosis of Chronic Kidney Disease (N18, N19) according to International Classification of Diseases (ICD 10) in the hospital records. The difference in numbers between patients with eGFR < 60 mL/min and patients with CKD (ICD 10) as primary or secondary diagnosis is shown in first figure.

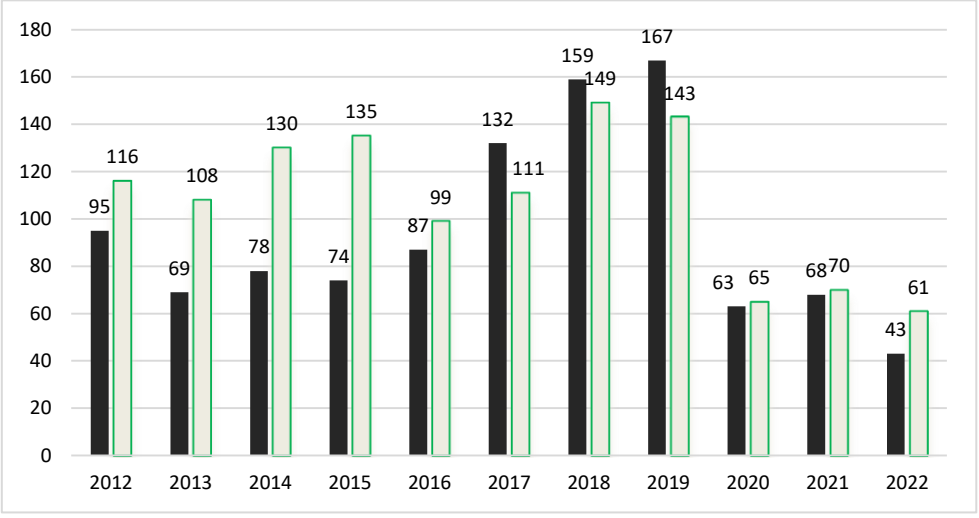

Fig. 1. Number of patients with eGFR < 60 mL/min (white) and patients with CKD diagnosis (ICD 10) (black), between 2012-2022.

Out of the 3,116 patients with at least one serum creatinine assessment, 1,655 patients had 2 or more creatinine values during different hospitalizations. Taking into account the first hospitalization of each patient during the 10-year interval, we found that 758 patients

had an eGFR < 60 mL/min of first assessment in the Geriatric Unit. The distribution of eGFR according to CKD-EPI for the first admission is described in first table.

TABLE I.
The distribution of eGFR according to CKD-EPI for the first admission

eGFR	Women	Men	Total
>90 mL/min	359	382	741
60-89 mL/min	1035	582	1617
45-59 mL/min	284	180	464
30-44 mL/min	163	71	234
15-29 mL/min	35	19	54
<15 mL/min	6		6
Total	**1,882**	**1,234**	**3,116**

According to ICD 10 diagnosis, in patients with eGFR < 60 mL/min, the most frequent comorbidity was heart failure (28.9%), followed by hypertension (20.7%), ischemic heart disease (15.2%), diabetes (10.5%), chronic respiratory disease (7.5%), atrial fibrillation (5.3%) and anemia (6.3%) (tab. II).

The distribution of patients with eGFR < 60 mL/min stratified by the number of comorbidities showed that 86.7% had ≥ 2 comorbidities. The largest CKD subgroup was the one that associated 3 comorbidities (315 patients, 31.5%), followed by two subgroups that had 2 comorbidities (249 patients, 24.9%), respectively 4 comorbidities (189 patients, 18.9%). Only a minority of patients with eGFR < 60 mL/min (24 adults, 2.4%) had no other chronic condition (tab. III).

TABLE II.
Distribution of main comorbidities according to eGFR

Comorbidities	eGFR>=60	eGFR<60	All patients (n= 3,116)
Heart failure	29.60%	28.90%	29.38%
Hypertension	21.10%	20.70%	21.00%
Ischemic heart disease	16.90%	15.20%	16.46%
Diabetes mellitus	8.30%	10.50%	8.90%
Chronic respiratory disease	8.30%	7.50%	8.12%
Atrial fibrillation	5.30%	5.30%	5.30%
Anemia	4%	6.30%	4.64%
Other comorbidities	6.40%	5.60%	6.21%

TABLE III.
The number of concurrent comorbidities in patients with eGFR < 60 mL/min.

Number of concurrent comorbidities	Percent of patients with eGFR < 60
0	2.40%
1	10.90%
2	24.90%
3	31.50%
4	18.90%
5	8.00%
6	2.90%
7	0.30%
8	0.10%

DISCUSSION

The analysis of this representative sample of the Romanian older hospitalized patients shows that the prevalence of eGFR <60 mL/min/1.73 m^2 is high (38 %). The identified prevalence is higher than the one previously reported by Mota *et al.* (8). In that study, researchers analyzed data from 2,717 Romanian adults and found an overall age- and sex-adjusted prevalence of CKD of 6.74%. The prevalence of CKD increased with age from 3.69 % in the 20–39-year age group to 14.35 % in the 60–79-year age group. In the Kidney Early Evaluation Program (KEEP) and National Health and Nutrition Examination Survey (NHANES) in the United States, which included more than 32,000 patients aged at least 65 years, the prevalence of CKD was 44% (9), which is similar to our findings.

In a systematic review of 26 population-based studies, the median prevalence of CKD was 7.2% in people aged ≥ 30 years, but it ranged from 23.4% to 35.8% in people aged ≥ 64 years (10).

An important section of our analysis was the comparison between the number of patients with a diagnosis of CKD according to International Classification of Diseases (ICD 10) and the number of patients with eGFR < 60 mL/min/1.73 m^2, using CKD-EPI equation. This comparison was made for each of the 11 years of the observation period. In the first 4 years of the selected period (2012-2015), there seems to be a greater difference between the CKD diagnosis and patients with eGFR < 60 mL/min/1.73 m^2, with less patients labeled as having CKD (fig. 1). This suggests that there might have been an under-recording of CKD diagnoses in healthcare records. The accuracy of CKD diagnosis using electronic health records (EHR) ultimately depends on the integration and interpretation of data by healthcare providers and the quality of the healthcare system's electronic records.

The difference between the CKD diagnosis and patients with eGFR < 60 mL/min/1.73 m2 is smaller starting from 2016 onward, with a slightly higher number of patients with CKD diagnosis. The number of patients with eGFR < 60

mL/min/1.73 m^2 is constantly increasing until 2019. Since the beginning of the COVID-19 pandemic (2020), both categories of patients are significantly decreased. The main reason could be the significant initial restrictions in the referrals to the Geriatric Unit, followed probably by an increased reluctance for inpatient vs outpatient care in the elderly population.

When examining the number of patients with eGFR < 60 mL/min/1.73 m^2, we can conclude that during the 11-year period, the prevalence has increased from 758/3116 patients (low eGFR on first admission) to 1,187/3,116 patients (taking into account the eGFR progression).

In our study, cardio-vascular pathology was a frequent characteristic of the population, with approximately one third of the patients that have heart failure and one fifth associating hypertension. The extent of the investigated comorbidities was similar across eGFR groups (>/= 60 mL/min *vs.* < 60 mL/min).

We also emphasized that in older adults, CKD is not an isolated entity, which makes the caring for these patients challenging, especially if we add the negative impact of other superimposed geriatric syndromes like frailty and cognitive decline.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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CORRELATION BETWEEN OCT MEASUREMENTS AND OXIDATIVE STRESS BIOMARKERS IN PATIENTS WITH AGE-RELATED MACULAR DEGENERATION

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CORRELATION BETWEEN OCT MEASUREMENTS AND OXIDATIVE STRESS BIOMARKERS IN PATIENTS WITH AGE-RELATED MACULAR DEGENERATION (Abstract): Age-related macular degeneration (AMD) is a neurodegenerative disease that affects the central retina and is the most frequent cause of irreversible blindness in the elderly population. **Aim:** to establish correlations between functional and structural changes and oxidative stress markers before and after treatment in patients diagnosed with AMD. We wanted to estimate the association of the oxidative stress with the occurrence of age-related macular degeneration. **Materials and methods:** This are a case-control study that included a group of 59 patients diagnosed with age related macular degeneration (23 dry AMD and 36 exudative AMD), and a control group that included 62 patients. They were followed over a period of 12 months by assessment of optical coherence tomography measurements and oxidative stress markers. The oxidative stress markers included superoxide-dismutase (SOD) and thiobarbituric acid reactive substances (TBARS). **Results:** The mean values of SOD and TBARS were significantly higher in the study group compared to the control group. We observed higher values in patients with exudative form of AMD. Even after treatment, the mean values remained higher than in controls. The standard linear regression analysis of the mean retinal thickness values for each individual test, confirmed that the connection between SOD, TBARS and mean retinal thickness meets the requirements of a valid linear regression and SOD and TBARS can be considered to be predictors. Conclusions**:** Increased oxidative stress, which causes oxidative damage to lipids and proteins, may lead to irreversible damage in AMD. Investigating the neuroprotection provided by antioxidants against AMD may be helpful to further entrench the significant role of oxidative stress in AMD. **Keywords**: AGE-RELATED MACULAR DEGENERATION, OPTICAL COHERENCE TOMOGRAPHY, OXIDATIVE STRESS MARKERS.

Age-related macular degeneration (AMD) is a neurodegenerative disease that affects the central retina of elderly patients. This results in a progressive loss of vision, being a common cause of visual impairment in the aging population (1). Based on the disease's clinical and pathological features, there are two subtypes of late AMD: atrophic (dry) and neovascular (wet), both of which can lead to significant visual loss.

Despite the severity of the condition, the etiology and pathogenesis of AMD are poorly understood and current treatment options are not satisfactory (2, 3). The most recent studies point to the key role of oxidative stress in the pathogenesis of AMD (4). Excessive generation of free radicals and other reactive oxygen species (ROS) and imbalance between their generation and the possibility of their degradation by the antioxidant defense system seem to be the most significant factors leading to AMD (5, 6).

The aim of this study is to establish correlations between functional and structural changes and oxidative stress markers before and after treatment in patients diagnosed with age-related macular degeneration. It is also meant to highlight the role of blood markers in early diagnosis of oxidative stress and in early identification of retinal lesions. We wanted to determine whether there is a covariation of the average thickness of the retina and the oxidative stress tests performed in the group of AMD patients, that is, to check whether they can be considered predictors of the variation of the average thickness of the retina.

MATERIALS AND METHODS

This is a case-control study that included a group of 59 patients diagnosed with AMD (23 dry AMD and 36 exudative AMD) and a control group that included 62 patients. Patients with diabetes, inflammatory and infectious diseases or vascular occlusions were excluded. Our inclusion criteria were: the presence of dry and exudative AMD, age between 55-86 years old. All subjects completed a questionnaire confirming the following information: age, gender, non-smoker status, non-consumption of supplements, such as vitamins and antioxidants. In accordance with the patients' rights act, they signed an informed consent. Patients were treated with antioxidant medication and intravitreal injections with antiangiogenic factors - anti vascular endothelium growth factor anti-VEGF - bevacizumab. We included patients that did not have any previous intravitreal IVT injections before enrollment. As for protocols, patients underwent one IVT bevacizumab every month for three months, and after that, one IVT every 2 months.

All patients underwent a comprehensive ophthalmic examination. Choroidal neovascularization CNV was diagnosed by slit-lamp biomicroscopic of the fundus and optical coherence tomography OCT. We measured central retinal thickness-from inner limiting membrane ILM to retinal pigment epithelium RPE, using OCT Spectral OPTOPOL, the Cube scan and Raster scan. The control subjects had a fully normal ophthalmic condition. The follow-up period was for a median of 12 months. Here we include the results at 3 months and 12 months of follow-up. As for oxidative stress markers, we studied the reactive substances of thiobarbituric acid TBARS and antioxidant enzyme - superoxide dismutase SOD. Blood harvested on heparin was taken in the morning. TBARS can be quantified by the colorimetric method. SOD activity, one of the main antioxidant systems in the retina, was determined by the xanthine-oxidase method.

The statistical analysis was intended to highlight significant correlations between the structural and functional changes and the findings of the blood tests. We also wanted to assess the effects of our therapy by comparing data collected before and after treatment. The statistical comparison was drawn using the "difference tests" of the sub-set of descriptive statistic programs; the differences which stood at a safe threshold of 95% ($p < 0.05$) were considered significant.

Another goal was to determine whether there is a covariation of the average thickness of the retina and the oxidative stress tests performed in the group of AMD patients, that is, to check whether they can be considered predictors of the variation of the average thickness of the retina. For this, we studied the statistical relationship between the average thickness of the retina and the oxidative stress tests.

RESULTS

We investigated a group of 59 patients with dry and exudative AMD, 29 men and 30 women, aged between 55-86 years old. We included 23 patients with dry AMD and 36 patients with exudative AMD. The study comprised a control groups (CG) that included 62 subjects, ranging from 53 to 80 years old. The study lasted 12 months. Patients with the exudative form were treated with intravitreal injections with anti-VEGF - bevacizumab, systemic antioxidants, while patients with dry AMD were treated with systemic antioxidants and neuroprotectors, alone or in combination, according to customized treatment plans. We compared the retinal thickness measurements and the blood tests findings before and after treatment.

In the group with neovascular AMD, the mean central retinal thickness decreased continuously and significantly during the follow-up of the patients, from above-normal readings of 420.417 µm on the first examination, to 370.139 µm (p = 0.0002) three months after treatment, reaching a mean value close to the normal reading of 351.083 µm one year after treatment (p = 0.088) (fig. 1).

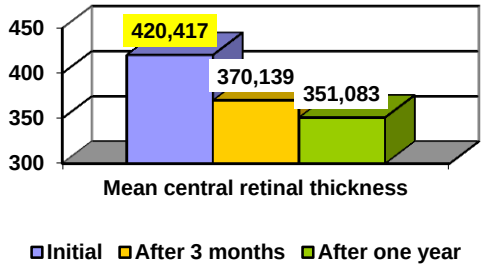

Fig. 1. Evolution of mean central retinal thickness in the neovascular AMD group

A significant reduction in retinal thickness was noted both 3 months and 1 year after treatment. We also noted a decrease in the frequency of edema on the quadrants and on average (fig. 2).

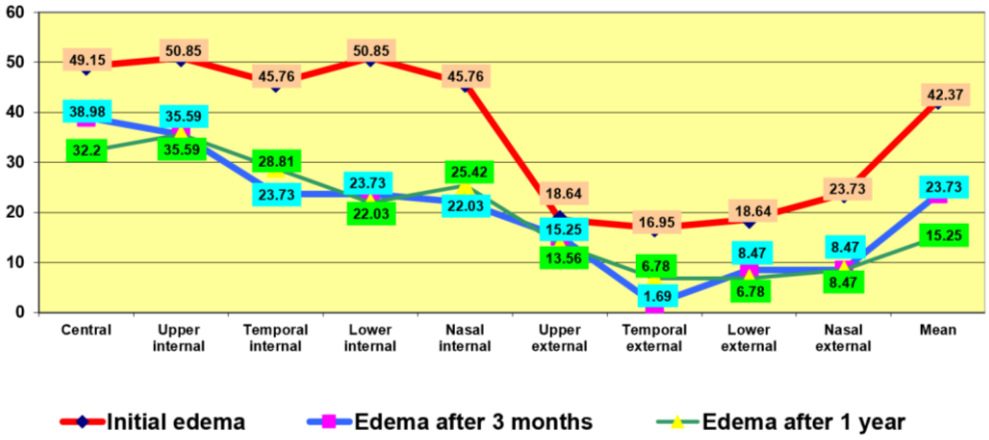

Fig. 2. Frequency of edema before and after treatment

We analyzed the values of SOD and TBARS in patients with dry and wet AMD at baseline and compared them with the control group (tab. I).

TABLE I.

Tests findings in the AMD groups and in the control groups before treatment

Tests	N	Mean	Lowest	Highest	Std. dev.
SOD (U/mL) dry AMD	23	271.91	241.00	296.00	15.46
SOD (U/mL) wet AMD	36	**337.78**	287.00	387.00	25.49
Difference significance (p)	**< 0.00001**				
TBARS (nmols/mL) dry AMD	23	3.95	3.30	4.36	0.26
TBARS (nmols/mL) wet AMD	36	**4.72**	4.13	5.59	0.27
Difference significance (p)	**< 0.00001**				
SOD (CG)	62	**269.55**			13.78
TBARS (CG)	62	**3.91**			0.16

We compared the mean values of SOD and TBARS in patients with dry and exudative AMD, with the mean values of these tests in the control group. We found that the mean value of SOD was significantly higher in patients with exudative form (337.78 U/mL ± 25.49) compared to the dry form (271.91 U/mL ± 15.46, p <0.00001). We also noticed that in patients with exudative AMD the mean value of TBARS was higher (4.72 nmol/mL ± 0.27), compared to the patients with dry AMD (3.95 nmol/mL ± 0.26, p <0.00001). The mean values of SOD and TBARS in the dry form do not differ significantly from those in the control group.

Three months after treatment, the differences of the mean values of the tests were statistically significant. The mean SOD value was significantly higher in exudative AMD patients (319.7 ± 20.8 U/mL) compared to the dry form (261.6 ± 14.5 U/mL, p < 0.00001). Patients with exudative form presented a higher value of TBARS (4.3 ± 0.2 nmol/mL versus 3.7 ± 0.2 nmol/mL in dry form patients, p < 0.00001).

The mean SOD value did no longer show any statistically significant difference one year after treatment. The mean TBARS value was significantly higher in exudative AMD patients (4.2 ± 0.31 nmol/mL), compared with the values in dry AMD (3.69 ± 0.31 nmol/mL, p < 0.00001) (figs. 3 and 4).

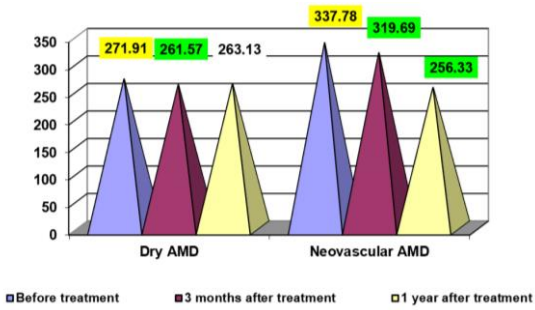

Fig. 3. Mean SOD (U/mL) value

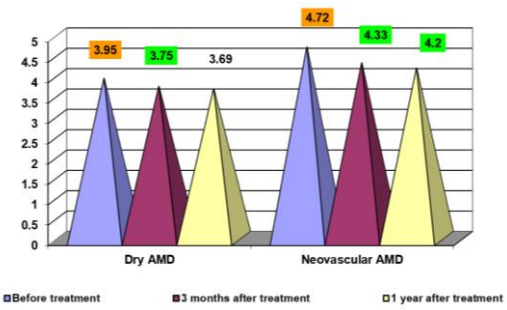

Fig. 4. Mean TBARS (nmols/mL) value

We proceeded to a standard linear regression analysis of the mean retinal thickness values for each individual test (figs. 5 and 6).

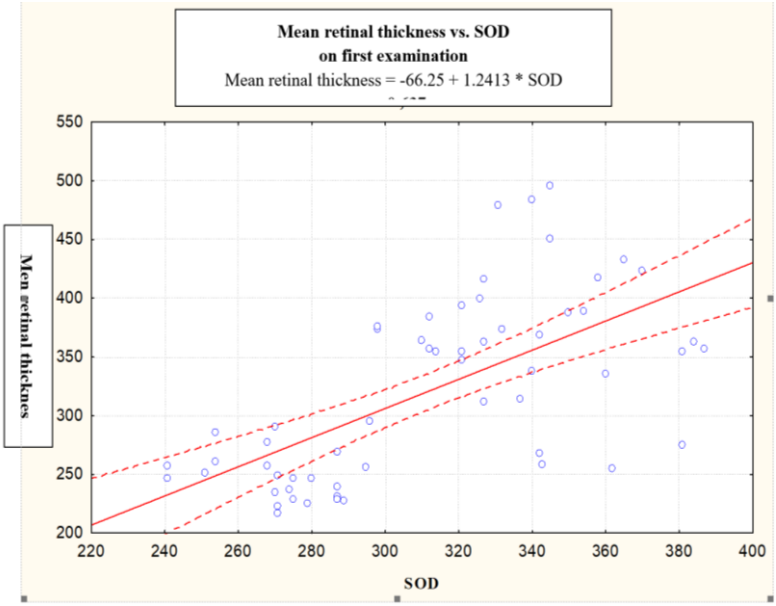

Fig. 5. Regression line of mean retinal thickness in the total group of AMD patients, according to SOD, before treatment

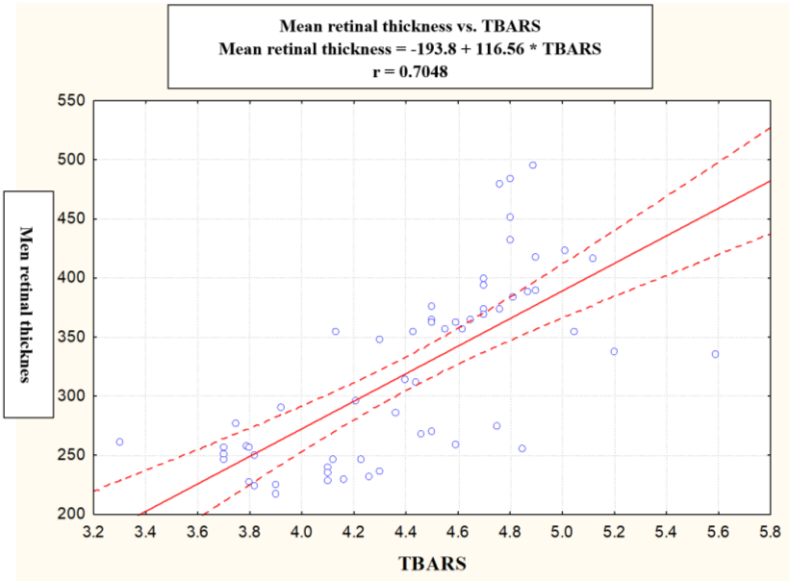

Fig. 6. Regression line of mean retinal thickness in the total group of AMD patients, according to TBARS values, before treatment

The test used to analyze regression is a global significance test of the set of coefficients, *test F* (tabs. II and III).

The fact that the value of the ***F*** test in this case is statistically significant confirms that the relationship between SOD and the mean retinal thickness meets the requirements of a valid linear regression. Therefore, SOD can be considered to be a predictor

TABLE II.

Variance of mean retinal thickness values with SOD values

Effect	Sum of squares	Degrees of freedom	Mean squares	F	P
Regression	**136947.5**	**1**	**136947.5**	**38.92870**	**<0.,0000001**
Residual	200520.7	57	3517.9		
Total	337468.2				

TABLE III.

Variance of mean retinal thickness values with TBARS values

Effect	Sum of squares	Degrees of freedom	Mean squares	F	P
Regression	**167632.1**	**1**	**167632.1**	**56.26029**	**< 0.0000001**
Residual	169836.1	57	2979.6		
Total	337468.2				

As one may notice, before treatment, just like SOD, TBARS may be a mean retinal thickness predictor, perhaps even the best predictor, considering that it has the highest correlation coefficient, r= 0.705.

DISCUSSION

Studies suggested that oxidative stress plays an important role in the pathogenesis of AMD. There is some evidence that oxidative stress and vascular dysfunction may both contribute to the pathology of neovascular AMD (7, 8, 9). Recent studies suggest that the association of oxidative stress, hypoxia and impaired autophagy may be a stimulus for VEGF secretion in RPE cells, thus inducing the appearance of new vessels, the hallmark of exudative AMD (10, 11).

Blood serum from AMD subjects showed increased levels of oxidative stress proven by high malondialdehyde (MDA) levels compared to normal non-AMD cohorts, thus suggesting that systemic oxidative stress is related to AMD (12).

In our research, the mean values of the performed tests did not have a statistically significant difference according to gender in any examination. The mean SOD value decreases significantly three months after treatment in patients with dry AMD - 271.91 U/mL to 261.57 U/mL. Three months after treatment, as well as 12 months after treatment, the mean SOD value is significantly lower in exudative form of AMD (319.69 U/mL and 256.33 U/mL).

According to previous studies, the plasma MDA levels were higher in AMD patients than in controls (13, 14, 15, 16). The more intense SOD activity may be related to higher MDA levels, as a compensatory regulation in response to oxidative stress in AMD patients. SOD lowers the superoxide level, trying to maintain the balance between antioxidants factors and free radicals. Dong *et al.* used a murine model deficient in SOD1 to study how a compromised antioxidant defense system could create a pro-angiogenic environment, and observed that deficient Sod1 mice had a significantly lower expression of VEGF and more neovascularization (17). A systematic review and meta-analysis, involving twelve case-control studies with a total of 634 AMD patients and 656 controls without AMD, concluded that there is some evidence of higher levels of MDA in AMD patients compared to healthy controls (18).

In their murine model designed to study the effects of oxidative stress on retinal tissues, Kim *et al.* noted the progressive accumulation of activated microglia and inflammatory cells in the subretinal space, followed by the appearance of new vessels, along with a significant increase in VEGF levels in RPE cells (19).

Joe Hollyfield identified modified proteins and products of carbohydrate oxidation in drusen composition. Carboxyethyl pyrrole products were identified in drusen, being much more frequent in AMD patients compared to healthy subjects (20). Protein oxidation is also a useful marker for in vivo oxidative stress assessment.

Even if TBARS is an early marker of oxidative stress, it was less studied in relation to AMD. In our study, we found significantly higher values of TBARS in patients with AMD, compared to the values found in the control group. The mean value remained higher after three months of treatment, compared to the control group. By comparing the TBARS mean value in the beginning of the study with the one after treatment in the total study group, we noticed that TBARS mean value decreased significantly. After treatment, the mean TBARS value showed a significantly lower level in patients with exudative form.

We assumed that there is a relationship between AMD and oxidative stress. Therefore, we carried out the standard linear regression analysis of the mean retinal thickness values for each individual test, SOD and TBARS, which confirmed that the connection between SOD and mean retinal thickness meets the requirements of a valid linear regression and SOD can be considered to be a predictor. There is a significant positive correlation between the mean retinal thickness and SOD and TBARS values. Following treatment, the relationship between oxidative stress and the change in retinal thickness diminishes, the effect of the treatment on the reduction of oxidative stress leading to a reduction of its negative influence. This study reveals the need to monitor the progression of AMD patients by assessing oxidative stress markers, alongside OCT.

Anti-VEGF agents are currently the standard of care for exudative AMD, but their role against oxidative damage has been poorly researched. The Polish National AMD Therapeutic Program estimated the approximate need for treatment in a given community to be 0.55-0.66% (21). In a recent study, the authors examined the effects of aflibercept and ranibizumab on in vitro oxidative stress on cultured ARPE-19

cells. In the presence of oxidative stress, these anti-VEGF were able to reduce nitric oxide release induced by H_2O_2 (22). Sheu *et al.* suggested that anti-VEGF agents could be protective in mitochondria, preserving their energetic metabolism, and might reduce cell damage caused by oxidative stress (23). De Cilla *et al.* showed that both aflibercept and ranibizumab inhibited the release of nitric oxide and ROS induced by H_2O_2 and modulated the activation and expression of nitric oxide synthase NOS eNOS and iNOS isoforms in RPE cells (24).

CONCLUSIONS

The results of this study suggest that an impaired antioxidant defense system and increased oxidative stress may play a role in the pathogenesis of AMD. In conclusion, increased oxidative stress, which causes oxidative damage to lipids and proteins, may lead to irreversible damage in AMD. Investigating the neuroprotection provided by antioxidants against AMD may be helpful to further entrench the significant role of oxidative stress in AMD as existing literature is inconclusive. Further research is needed to study new treatments, with the purpose of better counteracting the damaging effect of oxidative stress in AMD.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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COMORBIDITIES OF AUTOIMMUNE BULLOUS DERMATOSES IN NORTH-EASTERN ROMANIA

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COMORBIDITIES OF AUTOIMMUNE BULLOUS DERMATOSES IN NORTH-EASTERN ROMANIA (Abstract): Autoimmune bullous dermatoses (ABDs) are associated with a variety of systemic comorbidities, determined either by skin pathology or by the effects of systemic corticosteroid treatment. The most important comorbidities encountered in autoimmune bullous dermatoses belong to the cardiovascular, neurological, psychiatric, metabolic or infectious sphere. **Materials and methods:** We performed a retrospective observational study used the Minimum Basic Data Set of hospital discharges to review records of patients admitted to Sf Spiridon Iasi public hospital between 2015 and 2019 with a diagnosis of any autoimmune bullous disorder. The objectives were to describe the triggering factors and the comorbidity profile of patients with autoimmune bullous dermatoses. **Results:** The study included 225 patients, the most common autoimmune bullous dermatosis being pemphigus vulgaris. Some comorbidities, diabetes mellitus, or high blood pressure are more often found in patients with bullous pemphigoid, while vulgar pemphigus is more commonly associated with other diseases of autoimmune etiology (autoimmune thyroiditis, vitiligo). **Conclusions:** Evaluation of triggering factors and comorbidities before starting treatment may assist in controlling the disease more swiftly and reducing the side effects of treatment in the management of ABDs. **Keywords:** AUTOIMMUNE BULLOUS DERMATOSES, COMORBIDITIES.

Autoimmune bullous dermatoses are a heterogeneous group of rare dermatological diseases, characterized by vesicular bullous lesions in the skin or mucous membranes, caused by the formation of autoantibodies directed against target antigens in the skin tissue or mucous structures (1, 2).

Depending on the target cellular component, autoimmune bullous dermatoses are divided into intraepidermal diseases, which have as main representative the vulgar pemphigus (VP) and are characterized by the formation of autoantibodies against some intercellular adhesion molecules of keratinocytes - desmogleins, respectively subepidermal, represented by the bullous pemphigoid (BP), herpetiform dermatitis (HD)and acquired bullous epidermolysis, in which the autoantibodies target components of the basement membrane, with the subsequent disorganization of the dermal-epidermal junction (2).

Autoimmune bullous dermatoses (ABDs) are associated with a variety of systemic comorbidities, determined either by skin pathology or by the effects of systemic corticosteroid treatment. The most important comorbidities encountered in autoimmune bullous dermatoses belong to the cardiovascular sphere (high blood pressure, rhythm disorders, heart failure), neurological (stroke), psychiatric (sleep disorders, symptoms of anxiety or depression), metabolic (abdominal obesity, diabetes mellitus, metabolic syndrome, dyslipidemia), ocular (dry eye syndrome, corneal opacity) or infectious (bacterial, viral, fungal infections) (1, 3).

A special correlation between certain types of comorbidities and different autoimmune bullous dermatoses was observed. Some comorbidities, such as neoplastic pathology (solid metastases, malignant lymphoma), diabetes mellitus or osteoporosis, are more often found in patients with vulgar pemphigus, while cardiovascular, renal, pulmonary (sleep apnea syndrome, chronic obstructive pulmonary disease) or neuropsychiatric diseases (epilepsy, dementia, Parkinson's disease, cerebrovascular diseases, multiple sclerosis, schizophrenia) are predominantly present within the bullous pemphigoid. Also, pemphigus vulgaris is more commonly associated with other diseases of autoimmune etiology (autoimmune thyroiditis, type 1 diabetes, rheumatoid arthritis, inflammatory bowel disease) compared to the general population (2, 4).

Autoimmune diseases have a higher incidence in patients with a personal pathological history of autoimmunity. Thus, once an autoimmune pathology has developed, the risk of developing a new disease with similar pathogenesis is 25% of cases, usually at a certain time frame (5).

Vulgar pemphigus may occur in patients known with thymoma, myasthenia gravis, or systemic lupus erythematosus. It has also been reported to be associated with HIV disease, and immunosuppressive treatment used in bullous pathology can cause progression of viral disease. A small number of studies show associations of pemphigus vulgaris with other dermatological diseases such as psoriasis vulgaris, lichen planus, alopecia areata, vitiligo or Darier's disease (5). Due to the damage of the mucous barrier in pemphigus vulgaris, an increase in the incidence of infections is observed in this category of patients, namely those of viral (herpes simplex), bacterial (impetigo) or fungal (oral candidiasis), respectively urinary tract and lung infections (4).

Less common variants of pemphigus vulgaris may be associated with other autoimmune or neoplastic diseases. *Pemphigus vegetans* is correlated with pulmonary, gastric, colonic carcinomas or lymphosarcoma, the herpetiform form may develop in association with prostate or lung cancer, while foliaceous and erythematous subtypes can be identified in patients with thymomas, myasthenia gravis or rarely, with pre-existing dermatological pathologies (vulgar psoriasis, mycosis fungoides, oral lichen planus) (5).

Paraneoplastic pemphigus is an autoimmune syndrome, predominantly observed in association with other benign or malignant neoplastic disorders. The most common neoplasms present in a patient with paraneoplastic pemphigus are hematologic (84% of cases) such as non-Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), Castleman's disease, or Waldenstrom's macroglobulinemia. Other tumors associated with this autoimmune bullous dermatosis are pancreatic, uterine, renal, or hepatocellular cancer. There are a

number of neoplasms without direct association with paraneoplastic pemphigus, such as breast, intestinal, lung adenocarcinoma or basal cell and squamous cell skin carcinomas (5).

Bullous pemphigoid is predominantly found in the geriatric population over the age of 60, on average 80 years, which supports its possible association with oncological diseases (3, 4). A correlation has also been described between bullous pemphigoid and other types of systemic autoimmune diseases, such as SLE, vitiligo, dermatomyositis, polymyositis, Graves' disease, rheumatoid arthritis, Hashimoto's thyroiditis, HIV infection, autoimmune neutropenia or thrombocytopenia, or glomerulonephritis with immune complexes. Other pathologies associated with bullous pemphigoid include pernicious anemia, ulcerative colitis, polymyalgia rheumatica, primitive biliary cirrhosis, Kaposi's sarcoma, nephritis and glomerulonephritis, morphea, and acquired hemophilia (5). In addition, cross-reactions between common sequences of target antigen isoforms in the skin (BPAG1) and the nervous system play a key role in the association of bullous pemphigoid with neurological diseases such as schizophrenia, Parkinson's disease, multiple sclerosis, or epilepsy (4).

Among representatives of subepidermal autoimmune bullous dermatoses, herpetiform dermatitis is associated with the presence of diabetes mellitus and thyroid pathology (hypothyroidism) in 10% - 49% of cases, but also mild or subclinical Crohn's disease (3.3-18%). In patients with untreated underlying bullous dermatosis, an increase in the frequency of non-Hodgkin's lymphomas and T-cell lymphomas involving the gastrointestinal tract is observed. In addition, the neurotoxic effect of gluten predisposes to the association of herpetiform dermatitis with neuropsychiatric disorders (epilepsy, dementia, ataxia) in 25% of cases with gluten enteropathy (4).

As regards cardiovascular comorbidities, the most common risk factors in autoimmune bullous dermatoses are high blood pressure, dyslipidemia characterized by hypertriglyceridemia and hypercholesterolemia, and diabetes mellitus, respectively. Abdominal obesity, ischemic heart disease, peripheral vascular disease, cerebrovascular disease, or heart rhythm disorders have a significantly lower association. On the one hand, the link between cardiovascular and dermatological pathology is supported by chronic systemic inflammation that underlies the autoimmune response in bullous dermatoses. On the other hand, systemic and topical corticosteroids used in the treatment of these skin pathologies have multiple adverse effects, including increased risk of hypertension and diabetes mellitus among patients with autoimmune bullous dermatoses (1, 4).

Eye disorders in patients with autoimmune bullous dermatoses are frequently underdiagnosed and underestimated, but are found in up to 77.3% of cases. The most important ophthalmological comorbidity within bullous dermatological pathologies is represented by dry eye syndrome. Other less common complaints include local eye irritation, pain, burning sensation, decreased visual acuity, conjunctival scars, and simblefaron. In untreated bullous dermatoses, important eye complications such as conjunctival inflammation, trichiasis to complete corneal opacity and perforation can occur, which substantially compromises the quality of life of patients (3).

Long-term use of systemic corticosteroids to control disease activity, decreased

mobility in the elderly, and persistence of chronic inflammatory status are risk factors contributing to osteoporosis (40.4%) and bone disease in patients with autoimmune bullous dermatoses. Mild forms of pemphigus vulgaris are correlated with osteopenia, while moderately severe disease is associated with osteoporosis (6).

MATERIALS AND METHODS

We performed a retrospective study on the patients with autoimmune bullous dermatoses in evidence of the clinical hospitals from Northeastern Romania, mostly from "Sf. Spiridon" County Clinical Emergency Hospital of Iasi. Two hundred and twenty-five patients with autoimmune bullous diseases residing in the cities of the northeast region of Romania were enrolled in the present retrospective study between January 2014 and December 2019. Data were collected from the electronic database of the University Hospitals. In this analysis, inclusion criteria were a diagnosis of pemphigus vulgaris, bullous pemphigoid, or dermatitis herpetiformis confirmed by histopathology or/and direct immunofluorescence (DIF). Data on risk factors and comorbidities were analyzed. The study protocol was approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy in Iasi.

Statistical analysis was performed using the *SPSS version 29.0* software package (SPSS Inc., Chicago, IL, USA).

RESULTS

Most of the patients (81.3%) are non-smokers (tab. I); however, the percentages of smokers are statistically significantly different between the 4 diagnostic categories ($p = 0.002$). The highest percentage of smokers is found among patients with HD (35.1%); in contrast, only 20.5% of those with pemphigus smoke, and only isolated cases of those with bullous pemphigoid (3.7%). Epidemiological evidence suggests that smoking cigarettes may be beneficial in pemphigus, but no systematic evaluation exists to corroborate this assumption (7).

Similarly, the majority of patients (82.7%) do not consume alcohol (tab. II); and in this case the percentages of alcohol drinkers are statistically significantly different between the 4 diagnostic categories ($p = 0.043$). The highest percentage of alcohol users is also found among patients with HD (29.7%); this percentage decreases significantly among those with pemphigus (18.2%), and reaches isolated cases of those with bullous pemphigoid (7.4%).

TABLE I.

Smoking

		Pemphigus		Bullous pemphigoid		Mucous membrane pemphigoid		Herpetiformis Dermatitis		Total	
		N	%	N	%	N	%	N	%	N	%
		Pearson's Chi-square = 15.310; p = 0.002**									
	0	105	79.5%	52	96.3%	2	100.0%	24	64.9%	183	81.3%
	1	27	20.5%	2	3.7%			13	35.1%	42	18.7%
Total		132	100.0%	54	100.0%	2	100.0%	37	100.0%	225	100.0%

TABLE II.

Alcohol consumption

		Pemphigus		Bullous pemphigoid		Mucous membrane pemphigoid		Herpetiformis Dermatitis		Total	
		N	%	N	%	N	%	N	%	N	%
		Pearson's Chi-square = 8.167; p = 0.043*									
	0	108	81.8%	50	92.6%	2	100.0%	26	70.3%	186	82.7%
	1	24	18.2%	4	7.4%			11	29.7%	39	17.3%
Total		132	100.0%	54	100.0%	2	100.0%	37	100.0%	225	100.0%

62.2% of patients have various associated diseases (tab. III), the percentages being statistically significantly different between the 3 investigated diagnosis categories (p = 0.037). The highest percentage of patients with associated diseases is found among those with bullous pemphigoid (77.8%); the percentage decreases in patients with DH (64.9%) and in those with pemphigus (55.3%), reaching 50% of those with mucous membrane pemphigoid.

Comparative analysis by type of associated diseases, however, reveals statistically significant differences only in the case of diagnoses of Essential Hypertension (EH) and diabetes mellitus (DM). Thus, the incidence of EH is significantly higher in patients with bullous pemphigoid (50.0%) compared to the other diagnostic categories, for which it is around 25% - while none of the patients with pemphigoid mucous membranes have EH. Similarly, the incidence of DM is also significantly higher in patients with bullous pemphigoid (29.6%) compared to the other diagnostic categories: HD (18.9%) and pemphigus (7.6%) - while none of the patients with mucous membrane pemphigoid has DM. The other associated diagnoses usually recorded a low case, and relatively uniformly distributed between patients with pemphigus, bullous pemphigoid and HD – the two patients with scar pemphigoid generally do not have associated diseases.

TABLE III.

Associated diseases

		Pemphigus		Bullous pemphigoid		Herpetiformis Dermatitis		Total	
		N	%	N	%	N	%	N	%
	0	49	44.7%	2	22.2%	3	35.1%	54	37.8%
	1	63	55.3%	32	77.8%	24	64.9%	119	62.2%
Total		112	100.0%	34	100.0%	27	100.0%	173	100.0%

A predilect correlation between certain types of comorbidities and different autoimmune bullous dermatoses is observed. Some comorbidities, diabetes mellitus, or high blood pressure are more often found in patients with bullous pemphigoid, while

vulgar pemphigus is more commonly associated with other diseases of autoimmune etiology (vitiligo), in comparison to the general population. Diabetes mellitus occurs as an autoimmune response against BP antigens, which is transformed by the glycosylation of proteins at the dermo-epidermal junction in pemphigoid patients (8).

TABLE IV.
Associated pathologies - disease categories

		Pemphigus		Bullous pemphigoid		Mucous membrane pemphigoid		Herpetiformis dermatitis		Total	
		N	%	N	%	N	%	N	%	N	%
Associated diseases											
High Blood Pressure		Pearson's Chi-square = 14.680; p = 0.002**									
	0	102	77.3%	27	50.0%	2	100.0%	27	73.0%	158	70,2%
	1	30	22.7%	27	50.0%			10	27.0%	67	29.8%
Ischemic cardiopathy		Pearson's Chi-square = 2.022; p = 0.568									
	0	130	98.5%	52	96.3%	2	100.0%	35	94.6%	219	97.3%
	1	2	1.5%	2	3.7%			2	5.4%	6	2.7%
Chronic venous insufficiency		Pearson's Chi-square = 0.395; p = 0.941									
	0	112	84.8%	46	85.2%	2	100.0%	31	83.8%	191	84.9%
	1	20	15.2%	8	14.8%			6	16.2%	34	15.1%
Diabetes mellitus		Pearson's Chi-square = 15.841; p = 0.001**									
	0	122	92.4%	38	70.4%	2	100.0%	30	81.1%	192	85.3%
	1	10	7.6%	16	29.6%			7	18.9%	33	14.7%
Gastro-esophageal reflux disease		Pearson's Chi-square = 4.343; p = 0.227									
	0	126	95.5%	54	100.0%	2	100.0%	37	100.0%	219	97.3%
	1	6	4.5%							6	2.7%
Asthma		Pearson's Chi-square = 1.317; p = 0.725									
	0	130	98.5%	54	100.0%	2	100.0%	36	97.3%	222	98.7%
	1	2	1.5%					1	2.7%	3	1.3%
Vitiligo		Pearson's Chi-square = 1.709; p = 0.635									
	0	128	97.0%	54	100.0%	2	100.0%	36	97.3%	220	97.8%
	1	4	3.0%	0				1	2.7%	5	2.2%
Tinea infection		Pearson's Chi-square = 1.462; p = 0.691									
	0	122	92.4%	49	90.7%	2	100.0%	32	86.5%	205	91.1%
	1	10	7.6%	5	9.3%			5	13.5%	20	8.9%
Neoplasia		Pearson's Chi-square = 2.201; p = 0.532									
	0	128	97.0%	50	92.6%	2	100.0%	36	97.3%	216	96.0%
	1	4	3.0%	4	7.4%			1	2.7%	9	4.0%
Total		132	100.0%	54	100.0%	2	100.0%	37	100.0%	225	100.0%

DISCUSSION

The incidence of autoimmune bullous dermatoses is constantly increasing.

Smoking and alcohol consumption are not considered trigger factors in the occurrence of autoimmune bullous dermatoses, but as a percentage, most patients who drink alcohol and smoke are those diagnosed with herpetiform dermatitis. There are studies indicating that smoking may be a possible protective factor in autoimmune bullous dermatoses (9).

The most important comorbidities encountered in autoimmune bullous dermatoses belong to the cardiovascular sphere (high blood pressure, rhythm disorders, heart failure), neurological (stroke), psychiatric (sleep disorders, symptoms of anxiety or depression), metabolic (diabetes mellitus, metabolic syndrome), or are of an infectious nature (bacterial, viral, fungal infections), given in accordance with the literature.

A predilect correlation between certain types of comorbidities and different autoimmune bullous dermatoses is observed. Some comorbidities, diabetes mellitus, or high blood pressure are more often found in patients with bullous pemphigoid, while vulgar pemphigus is more commonly associated with other diseases of autoimmune etiology (vitiligo), in comparison to the general population.

Bullous pemphigoid is predominantly found in the geriatric population over the age of 60, on average 80 years, which supports its possible association with oncological diseases. In our study, the presence of neoplasms in relatively equal percentages is observed, both in patients diagnosed with bullous pemphigoid and in those with vulgar pemphigus. In addition, cross-reactions between common sequences of target antigen isoforms in the skin (BPAG1) and the nervous system play a key role in the association of bullous pemphigoid with neurological diseases such as schizophrenia, Parkinson's disease, multiple sclerosis, or epilepsy.

As regards cardiovascular comorbidities, the most common risk factors in autoimmune bullous dermatoses are high blood pressure, dyslipidemia characterized by hypertriglyceridemia and hypercholesterolemia, and diabetes mellitus, respectively. On the one hand, the link between cardiovascular and dermatological pathology is supported by chronic systemic inflammation that underlies the autoimmune response in bullous dermatoses. On the other hand, systemic and topical corticosteroids used in the treatment of these skin pathologies have multiple adverse effects, including increased risk of hypertension and diabetes mellitus among patients with autoimmune bullous dermatoses (9).

Thus, the presence of a variety of comorbidities involving all systems and organs associated with autoimmune bullous dermatosis requires an early diagnosis of bullous dermatological pathology, extensive paraclinical examinations and an exhaustive screening of possible concomitant conditions for optimal therapeutic management.

CONCLUSIONS

Evaluation of triggering factors and comorbidities before starting treatment may assist in controlling the disease more swiftly and reducing the side effects of treat-

ment in the management of ABDs. Therefore, since autoimmune bullous disorders are associated with various comorbidities and their incidence has risen in recent years, the establishment of strategies to prevent the main comorbidities in these patients is justified.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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THE PARTICIPATION OF THE SUPERIOR AND INFERIOR THYROID ARTERIES IN THE VASCULARIZATION OF THE THYROID GLAND

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THE PARTICIPATION OF THE SUPERIOR AND INFERIOR THYROID ARTERIES IN THE VASCULARIZATION OF THE THYROID GLAND (Abstract): **Background and aim:** This study aims to evaluate the level of vascularization within the thyroid gland by analyzing the diameters of the Romanian population's superior and inferior thyroid arteries. **Materials and methods:** Our study was performed on angiographies by computed tomography on a LightSpeed VCT64 Slice CT on 317 thyroid arteries, 168 arteries on the right side, and 149 on the left side. The measurements of the diameters of the superior and inferior thyroid arteries were compared between sides (left/right) and sexes. **Results:** The diameter of the superior thyroid artery, right and left, we're observed on a number of 176 cases, finding it to be between 1.6-4.3 mm. The right superior thyroid artery was between 1.8-4.3 mm in diameter while the left artery was between 1.6-2.8 mm in diameter. The diameter of the inferior thyroid artery, right and left, on a number of 141 cases was found to be between 1.2-4.1 mm, the right one was between 1.7-3.9 mm and the left was between 1.2-4.1 mm. **Conclusions:** The variances in the diameters of the two arteries result in fluctuation in their respective contributions to the vascular supply of the thyroid gland. **Keywords:** THYROID ARTERIES, SUPERIOR THYROID ARTERY, INFERIOR THYROID ARTERY, MORPHOMETRY.

INTRODUCTION

The thyroid gland is most commonly vascularized by two thyroid arteries: the superior thyroid artery (STA), with its origin in the carotid system (external carotid and common carotid, either at the level of its terminal branch or from the arterial trunk below the terminal branch) and the inferior thyroid artery (ITA) with its origin in the subclavian artery (either directly from the arterial trunk or via a thyrocervical trunk). Occasionally, there may be a third thyroid artery, Neubauer's thyroid ima, present in about 10% of cases according to Paturet (1) and Moore (2). According to Jelev (3) and Roshan (4), the STAs were unique and present in all cases, and Jelev (3), finds the absence of the ITA only in the right side, without being replaced by the ima, resulting in the vascular compensation of the organ being provided by the STA, which therefore has a larger caliber. Esen (5) could not identify the right ITAs in 14 cases (2.2% of cases) and left ITAs in

45 cases (7% of cases), with both ITAs absent in six cases (0.9% of cases). According to Lippert (6), the inferior thyroid artery may be missing in 3% of cases, being supplanted by the superior thyroid.

The research was conducted to identify variances in the arterial vascularization of the thyroid gland and to emphasize the differences in arterial patterns between the left and right sides, as well as between the superior and inferior.

MATERIALS AND METHODS

The angiographies conducted via computed tomography (CTA) were reviewed retrospectively. The study focused on the Romanian Caucasian population.

The study has the accord of the local Ethics Commission (34490 / 08.08.2019) within the institution of "Sf. Apostol Andrei" Clinical County Emergency Hospital from Constanța and respects the principles of the General Regulation on Personal Data Protection (EU) 679/2016. The research was conducted following the guidelines outlined in the Helsinki Declaration and adhering to all applicable national regulations concerning patient studies. Additionally, informed consent was obtained from all participants involved in the study.

Our study was performed on a number of 317 thyroid arteries, 168 arteries on the right side, and 149 on the left side. In male subjects, there were 158 thyroid arteries (49.84% of cases), 82 cases on the right side (51.90% of cases), and 76 on the left side (40.10% of cases). In female subjects, there were 159 thyroid arteries (55.43% of cases), 86 cases on the right side (54.09% of cases), and 73 arteries on the left side (45.91% of cases).

The study involved measuring the diameters of the superior and inferior thyroid arteries, comparing them bilaterally and superior with inferior, as well as by sex. It is noted that not all anatomical landmarks were consistently observed in the cases studied, with each landmark being observed in a specific number of cases as outlined in the results. Diameters were not measured in cases with an ima thyroid artery, and the origin of the thyroid arteries was not factored into the measurements.

The inclusion criterion in the study was disease-free arteries, while patients after neck surgery and with atherosclerosis were excluded. The mean ages of the males and females were 66.5 ± 17.5 and 51.5 ± 30.5 years, respectively.

RESULTS

For the diameter of the STA, on the right and left sides, we followed 176 cases and found it to be between 1.6-4.3 mm. The right STA in 90 cases was between 1.6-3.9 mm in diameter and the left STA in 86 cases was between 1.8-4.3 mm in diameter. In 84 cases in the male gender, the diameter of the STA was between 1.8-3.3 mm, in 44 cases the diameter of the right STA was between 1.8-3.3 mm, and in 40 cases the diameter of the left STA was between 1.8-2.8 mm. In female subjects, in 92 cases, the diameter of the STA was between 1.6-4.3 mm, in 46 cases the diameter of the right STA was between 1.6-3.9 mm, and in 46 cases the diameter of the left STA was 1.8-4.3 mm.

The diameter of the right and left ITA in 141 cases was found to be between 1.2-4.1 mm. The right ITA in 78 cases was between 1.7-3.9 mm in diameter and the left ITA in 63 cases was between 1.2-4.1 mm in diameter. In 74 cases of male subjects, the ITA diameter was between 1.2-4.1 mm, in 38 cases the right ITA diameter was between 1.7-3.5 mm, and in 36 cases the left ITA diameter was between 1.2-4.1

mm. In female subjects, the ITA diameter was between 1.7-3.9 mm in 67 cases, for the right ITA it was between 1.7-3.9 mm in 40 cases and for the left ITA, it was between 1.9-3.6 mm in 27 cases.

DISCUSSION

On a total of 80 CTA, we compared right/left STA diameters, finding that in 30 cases (37.50% of cases) the right STA diameter was larger than the left STA diameter with differences ranging from 0.1-1.5 mm. In 44 cases (55.0% of cases) the left STA diameter was larger with differences ranging from 0.1-1.4 mm. In 6 cases (7.5% of cases) the diameters of the two arteries were equal.

In males, from 44 cases (55.0% of cases), the diameter of the right STA was larger in 14 cases (31.82% of cases), with differences ranging from 0.1-1.3 mm.(fig. 1). In 28 cases (63.64% of cases), the diameter of the left STA was larger, with differences ranging from 0.1-1.0 mm. In 2 cases (4.55% of cases) the diameter of the right STA was equal to that of the left STA. (fig. 2).

In females, out of 36 cases (45.0% of cases), the diameter of the right STA was larger in 16 cases (44.44% of cases), with differences ranging from 0.1-1.5 mm. Also, in 16 cases (44.44% of cases), the diameter of the left STA was larger, with differences ranging from 0.1-1.4 mm. (fig. 3). In 4 cases (11.11% of cases) the diameter of the right STA was equal to the diameter of the left STA.

For Gursharan (7) and Ozgur (8), the STA is the main source of arterial blood for the thyroid gland. According to Paturet (1), the superior thyroid artery is relatively bulky, and when the superior thyroid artery is very thin, the one on the opposite side is usually larger, supplanting it.

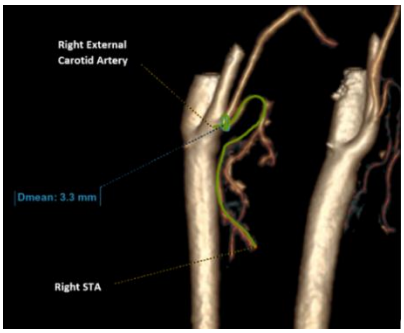

Fig. 1. The diameter of the right STA is 3.3 mm (male subject)

Fig. 2. The diameter of the right STA is 2.2 mm, equal to the diameter of the left STA; the diameter of the left ITA is 2.0 mm larger than the diameter of the right ITA (1.8 mm) by 0.2 mm (male subject)

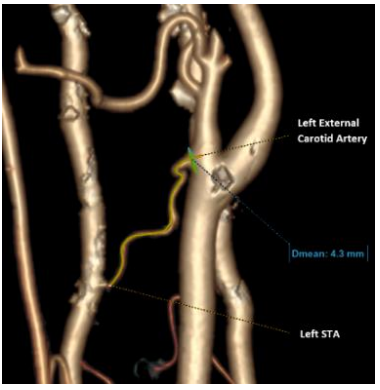

Fig. 3. The diameter of the left STA is 4.3 mm (female subject)

Different authors have identified differences in the caliber of the STA in cadavers, ranging from 1.04-6.1 mm (tab. I).

Comparing the results found by us with those reported in the literature, it appears that at the minimum measure level, it is smaller than that found by several authors (4, 10, 13, 14) with differences of 0.1-4.3 mm, larger than Ozgur (8 (right side)) and Troupis (12) with 0.3-0.74 mm and equal to the diameter found by Ozgur (8 (left side)). The maximum diameter found by us is smaller than that found by some authors (4, 7 (right and left), 10) with differences of 0.1-1.8 mm and larger than the diameter found by various authors (1, 12, 13, 14) with differences of 1.0-1.8 mm.

TABLE I.

Diameter of the superior thyroid artery in literature

Authors	Type of study	Diameter of STA (mm)
Fujimoto (1974) (9)	cadaver	3.3 (R); 2.7 (L)
Zhao (1999) (10)	cadaver	1.9
Jelev (2001) (3)	cadaver	4.4
Ozgur (2008) (8)	cadaver	1.5-6.1 (R); 1.8-5.5 (L)
Vazquez (2009) (11)	cadaver	2.6
Troupis (2014) (12)	cadaver	1.04
Sung-Yoon (2016) (13)	cadaver	4.4
Personal cases	CTA	Male subjects (1.8-3.3) R: 1.8-3.3; L: 1.8-2.8 Female subjects: (1.6-4.3) R: 1.6-3.9; L: 1.8-4.3

On a total of 91 CTA, we compared the diameters of the ITA right/left, finding that in 30 cases (32.97% of cases) the right ITA diameter was larger than the left ITA diameter with differences ranging from 0.1-1.5 mm. In 56 cases (61.54% of cases) the diameter of the left ITA was larger with differences ranging from 0.1-1.4 mm. In 5 cases (5.49% of cases) the diameters of the two arteries were equal. In male subjects, in 45 cases (49.45% of cases), the right ITA diameter was larger in 22 cases (48.89% of cases), with differences ranging from 0.1-1.3 mm. Also, in 22 cases (48.89% of cases), the left ITA diameter was larger with differences ranging from 0.1-1.0 mm (fig. 4)In only one case (2.22% of cases) was the right ITA diameter equal to the left ITA diameter. In female subjects, in 46 cases (50.55% of cases), the diameter of the right ITA was larger in 8 cases (17.39% of cases), with differences ranging from 0.1-1.5 mm (fig. 5). In 34 cases (73.91% of cases) the diameter of the left ITA was larger, with differences ranging from 0.1-1.4 mm. In 4 cases (8.70% of cases) the diameter of the right ITA was equal to that of the left ITA.

In the literature, we did not find the value of the ITA diameter, but the size of the artery was only mentioned in general, Testut (14) states that the ITA volume varies greatly, depending on the development of the thyroid gland and being the inverse of the ITA on the opposite side. Roshan (4), found that the ITA is usually the largest artery of the normal thyroid gland, ITA at the entrance to the hilum, representing the main functional blood source of the gland.

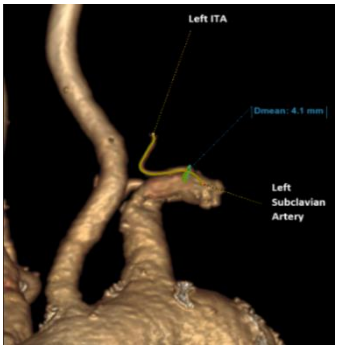

Fig. 4. The left ITA diameter is 4.1 mm (male subject)

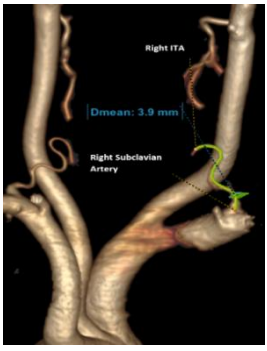

Fig. 5. The diameter of the right ITA is 3.9 mm (female subject)

We compared the diameter of the STA with the diameter of the corresponding ITA in 133 cases and found that in 66 cases (49.62% of cases) the diameter of the STA was larger by 0.1-2.0 mm (fig. 6). In 64 cases (48.12% of cases) the diameter of the ITA was larger by 0.1-2.2 mm, and in 3 cases (2.26% of cases) the diameter of the two arteries was equal. In male subjects, in 76 cases (57.14% of cases), the diameter of the STA was larger in 38 cases (50.0% of cases) with differences of 0.1-1.6 mm. In 36 cases (47.37% of cases), the diameter of the ITA was larger with differences of 0.1-2.3 mm. In 2 cases (2.63% of cases) the diameter of the two arteries was equal. In female subjects, in 57 cases (42.86% of cases), the diameter of the STA was larger in 28 cases (49.12% of cases), with differences between 0.1-2.3 mm. Also, in 28 cases (49.12% of cases) the ITA diameter was larger with differences between 0.2-1.7 mm. In only one case (1.75% of cases) the diameter of the two arteries was equal.

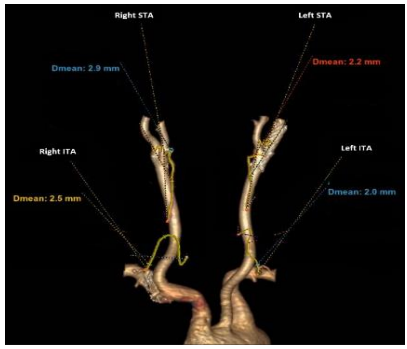

Fig. 6. The diameter of the right STA is 2.9 mm and the right ITA diameter is 2.5 mm, being 0.4 mm smaller; the diameter of the left STA is 2.2 mm, and the left ITA diameter is 2.0 mm, being 0.2 mm smaller; the diameter of the right STA is 0.5 mm larger than the diameter of the left STA; the diameter of the right ITA is 0.5 mm larger than the diameter of the left ITA (female subject)

In reviewing scientific literature, we did not come across specific citations regarding the morphometric variances in external diameter values between the superior and inferior thyroid arteries. However, we did find general information on this topic. Thus, Paturet (1) and Testut (14) noted that the volume of the inferior thyroid artery is inversely proportional to that of the superior thyroid artery on the corresponding side, as well as the inferior thyroid artery on the opposite side. As noted by Paturet (1), the superior thyroid artery typically has a significant size, with its caliber usually being slightly smaller than or equivalent to that of the inferior thyroid artery. A balancing effect is observed between the calibers of these arteries and the two superior thyroid arteries. In cases where one superior thyroid artery is thin, the one on the opposite side tends to be larger to ensure proper arterial blood supply to the gland. In a study conducted by Lippert (6), it was observed through dissections that the inferior thyroid artery typically has a larger size in comparison to the superior thyroid artery, indicating that it serves as the main artery supplying the gland. Additionally, Testut (14) notes that the volume of the inferior thyroid artery is inversely proportional to that of the superior thyroid artery on the same side.

CONCLUSIONS

There is consistent variability in percentages among different authors, often showing significant differences over the same or similar time periods. We did not come across any literature analyzing the origin of the artery based on sex, despite known differences between males and females. Few authors provide detailed percentages on the origin of thyroid arteries comparing right to left, also disparities between the two are present. It should be noted that the percentages reported may vary depending on the methodology employed.

We have not observed any instances of missing or duplicated thyroid arteries (superior or inferior) in our study. The diameters of the thyroid arteries we identified showed variations, indicating that these arteries' roles in the thyroid gland's vascular supply may vary as well. Thus, of the total cases, the right ITA was larger in diameter in 10.57% of cases and the left ITA was larger in diameter in 6.90% of cases. In the male subjects, in the right thyroid arteries, the diameter of the right STA was larger than the diameter of the right ITA in the same proportion that the diameter of the right ITA was larger than the diameter of the right STA. In female subjects, the right ITA diameter was larger than the right ITA diameter in 5.36% of cases, and the right ITA diameter was larger than the left ITA diameter in 10.52% of cases. The equality of the diameters of the two thyroid arteries, superior and inferior, was greater in females in 2.70% of cases. The minimum and maximum diameters were frequently found in only one case.

CONFLICT OF INTEREST AND FUNDING

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RISK FACTORS AND ANTIBODY RESPONSE ASSOCIATED WITH LONG COVID: LONGITUDINAL COHORT STUDY

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RISK FACTORS AND ANTIBODY RESPONSE ASSOCIATED WITH LONG COVID: LONGITUDINAL COHORT STUDY(Abstract): **Aims:** Although numerous studies assess risk factors for long COVID, data on humoral response are scarce and discordant. This study aimed to determine if serum antibody titers are associated with long COVID and to analyze demographic characteristics, comorbidities, treatment, and disease severity influencing its development. **Materials and methods:** We included 80 unvaccinated COVID-19 patients who contracted the disease in early 2020. Clinical data and serum samples were collected at inclusion, 33±10 days (visit 1), and 91±19 days (visit 2) post-symptom onset for symptomatic patients, and post-diagnosis for asymptomatic patients. Symptoms were tracked via questionnaires at each visit and one month after the second visit by telephone. Serum samples were analyzed to measure IgG antibodies using indirect chemiluminescent immunoassay technology. **Results:** We found 53.7% (CI95%, 42.2-65; p=0.57) of patients experienced long COVID. Female gender (OR, 1.95; CI95%, 1.12-3.41; p=0.01) and pre-existing comorbidities (OR, 1.56; CI95%, 1.02-2.38; p=0.04) were significantly associated with long COVID symptoms. Importantly, patients with long COVID had higher overall median antibody levels at both follow-up visits. While this difference was not statistically significant, it suggests a trend worth further investigation. **Conclusions:** Understanding the risk factors associated with long COVID could provide valuable insights for managing and potentially decreasing its incidence in the future. **Keywords:** LONG COVID, RISK FACTORS, GENDER, COMORBIDITIES, ANTIBODY TITER.

Long COVID, also known as chronic COVID syndrome or prolonged COVID-19 symptoms, refers to persistent health issues experienced by individuals following a COVID-19 diagnosis. These symptoms typically emerge within a few months and can significantly affect quality of life. While various definitions exist, the World Health Organization defines long COVID as the persistence or emergence of new symptoms at least three months after initial SARS-CoV-2 infection, lasting for a minimum of two months without any other clear cause (1). Estimates suggest 10–20% of

individuals may experience such symptoms (1), which encompass over 200 reported manifestations including fatigue, shortness of breath, cognitive difficulties, chest pain, and joint pain, among others (2).

Similarities to postviral syndromes observed in previous coronavirus diseases like MERS and SARS highlight the long-term impact of such infections, with symptoms such as fatigue and psychiatric impairments persisting for years (3, 4). Identifying risk factors, both pre-infection (e.g., age, sex, comorbidities) and infection-associated (e.g., disease severity, viral load), is crucial for predicting, preventing, and managing long COVID (5).

Understanding the role of antibody response dynamics is pivotal for developing effective treatments and interventions (6). However, studies on humoral responses in long COVID have yielded conflicting results, with some indicating higher antibody titers associated with persistent symptoms (7,8), while others suggesting lower titers (9).

This study aimed to investigate the association between serum antibody titers and long COVID, while analyzing demographic characteristics, comorbidities, treatment, and disease severity as potential risk factors for its development. By elucidating these factors, this research contributes to ongoing efforts in managing and potentially reducing the incidence of long COVID in the future.

MATERIALS AND METHODS

Patients and collection of serum samples and clinical data

Adult patients aged 18 years or older diagnosed with SARS-CoV-2 infection via positive reverse transcription polymerase chain reaction (PCR) tests from nasopharyngeal swabs were consecutively recruited for this study. The study population was drawn from a larger investigation conducted at the onset of the COVID-19 pandemic, focusing on the clinical performance of six commercially available assays, including the chemiluminescent immunoassay (CLIA) IgG antiS1/S2 (10). Enrollment commenced in May 2020, with baseline blood samples collected during hospitalization: 6+/-10 days post-symptom onset (PSO) for symptomatic patients, and post-day of diagnosis (PDD) defined as the first positive PCR for asymptomatic patients. Subsequent serum samples were obtained at 33+/-10 days (visit 1) and 91+/-19 days (visit 2) PSO for symptomatic patients, and PDD for asymptomatic patients. Demographic and clinical data were collected concurrently. Only patients who completed visit 2 were included in the analysis. Specimens were processed within 5 days of collection: stored at 4 °C, centrifuged, aliquoted, and frozen at −20 °C until analysis. Data on demographic characteristics (age, gender), comorbidities, and COVID-19 clinical classifications (mild, moderate, severe) were collected using a questionnaire based on WHO criteria. Persistent or new symptoms were assessed at each visit, with a follow-up telephone questionnaire (Annex 1- Post-COVID Questionnaire) conducted one month after visit 2 for symptomatic patients.

Long COVID was defined as the persistence or emergence of signs, symptoms, or conditions beyond three months post-acute COVID-19 infection, lasting over one month without an apparent cause. Patients reporting at least one persistent symptom fitting these criteria were classified as having long COVID.

Ethical approval was obtained from the hospital's local ethics committee, and all participants provided written informed consent for serial blood sampling.

Assays. Nasopharyngeal swabs were analyzed for SARS-CoV-2 RNA using the Allplex™ SARS-CoV-2 Assay and the CFX96 Real-Time PCR Detection System. Serum samples were tested for IgG antibodies against SARS-CoV-2 S1/S2 using the LIAISON® SARS-CoV-2 S1/S2 IgG assay on the Liaison XL platform, with results reported in arbitrary units (AU/mL). Samples with a value of ≥15 AU/ml were interpreted as positive, 12.0 ≤ AU/mL < 15.0 equivocal, and < 12.0 AU/mL as negative.

Statistical analysis employed IBM® SPSS® Statistics version 26.0. Descriptive statistics included median values with interquartile range (IQR). Results are presented as total values and percentages with 95% confidence intervals. Mann-Whitney U test compared categorical variables, and Fisher's exact test analyzed categorical variables between patients with and without long COVID symptoms. Odds ratios (ORs) with 95% confidence intervals were calculated. A p value <0.05 indicated significance.

RESULTS

A total of 93 patients were initially recruited in the study, but by the end of the study two patients died and 11 were lost to follow-up. Among them, 44 were males (55%, p=0.43), median age 49.5 (IQR 38.2-57). Five patients (6.25%, p=0) had been asymptomatic, 32 (40%, p=0.09) had a mild form of the disease, 25 (31.25%, p=0.001) had moderate disease and 18 of them (22.5%, p=0) had severe disease. Comorbidities were recorded in 45 patients (56.25%, p=0.31) as illustrated in Table 1, with arterial hypertension being the most common one.

Forty-three patients (53.75%, p=0.57) had long COVID symptoms; refer to Table 2 for specific symptoms.

Other Long Covid symptoms: diaphoresis, arterial hypertension, alopecia, headache, visual acuity disturbances (hypermetropia, myopia), ulnar neuropathy, sneeze, and rash

TABLE I.

Associated comorbidities

Comorbidities	N (%)
Arterial hypertension	26 (32.5)
Diabetes mellitus	12 (15)
Dyslipidemia	9 (11.2)
Obesity	8 (10)
Chronic Liver Disease	7 (8.7)
Thyroid Dysfunction	6 (7.5)
Asthma	3 (3.7)
Neoplasia	3 (3.7)

TABLE II.

Long COVID symptoms

Symptoms	N (%)
Fatigability	29 (67.4)
Arthralgia	11 (25.5)
Lack of concentration memory attention	11 (25.5)
Dyspnea	11 (25.5)
Smell disorders	8 (18.6)
Anxiety	7(16.2)
Cough	6(13.9)
Sleep disorders	5 (11.6)
Palpitations	4 (9.3)
Mialgii	4 (9.3)
Taste disorders	3 (6.9)
Emotional lability	3 (6.9)
Lack of interest/Social isolation	1 (2.3)
Others	11 (25.5)

Female gender, OR 1.95 (95% CI 1.12-3.41, p 0.01), severe disease, OR 2.23 (95% CI 0.88-5.68, p 0.07), and comorbidities, OR 1.56 (95% CI 1.02-2.38, p 0.04), especially dyslipidemia, OR 6.88 (95% CI 0.90-52.51, p 0.03), were associated with long COVID. Table III illustrates factors linked to long COVID symptoms.

Steroid treatment and Tocilizumab did not correlate with long COVID onset (OR 1.28 (95% CI 0.74-2.21), p 0.53 and OR

1.15 (95% CI 0.51-2.57), p 1, respectively). Patients with severe disease showed elevated IgG anti-S1/S2 titers, but no significant difference was found between groups or disease severity at any evaluation point (tab. IV).

TABLE III.

Factors associated with developing long COVID

Characteristics	Long COVID N=43 / N (%)	Non-long COVID N=37 / N (%)	OR (95% CI)	p
Sex (F)	25 (58.1)	11 (29.7)	1.95 (1.12-3.41)	0.01
Age (≤50 years)	26(60)	14(37)	1.64 (0.99-2.70)	0.07
Disease form				
Asymptomatic	1 (2.3)	4 (10.8)	0.21 (0.02-1.84)	0.12
Mild	17 (39.5)	15 (40.5)	0.97 (0.57-1.66)	0.92
Moderate	12 (27.9)	13 (35.1)	0.79 (0.41-1.52)	0.49
Severe	13 (30.2)	5 (13.5)	2.23 (0.88-5.68)	0.07
Presence of comorbidities	29 (67.4)	16 (43.2)	1.56 (1.02-2.38)	0.04
Arterial Hypertension	13 (44.8)	13 (81.2)	0.86 (0.45-1.61)	0.81
Dyslipidemia	8 (27.5)	1 (2.7)	6.88 (0.90- 52.51)	0.03
Diabetes mellitus	8 (27.5)	4 (10.8)	1.72 (0.56-5.25)	0.36
Obesity	5 (17.2)	3 (8.1)	1.43 (0.36-5.60)	0.71
Chronic liver disease	5 (17.2)	2 (5.4)	2.15 (0.44-11.44)	0.44
Thyroid dysfunction	4 (13.8)	2 (5.4)	1.72 (0.33-8.86)	0.68
Asthma	2 (6.9)	1 (2.7)	1.72 (0.16-18.22)	1.00
Neoplasia	1 (3.4)	2 (5.4)	0.43 (0.41-4.55)	0.60

TABLE IV.

Median CLIA detected IgG antiS1/S2 concentration according to disease severity and time PSO for both Long COVID and non-Long COVID groups

Disease form	Median CLIA IgG antiS1/S2 (AU/mL), IQR								
	Baseline (6+/-10 days) N=80			Visit 1 (33 +/-10 days) N=80			Visit 2 N=74		
	Long COVID N=43	Non-Long COVID N=37	p	Long COVID N=43	Non-Long COVID N=37	p	Long COVID N=40	Non-Long COVID N=34	p
Asymptomatic / Mild	3.8 3.8-4.9	3.8 3.8-4.7	0.82	48.7 33.2-76.3	63.7 19.1-104	0.61	59.4 26.2-91.4	37.8 11.4-79.3	0.61
Moderate	3.8 3.8-9.2	3.8 3.8-8.9	0.92	76.6 42.7-160	80.6 20.6-115	0.90	91.4 41.6-177.7	69.2 17-92.6	0.25
Severe	3.8 3.8-5.3	3.8 35-21	0.41	76.4 42.3-108.7	80 22.9-107.7	0.61	71.6 51.9-127	72.6 22.7-101.1	0.89
Overall	3.8 3.8-9.2	3.8 3.8-17.8	0.56	95.2 44.5-159	82.8 27.5-121.5	0.26	91.4 56.3-171.7	77.1 25.8-137.7	0.47

DISCUSSION

Long COVID poses challenges for healthcare systems, demanding a multidisciplinary approach (5). Ongoing research has identified risk factors like older age, female gender, and pre-existing conditions (11), but much remains unknown about why symptoms persist variably among individuals. Literature on chronic COVID reflects diverse symptom persistence due to

sample characteristics, study methodologies, and the fact that long COVID exhibits significant heterogeneity.

Long COVID's mechanisms may involve sustained tissue damage and inflammation (5). Survivors often experience multi-organ complications, such as pulmonary (12), neurological (13), cardiac (14), and others (15). Pathological inflammation, including prolonged virus shedding (16), immune dysregulation involving T and B cells (17, 18), and disrupted gut microbiome (19), likely contributes to persistent symptoms.

Ideal for long COVID research is a diverse cohort of individuals recovering from SARS-CoV-2 infection, ranging from mild to severe cases, with persistent symptoms post-acute phase. Longitudinal tracking is crucial to monitor symptom evolution across organ systems. Including hospitalized and non-hospitalized patients, diverse demographics, and comorbidities would capture comprehensive long COVID manifestations and risk factors. Our cohort includes 80 patients recovering from various SARS-CoV-2 clinical forms, from asymptomatic to severe, with ongoing symptoms three to four months post-acute phase. It features balanced gender representation and diverse comorbidity profiles.

Regarding long COVID symptoms in survivors, fatigue was prominently reported in our patient cohort, consistent with findings from other studies (20, 21). Approximately half of our patients experienced fatigue three months post-SARS-CoV-2 infection. Dyspnea, cognitive impairment, and arthralgia affected nearly a quarter of our cohort. Studies with higher dyspnea prevalence, up to 61%, likely included more severe cases and ICU admissions (20, 22), unlike our cohort where severe cases were 22.5% and no ICU admissions occurred. Other symptoms like anosmia, anxiety, and cough were less frequent (23, 24).

Numerous studies are focused on demonstrating that specific epidemiological and clinical risk factors are associated with an increased likelihood of developing long COVID symptoms.

Our study identified a significant association between female gender and increased risk of long COVID symptoms compared to males (OR, 1.95; CI95%, 1.12 to 3.41; p =0.014). This finding is consistent across several individual studies (8, 25-27,) and meta-analyses (11, 28). Studies such as Bai *et al.* (25) reported females having 2.7 times higher odds of long COVID symptoms (CI95%, 1.68 to 4.62), while Asadi-Pooya *et al.* (26), Chudzik *et al.* (27), and Peghin *et al.* (8) reported odds ratios of 1.26 (CI 95%, 1.12 to 1.43), 1.44 (CI95%, 1.20 to 1.70), and 1.55 (CI95%, 1.05 to 2.27), respectively. Meta-analyses by Kin Israel Notarte *et al.* (11) and Maglietta *et al.* (28) also showed significant associations with approximately 50% higher risk for females. The reasons for this gender disparity in long COVID risk are not fully understood, but potential factors include the influence of female hormones on sustaining post-recovery inflammation (29) and higher acute phase mortality rates among males during COVID-19 (30).

The influence of age on long COVID is still debatable, despite the well-established understanding that older age is a significant risk factor for adverse outcomes during COVID-19 hospitalization (31). In our study as well as in the study conducted by Peghin *et al.* (8), who compared young adults under 40 with those older than 40, no association between age and long-COVID was observed. Furthermore, no such association was found in the meta-analysis conducted by Kin Israel Notarte *et*

al. (11) (OR, 0.86; 95%CI 0.73 to 1.03, p = 0.17) who compared individuals under the age of 60 with those aged 60 and above. One possible explanation for this discrepancy includes the inadequate control of confounding factors observed in older individuals, such as a higher prevalence of medical comorbidities or longer hospitalization stays, which may also contribute to developing long COVID (11).

The severity of acute COVID-19 illness does not appear to predict the development of long COVID. Research indicates that long COVID can impact individuals with mild-to-moderate cases, including younger adults treated as outpatients (5). Consistent with other studies (32, 33), we found no association between initial disease severity and long COVID. In contrast, Tsampasian *et al.*'s meta-analysis highlighted severe illness as a significant risk factor (34). ICU survivors may experience post-intensive care syndrome, overlapping with long COVID symptoms (35). Our study, with few severe cases and none in the ICU, underscores the need for further exploration into these complexities.

Comorbidities are recognized as potential risk factors for long COVID. Studies have linked conditions like diabetes (36), obesity (34, 37), cardiovascular disease (36, 37), and respiratory disorders (38) to a higher likelihood of persistent symptoms post-COVID-19 recovery. Consistent with this research, our study found that having comorbidities significantly increased the risk of long COVID (OR, 1.56; CI95% 1.02 to 2.38; p 0.04). Dyslipidemia showed a particularly strong association (OR, 6.88; CI 95% 0.90 to 52.51; p 0.03), though our cohort size limited statistical significance for other conditions. Pretorius *et al.* also noted elevated cholesterol and conditions like hypertension and type 2 diabetes as significant in long COVID (36). Lipid metabolism disorders and obesity represented age-independent risk factors for long COVID syndrome indicating that metabolic changes play a significant role in determining the severity of the disease throughout all stages of COVID-19 in another study conducted by Loosen *et al.* (37).

Similar to other research findings, we found no association between the treatment administered during the acute phase of SARS-CoV-2 infection and long COVID (39).

The long COVID group revealed overall higher median titers of IgG anti-S1/S2 antibody at visit 2 and visit 3, compared to baseline where no disparities were noticed, with no statistically significant differences observed at any time point. Also, when comparing median antibody antiS1/S2 concentration according to the disease severity and time PSO between the two groups, no statistically significant differences were observed. Similar to our results, no association was observed between increased specific immunity evaluated by qualitative IgG antibodies directed against S1 protein using in vitro SARS-CoV-2 ELISA assay and long-term complications in a study conducted by Rank *et al.* (7). Interestingly, Klein *et al.*, found that the long COVID state positively predicted the anti-spike humoral response with significant differences among the various epitopes (40). Potential explanations suggest that long-term complications following COVID-19 may be due to virus-specific pathophysiological changes, immunological dysregulation, and inflammatory damage initiated by the acute infection. However, the involvement of the SARS-CoV-2 specific humoral immune response in post-COVID syndrome remains unclear, given the incomplete and sometimes conflicting data, potentially arising from differences in

methodological approaches.

Our study's strength lies in its focus on unvaccinated patients recruited early in the pandemic, ensuring antibody responses were not influenced by prior COVID-19 infection or vaccination. Further studies are needed to determine the usefulness of measurement of antibody titers for prognosis of long COVID symptoms.This research surpasses the examination of recognized risk factors and extends its focus to explore the complex interaction between antibody titers and the emergence of long COVID over different timeframes. A limitation of this study is its small sample size, although it included participants with varying disease severities from asymptomatic to severe cases. Due to the limited number of patients, not all potential long COVID symptoms may have been captured. While we followed up with patients for at least three months, longer-term observation is needed to understand symptom persistence fully. Another limitation is the use of a single serological method, LIAISON® SARS-CoV-2 S1/S2 IgG (DiaSorin), chosen based on its superior sensitivity and specificity compared to other serological assays, as supported by recent literature (10, 41).

CONCLUSIONS

The study identified female gender and pre-existing medical conditions as potential risk factors for long COVID symptoms. Factors such as age, disease severity during acute COVID-19, immunomodulatory treatment, and antibody levels were not associated with long COVID symptoms. Nonetheless, the current literature does not conclusively identify the risk factors associated with long COVID symptoms. Through the inclusion of analyses conducted at various intervals, the study provides insight into the possible role of antibody dynamics in the development of this condition.

CONFLICT OF INTEREST AND FUNDING

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THE CLINICAL FACTORS OF EVOLUTION AND PROGNOSIS IN CUTANEOUS SEPSIS: DERMATOLOGIST'S PERSPECTIVE IN SEVEN BASIC STEPS

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THE CLINICAL FACTORS OF EVOLUTION AND PROGNOSIS IN CUTANEOUS SEPSIS: DERMATOLOGIST'S PERSPECTIVE IN SEVEN BASIC STEPS (Abstract): Disturbances of the skin microbiome characterize a variety of infectious and inflammatory dermatoses with important impact on the quality of life. Most of these have presents a skin manifestation one or more lesions with loss of tissue, which are infected with microorganism. Increased mortality because of sepsis and increased incidence of organ dysfunction are a global health problem. Pathophysiological mechanism from skin infection to sepsis evolves rapidly, by triggering a complex cascade involving inflammatory and coagulation processes. **Materials and methods:** Clinical factors of evolution and prognosis, together with skin changes, are essential elements in the management of cutaneous sepsis. This retrospective observational study aims to assess clinical risk factors in cutaneous sepsis and their involvement in the evolution and prognosis of this disease. A total of 441 medical documents of patients with skin lesions with loss of tissue, in various stages of evolution, admitted to dermatology over a period of 3 years were analyzed. Out of the 441 infectious dermatoses, 363 had a favorable evolution, without septic complications and 78 presented sepsis. The most frequent skin infections were: venous ulcers, microbial eczema, cellulitis, superinfected bullous dermatoses (pemphigus, bullous pemphigoid, dermatitis herpetiformis), erysipelas, erythroderma. **Results**: Skin semiology was correlated with risk factors such as smoking, obesity, using invasive devices and with comorbidities - cardiovascular, metabolic and neuropsychiatric. This way it was described the correlation between skin-sepsis in seven basic steps. **Conclusions:** The study illustrates the importance of skin manifestations, which although they are initially not important, should not be neglected, as they can become bacterially infected and progress to severe infections, even sepsis, with a fatal prognosis. At the same time, this study encourages further research for a prompt and appropriate approach of cutaneous sepsis. **Keywords**: CUTANEOUS SEPSIS, RISK FACTORS, SKIN INFECTIONS.

INTRODUCTION

Over the last 30 years, sepsis has been the focus of research and clinical debate, with multiple dynamic challenges, repre-

senting one of the major life-threatening health problems in the absence of urgent and appropriate intervention.

Throughout this time, the concept of *sepsis* was modified as a result of advances in understanding the mechanisms that produce it. These highlights that the clinical manifestations of sepsis are attributed to the infectious agent, the immune response it generates, changes in coagulation parameters, immunosuppression and organ dysfunction (1).

Currently, according to the third international consensus, sepsis is known as *sepsis-3* and is a life-threatening organ dysfunction, recognizing the severity and lethality triggered by a pathogen that invades the body (2, 3). Organ dysfunction is defined as an acute change in the total Sequential Organ Failure Assessment (SOFA) score of at least 2 points as a result of infection (4).

The current available data on the incidence of cutaneous-onset sepsis is limited. Some reports indicate that cutaneous and soft tissue infections cause 14 million visits per year in the US and approximately 900,000 hospitalizations annually. In Europe the situation is similar, with 1,250 episodes of acute skin infections reported in emergency department, of which 3.3% had sepsis (5, 6, 7).

Skin and soft tissue infections (SSTIs) are prevalent in the elderly, with a varied clinical spectrum from mild infections to septic complications. The prognosis becomes severe depending on comorbidities such as heart failure, diabetes mellitus, anemia. SSTIs represents a management challenge, especially in emergency hospitals and chronic care institutions, where their prevalence is significant - 10.9% and 17% (8).

Clinical risk factors which are influencing the prognosis are: age - patients over 65 years of age are vulnerable, due to a inefficient immune system, to act appropriately on the bacterial invasion; comorbidities - cardiac, oncological, renal or any other medical condition that compromises immunity and decreases the body's ability to defend itself (diabetes, AIDS, transplant, immunosuppressive drugs, hypoalbuminemia); invasive devices: peripheral or central venous catheters, urinary catheters, mechanical ventilation; antibiotic resistance may favor the occurrence of sepsis (9).

MATERIALS AND METHODS

This retrospective observational study included 441 patients with acute bacterial skin infections admitted to the Dermatology Clinic of “Sf. Spiridon” County Clinical Emergency Hospital Iasi, between January 2020 and December 2022. The study was conducted after obtaining the approval of the Institutional Ethics Committee. The main objective is to illustrate the importance of skin manifestations in correlation with risk factors, all of there influencing the evolution and prognosis of the disease.

363 had not complicated skin infections and 78 had sepsis. The risk factors followed were: age, gender, smoking, obesity, thrombotic history, usage of medical devices (probes, catheters). Inclusion criteria: patients over 18 years of age, local and/or systemic clinical signs of infection; skin manifestations with loss of tissue (ulcerations, erosions, fissures, excoriations) associated with inflammatory phenomena - swelling, erythema, pain; bacteriological examination of the skin lesion with antibiogram on admission to the dermatology department. Exclusion criteria: patients

under 18 years of age, with SARS-Cov2 infection, patients with sepsis other than skin sepsis, severe alteration of mental status and patients with negative bacteriological examination.

Sepsis was defined based on SIRS at infection (minimum 2 criteria from: temperature > 38°C or < 36°C, tachycardia, tachypnea, leukocytosis/leukopenia) and the confirmation of bacterial agents in cultured samples from the skin lesions. The bacterial agent was identified by bacteriological examination collected (on admission to the clinic) on single-use tube swab with transport medium (Amies). The collected sample was transported within the first 4 hours to the hospital microbiology laboratory, where culture and antibiotic susceptibility testing was performed.

Statistical analysis was performed using the *SPSS version 29.0* software package (SPSS Inc., Chicago, IL, USA). Categorical variables were presented as frequencies and percentages, and continuous variables as mean ± standard deviation. Categorical variables were compared between groups using the Pearson Chi-squared test.

RESULTS

Demographic characteristics

In our study, the average age of the patients with sepsis is 61.33 ± 15.022 years. There are no statistically significant differences in terms of gender distribution (52.6% are male and 47.4% are female) and in terms of distribution by environment (rural patients predominate, but the percentages are close - 53.8% rural and 46.2% urban).

TABLE I.

Demographic characteristics

	Total		Acute Skin lesions with Sepsis		Acute Skin lesions without Sepsis	
	N	%	N	%	N	%
Sex						
male	248	56.2%	41	52.6%	207	57.0%
female	193	43.8%	37	47.4%	156	43.0%
Environment						
urban	188	42.6%	36	46.2%	152	41.9%
rural	253	57.4%	42	53.8%	211	58.1%
Total	441	100%	78	100%	363	100%

TABLE II.

Mean age of patients with acute skin infections with sepsis *versus* without sepsis.

Patients of Study	N	Mean	St. dev.	St. error	Min	Max	Median
Acute skin infections with sepsis	78	61.33	15.022	1.701	18	88	62.0
Acute skin infections without sepsis	363	65.23	14.056	0.738	18	92	65.0

Sepsis present/absent: Mann-Whitney U = 12122.000; p = 0.046*.

Lifestyle

Among the risk factors (table 3, fig.1), smoking is notable in the majority of patients included in the study, with no increased risk of developing sepsis in smokers with acute infections. Chronic staphylococcal carrier status does not predispose to an additional risk for sepsis, as there are

numerous species of the skin microbiome that are harmless as long as the skin barrier is not disrupted. In contrast, acute infections in chronic carriers of invasive medical devices, such as urinary catheters, associate a 9.757-fold increased septic risk. The same is for patients with a history of thromboembolic disease, where the risk of sepsis is 2.165 times higher, as found in 24.4% of patients with sepsis, compared with 12.9% of acute skin infections.

10.3% of patients with sepsis had prolonged immobilization and only 5,5 % of patients without sepsis, a difference that is not statistically significant. 24.4% of patients with sepsis had PTE or a history of thrombosis compared to only 12,9% of the group without sepsis, a difference that is statistically significant and is associated with a 2.165-fold increased risk in patients with PTE or a history of thrombosis of developing sepsis.

TABLE III.
Risk factors

	Total		Acute Skin lesions with Sepsis				Pearson Chi-squared		OR (OddsRatio) 95% CI / RR
	N	%	N	%	N	%	Chi^2	p	
Smoking							.391	.532	-
present	246	55.8%	46	59.0%	200	55.1%			
absent	195	44.2%	32	41.0%	163	44.9%			
Obesity							.047	.828	-
present	148	33.6%	27	34.6%	121	33.3%			
absent	293	66.4%	51	65.4%	242	66.7%			
Chronic carrier of *S.aureus*							1.700	.192	-
present	69	15.6%	16	20.5%	53	14.6%			
absent	372	84.4%	62	79.5%	310	85.4%			
Urinary catheter carrier							10.023	.010*	9.757 (1.755÷ 54.253)
present	6	1.4%	4	5.1%	2	0.6%			
absent	435	98.6%	74	94.9%	361	99.4%			
Prolonged immobilization							2.433	.127	-
present	28	6.3%	8	10.3%	20	5.5%			
absent	413	93.7%	70	89.7%	343	94.5%			
Pulmonary thromboembolism (PTE) / Thrombosis History							6.570	.010*	2.165 (1.187÷ 3.949)
present	66	15.0%	19	24.4%	47	12.9%			
absent	375	85.0%	59	75.6%	316	87.1%			
Total	441	100.0%	78	100.0%	363	100.0%			

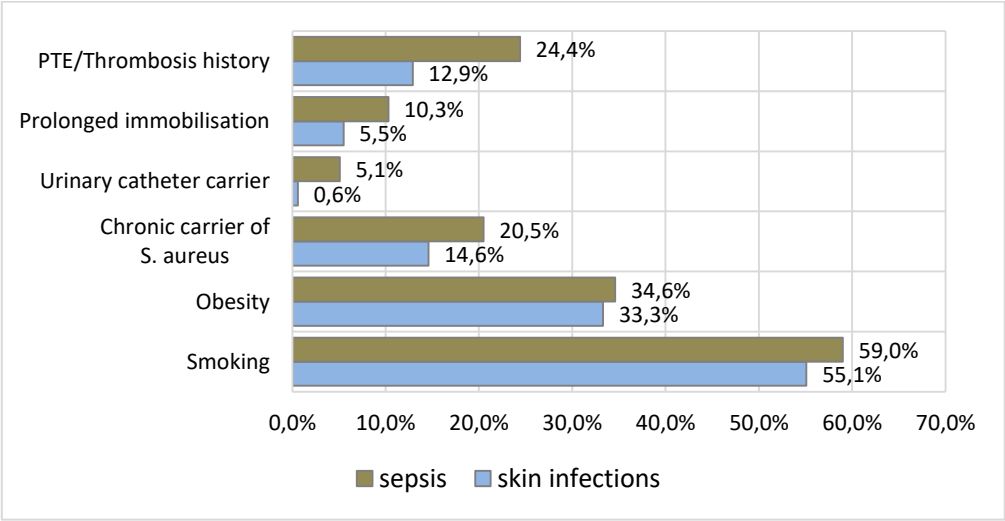

Fig. 1. Risk factors for skin sepsis *versus* acute skin infections

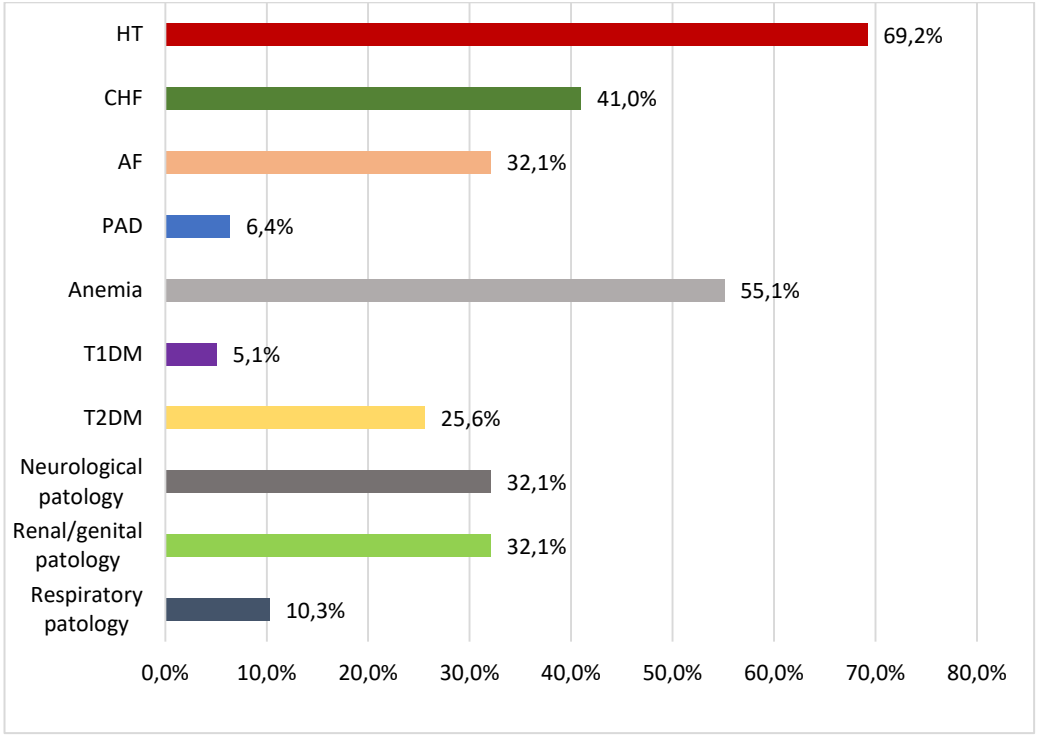

Fig. 2. Comorbidities of patients with sepsis.

Regarding the associated pathologies of patients with sepsis (fig. 2), it was found that cardiovascular diseases: hypertension (HT), chronic heart failure (CHF), atrial

fibrillation (AF), extremity peripheral arterial disease (PAD), have a significant percent. Essential hypertension occurs in 69.2% of patients, atrial fibrillation in 32.1% and heart failure in 41%. Type 1 and type 2 diabetes mellitus are common among the studied population: 5.1% of patients with sepsis have type 1 diabetes mellitus compared to 25.6% of patients with type 2 diabetes mellitus. Microcytic hypochromic anemia was also present for 55.1% of all cases. Extremity peripheral arterial disease was found in 6.4% while the proportion of neuro-psychiatric disorders (such as stroke or depressive disorders and their consequences) was of 32.1%.

The polymorphous spectrum of the cutaneous and systemic manifestations in patients with sepsis (Fig. 3) illustrates in 89.7% of cases ulcerations, erosions, fissures or excoriations. Pain was discovered in 96.2%, tumefaction in 92.3% and edema was observed in 80.8% of septic cases. The presence of bullae or vesicles is not systematically associated with sepsis, as only 21.8% of patients have this symptomatology. Pruritus was observed in 29.5% of cases.

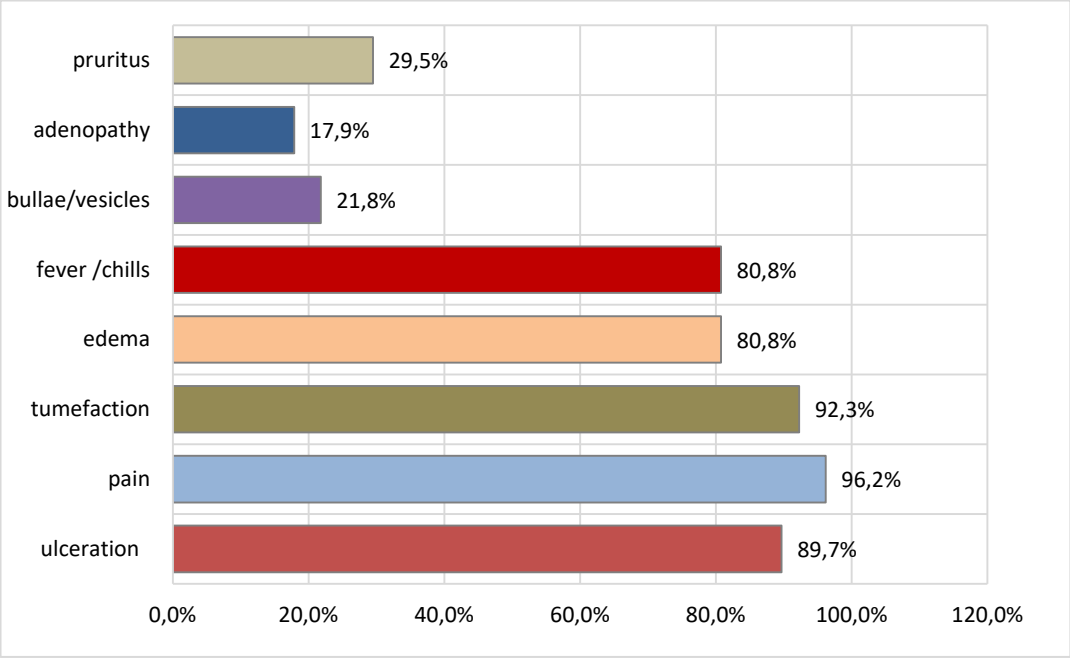

Fig. 3. Local and systemic clinical manifestations.

Another element investigated in patients with sepsis was the pathogen agent. Among the bacteria identified by culture from the cutaneous lesions (fig. 4), the data analysis shows that in 34.6% the culture is positive for *P. aeruginosa*; in 37.2% cases the culture reveals *S. aureus*, in 17.9% - *E. coli* and in 32.1% shows other bacterial species such as *P. Mirabilis*, *Serratia marcescens*, *Citrobacter freundi*, *Providencia stuartii*, *Stenotrophomonas maltophilia*, *Morganella morganii and Enterobacter*.

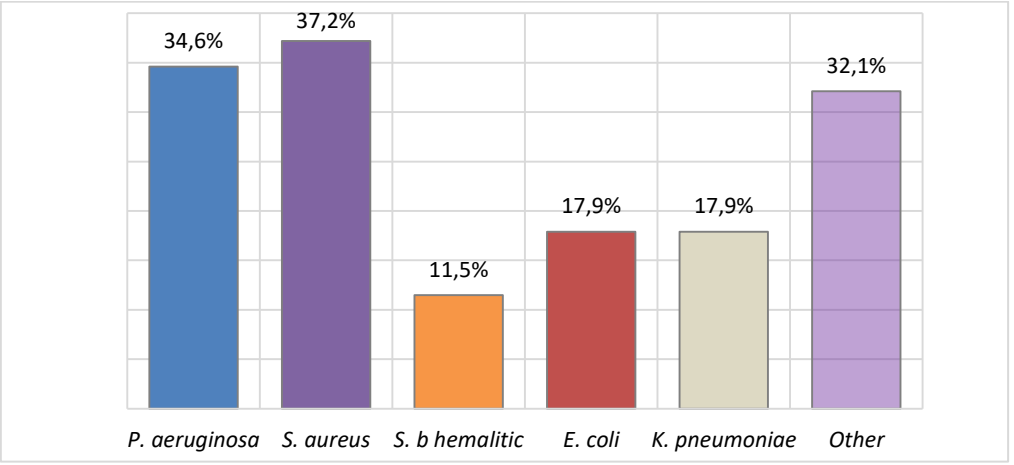

Fig. 4. Distribution of bacterial etiological agents.

DISCUSSION

Although there is lot of documented information on acute infectious dermatoses, the medical literature is poor in data related to the incidence of cutaneous-onset sepsis. By analyzing data from 441 patients with skin infections - some with septic complications - the skin-sepsis correlation was established, based on clinical risk factors grouped into seven essential steps.

Analysis of demographic characteristics

Age is a key parameter in the sepsis evolution, but recent research shows that the severity of comorbidities and organ dysfunction are closely correlated with severe prognosis (7, 10). Sepsis affects all age groups, but its incidence and mortality increase considerably with age. The complex interaction of environmental, genetic and lifestyle factors contributes to the development of chronic diseases and geriatric syndromes that decrease the length and quality of life. An emerging risk factor for age-related diseases is the microbiome. Elderly people are vulnerable to developing sepsis due to comorbidities, compromised immune function, sarcopenia, diminished physiological reserves, malnutrition and polymedication. Immunosenescence and inflammation are key factors that increase susceptibility to sepsis. Immunosenescence involves a gradual decrease in the immune system, especially T lymphocyte function (11).

There are numerous studies showing that sepsis is more prevalent among men than women. The differences between men and women in sepsis prevalence is explained on the basis of physiological characteristics, men's greater exposure to physical activity, but also biological aspects, concerning hormonal peculiarities and the immune system. Estrogens induce effective cellular and humoral mediated immune responses, while androgens have suppressive effects (12).

People from rural areas are known to have limited and delayed access to health services, such as primary and secondary health care. Limited access on low developed areas contributes to delays in the

management of sepsis and comorbidities, leading to higher mortality rates (13).

Lifestyle assessment

Smoking influences the body's inflammatory response through increased release of pro-inflammatory factors. In addition, smoking stimulates oxidative stress, which induces systemic inflammation. Smoking is also associated with immunosuppressive effects, reducing the patient's resistance to infection (14). Given the reported effects of smoking on proinflammatory mediators, including TNF-alpha, interleukin 6 and 8, severe cases of sepsis with higher mortality in smoking patients have been described, due to the immunosuppressive influences of smoking. All these are making the host less able to fight infection (15).

Hwa Lee *et al*. showed that the incidence of sepsis was higher in active smokers (especially those who smoked more than 30 pack-years) than in former smokers and non-smokers (16).

Obesity - a clinical condition in which weight is significantly greater than recommended (body mass index (BMI) > 30 kg/m^2, according to the World Health Organization definition) is usually attributed to excessive fat accumulation in the body. Statistically, it is involved in the pathophysiology of many diseases, impacting on global health (17).

Currently, the relationship between obesity and sepsis is incompletely elucidated. On the one hand, some studies show that obesity increases the risk of sepsis or other acute infections (17,18). On the other hand, study findings illustrate the *obesity paradox* for which the mortality rate from sepsis in obese patients is lower than in similar patients with lower BMI. (19, 20). The concept of obesity paradox is explained by three pathophysiological hypotheses: a) excess adipose tissue and stored energy support the catabolic state induced by sepsis; b) the systemic inflammatory response may be different in obese patients than in normal weight patients, with an important role for visceral fat, which is associated with metabolic syndrome; c) adipocytes may be immunomodulatory, as obese patients have lower levels of proinflammatory cytokines than expected during severe infections (21, 22). Increased levels of lipoproteins and adipose tissue may also bind and inactivate lipopolysaccharides, which are involved in septic pathophysiology. Also, levels of the proinflammatory cytokine interleukin-6 are reduced in obese patients (23, 24).

Patients with sepsis are affected by coagulation disorders. They present an increased risk of thrombotic complications, which may vary from microvascular changes such as disseminated intravascular coagulation to venous thromboembolism in the form of deep vein thrombosis or pulmonary embolism. The pathophysiological processes in sepsis occur simultaneously, and the correlation between the mechanisms of coagulation and inflammation has been described by Engelmann and Massberg as *immunothrombosis*. This explains the complex and mutual interaction from which the activation of the coagulation cascade triggers the immune system and innate immune cells stimulate thrombus development (25).

Prolonged bed rest and lack of physical activity have significant consequences for the body's well-being, with disorders on the musculoskeletal, cardiovascular, respiratory, integumentary and cognitive systems (26).

Identification of invasive medical devices

The use of any type of invasive device increases the risk of infection. Most commonly used are intravascular catheters (peripheral and central), urinary catheters, endotracheal catheters. The risk of infection increases with the duration of exposure. It has been estimated that approximately 50% of urinary tract infections (UTIs) are associated with the presence of a urinary catheter, and up to 64% of bloodstream infections are associated with a vascular device (27). In our study there were also patients with sepsis, who had urinary catheters, but sepsis was not due to urinary causes but to the skin one, an aspect highlighted by the bacteriological examination from the skin lesion (one of the inclusion criterion).

Clinical examination of skin lesions

Acute infectious dermatoses most of the time start suddenly with soft tissue erythema, induration, local heat, pain, erosions or fissures, to which general symptoms such as fever, deterioration of vital functions can be added, up to multiple organ dysfunction in case of septic complications. Detailed analysis of local and systemic manifestations allows a correct diagnosis to be made and appropriate treatment to be rapidly instituted (7). In the studied groups, skin semiology revealed the presence of lesions with loss of tissue: erosions, fissures, ulcerations, excoriations, bullae/blisters. These completed the clinical picture of dermatological pathologies such as venous ulcers, microbial eczema, cellulitis, erysipelas, pemphigus, pemphigoid, polymorphic erythema, Steaven Johnson's disease, erythroderma.

The appearance of lesions with loss of tissue and their superinfection are the main clinical warning signs of cutaneous sepsis. Dynamic observation and assessment by the dermatologist allow therapeutic measures to be applied before sepsis occurs (fig. 5). These could be essential steps in the clinical algorithm of the skin changes.

Identification of the microbial agent in skin lesions

Any disturbance of the skin microbiome exposes the skin to microbial invasions - some of these concerns the superficial layers (ecchymoses, boils, folliculitis, etc.), others progress deeper, as in cellulitis, erysipelas, erythroderma, necrotizing fasciitis. Pathogen agents enter into the skin through hair follicles or through minor skin lesions with loss of tissue. Among common bacterial species there are penicillin-sensitive or penicillin-resistant S. aureus and group A Streptococcus β- hemolytic (28). Streptococcal species are involved in the pathogenesis of cutaneous infections through activation of superantigen T cells by streptococcal toxins, followed by a specific T cell response (29). Some bacterial infectious agents can induce an altered immune response that underlies the etiopathogenesis of autoimmune diseases, such as systemic sclerosis. The presence of increased titers of specific autoantibodies causes immunosuppression (30, 31).

Assessment of comorbidities

Associated pathologies have been carefully assessed as they influence the progression of skin infections and sepsis, both by accelerating inflammatory mechanisms and by involving coagulation mechanisms. The patients of our study presented a series of typical representative systemic diseases, which can be systematized into cardiovas-

cular, metabolic, neurological, respiratory, renal and genital diseases.

Acute inflammatory mechanisms of infections increase the risk of cardiovascular events. The activation of inflammatory molecules and platelets, increased inflammation in atheromatous plaques, endothelial dysfunction and increased activity of the sympathetic nervous system with catecholamine release contribute to the electrical instability of the heart and atherosclerotic plaque but also to a concomitant hypercoagulable state (32).

Data published by Bohula *et al*. illustrate that the most common cardiovascular comorbidities in patients with sepsis are heart failure, valvular disease, arrhythmias and ischemic heart disease (33). Consistent with this, our study highlights the increased proportion of hypertension, atrial fibrillation, heart failure and obliterating arteriopathy of the lower limbs for patients with cutaneous sepsis.

Acute skin infections of patients with diabetes include multiple diseases, from folliculitis to more severe infections involving deep tissues, diabetic foot and sepsis. They are the leading cause of morbidity and mortality. Poor blood glucose control is closely correlated with infectious risk. Elevated glucose levels are associated with tissue damage, through irreversible, non-enzymatic glycation of proteins, impaired immune response and generation of reactive oxygen species, which play a critical role in cellular homeostasis (34).

The results of our study illustrate that diabetes is a common comorbidity in patients with cutaneous sepsis, particularly for type 2 diabetes. However, the impact of diabetes on the evolution and prognosis of sepsis is controversial, and there is sufficient argument to confirm that the presence of diabetes does not increase the risk of mortality in patients with sepsis, but is associated with an increased risk of acute renal failure (35).

Altered mental status in patients with sepsis is an acute alteration in cognitive and psychological function and level of consciousness, which may manifest as confusion, behavioral changes and changes in alertness. Sepsis-associated encephalopathy (SAE) is a complex neurological syndrome contributing to diffuse brain dysfunction. It can manifest itself through a range of symptoms, from agitation and restlessness to delirium and coma. Although SAE appears to be a reversible pathophysiological process centered on systemic inflammation, there is sufficient evidence that sepsis can cause structural brain damage and long-term neurological consequences (36).

The current definition of sepsis (sepsis-3), clarifies the altered mental status of sepsis, measured by the Glasgow Score (GSC), which is found in both the Sequential Organ Failure Assessment (SOFA) score and the Quick SOFA (qSOFA). The SOFA score is used to assess organ failure, which evaluates the degree of respiratory, renal, cardiovascular, neurological, hepatic and hematological impairment. The qSOFA is used in the emergency department for the rapid calculation of organ failure, considering respiratory rate, hypotension and Glasgow score (37, 38).

Other concomitant systemic conditions that patients with sepsis presented with were renal and genital diseases such as lower urinary tract infections and chronic kidney disease. Although the percentage was 10.3% for respiratory comorbidities, these included chronic obstructive pulmonary disease (COPD) and asthma.

Clinical algorithm for cutaneous lesions

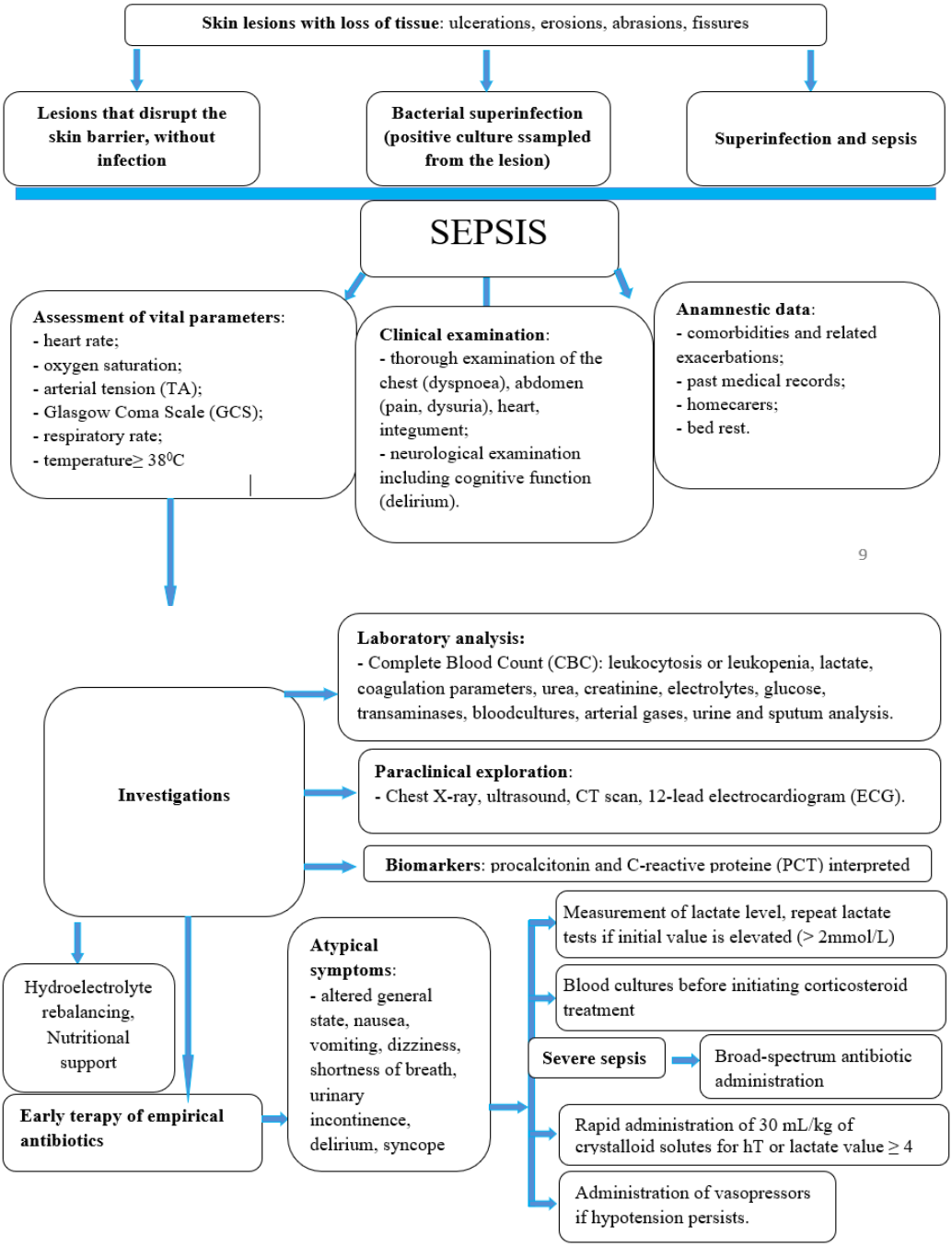

Fig. 5. Clinical algorithm for skin lesions

CONCLUSIONS

The skin, through changes related to the disruption of the skin microbiome, provides many clues to severe conditions, which can be prevented by strategies targeted on early miscellaneous skin disorders. A detailed analysis of over 400 patients with acute skin infections admitted to the dermatology clinic showed that minor skin disorders (fissures, erosions, etc.) can lead to serious systemic complications with a negative impact on life quality. A simple but careful inspection of the skin surface performed by the dermatologist provides useful information in assessing the extent and depth of the lesion, local microcirculation and hydration. Erosions, fissures, excoriations are skin lesions with loss of tissue, which were present on the patients included in the study (one of the inclusion criteria). They represent the coutaneous-onset sepsis and therefore the proper management of these lessions has an important role in preventing sepsis. The number of skin lesions, their surface are important dermatological characteristics, but the severity of the acute infection depends on the aggressiveness of the bacterial agent which invades the body, depends on the host's immunity and associated pathologies. Correlation with lifestyle and systemic symptoms - cardiovascular, metabolic, neuropsychiatric - are essential prognostic indicators in cutaneous sepsis. The usage of the qSOFA score in dermatological practice is an effective and rapid measure for detecting cutaneous sepsis when correlated with the cutaneous semiology. Most of the time skin lesions are not just a dermatological condition, but are the clinical expression of internal organ imbalances. The dermatologist's involvement in the management of the critically ill patient, who also associates cutaneous lesions, is necessary in order to establish their primary or secondary nature and makes an important contribution in differentiating cutaneous sepsis from other types of sepsis.

CONFLICTS OF INTEREST AND FUNDING

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ARTERIAL STIFFNESS ASSESSMENT IN ABDOMINALLY OBESE INDIVIDUALS: INSIGHTS FROM CLINICAL OBESITY PHENOTYPES

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ARTERIAL STIFFNESS ASSESSMENT IN ABDOMINALLY OBESE INDIVIDUALS: INSIGHTS FROM CLINICAL OBESITY PHENOTYPES (Abstract): Precise cardiovascular risk assessment remains challenging, especially amidst the dynamic interplay of factors like abdominal obesity, inadequate physical activity and imbalanced diet. This study aims to assess subclinical atherosclerosis in apparently healthy individuals with excess weight, using arterial stiffness as a parameter for cardiovascular risk assessment. **Materials and methods**: A cohort of 67 patients aged 35-75 years, without any prior atherosclerotic pathology, with abdominal obesity and no known chronic illnesses, underwent evaluation. The cohort was stratified based on obesity (BMI≥30kg/m^2), metabolic syndrome (MetS NCEP-ATP III criteria), BMI class, total or trunk adipose tissue percentage, and clinical phenotypes of obesity (metabolically healthy obese - MHO, metabolically unhealthy obese - MUO, metabolically healthy non-obese - MHNO, metabolically unhealthy non-obese - MUNO), where unhealthy is defined by the presence of MetS. Biochemical analysis was performed to assess glucose, TG and HDL-c levels, aiming to detect potential undiagnosed cases of MetS. Adiponectin and leptin measurements were also performed. Hologic QDR Delphi A determined whole body composition with dual-energy-X-ray absorptiometry (DEXA) and Arteriograph® Tensiomed measured parameters of subclinical atherosclerosis. **Results**: Pulse wave velocity (PWV) values did not significantly differ across study groups, including obese versus non-obese individuals, clinical phenotypes of obesity, presence or absence of MetS, groups defined by BMI class, or adipose tissue percentages. Nevertheless, participants with excess weight or with MetS presented lower AIXa values. The groups that did not present MetS (obese and non-obese) showed the highest proportion of patients with PWV values under the age-adjusted theoretical threshold (46.15% and 44.4%, respectively), suggesting a potentially lower vascular age than the biological one. Conversely, the groups with MetS (obese and non-obese) had smaller percentages (26.67% and 16.66%, respectively). Around 30% of patients across clinical obesity phenotypes exhibited PWV values exceeding the theoretical threshold, but under 10 m/s. **Conclusions**: AIXa values are lower in the presence of MetS and in higher BMI subjects, whereas PWV values do not change significantly. Healthy obese individuals demonstrate a vascular age similar to the healthy non-obese group, suggesting enhanced cardiovascular protection. On the other hand, arterial stiffness is reported in a higher percentage of subjects that present MetS (obese or non-obese) compared to subjects without MetS, even though the syndrome is at an early stage. **Keywords**: OBESITY, ADIPOSE TISSUE, ARTERIAL STIFFNESS, SUBCLINICAL ATHEROSCLEROSIS, METABOLIC SYNDROME.

INTRODUCTION

Chronic inflammation, a key feature of the pathophysiology of obesity, is associated with increased arterial stiffness, along with other known cardiovascular risk factors including age or systolic blood pressure (1-3). Nevertheless, studies are conflicting concerning the relationship between adipose tissue and vascular rigidity. Several analyses reported higher pulse wave velocity (PWV) values in patients with obesity measured by BMI or trunk adiposity (4-6), while other studies support the inversed relationship, commonly referred to as the "obesity paradox". Without any doubt, obesity as defined by BMI increases the risk of cardiovascular events. However, this does not consider the percentage of whole-body or trunk adipose tissue, nor the visceral adipose tissue. Therefore, this seemingly paradoxical relationship may be explained by the fact that BMI primarily reflects lean body mass rather than adiposity and the latter is a more robust predictor of cardiovascular mortality and morbidity (7).

After a cardiovascular event a trend toward better prognosis has been observed in the overweight and obese population, linked to the same phenomenon of obesity paradox (8-10). Vascular function has been shown to be unexpectedly better in severely obese individuals compared to moderate or even normal-weight subjects. Even though they exhibit high inflammation, they may still experience partial protection against atherosclerosis, and one possible explanation is enhanced mobilization of endothelial progenitor cells (11).

Accurate estimation of cardiovascular risk remains a challenge, particularly in the context of dynamic changes in metabolic pathways influenced by the presence of modifiable factors like smoking, low dietary intake of fruits and vegetables, insufficient physical exercise, abdominal obesity, diabetes, hypertension, dyslipidemia or non-modifiable factors like age, sex, personal or family history of cardiovascular disease, ethnicity, genetic evidence. This study aims to examine the relationship between arterial stiffness and different measures of obesity in apparently healthy subjects, with no known chronic diseases, in order to reduce at a minimum the bias of factors.

MATERIALS AND METHODS

This prospective study was conducted over a two-year period and included 67 participants recruited based on the following criteria: no history of chronic disease and no prior atherosclerotic events. Pregnant women and individuals who declined to provide informed consent prior to enrolment were not included in the study. All investigations were undertaken by trained personnel within the same clinical and laboratory setting, utilizing standardized equipment and analytical methods throughout the study duration.

Anthropometric parameters were measured using a tape meter at the umbilical (waist circumference) and greater trochanter (hip circumference) level. Whole body composition measurements were assessed with Dual-Energy X-ray Absorptiometry (DEXA) technique, using Hologic QDR Delphy A fan-beam (Hologic Inc., Massachusetts). The biochemical profile for each participant at the study was performed using the spectrophotometric method for

triglycerides, HDL-c and glucose, enzyme-linked immunosorbent assay (ELISA) for adiponectin and leptin, chemiluminescence technique for insulin.

Arterial stiffness was assessed in participants using the Arteriograph® Tensiomed (Budapest, Hungary). The following parameters were measured: pulse wave velocity (PWV), augmentation index (AIXa), aortic pulse pressure (PPao), aortic systolic blood pressure (SBPao), mean arterial pressure (MAP), reflection time (RT), and ejection time. The device's cuff was positioned on the dominant arm, and the length of the aorta up to the iliac bifurcation was estimated based on anthropometric measurements (the distance between the pubic symphysis and the suprasternal notch) (12). To ensure standardized conditions, participants were instructed to rest quietly in a controlled environment prior to the examination, with no alcohol, tobacco or caffeine in the last 10 hours. Talking or movement during measurements was not allowed (13). After recording blood pressure, the device applied an additional pressure of 35 mmHg to facilitate the analysis of pulse wave reflections. The accompanying software calculated the PWV and other hemodynamic parameters.

The study cohort was divided in groups dependent on the obesity status, MetS status and body composition. Four obesity clinical phenotypes were described: metabolically healthy obese (MHO) or non-obese (MHNO) and metabolically unhealthy obese (MUO) or non-obese (MUNO). The obesity status was present if participants presented BMI≥30 kg/m^2. The metabolically unhealthy status was present if the participant presented MetS, which was defined in patients with waist circumference > 88 cm (women) / 104 cm (men) and that presented at least 2 criteria: glucose ≥ 100 mg/dL, HDL-C < 40 mg/dL (men) / < 50 mg/dL (women), TG ≥ 150 mg/dL, TAS/TAD ≥ 130/85 mmHg (14).

The process of data was handled with Microsoft Excel v.16.64 (Microsoft Corporation, USA) and *SPSS version 23.0* (IBM Corporation, USA). Kolmogorov-Smirnov/ Shapiro-Wilk tests were used for assessing data distribution. For normally distributed variables, values of the mean±standard deviation were reported and for non-normally distributed variables, values of the median, along with quartiles. Categorical variables are reported as percentages. For comparative analysis between continuous variables, the independent parametric test one-way ANOVA was used for a normal data distribution and the non-parametric test Mann-Whitney U for a non-normal distribution. For correlation analyses, the Pearson, Kendall's tau-b and Spearman correlation tests were performed. Values of $p<0.05$ were considered statistically significant.

RESULTS

This study included 67 apparently healthy but overweight patients, in whom subclinical atherosclerosis indicators were measured to assess the cardiovascular risk. Among the arterial stiffness parameters, only AIXa, RT, MAP and ejection duration showed a normal distribution according to Shapiro-Wilk test. Table I presents the general data of the parameters evaluating the biochemical profile and subclinical atherosclerosis in the study group. The comparative analysis of the PWV value

between groups did not reveal statistically significant differences, but significant differences were observed regarding the AIXa values (fig. 1).

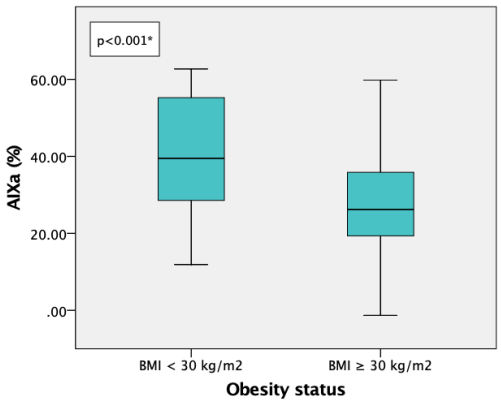
Within the entire sample, of all the anthropometric, biochemical and adipose tissue-related parameters, PWV correlated only with the waist-to-hip ratio. Conversely, AIXa was negatively associated with anthropometric parameters (tab. II).

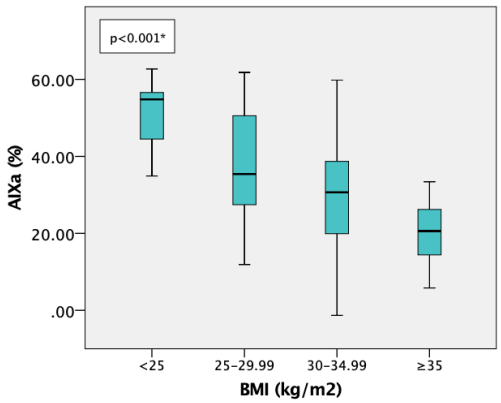
TABLE I.

Descriptive parameters of the study group

Parameter	Mean±S.D.	Min	Max	Median (Q3-Q1)
Parameters of subclinical atherosclerosis				
PWV	9.90±1.76	7.2	14.9	9.7 (2.2)
AIXa	31.87±15.36	-1.3	62.7	30.4 (22.1)
PP	50.09±15.05	29.5	103.1	45.4 (14)
SBPao	138.23±20.94	109.9	233.1	134.8 (21.6)
PPao	52.09±12.65	33	92	50 (12)
MAP	105.49±13.43	83	161	105 (17)
RT	113.84±18.35	71	153	115 (20)
Ejection time	305.22±31.01	215	370	305 (40)
Biochemical parameters				
TG	154.70±92.53	49	488	125 (100)
HDL-c	52.78±14.47	20	85	52 (20)
Glucose	113.43±39.73	76	336	103 (22)
Insulin	18.98±12	2.13	52	16.10 (12.01)
HOMA-IR	5.56±4.74	0.49	27.25	3.85 (3.51)
Adiponectin	13.80±5.41	4.53	28.53	12.96 (7.14)
Leptin	22.93±13.77	2.60	48.98	18.72 (24.05)

*SD= standard deviation, Q3-Q1=difference between superior quartile and inferior quartile.





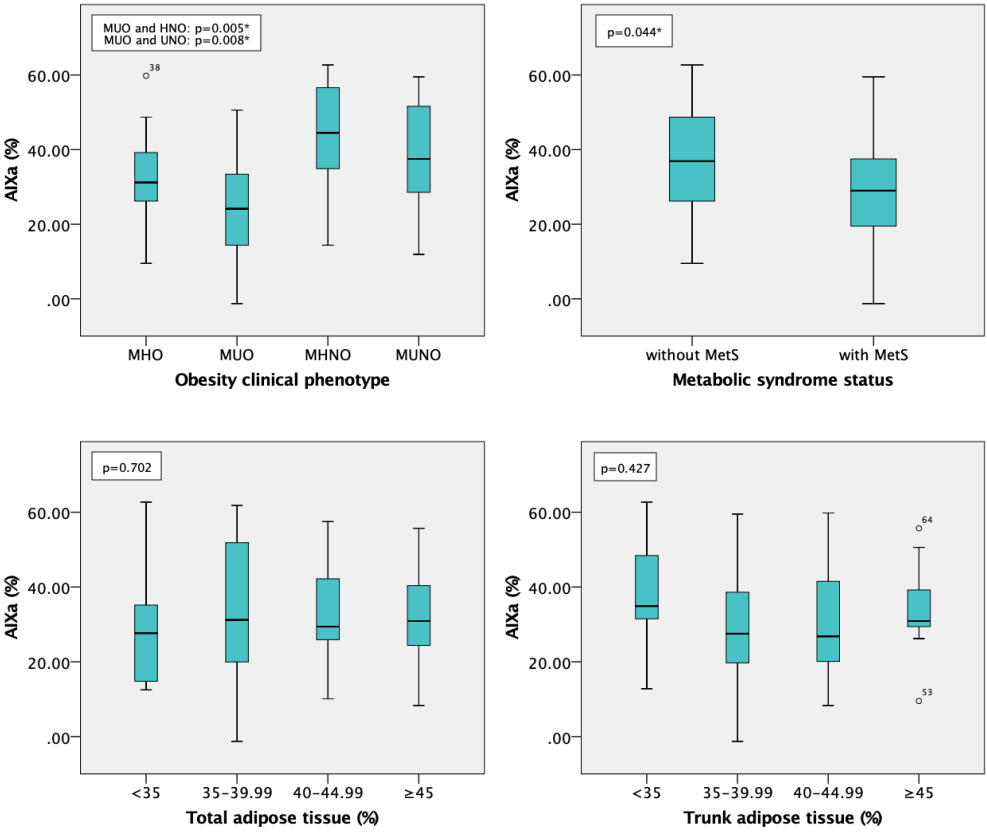


Fig. 1. Comparison of AIXa parameter value across the study groups.

TABLE II.

Correlation between adipose tissue parameters and cardiovascular risk

		PWV	AIXa*	PPao	SBPao
BMI*	r	0.03^{K}	-0.52^{P}	-0.19^{K}	-0.8^{K}
	p	0.75	<0.001	0.03	0.33
Waist circumference	r	0.20^{S}	-0.30^{K}	-0.17^{S}	0.03^{S}
	p	0.10	<0.001	0.16	0.80
Waist-height ratio	r	0.16^{S}	-0.26^{K}	-0.18^{S}	-0.02^{S}
	p	0.19	0.002	0.14	0.85
Waist-hip ratio	r	0.16^{K}	-0.25^{P}	-0.05^{K}	0.13^{K}
	p	0.02	0.04	0.51	0.12
Total adipose tissue (%)	r	-0.08^{S}	0.05^{K}	0.01^{S}	-0.13^{S}
	p	0.49	0.51	0.93	0.29
Trunk adipose tissue (%)*	r	-0.01^{K}	-0.12^{P}	-0.04^{K}	-0.07^{K}
	p	0.87	0.33	0.63	0.42

*normal distribution, PWV=pulse wave velocity, AIXa=aortic augmentation index, PPao=aortic pulse pressure, SBPao=aortic arterial systolic pressure, P=Pearson, S=Spearman, K=Kendall's tau.

From the entire cohort of patients, 61.20% exhibited normal PWV values. Within this subgroup, nearly half presented values below the theoretical PWV, derived from a database of approximately 10,000 measurements from a Central European population. Table III details the distribution of patients and corresponding percentages, stratified by clinical obesity phenotypes.

No statistically significant differences in biomarkers, anthropometric measurements, or adipose tissue parameters were observed between patients with normal PWV values and those with PWV values exceeding 10 m/s within the MHO, MHNO, MUNO subgroups. Statistically significant data for the MUO subgroup are presented in table IV.

TABLE III.

Comparative analysis of real PWV with theoretical PWV across study groups

	MHO	MUO	MHNO	MUNO	Total
Patients with PWV < theoretical value	6	5	4	4	19
Patients with PWV > theoretical value. but under 10 m/s	3	11	3	5	22
% patients with PWV > theoretical value. but under 10 m/s	33.33%	68.75%	42.86%	55.56%	53.66%
Patients with PWV < 10 m/s	9	16	7	9	41
Patients with PWV ≥ 10 m/s	4	14	2	6	26
% patients with PWV ≥ 10 m/s	30.77%	46.67%	22.22%	40%	38.80%

TABLE IV.

Parameters exhibiting statistically significant differences between the MUO group with vascular stiffness and the MUO group without vascular stiffness.

	PWV < 10 m/s			PWV ≥ 10 m/s			
Results of the one-way ANOVA parametric test							
Parameter	Mean ± S.D.	C.I. 95%		Media ± S.D.	C.I. 95%		p
		I.L.	S.L.		I.L.	S.L.	
Adiponectin	13.43±4.30	11.14	15.72	9.92±3.77	7.74	12.10	0.02
Waist circumference	109.19±10.34	103.68	114.69	117.86±8.81	112.77	122.95	0.02
Total adipose tissue (%)	40.39±5.50	37.46	43.32	36.33±5.12	33.37	39.28	0.05
Results of the one-way Mann-Whitney U non-parametric test							
Parameter	Median	Q1-Q3		Median	Q1-Q3		
Insulin	14.45	18.7-21.45		17	30.65-45.22		0.05
HOMA-IR	3.46	4.56-5.26		4.45	10.24-12.77		0.04

*S.D.= standard deviation, C.I.=confidence interval, I.L.= inferior limit, S.L.= superior limit, Q1= inferior quartile, Q3= superior quartile.

DISCUSSION

The present study reports new data on the association between the four obesity clinical phenotypes and cardiovascular risk, also exploring the correlations between markers of subclinical atherosclerosis and

adiposity parameters. Arterial stiffness, as assessed by PWV, AIXa, and PP, is an independent risk factor for cardiovascular mortality (15). Our results suggest that while PWV and PP do not significantly discriminate between groups regardless of obesity status, clinical phenotype of obesity, presence of metabolic syndrome, or body composition, AIXa reports statistically different values. These findings emphasize the need for further studies to clarify the role of AIXa in the assessment of cardiovascular risk. This marker is not as well studied as PWV, but it is associated with known risk factors for cardiovascular disease (16, 17).

In obesity, AIXa has been reported to have both elevated (18) and decreased values (19, 20). In our study, obese patients overall have lower AIXa values than non-obese patients, as well as those with MetS compared to those without MetS. In particular, at the clinical phenotype level, only MUO report significantly lower values than MHNO and MUNO. Consequently, we can assume that obesity together with MetS represents an association that reports low AIXa values in the studied population. Our findings are contrary to those of several previous investigations included in the systematic review and meta-analysis performed by Li P., *et al.* that showed increased PWV and AIX values in obese or overweight subjects (21). Nevertheless, our results are consistent with other investigations that emphasize a better outcome after percutaneous coronary interventions in subjects with high BMI (22) or even improved vascular function in healthy but obese individuals (11).

The decrease in AIXa values could be explained by the effect of insulin. It has a vasodilatory role by stimulating nitric oxide synthesis (23, 24), leading to increased vascular wall elasticity and altered aortic wave reflection. Several studies have reported lower arterial stiffness in obese and/or MetS patients compared to normal-weight patients, due to insulin (25-28). At the same time, metabolic insulin resistance may have vascular effects through non-enzymatic glycation processes leading to collagen cross-linking or through sympathetic activation and smooth muscle cell proliferation, leading to stiffness (29, 30). We cannot establish a cause-and-effect relationship in our study, but insulin and HOMA-IR values are higher in obese patients, with MetS or MetS clinical phenotypes. We can consider the acceptable quality of endothelial function observed in these patients as a consequence of the diagnosis of an early stage of MetS in the study group, with minimal metabolic changes, given that the patients included in the study were apparently healthy. Moreover, we must also consider the fact that the AIXa parameter is influenced by heart rate and does not represent such a reliable parameter of vascular stiffness as PWV.

In the studied population, PWV value is associated with MetS and not with obesity, since the prevalence of patients with abnormal PWV values is higher in MetS patient groups, regardless of the presence of obesity. Recent studies have demonstrated that there is no association between PWV level and obesity (31, 32), but a significant impact of MetS on PWV was confirmed in a prospective, multicenter study with patients from 18 European countries (33). Furthermore, another study identifies PWV as a more effective parameter than SCORE-2 or other grids that measure the risk of cardiovascular disease, to estimate vascular age in patients with MetS (34).

Adiponectin is lower in patients with

abnormal PWV values, although the percentages of total and non-adipose tissue at the trunk level show similar values to those of patients in the normal PWV group. Hypoadiponectinemia has been identified in coronary atherosclerosis and acute coronary syndrome and has been associated with increased arterial stiffness (35, 36).

Several studies have reported a higher cardiovascular risk in MUO patients than in MHO (37, 38). The MHO phenotype in our study presents the highest percentage of patients with PWV below the theoretical value, more precisely below the mean value corresponding to the patient's age. This result implies a lower vascular age than that associated with the patient's biological age, suggesting additional cardiovascular protection. A study conducted on 2,076 participants supports our results, concluding that the progression of arterial stiffness was not increased in MHO patients, contrary to the result obtained for MetS groups, where regardless of the presence or absence of obesity, the evolution of arterial stiffness was significantly unfavorable (39).

CONCLUSIONS

PWV values do not differ significantly in apparently healthy individuals with abdominal obesity, regardless of BMI, MetS, adipose tissue percentage or clinical obesity phenotypes. AIXa values are lower in the presence of MetS and in subjects with higher BMI, however its values are influenced by different factors and therefore it is not such a reliable parameter of vascular stiffness as PWV. Healthy obese individuals demonstrate a vascular age similar to the healthy non-obese group, meanwhile there is an important increase in the number of subjects with MetS-associated arterial stiffness, even though the MetS is at an early stage. Therefore, as long as healthy obese do not develop MetS, their vascular rigidity is considered more likely to be at the level of that for non-obese.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest or funding.

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A QUALITATIVE STUDY ON THE CAUSES OF MEDICAL MALPRACTICE COMPLAINTS

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A QUALITATIVE STUDY ON THE CAUSES OF MEDICAL MALPRACTICE COMPLAINTS (Abstract): Medical malpractice complaints represent a genuine challenge for both medical and legal scholars. In recent years, there has been a notable increase in the number of complaints filed against physicians for alleged acts of medical malpractice. **Materials and methods:** To achieve our objective, we conducted a qualitative study in which data were collected through semi-structured interviews. The participants in our study were lawyers with experience in handling medical malpractice cases. **Results:** A total of 14 lawyers participated in the study. Thematic analysis of the collected data led to the identification of 9 main themes and 21 subthemes. The central focus of this article is the investigation of the causes underlying medical malpractice complaints. According to the lawyers interviewed, these causes include dysfunctions in the physician-patient relationship, professional errors, and systemic shortcomings. **Conclusions:** Our research indicates that the reasons behind medical malpractice complaints are multifaceted. The results of this study may serve as a foundation for developing strategies aimed at reducing the number of malpractice complaints in the healthcare sector. **Keywords:** MEDICAL MALPRACTICE, DOCTORS, LAWYERS, MEDICAL LIABILITY.

INTRODUCTION

The healthcare sector is considered a top priority within the broader framework of social concerns (1). In contemporary medicine, patients are increasingly regarded as active participants in the provision of care, holding both rights and responsibilities (2). According to the specialized literature, the causes underlying medical malpractice complaints are diverse, including misdiagnosis, procedural errors (3), and other similar factors.

Filing a complaint against a healthcare professional is viewed as a normal and legitimate mechanism in a democratic society. Although recent technological advancements in the medical field have been significant, the number of malpractice complaints against physicians continues to rise (4). Identifying the causes of medical malpractice complaints is of critical importance, as such insights can help reduce litigation within the healthcare system (5).

The aim of our study was to determine the reasons why patients file medical malpractice complaints against physicians.

MATERIALS AND METHODS

To achieve the objectives of our research, we employed a qualitative methodology, with data collected through semi-structured interviews. The target population consisted of lawyers who had handled at least one case involving a medical malpractice complaint.

Both male and female lawyers participated in the study, regardless of their years of professional experience. Prior to participation, the lawyers were informed about the purpose, objectives, and procedures of the study, and each provided written informed consent.

The interview began with introductory questions related to the participants' legal specialization, their motivation for choosing this area of practice, as well as questions concerning their experience in handling medical malpractice cases. In addition to these, the interview included 19 open-ended questions. Among the topics explored were the reasons patients file complaints against physicians, as well as the lawyers' perspectives on alternative methods of dispute resolution in medical malpractice cases.

The interviews were fully transcribed, and the data obtained were analyzed using NVivo 14 software (trial version). The responses were grouped into nine areas of interest, which were initially coded through a review of the interview transcripts. These codes were subsequently organized into subthemes. The subthemes were identified based on their frequency of occurrence among multiple participants. Responses were labeled with the letter "A" followed by Arabic numerals corresponding to the order in which the interviews were conducted.

This study was approved by the Research Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy of Iași, through Decision No. 135/23.01.2022.

RESULTS

1. Causes of Medical Malpractice Complaints

In Romania, there are no predefined legal grounds for which a physician can be accused of medical malpractice. However, judicial practice has identified several common causes of malpractice complaints, including misdiagnosis, incorrect treatment, failure to intervene, and so on.

The lawyers who participated in our interviews provided detailed insights regarding both the physician-patient relationship and the broader systemic issues within the healthcare sector. Based on their responses, two main subthemes emerged: one related to the therapeutic relationship and the other concerning systemic dysfunctions in the medical system.

1.1. Dysfunction in the Physician-Patient Relationship

Physicians represent a cornerstone of the healthcare system, alongside other professionals involved in medical care. In the course of their daily activities, they come into contact with a significant number of individuals, each of whom is affected by a medical condition. The main relational issues identified by the interviewed lawyers that lead patients to file complaints against physicians revolve around a breakdown in communication.

The physician-patient relationship is fundamentally based on mutual trust and

respect. In fact, the Medical Code of Ethics issued by the Romanian College of Physicians states, under Article 14 titled "Prior and Appropriate Information of the Patient," that "The physician shall request and obtain informed consent only after the person concerned, or the person legally entitled to give consent for the medical intervention, has been adequately informed about the purpose and nature of the intervention, as well as the foreseeable and generally accepted risks and consequences, in accordance with medical standards."

Similarly, Article 8 of the Patient Rights Law No. 46/2003 stipulates: "Information shall be communicated to the patient in a respectful and clear manner, minimizing the use of medical terminology. If the patient does not understand the Romanian language, the information shall be provided in their native language or a language they understand, or, if necessary, through alternative means of communication."

In practice, when it comes to informing the patient, legal provisions are clearly in place. This means that failure to comply with these requirements may result in disciplinary or legal sanctions for the physician. In this regard, issues related to lack of transparency and communication breakdowns were frequently reported by the lawyers we interviewed.

"This lack of transparency is about the fact that, in the medical field, no one really has time to explain things like, 'we're about to perform a procedure that involves certain risks, certain issues.' They just put that informed consent form in front of you, which obviously needs to be signed. They rely on the assumption that the patient will read it… but let's be honest-out of ten patients, nine don't read what they sign; they just want the medical problem to be resolved. I think it should be like in the movies, where the doctor always explains to the patient, 'Look, there are some risks involved; we want you to make an informed decision.' Basically, that's what informed consent is supposed to mean, but expressed in a much more accessible, down-to-earth way that everyone can understand. Many people who come to the hospital or to the doctor don't have a formal education, they don't understand medical terminology, so it's absurd to just say, 'Here's the paper, you signed it, we're no longer responsible.'" (A2)

"There are cases where the patient's next of kin or family members are completely unaware of what's happening with the patient, and that's very… maybe it has some legal justification to a certain extent, but it's really hard to explain to someone that it's okay not to know anything about their mother or father who is in the hospital." (A2)

"After an error happens-or is suspected to have happened-or when the treatment doesn't yield the expected outcome, or there's disappointment regarding the result, the patient or family tries to contact the doctor or the medical facility. And at that point, the doctor shuts down all communication. In general, 90% of cases are like this. They stop responding to patients, or if they do meet them, they immediately start saying it's not their fault. They're unable to look at the situation from the perspective that they might have made a mistake. This violation of the patient's right to be informed-even after the medical act has taken place, about what happened and why-is deeply harmful. It's a non-material value that gets violated, and this happens in 99.9% of cases. When patients

hire us, I ask them: 'Why?' And they tell me, very clearly, 'I called, they wouldn't answer. They told me to stop calling, to do whatever I want.' It's a violation of human dignity, and that is what actually drives them to move forward with legal action." (A6)

1.2. Professional Errors

According to the opinions expressed by the study participants, another significant cause of medical malpractice complaint is the professional error committed by the physician.

"Patients are dissatisfied with the quality of medical care and with the refusal to provide medical assistance." (A9)

"In this case, medical protocols were violated, and the doctor essentially confused one illness for another. My client was repeatedly treated for adnexitis and pelvic inflammatory disease. But in reality, she had cancer. The symptoms are similar, but the most serious mistake the doctor made was that-after about seven hospitalizations-on the final lab report, the laboratory personnel explicitly recommended further in-depth testing. The doctor failed to inform my client about this and did not refer her for the additional tests." (A14)

There is no medical system in which those practicing within it do not make mistakes, regardless of how advanced or efficient that system may be. More importantly, the core issue does not lie in attempting to eliminate all errors-which is virtually impossible-but rather in how the consequences of such errors are managed and addressed.

1.3. Deficiencies in the Healthcare System

Other causes of complaints filed by patients are related to hospitalization conditions. The state of hospital infrastructure appears to contribute significantly to patients' dissatisfaction, many of whom hold physicians accountable for the overall situation. Although substantial investments have been made in Romania's healthcare system in recent years, these efforts have not been sufficient to meet patient expectations or ensure a satisfactory level of care.

"They [the patients] are primarily dissatisfied with the hospital conditions." (A4)

DISCUSSION

1. Causes of Medical Malpractice Complaints

Medical malpractice complaints can be filed by any individual, as the legislator grants patients the freedom to exercise this right. From a legal standpoint, there are no sanctions imposed on individuals who choose to submit such complaints.

To better understand the magnitude of medical malpractice causes, attention should be given to a study conducted in 2010 in the United States, which reported that, by the end of 2008, the Physician Insurers Association of America (PIAA) database contained information on approximately 239,756 resolved malpractice claims filed between 1985 and 2008.

The same study highlighted the most common medical errors, which included diagnostic mistakes, lack of physician knowledge, failure to properly monitor the patient, medication administration errors, failure or delay in referring patients to a specialist, failure to recognize adverse treatment effects, omission of clear instructions, and ineffective communication with the patient (6, 7).

1.1. Dysfunction in the Physician-Patient Relationship

The physician-patient relationship represents a key aspect of our research. The use of communication techniques that facil-

itate interaction between doctor and patient may be a decisive factor distinguishing physicians who have been subject to malpractice complaints from those who have not-primarily due to the loss of patient trust resulting from poor communication (8).

Effective communication between physicians and patients directly influences patient behavior and reduces the likelihood of filing medical malpractice complaints (9). This idea is also supported by the participants in our study, who indicated that one of the main causes of malpractice complaints is indeed the dysfunctionality of the physician-patient relationship.

Another viewpoint found in the specialized literature emphasizes that, in the modern world, medical malpractice complaints are less often rooted in the quality of care provided, and more frequently stem from the nature of the relationship between the two key actors in medical care-the physician and the patient (10). A lack of communication skills on the part of the physician increases the probability that the patient will file a complaint, regardless of whether a medical error actually occurred (11).

Similarly, a point highlighted by participants in our study is that patients tend to file complaints against physicians they do not feel a personal affinity with, as they often experience a sense of being ignored. Consequently, when a healthy, trust-based relationship is established between physician and patient, it serves as an important preventive factor against potential complaints (12). Moreover, a relationship built on trust necessarily involves effective communication, which strengthens the patient's sense of being respected-ultimately reducing the likelihood of initiating a malpractice claim (13)

1.2. Professional Errors

In line with the perspectives expressed in the specialized literature, the most common causes of medical malpractice are diagnostic errors, as well as delays in establishing a diagnosis (14). The literature, consistent with the views of the participants in our study, emphasizes that professional errors-particularly misdiagnosis-rank as the primary reason for filing medical malpractice complaints (15).

This finding is further supported by another study indicating that among the most frequent reasons patients are encouraged to file complaints against medical personnel are misdiagnosis, improper execution of medical procedures, and medication errors (16)-all of which fall under the category of professional mistakes. A separate study conducted in 2012 demonstrated that medical errors occurred both during the anamnesis phase and during the period in which the patient was under clinical observation (17).

1.3. Deficiencies in the Healthcare System

According to the participants in our study, one of the frequent reasons for filing medical malpractice complaints is represented by systemic shortcomings. This observation is supported by the specialized literature, with studies indicating that certain healthcare systems face ongoing challenges related to patient safety and the quality of care provided (18).

The literature further suggests that insufficient investment in the healthcare system can lead to medical errors, which undoubtedly reflect structural deficiencies within the system (19). Additionally, research shows that one of the causes for filing medical malpractice complaints is the violation of regulations by healthcare insti-

tutions (20). For example, a study conducted in the United States estimates that between 44,000 and 98,000 hospital deaths annually are attributable to medical management issues (21), which supports the claims made by participants in our research. Moreover, an important insight derived from another study indicates that harm resulting from "error," in its broadest sense, also encompasses systemic issues related to the organization and delivery of healthcare services (22)

CONCLUSIONS

Our research indicates that the underlying reasons for medical malpractice complaints are complex. Thus, patient complaints filed against physicians are not solely based on clinical errors, but also stem from dysfunctional relationships between doctors and patients, as well as from broader issues affecting the healthcare system as a whole.

The findings highlight the need for physicians to pay particular attention to the way they communicate and interact with patients, as this significantly influences the likelihood of a complaint being filed. Even though our national healthcare system is not among the most advanced globally, medical professionals continue to make substantial efforts to provide healthcare services that meet international standards.

The results of our study can serve as a foundation for developing strategies aimed at reducing the number of complaints in the medical sector.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding.

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COMPARATIVE SURGICAL APPROACHES FOR EARLY-STAGE HEPATOCELLULAR CARCINOMA. A SINGLE-CENTER EXPERIENCE

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COMPARATIVE SURGICAL APPROACHES FOR EARLY-STAGE HEPATOCELLULAR CARCINOMA. A SINGLE-CENTER EXPERIENCE (Abstract): Hepatocellular carcinoma (HCC) remains a major global health concern, ranking among the top causes of cancer-related mortality. This study aims to assess and compare the outcomes of three curative surgical approaches-hepatic resection (HR), radiofrequency ablation (RFA), and liver transplantation (LT)-in patients with early-stage HCC. **Materials and methods:** We conducted a prospective analysis of 105 patients with BCLC stage 0-B HCC treated between 2015 and 2020 at a tertiary center in Romania. Patients were divided into three treatment groups: Group I (n=64) underwent HR, Group II (n=29) received RFA, and Group III (n=12) underwent LT according to Milan Criteria. Baseline liver function, tumor characteristics, operative details, and postoperative outcomes were recorded. The HR group was further stratified by transection technique (Habib device vs. crush-clamping). **Results:** The mean age differed significantly across groups (p=0.013), with LT patients being younger. Child-Pugh class A was predominant in HR (84.38%) and RFA (72.41%) groups, while B and C were more frequent in LT. Blood loss was significantly lower with the Habib device compared to crush-clamping (p=0.003). No recurrence was recorded in the LT group during follow-up, while recurrence rates were 56.52% for RFA and 31.67% for HR (p=0.003). The overall postoperative complication rate did not differ significantly among groups. Early mortality rates were 6.25% (HR), 10.34% (RFA), and 8.33% (LT). Our findings suggest that all three treatment modalities can be effective for early-stage HCC when selected according to patient-specific liver function and tumor burden. HR and RFA remain viable first-line options in compensated liver disease, while LT provides the most durable oncologic outcomes but is limited by eligibility and organ availability. The use of the Habib device, may reduce intraoperative blood loss in hepatic resections. **Conclusions:** Hepatic resection and radiofrequency ablation remain valid treatment options for early-stage HCC in patients with well-compensated liver function, while liver transplantation offers the most favorable long-term oncologic outcomes when applied within established criteria. A multidisciplinary, patient-centered approach is essential to maximize survival and minimize recurrence in HCC management.
Keywords: HEPATOCELLULAR CARCINOMA, LIVER RESECTION, RADIOFREQUENCY ABLATION, LIVER TRANS-PLANTATION, BCLC STAGE, SURGICAL OUTCOMES.

Hepatocellular carcinoma (HCC) is the most prevalent form of primary liver cancer and represents a major global health burden, both in terms of incidence and mortality. According to the latest global cancer statistics, liver cancer ranks as the sixth most commonly diagnosed malignancy and the third leading cause of cancer-related deaths worldwide, accounting for over 840,000 new cases and more than 800,000 deaths annually (1). Among these, HCC constitutes approximately 75% to 85% of all primary liver malignancies (2). The burden of HCC is not uniformly distributed; its incidence is highest in East and Southeast Asia, sub-Saharan Africa, and increasingly in Western countries, where lifestyle-related liver diseases are on the rise (3).

The pathogenesis of HCC is closely linked to chronic liver injury and inflammation, with cirrhosis being the most significant risk factor (4). Cirrhosis often results from chronic hepatitis B or C infection, excessive alcohol consumption, nonalcoholic steatohepatitis (NASH), autoimmune hepatitis, or genetic conditions such as hemochromatosis (5). In recent years, a paradigm shift in the etiology of HCC has been observed. While viral hepatitis remains a dominant cause in endemic regions, there is an alarming increase in HCC cases associated with metabolic disorders, particularly NAFLD and NASH, in both developed and developing countries (6). This trend is anticipated to continue, with the declining incidence of HCV-related HCC due to the widespread availability of direct-acting antivirals (DAAs) being counterbalanced by a growing prevalence of obesity, insulin resistance, and type 2 diabetes (7).

Despite notable advancements in diagnostic modalities and therapeutic interventions, the overall prognosis of HCC remains dismal. The asymptomatic nature of early-stage HCC and the lack of universally adopted screening programs contribute to late diagnoses in many patients(8). Consequently, only a small fraction of patients is eligible for curative treatments at the time of diagnosis(9). The cornerstone of curative therapy in HCC comprises three main strategies: hepatic resection (HR), liver transplantation (LT), and local ablative therapies such as radiofrequency ablation (RFA) (10, 11). The selection among these modalities depends on a delicate balance between tumor characteristics (number, size, location, and vascular invasion), liver function reserve, performance status, and comorbidities.

The Barcelona Clinic Liver Cancer (BCLC) staging system is widely endorsed, especially in Europe and Latin America, as a comprehensive framework for stratifying patients and guiding therapeutic decision-making (12). The BCLC system classifies HCC into five stages (0, A, B, C, and D) and recommends treatment options accordingly. Curative approaches are typically reserved for very early (stage 0) and early-stage (stage A) disease, which includes patients with preserved liver function, a solitary lesion or up to three nodules less than 3 cm, and an absence of portal hypertension or vascular invasion. For such patients, HR, LT, and RFA are considered appropriate curative strategies (9).

Hepatic resection remains the first-line treatment for patients with a single tumor and preserved liver function (typically Child-Pugh A), especially when the tumor is located in a favorable anatomical position and sufficient functional hepatic reserve is maintained (13).

Modern surgical techniques, enhanced imaging modalities, and better perioperative management have contributed to decreased morbidity and mortality following liver resection. However, recurrence remains a significant challenge, with reported rates of intrahepatic recurrence ranging from 50% to 70% within five years postoperatively (14).

RFA, a minimally invasive technique, has gained widespread acceptance as an alternative to resection in selected cases. It is particularly suitable for small tumors (<3 cm), especially in patients unfit for surgery due to comorbidities or poor liver function. RFA offers the advantages of shorter hospitalization, lower complication rates, and repeatability. However, its limitations include incomplete tumor necrosis, the heat-sink effect in perivascular tumors, and higher recurrence rates compared to surgical resection (15, 16).

Liver transplantation is considered the ideal treatment for patients with early-stage HCC and advanced liver disease, as it simultaneously removes the tumor and treats the underlying cirrhotic liver. The Milan Criteria-defined as a solitary tumor ≤ 5 cm or up to three nodules ≤ 3 cm without vascular invasion or extrahepatic spread-have become the benchmark for selecting transplant candidates due to their association with excellent post-transplant outcomes (17). Several centers have proposed expanded criteria (e.g., UCSF, up-to-seven, and Toronto criteria), which may further extend access to LT while maintaining acceptable survival outcomes (18-20). Despite its benefits, LT is limited by organ shortage, waiting list mortality, and complex perioperative management. In countries with underdeveloped organ donation systems, such as Romania, access to transplantation remains restricted (21).

The clinical decision-making process in HCC is further complicated by the coexistence of tumor progression and liver dysfunction. Scoring systems such as the Child-Pugh score and the Model for End-Stage Liver Disease (MELD) score are commonly used to assess liver function and determine surgical candidacy (22). The Child-Pugh score, although widely used, incorporates subjective elements such as ascites and encephalopathy. In contrast, the MELD score is an objective, continuous variable based on serum creatinine, bilirubin, and INR, and is particularly useful in organ allocation for transplantation (23).

The role of advanced imaging modalities-contrast-enhanced ultrasonography, multi-detector CT, and liver-specific MRI-cannot be overstated in the accurate diagnosis and staging of HCC (24). These modalities not only guide treatment planning but also reduce the need for invasive biopsies. Nonetheless, biopsy remains necessary in cases of atypical imaging patterns or when differentiating HCC from cholangiocarcinoma or metastases is clinically relevant (25).

In the Romanian healthcare system, characterized by limited resources and evolving transplant infrastructure, treatment decisions are influenced by institutional capabilities and availability of therapeutic modalities. Our center, located in Northeastern Romania, represents a tertiary referral institution where HR and RFA have been established for over a decade, whereas LT was only introduced in 2016. This context presents a unique opportunity to evaluate and compare outcomes across all three curative modalities for early-stage HCC within a single center, using standardized staging and inclusion criteria.

Given these considerations, the present study aims to provide a comprehensive analysis of clinical outcomes associated with hepatic resection, radiofrequency ablation, and liver transplantation in patients with BCLC stage 0-B hepatocellular carcinoma. Furthermore, the study evaluates technical aspects such as blood loss, resection margins, ischemia times, and intraoperative incidents, thereby contributing to the refinement of surgical strategies in early-stage HCC.

MATERIALS AND METHODS

The study was assessed and approved by the ethics committee of the "Sf. Spiridon" County Clinical Emergency Hospital from Iasi (No. 479/07.10.2024), and written informed consent was obtained from all patients. A prospective analysis was conducted, related to 105 patients selected in our Department of General Surgery and Liver Transplantation between 2015 and 2020, for surgical treatment, for stages BCLC 0-B. The inclusion criteria were: age ≥ 18 y.o., primary malignant tumor (histologically proved or overt imaging and laboratory HCC criteria), no macroscopic vascular invasion, no extrahepatic extension.

The 105 patients were divided into 3 groups: Group I, 64 patients who underwent LR, with 2 subgroups: I A, to whom we used the Habib US device liver transection, I B to whom we used crush clamping transection technique, Group II, 29 patients who had RF, and group III, 12 patients who underwent LT, all fulfilling Milan Criteria. This last group has been considered since October 2016 when we started the LT program. None of the resection or RF groups presented with previous surgery or synchronous malignancies so that they received a primary surgery. In the LT group,5 patients had previously one or more seances of TACE.

Prior to surgery, all patients underwent an accurate clinical assessment, laboratory work-up and imaging evaluation, including thorax and abdominal imaging (US, contrast-enhanced CT, MRI with liver specific contrast agent), percutaneous biopsy. Diagnostic laparoscopy and biopsy were necessary for indeterminate lesions non-accessible to percutaneous biopsy. Data about age distribution, etiology of the underlying chronic liver disease, Child-Pugh score, αFP, number and distribution of the tumors were recorded for all patients.

Specific data included the type of hepatectomy, intraoperative blood loss, tumor histology, and study of the resection margin for the resection group, as well as the waiting time on the list, the number and size of the nodules and operative parameters (cold ischemia time, warm ischemia time, blood loss, intraoperative transfusions, intraoperative incidents) in the LT group. Postoperative morbidity, surgical revision, early postoperative mortality, tumor recurrence, distant metastases, survival and hospital stay were evaluated for the three groups.

RESULTS

The male/female ratio in the overall group was 74/31. Patient characteristics over the 3 groups are seen in table 1. The mean age was higher in the resection group and as predicted was the smallest in the liver transplantation group due to the limit of age on the waiting list (< 65 years). The etiology of the underlying liver disease was mostly alcoholic and viral for all the three groups. The HBV infection was more frequent in the resection group, but in the radiofrequency group the infection with

HCV had higher incidence. Less than 20% of the patient had antiviral therapy in the resection group and less than 10% in the other 2 groups (tab. I).

TABLE I.
Patient characteristics

No patients (%)		Group I (HR)	Group II (RF)	Group III (LT)	
		64	29	12	
Mean age (years)		63.53	62.79	50.33	ANOVA test - F (2,102) =4.355; p=0.013
Etiology n, (%)	HBV	14 (21.8)	8 (27.58)	2 (16.66)	Pearson-χ2 - χ2=8.746; df=4; p=0.064
	HCV	13 (20.3)	9 (31.03)	3 (25)	
	HBV+ D	7 (10.93)	2 (6.89)	2 (16.66)	
	Toxic	23 (35.9)	7 (24.13)	4 (33.33)	
	Metabolic syndrome	6 (9.3)	3 (10.34)	1 (8.33)	
Antiviral therapy n, (%)	Entecavir	6 (9.3)	1 (3.44)	1 (8.33)	Pearson-χ2 - χ2=9.835; df=4; p=0.072
	IFN	2 (3.12)	0 (0)	0 (0)	
	IFN-Free	4 (6.25)	1 (3.44)	0 (0)	
Child-Pugh score n, (%)	A	54 (84.38)	21 (72.41)	2 (16.66)	Pearson-χ2 - χ2=31.997; df=4; p<0.001
	B	8 (12.5)	7(2414)	5 (41.67)	
	C	2 (3.12)	1 (3.45)	5 (41.67)	
AFP n, (%)	1-9 ng/mL	29 (45.31)	7 (25)	9 (75)	Pearson-χ2 - χ2=9.396; df=4; p=0.052
	10-99 ng/mL	25 (39.06)	15 (53.57)	3 (25)	
	>100 ng/mL	10 (15.63)	6(21.43)	0	

The main symptoms encountered within the clinical presentation were: pain, weight loss, loss of appetite, asthenia and fever. Abdominal US identified suspect nodule/s in all cases (100%), which were further assessed as malignant / HCC by MDCT - 93.33% and MRI - 53.33%; PET-CT was used in 4 patients (3.8%) to exclude distant metastases. The percutaneous biopsy was performed in39 cases (37.14%) with non-specific or indeterminate imaging. In 21 patients (20%), laparoscopic biopsy was necessary.

For HR group, the type of resection, intraoperative blood loss and the study of the resection margin are presented in table 2, either for the use of Habib device transection technique or crush clamping procedure. The compared parameters revealed a statistically significance regarding the intraoperative blood loss between the Habib group and the crush clamping group (p= 0.003).

Twenty-nine patients underwent RF, by open approach -17 (58.6 %), laparoscopic - 10 (34.48%) and percutaneous - 2 (6.89%).

For the LT group, the waiting time on the list was 29-456 days (mean 177.5 days). The number of tumors were: single in 7 patients, two in 3 patients and three in 2 patients (mean number of lesions 1.58), with a maximal diameter of 15-50 mm (mean 24.16 mm).

Regarding the intraoperative parameters, cold ischemia time was 90 to 330 minutes (mean 206.25 minutes) and warm ischemia time 33 to 60 minutes (mean 41.25 minutes). The blood loss during the

LT procedure varies between 1800 mL and 9000 mL (mean 4908.33 mL).

The necessary of transfusion according to the intraoperative blood loss and intraoperative incidents are disclosed in table III.

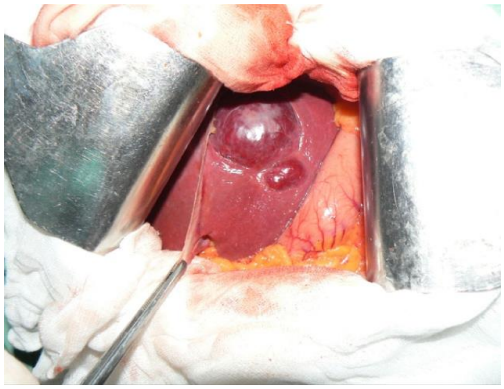

Fig. 1. Intraoperative aspect of hepatocarcinoma in the left hepatic lobe

Fig. 2. Intraoperative aspect of atypical hepatectomy with Habib

TABLE II.

Hepatic resection- operative parameters

Group I - HR: Operative parameters		Habib	Crush clamp	p
Types of hepatectomy	Segmentectomy	15 (51.72%)	14 (48.28%)	p=0.821
	Bisegmentectomy	6 (66.67%)	3 (33.33%)	p=0.356
	Sectionectomy	5 (45.45%)	6 (54.55%)	p=0.545
	Left hepatectomy	1 (20%)	4 (80%)	p=0.892
	Right hepatectomy	2 (20%)	8 (80%)	p=0.781
Intraoperative blood loss	Mean (mL)	344.48	1015.71	p=0.003
	Median (mL)	300	560	p=0.072
	SEM	54.58	204.66	p=0.081
	Minimum (mL)	50	140	p=0.340
	Maximum (mL)	1500	6000	p=0.280
Resection margin	R_0	27	33	p=0.432
	R_1	2	2	p=0.057

TABLE III.

LT-Operative parameters

	Blood loss (mL)	Transfusions	Intraoperative incidents
1	1800	500 mL RCM	
2	6500	1400 mL RCM+ 2850 mL FFP; 1200 mL cell saver	
3	2000	500 mL RCM	
4	9000	2000 mL RCM 4 U. BP 1200 mL FFP	
5	3800	1000 mL RCM; 1600 mL autotransfusion (cell saver)	

	Blood loss (mL)	Transfusions	Intraoperative incidents
6	2400	1500 mL RCM	Portal thrombectomy
7	2200	750 mL RCM; 800 mL PCC; 1100 mL autotransfusion (cell saver)	Redoing arterial anastomosis (Incongruity donor -recipient HA)
8	5800	900 mL RCM; 1000 mL PCC; 1400 mL autotransfusion (cell saver)	
9	8100	1200 mL RCM; 600 mL PCC	
10	4300	600 mL RCM + 300 mL PCC; 600 mL autotransfusion (cell saver)	Eversion portal thrombectomy
11	8000	1500 mL RCM; 300 mL PCC 350 mL autotransfusion (cell saver)	Redoing the PV anastomosis due to the donor PV fragility
12	5000	800 mL RCM; 600 mL FFP + 1 g fibrinogen	Median arcuate ligament syndrome

RCM = red cell mass; PCC- prothrombin cellular concentrate; FFP: fresh frozen plasma

Tumor histology according to the pathology report for group I and III confirmed HCC in 71 cases representing 93,42% of 76 cases, the remaining cases being fibrolamellar carcinoma (3 cases in HR group) and intrahepatic cholangiocarcinoma (1 in HR group and 1 in LT group). All patients from group RF had a previous confirmation of HCC by biopsy.

Overall postoperative morbidity and the type of postoperative complication on each group are presented in table IV. The incidence of complications is not significantly different between the three groups (χ^2=0.533; df=2; p=0.766).

Surgical revision was required in the whole groupin5.71% cases, in6.25% cases in the resection group, 3.44% cases in the RF-ablation group and 16.67% in the LT group (χ^2=2,027; df=2; p=0,363).

TABLE IV.

Postoperative complications

Complications n, (%)	HR,19 (29.68%)	RF, 4 (13.7%)	LT 3 (25.00%)
Respiratory	10(15.62)	2 (6.89)	1 (8.33)
Liver failure	7 (7.81)	0 (0)	0 (0)
Ascites	12 (18.75)	2 (6.89)	3 (25.00)
Biliary fistula	3 (4.68)	0 (0)	0 (0)
Hemoperitoneum	2 (3.1)	1 (3.44)	1(8.33)
Liver abscess	1 (3.44)	0 (0)	0 (0)
Wound infection	8 (12.5)	0 (0)	0 (0)
Renal failure	3 (4.68)	1 (3.44)	1 (8.33)
MSOF	1 (3.44)	0 (0)	1 (8.33)

Mean hospital stay in groups LR/RF/LT was 12.63/6.69/28.58 days respectively. The difference was significant for groups II and III ($p<10^{-3}$).

Recurrence was significantly higher in the RF group (13 patients representing 56.52%) compared with HR group (19 patients -31.67%) (χ^2=11.575; df=2; p=0.003); no recurrence was noted in the LT group until the end of the follow-up

period. The mean interval until recurrence was 13.25±7.96 months.

Distant metastases were diagnosed in12 cases, representing 12.63% of patients of the whole study group, with the following location: lung - 6 cases (50%), bones - 4 (33.34%), both bone and lung and abdominal wall in one case each (8.33%). The mean period until diagnosis of distant recurrence was 13.33±5.68 months.

The overall early postoperative mortality (<30 days) was 6.67%, also higher in RF group (10.34%), compared with HR group (6.25%) and LT group (8.33%) (χ^2=1.506; df=2; p=0.471). The overall survival is presented in figure 2. The overall survival for the first two groups was influenced by the recurrence and the underlying liver disease, 49,9 months. For LT group, 11 patients were alive at the end of the follow-up period.

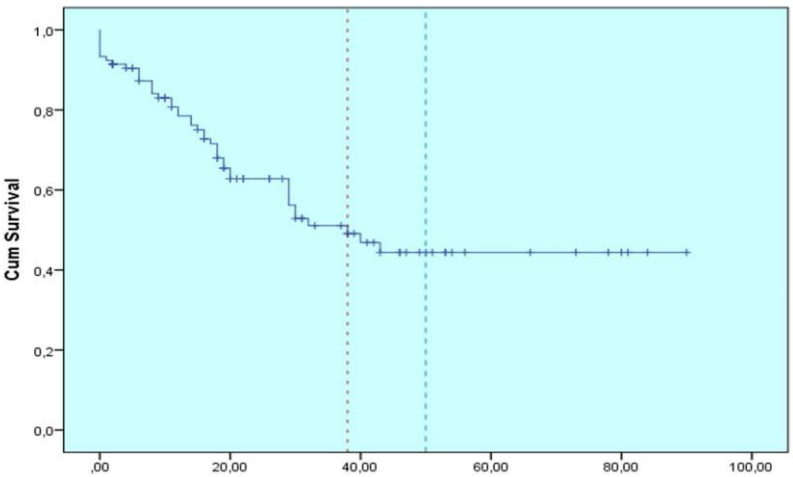

Fig. 2. Kaplan-Meier survival curve

DISCUSSION

Despite the steadfast progresses in the treatment of chronic liver disease, early tumor detection and oncological therapies, the HCC remains a challenge to early diagnose therefore the prognostic remains poor. About 85-90 % of all HCCs have a certain etiology, foremost the viral cirrhosis (26, 27). However, for the next few decades, a major shift is likely to appear in the etiology, with a decreasing of chronic hepatitis C disease, due to the fast spread of modern direct-acting antiviral drugs alongside a rise of non-alcoholic fatty liver disease (28).

In our series, the spectrum of etiology was dominated by metabolic cirrhosis and ethanol. Most of the patients in groups 1 and 2 in our series have Child-Pugh A score (84 % and 72 % in the HR and RF group respectively, unlike the LT group with 16 %. As consequence, most patients of the LT group had B and C Child-Pugh score (42 % of each). In Western Europe countries, the Barcelona Clinic Liver Cancer (BCLC) classification is the most widely employed prognostic staging system for HCC. It includes liver function, patient`s performance status and the extent of tumors and identifies 5 stages of disease (0, A-D) (9,29,30). All the patient in our series were included in stages 0-B BCLC (very early

and early disease).

Since the classical BCLC classification constitutes a landmark in decision-making approach, the BCLC classification-based therapy boundaries tends to blur. Hepatic resection represents the first-line therapy for hepatocellular carcinoma, with a 5-year survival rate exceeding 50%. However, postoperative outcomes are influenced by the presence of liver cirrhosis, requiring a parenchymal reserve of at least 40-50%, or by the multicentric nature of the tumor. The degree of fibrosis associated with liver cirrhosis is directly correlated with post-resection tumor recurrence (31, 32).

Liver resection should be the primary treatment option in patients with single HCC and well-compensated Child A cirrhosis (33, 34). Hepatic resection can be undertaken also in advanced HCC, RF is also applied in larger HCCs with good results (35). RFA represents a therapeutic option for elderly patients with multiple comorbidities, poor general condition, small tumor formations, or multicentric disease, as a single session can achieve complete ablation of lesions smaller than 3 cm with a 1 cm safety margin (36, 37). Multiple procedures can be performed in the case of large tumors (38).

Consequently, individual patient evaluation, biology of HCC and underlying liver disease understanding, are mandatory to achieve maximal oncological radicality without significantly endanger the liver function (Bruis J, WJG). It has been revealed that the 5-year survival excides 50% (39). According to the size and location, vascular invasion as well multifocal disease, segmentectomy, bisegmentectomy, sectionectomy or even major hepatectomy can be performed (40).

Major hepatectomy can be safely performed nowadays due to better understanding of the liver anatomy, advances in surgical instruments and refinements of surgical approaches (41). In our series, we used most commonly the anterior approach to avoid liver mobilization and rupture of larger tumors, by crush clamping technique when performing major hepatectomy, unless segmentectomy and bisegmentectomy, whenever we have used most commonly the 4 electrodes Habib device.

New therapies in hepatocellular carcinoma and the growing experience in liver transplantation and resection have led to the extension of transplant criteria beyond those defined by the Milan Criteria; however, this has been associated with an increased rate of tumor recurrence (42,43). In our study, all patients who underwent liver transplantation met the Milan Criteria, with no tumor recurrence recorded at 4 years.

Another essential aspect of HCC management is prevention, a factor that should play a central role in the overall treatment strategy. Prevention is primarily based on the control of modifiable risk factors, such as viral hepatitis and metabolic syndrome, which can significantly contribute to the development and progression of HCC (44).

The implementation of effective measures to prevent hepatitis B and C virus infections-through vaccination, screening, and antiviral treatments-can significantly reduce the incidence and severity of the disease (45, 46).

CONCLUSIONS

The management of Hepatocellular Carcinoma requires a personalized approach that balances treating the tumor with preserving the patient's liver function. Our findings confirm that treatment selec-

tion—whether resection, ablation, or transplantation-is critically dependent on the severity of the underlying liver disease. As the cause of HCC shifts from viral hepatitis to metabolic conditions, strategies for prevention and surveillance must also evolve. The future of effective HCC management lies in moving beyond rigid guidelines toward individualized plans crafted by a multidisciplinary team, pairing advanced therapies with a renewed focus on preventing the root causes of liver disease.

This two-pronged approach-advancing treatment while preventing initial disease-offers the greatest promise for reducing the global burden of this formidable cancer.

CONFLICT OF INTERESTS AND FUNDING

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POSTOPERATIVE LUMBAR SPONDYLODISCITIS: INFLUENCE OF DISCECTOMY LEVEL AND COMORBIDITIES ON PAIN RELIEF AND HOSPITALIZATION DURATION

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POSTOPERATIVE LUMBAR SPONDYLODISCITIS: INFLUENCE OF DISCECTOMY LEVEL AND COMORBIDITIES ON PAIN RELIEF AND HOSPITALIZATION DURATION (Abstract): Postoperative lumbar spondylodiscitis, although uncommon affecting approximately 3-5% of spinal disc surgeries remains a serious complication. Unlike primary vertebral osteomyelitis, this condition generally results from intraoperative contamination or unfavorable postoperative circumstances. The current study investigates the influence of comorbidities and other clinical parameters on the course and prognosis of postoperative spondylodiscitis occurring after lumbar disc herniation surgery. **Materials and methods:** We performed a retrospective, cross-sectional evaluation involving 85 patients diagnosed with postoperative lumbar spondylodiscitis between 2018 and 2024 at two tertiary care hospitals in Bucharest. Clinical data were retrospectively collected from patient records and included demographic variables, comorbidity profiles, discectomy level, latency from surgery to symptom onset, hospitalization duration, and Visual Analogue Scale (VAS) scores pre- and post-treatment. Comparative statistical analyses were conducted across clinically relevant subgroups, with a focus on comorbidity status. **Results:** Cardiovascular disease (CVD) and type II diabetes mellitus (DM) emerged as the predominant comorbidities. The lumbar region was the most frequently involved anatomical site, regardless of comorbidity presence. Patients with comorbidities were significantly older and exhibited extended hospital stays (Welch's t-test, $p < 0.05$). While comorbidity status did not significantly influence the mean time to symptom onset, complications such as fever and motor deficits were strongly associated with prolonged hospitalization. VAS scores improved substantially following treatment across all subgroups ($p < 0.05$); however, intergroup comparisons revealed no significant differences in pain reduction outcomes between patients with and without comorbidities. **Conclusions:** Pre-existing comorbidities are associated with a higher likelihood of postoperative lumbar spondylodiscitis and correlate with increased patient age and lengthier hospital stays. Nonetheless, the condition demonstrates a favorable response to treatment, particularly regarding pain reduction. These findings underscore the importance of early diagnosis and coordinated multidisciplinary care to improve clinical outcomes and mitigate long-term morbidity. **Keywords:** POSTOPERATIVE SPONDYLODISCITIS, LUMBAR DISC HERNIATION, SPINAL INFECTION, COMORBIDITIES, VAS SCORE, HOSPITALIZATION DURATION, PAIN MANAGEMENT.

"Spinal infections remain elusive in early stages, yet devastating when diagnosis is delayed." (Modic & Ross, "Infectious Discitis and Vertebral Osteomyelitis", Radiology, 1985)

INTRODUCTION

Infections of the lumbar spine following disc surgery presenting as discitis, vertebral osteomyelitis, or combined spondylodiscitis are uncommon but carry serious consequences. They occur in approximately 3-5% of lumbar interventions and primarily affect the lumbar region, with ~65% single-level, ~20% contiguous multilevel, and ~10% noncontiguous involvement (1, 2). These complications can lead to neurological deficits, spinal deformities, and segmental instability, making them clinically significant (1, 3, 4).

Postoperative spinal infections are driven by various patient and procedural risk factors. Comorbid conditions such as type II diabetes mellitus (DM), cardiovascular disease (CVD), obesity, immunosuppression, alcohol abuse, chronic steroid therapy, liver or kidney disease, prior spine operations, and malnutrition all increase susceptibility (4-6). In our cohort of 85 patients treated between 2018 and 2024, 81% had at least one major comorbidity with DM and CVD being the most prevalent mirroring trends seen in broader epidemiological studies (4, 5, 7).

Accurate diagnosis necessitates a comprehensive, multidisciplinary approach leveraging clinical signs, laboratory markers (ESR, CRP), advanced imaging (MRI, CT), and bacteriological cultures obtained from disc aspirates (7, 8). Staphylococcus aureus remains the most frequently isolated pathogen; however, culture-negative results can occur in up to 50% of cases, complicating treatment and increasing reliance on clinical judgment (8, 9).

Management strategies span from conservative treatments such as prolonged intravenous antibiotics and immobilization to early surgical intervention involving debridement and spinal stabilization. A meta-analysis of over 10,000 patients found that early surgery reduces mortality by ~39%, relapse by ~40%, and shortens hospital stays by about 7.8 days (8, 11).

Epidemiological studies indicate regional differences in incidence: less than 1% in Western countries, compared to 4-10% in India (9, 10). Moreover, rising incidence trends have been observed globally, particularly over the past two decades, attributed to aging populations and increased chronic disease prevalence (12-14). In our Bucharest-based cohort, higher postoperative infection rates were noted among elderly patients with significant comorbidities.

Given the substantial morbidity of postoperative lumbar spondylodiscitis, diagnostic uncertainty, and variable prognostic influence of comorbidities and complications, this study aims to enhance clinical understanding. Our retrospective cross-sectional analysis of 85 patients across two tertiary hospitals in Bucharest evaluates associations between patient comorbidities, latency to symptom onset, pre- and post-treatment VAS pain scores, occurrence of complications and hospitalization duration.

MATERIALS AND METHODS

This descriptive, cross-sectional study was conducted using secondary data extracted from general clinical observation records between 2018 and 2024 at two tertiary care centers in Bucharest: the Bucharest University Emergency Hospital and the Dr. Victor Babeș Clinical Hospital for Infectious and Tropical Diseases. The study was approved by the Ethics Committees of both institutions, and written informed consent for data processing and participa-

tion was obtained from each patient or their legal guardian.

The study is based on the hypothesis that certain patient-associated factors, such as pre-existing comorbidities, age, and neurological complications may significantly influence the clinical course of postoperative osteodiscitis and contribute to the risk of prolonged hospitalization or treatment failure.

Objectives. Primary Objective: to evaluate the association between comorbidities and the occurrence of complications in patients diagnosed with postoperative osteodiscitis.

Secondary Objectives: to assess demographic and clinical patterns associated with osteodiscitis severity and recovery outcomes; to compare pre- and post-treatment pain levels (VAS scores) across clinical subgroups; to analyze neurological deficits in relation to specific recovery patterns and to evaluate hospitalization duration based on comorbidities and complication status.

Study Population. The sample included 85 consecutively hospitalized patients diagnosed with postoperative osteodiscitis following lumbar disc herniation surgery, involving the thoracic, lumbar, or lumbosacral spine. Of these, 64 were treated at the Bucharest University Emergency Hospital and 21 at the Dr. Victor Babeș Clinical Hospital.

Data were extracted from clinical records and compiled into a structured database. Recorded variables included:

- Demographics (age, sex, residence, education level);
- Clinical factors (type and presence of comorbidities, presence of fever, neurological deficits, time to symptom onset);
- Surgical site (discectomy level);
- Pain intensity (VAS scores before and after treatment);
- Hospitalization duration.

Statistical Analysis. Patients were grouped for analysis based on sex, age, comorbidity status, complication status, and discectomy level. Statistical methods included:

- Independent samples t-test for comparing VAS scores and time to symptom onset between subgroups;
- Welch's t-test for mean age and hospitalization duration when variances were unequal;
- Chi-square (χ^2) test for assessing relationships between categorical variables (e.g., education level and residence);
- Shapiro-Wilk test for testing normality;
- Levene's test for homogeneity of variances;
- A p-value < 0.05 was considered statistically significant.

Results were synthesized into comparative tables and graphical visualizations.

Ethical Compliance. All research procedures adhered to the Declaration of Helsinki. The study received formal approval from both participating hospitals' Ethics Committees. Data confidentiality and patient privacy were strictly maintained throughout the study.

RESULTS

Among the total cohort of 85 patients diagnosed with postoperative lumbar spondylodiscitis, a significant proportion were admitted to the Bucharest University Emergency Hospital (n = 64), while the remaining cases were documented at the Dr. Victor Babeș Clinical Hospital for Infectious Diseases and Tropical Medicine (n = 21). The gender distribution showed a relatively balanced profile, with 43 female and 42 male patients enrolled across both institutions.

Notably, the University Emergency Hospital recorded 30 female patients and

34 male patients, whereas the Dr. Victor Babeș Hospital admitted 13 women and 8 men. This distribution illustrates a slight male predominance across the cohort but without a disproportionate representation between institutions (fig. 1).

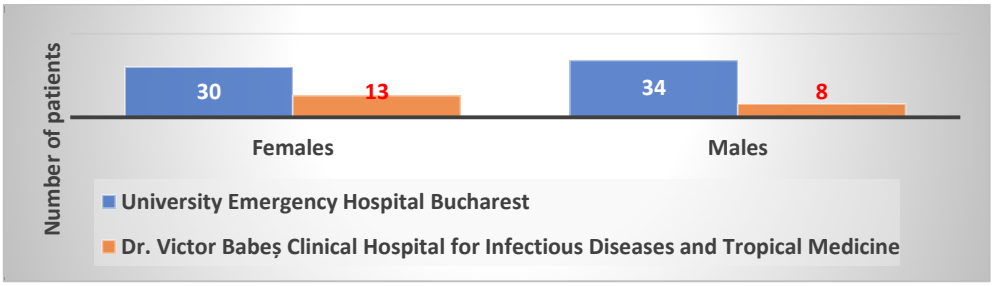

Fig. 1. Distribution of patients with postoperative osteodiscitis by sex and hospital of admission (University Emergency Hospital Bucharest - blue bars; Dr. Victor Babeș Clinical Hospital for Infectious Diseases and Tropical Medicine - orange bars)

An analysis of comorbidities within the study population revealed that cardiovascular diseases (CVD) were the most frequently encountered condition, affecting a total of 45 patients (26 women and 19 men). Type II diabetes mellitus (DM) represented the second most prevalent comorbidity, followed by cases of obesity, neoplasm, and other chronic conditions, which were relatively evenly distributed between sexes.

No statistically significant gender-based differences were observed in the distribution of any comorbidity category, as confirmed by the Chi-square test ($p > 0.05$), indicating a uniform comorbidity profile across male and female subgroups (fig. 2).

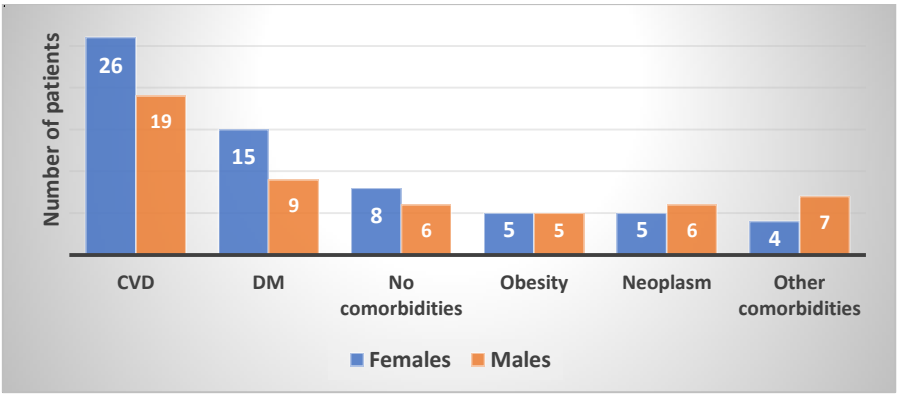

Fig. 2. Distribution of patients by comorbidities and gender.
(Blue bars = Females; Orange bars = Males.)

Among the 85 patients analyzed, a substantial majority - 69 individuals, accounting for 81.18%, had at least one documented comorbidity. As illustrated in Figure 3, cardiovascular diseases (CVD) were the most prevalent, affecting 45 patients, fol-

lowed by type II diabetes mellitus (DM), reported in 24 cases. Other notable comorbidities included oncologic conditions and various chronic pathologies grouped as "other", each present in 11 patients, while obesity was identified in 10 individuals.

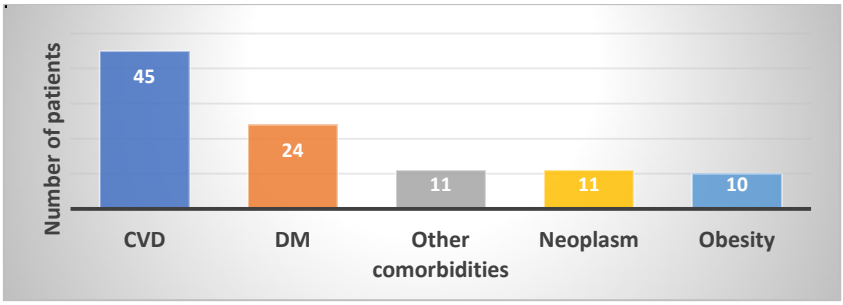

Fig. 3. Distribution of comorbidities among patients diagnosed with postoperative spondylodiscitis following lumbar disc herniation surgery.

The distribution presented in the first table reveals that the lumbar region was the most commonly affected site across all comorbidity categories, including in patients without any known comorbidities. For instance, among those with cardiovascular diseases, 33 out of 45 patients exhibited lumbar involvement, while 16 out of 24 diabetic patients showed similar localization. Thoracolumbar and lumbosacral levels were considerably less frequent.

Despite these observations, no statistically significant association was found between the presence of comorbidities and the level of osteodiscitis involvement. This was confirmed by both the Chi-square test and Fisher's exact test ($p \geq 0.05$ for both), indicating that comorbid status does not predict the anatomical level at which infection manifests.

Analysis of the second table highlights the variation for the mean time to symptom onset of postoperative osteodiscitis across different comorbidity groups. The longest average delay was observed in patients with obesity, who developed symptoms at approximately 12.9 weeks post-intervention (SD ±6.657). Conversely, the shortest interval was seen in patients with neoplastic conditions, with an onset average of 8.73 weeks (SD ±4.798).

Despite these numerical differences, none of the observed variations reached statistical significance when comparing presence versus absence of each comorbidity group (t-Student's test, $p \geq 0.05$ for all comparisons). The aggregated analysis of all comorbid patients *versus* those without comorbidities also revealed no significant difference in onset timing (mean 10.77 weeks *vs.* 9.19 weeks respectively).

Results showed a predominance of patients with comorbidities in the older age brackets, particularly between 60 and 79 years. The peak distribution of such cases was registered in the 60-64 age group, followed closely by the 70-74 cohort. In contrast, the number of patients without comorbidities was notably lower across all age categories, especially among those aged over 55 (fig. 4).

From a statistical standpoint, Levene's test indicated significant heterogeneity of variances between the two groups ($p < 0.05$), prompting the use of Welch's t-test, which confirmed that the mean age differed significantly between patients with

comorbidities and those without ($t(17.143) = 2.226$, $p < 0.05$). Though normality assumptions were not met (Shapiro-Wilk test, $p < 0.05$), the absence of extreme outliers (Z-score between -3 and +3) supports the reliability of this finding.

These observations underline the age-associated nature of comorbid conditions in the context of postoperative osteodiscitis, highlighting older individuals as a demographically vulnerable group in this cohort.

TABLE I.

Distribution of patients according to the presence of comorbidities and the level of discectomy

No.	Comorbidities	Discectomy level	Number of patients
1	Diabetes mellitus	Thoracolumbar	3
		Lumbar	16
		Lumbosacral	5
2	Cardiovascular diseases	Thoracolumbar	2
		Lumbar	33
		Lumbosacral	10
3	Obesity	Thoracolumbar	0
		Lumbar	9
		Lumbosacral	1
4	Neoplasm	Thoracolumbar	0
		Lumbar	9
		Lumbosacral	2
5	Other comorbidities	Thoracolumbar	1
		Lumbar	39
		Lumbosacral	12
6	No comorbidities	Thoracolumbar	1
		Lumbar	11
		Lumbosacral	0

TABLE II.

Distribution of patients according to the presence of comorbidities and the average time until onset of symptoms

No.	Comorbidities	Presence or absence	Median duration (weeks) until onset of symptoms	Number of patients
1	Diabetes mellitus	Yes	9.88 (SD±9.638)	24
		No	10.70 (SD±7.828)	61
2	CVD	Yes	10.84 (SD±9.436)	45
		No	10.05 (SD±6.969)	40
3	Obesity	Yes	12.90 (SD±6.657)	10
		No	10.15 (SD±8.510)	
4	Neoplasm	Yes	8.73 (SD±4.798)	11
		No	10.73 (SD±8.725)	74
5	Comorbidities (total)	Yes	10.77 (SD±7.901)	52
		No	9.19 (SD±8.451)	33

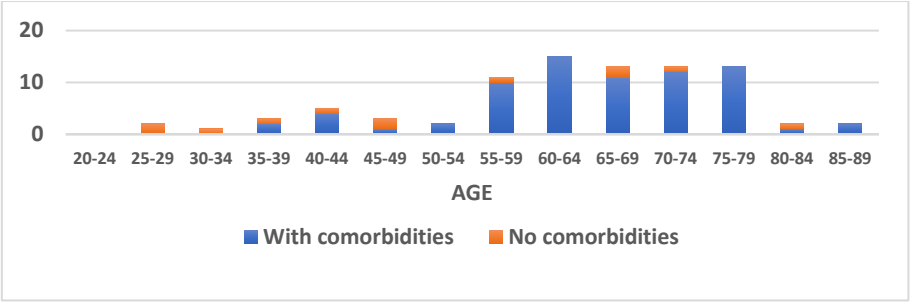

Fig. 4. Age group distribution of patients according to the presence or absence of comorbidities.

The preoperative VAS scores remain uniformly elevated across all categories, with minimal variance between patients with and without comorbidities, highlighting that baseline pain perception was not significantly influenced by comorbid status (fig. 5). This aligns with prior literature suggesting that the initial severity of symptoms in spondylodiscitis may be more a function of disease localization than of systemic factors (Modic & Ross, 1985).

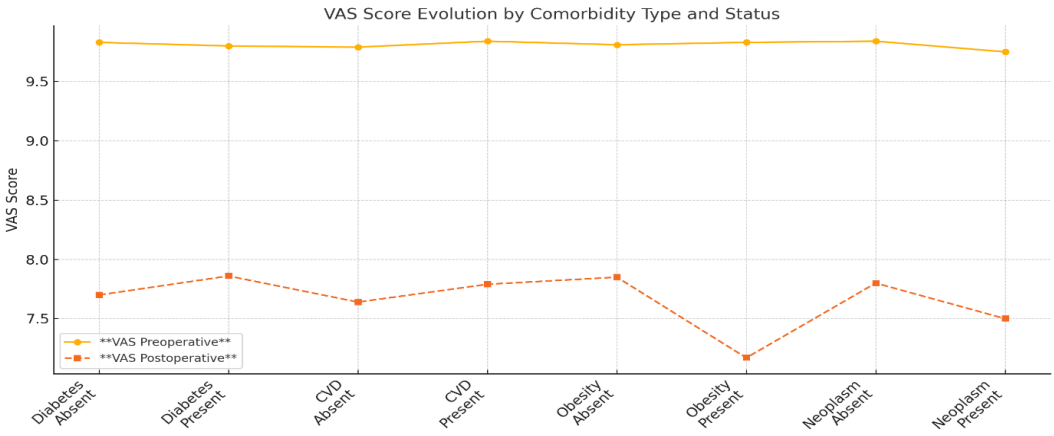

Fig. 5. Evolution of VAS scores by comorbidity type and status.

A marked reduction in VAS scores across all spinal levels following treatment is represented in figure 6, with the thoracolumbar subgroup showing the most pronounced improvement suggesting that anatomical site may influence analgesic response, independent of baseline symptom severity.

According to Welch's t-test, patients with pre-existing comorbidities experienced substantially longer hospital stays (mean = 16.46 days) than those without (mean = 3.75 days), a difference that remained statistically significant despite variance heterogeneity (Levene's test, $p < 0.05$; Welch's test, $p < 0.05$). This aligns with prior observations that systemic disease burden prolongs recovery time and complicates discharge planning.

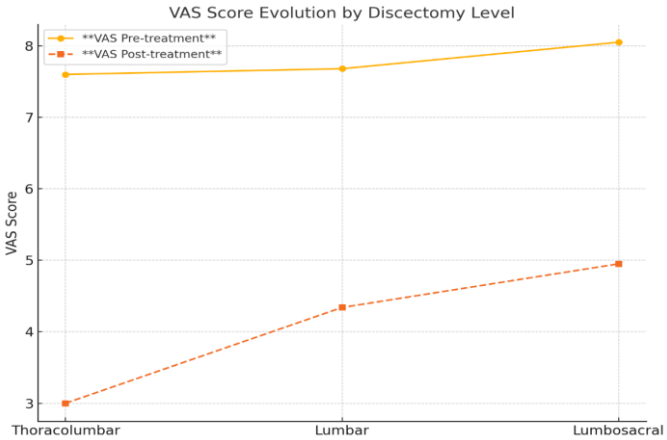

Fig. 6. Evolution of VAS scores before and after treatment, by discectomy level.

Similarly, the presence of postoperative complications-most notably infectious or wound-related-was associated with prolonged inpatient care (23.25 *vs.* 10.35 days; $p < 0.05$), even though Levene's test confirmed equal variances. Interestingly, fever did not influence the average length of stay ($p \geq 0.05$), possibly due to the limited number of febrile patients (n = 3) and overlapping management strategies in afebrile cases with other complications.

Notably, the analysis also identified a counterintuitive trend regarding motor deficits: patients with neurological impairment had shorter hospitalizations on average (8.83 *vs.* 22.67 days), a result that was statistically significant (Welch's test, $p < 0.05$) but potentially influenced by early discharge for rehabilitation or social factors (fig. 7).

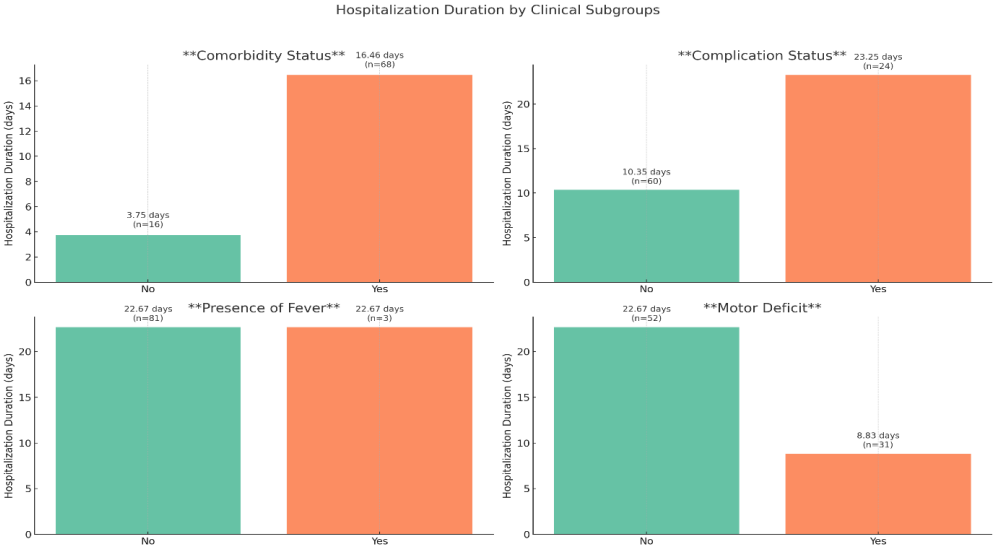

Fig. 7. Hospitalization duration by presence of comorbidities, complications, fever, and motor deficit

DISCUSSION

Multiple studies have confirmed that the presence of preoperative comorbidities, particularly diabetes and cardiovascular disease, correlates with extended hospitalization, increased complication rates, and delayed recovery (15). Our results support this, with statistically significant differences observed in length of stay between patients with and without comorbidities. Similar patterns have been described in a multicenter prospective analysis involving 102 patients, where comorbid individuals had a mean hospitalization duration 1.8 times longer than those without (16).

The relationship between infectious complications and hospitalization is particularly critical in the setting of spinal surgery. A recent cohort study emphasized that even minor postoperative infections can double the duration of hospital stay, particularly in immunocompromised populations (17). This finding resonates with our data, where patients experiencing complications such as fever or local infection exhibited an average hospital stay exceeding three weeks.

Regarding motor deficits, our findings revealed a paradoxical trend-patients with neurological impairment were discharged earlier than those without. Although initially counterintuitive, this pattern has been reported in prior literature and is hypothesized to result from early rehabilitation transfers or limited surgical benefit in advanced neurological decline (18). These cases often follow distinct clinical pathways not entirely captured by general length-of-stay metrics.

Pain relief, as assessed by the VAS score, demonstrated significant improvement post-treatment across all subgroups, regardless of sex or comorbidity profile. This is consistent with a recent retrospective series involving 89 patients, where over 85% experienced a clinically meaningful reduction in pain within the first 4 weeks following antibiotic and/or surgical intervention (19). Importantly, our results show that anatomical localization-particularly thoracolumbar involvement-is associated with superior analgesic outcomes, a finding also observed by Kim *et al*. in their comparison of thoracic versus lumbar infections (20).

There remains ongoing debate regarding the role of early surgical intervention in spondylodiscitis. Our dataset supports the notion that early, targeted debridement and stabilization can facilitate pain reduction and reduce relapse risk, consistent with contemporary meta-analytical evidence (21).

CONCLUSIONS

This retrospective cross-sectional study provides robust evidence on the clinical course, pain outcomes, and hospitalization burden associated with postoperative lumbar spondylodiscitis in a Romanian tertiary care context. Our cohort of 85 patients revealed key associations that may inform future diagnostic and management pathways for this complex condition.

One of the most significant findings relates to the evolution of pain intensity, quantified using the Visual Analogue Scale (VAS). Across the entire study population, the mean VAS score decreased from 7.76 pre-treatment to 4.40 post-treatment, a statistically significant improvement ($p < 0.05$, paired t-test). This analgesic benefit was consistently observed across all sub-

groups, regardless of sex, comorbidity presence, or discectomy level. Notably, patients with thoracolumbar discectomy experienced the most pronounced improvement, with a mean post-treatment VAS of 3.0, compared to 4.34 for lumbar and 4.95 for lumbosacral involvement. This anatomic variance reached statistical significance ($p < 0.05$), suggesting that spinal level may influence therapeutic responsiveness.

Equally important were the insights into hospitalization duration. Statistical comparisons revealed that patients with comorbidities had a mean hospital stay of 16.46 days, significantly longer than those without comorbidities (3.75 days; Welch's t-test, $p < 0.05$). Levene's test confirmed unequal variances ($p < 0.05$), validating the use of Welch correction. Additionally, the presence of postoperative complications was associated with markedly extended hospitalization (23.25 *vs.* 10.35 days; $p < 0.05$), despite homogeneity of variance. These findings reinforce the critical role that systemic health and postoperative course play in determining resource utilization and clinical recovery.

Interestingly, motor deficits often considered markers of severe disease, were paradoxically associated with shorter hospitalizations (8.83 *vs.* 22.67 days; Welch's test, $p < 0.05$).

This result may reflect early discharge for rehabilitation or divergent clinical pathways, underscoring the need for cautious interpretation.

Although sex and specific comorbidities (e.g., diabetes, cardiovascular disease) did not significantly influence the degree of pain relief, their presence clearly impacted hospitalization metrics. These results emphasize the importance of early identification of high-risk patients, as well as the need for coordinated care plans that account for both infectious and non-infectious drivers of morbidity.

This study confirms that while postoperative lumbar spondylodiscitis responds favorably to targeted treatment in terms of pain relief, its clinical trajectory is heavily modulated by the presence of comorbidities and surgical complications.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interests, and they received no specific funding regarding this scientific research.

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MEDICATION AND REGULATION OF ACUTE POSTOPERATORY PAIN, USING MULTIMODAL THERAPY

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MEDICATION AND REGULATION OF ACUTE POSTOPERATORY PAIN, USING MULTIMODAL THERAPY (Abstract): Surgical interventions and opioid exposure activate both peripheral (satellite glial cells) and central glia (microglia and astrocytes), contributing to peripheral and central sensitization and maintenance of pain. Specific molecules such as MMP-9 and IL-1β are involved. **Materials and methods**: We analyzed female patients who presented for emergency or elective abdominal surgery at the "Cuza-Voda" Hospital of Obstetrics and Gynecology Iași, Romania and who met the following inclusion criteria and did not present any of the exclusion criteria were included in the analysis. Inclusion criteria: Age over 18 years; Surgical obstetrical or gynecological indication. exclusion criteria: patients under 18 years of age; people with suspected sepsis or serious medical comorbidities such as heart failure, undiagnosed fever and instable state. The study was retrospective and prospective, during January 2024 to November 2025, starting from the clinical parameters, surgery indication, analyzing clinically and paraclinically, the importance of intra- and postoperative analgesic medication that was used. **Results:** Of the total study group, 52.3% of patients underwent cesarean sections (CST), 14.4% underwent total hysterectomy with bilateral adnexectomy (HT+AB) and 9% underwent biopsy curettage, the rest of gynecological surgeries consisted of Madden mastectomy, salpingectomy, ovarian cyst removal. Multimodal drug therapy and opioid treatments were required to achieve the control of post operatory pain. **Conclusions**: Current areas of interest in pain research include investigating the effect of mood on pain processing in the brain and the search for new drugs to block the channels involved in pain transmission, and the actual important post operatory pain therapy involves a combination of multimodal and opioid treatments. **Keywords***:* PAIN, RECEPTOR, POLYMORPHISM, OPIOID THERAPY, MULTIMODAL TREATMENT.

INTRODUCTION

Pain is one of the most subject symptoms concerning patient care in healthcare. Primary afferent fibers, Aδ and C fibers that carry noxious sensory information, are extra primary Aβ fibers that carry non-noxious stimuli (1). Each of these fiber types has different characteristics that allow the transmission of certain types of sensory information (2). Aβ fibers are heavily myelinated and of large diameter, thus allowing rapid signal conduction. They have a low activation threshold and usually respond to light touch and transmit innocuous stimuli. Aδ fibers are lightly myelinated and of smaller diameter and, therefore, conduct more slowly than Aβ fibers. They respond to mechanical and thermal stimuli. They interpret fast, sharp pain and are responsible for the initial re-

flex response to acute pain (3). C fibers are unmyelinated and are also the smallest type of primary afferent fiber. Therefore, they demonstrate slow conduction. C fibers are polymodal, responding to chemical, mechanical, and thermal stimuli. C fiber activation results in slow, burning pain. These reactions have been studied in orofacial surgery, neurological diseases, and general surgery (4, 5).

Peripheral sensitization is a state of hypersensitivity and hyperexcitability of nociceptors, caused by inflammation and injury. It involves activation of a variety of ion channels and protein kinases (6). Central sensitization: Persistent nociceptive stimulation leads to changes in the central nervous system, involving increased excitability of neurons in the spinal cord and brain. This contributes to chronic pain (7, 8, 9). Glial activation and neuroinflammation after surgery/opioid treatment: Surgical interventions and opioid exposure activate both peripheral (satellite glial cells) and central glia (microglia and astrocytes), contributing to peripheral and central sensitization and maintenance of pain. Specific molecules such as MMP-9 and IL-1β are involved and more research is required to understand this process (10, 11, 12).

MATERIALS AND METHODS

Study perspective. We analyzed 111 female patients who presented for emergency or elective abdominal surgery at the "Cuza-Voda" Hospital of Obstetrics and Gynecology Iași, Romania and who met the following inclusion criteria and did not present any of the exclusion criteria.

The study was retrospective and prospective, during December 2024 to December 2025, starting from the clinical parameters, surgery indication, analyzing clinically and paraclinically, the importance of intra- and post-operative analgesic medication that was used.

Inclusion criteria were formed by: age above 18 years; associated obstetrical or gynecological surgical intervention.

Exclusion criteria were patients under 18 years of age; persons with suspected sepsis or associated severe medical pathologies

The general objective was evaluating the effectiveness of the treatment of moderate to severe postoperative pain in patients who present major risk factors for having an unusually high level of acute postoperative pain.

Statistical analysis. Descriptive and analytical methods were used in the statistical analysis. After collecting the data, in an accessible form to ensure their informational character, their processing was carried out. The data were centralized in *Excel* and *SPSS* databases and processed with the statistical functions to which they lend themselves. In the presentation of the data, confidence intervals at the 95% significance threshold were used, and the χ^2 and t-Student tests were used to test the differences. The normality testing of quantitative value series was performed with the Kolmogorov-Smirnov test used to test large data sets. The threshold chosen in testing the null hypothesis is $p > 0.05$, therefore the variables are normally distributed. Normality tests Skewness or Kurtosis, which often involve skewness, assess whether the distribution of a data set resembles a normal distribution. Skewness measures the skewness of the Linear regression, by the method of least squares, was the modeling method used. It is the method called "regression", "linear regression", "multiple regression" or "least squares" when building a model.

RESULTS

Particularities of the drug treatment used before hospitalization

Before the surgical intervention, 8.6% of the patients with cesarean section (CSS) received Aspenter 75 mg, 100 mg, respectively 150 mg and 15.5% other medication such as Euthyrox for thyroid pathology, Metformin for diabetes pathology, while patients with interventions such as total hysterectomy with bilateral adnexectomy (HT+AB) did not receive medication before the surgical intervention ($p = 0.002$).

Type of surgery performed and surgical outcomes

Of the total study group, 52.3% of patients underwent cesarean sections (CST), 14.4% underwent total hysterectomy with bilateral adnexectomy (HT+AB) and 9% underwent biopsy curettage, the rest of gynecological surgeries consisted of Madden mastectomy, salpingectomy, ovarian cyst removal.

Cesarean section results (CST). Of the 58 patients with cesarean section, 79.3% were under 36 years of age, while 83% of patients with other types of surgery were over 36 years of age ($p = 0.001$).

In patients with cesarean section, the series of values for gestation, number of weeks of pregnancy, birth weight, Apgar and number of days of hospitalization are distributed with the values of the Kurtosis test > 2 and the values of the Kolmogorov-Smirnov test < 0.05, while the series of values for parity varies in the range 1-4; group mean 1.72 ± 0.76; median 2; Kurtosis test result 1.249 and $p > 0.05$ in Kolmogorov-Smirnov test, which suggests that statistical significance tests can be applied for continuous variables.

Before surgery, 8.6% of patients with cesarean section received aspirin and 15.5% other medication, while patients with HT+AB did not receive medication before surgery ($p = 0.002$).

Demographic features of analyzed patients according to the medication

Of the 17 patients treated intraoperatively with Mialgin, 88.2% were over 36 years old ($p=0.001$) and 64.7% came from urban areas ($p=0.424$). Of the 30 patients treated intraoperatively with Fentanyl, 73.3% were over 36 years old ($p = 0.003$) and 76.7% came from urban areas ($p = 0.219$). Of the 12 patients treated intraoperatively with Diazepam, all were under 36 years old ($p = 0.001$) and 66.7% came from urban areas ($p = 0.532$). The only patient treated intraoperatively with Phenobarbital was under 36 years old and came from rural areas, and the patient treated with Morphine was over 36 years old and came from rural areas.

Associated pathologies and the association with the medication

Of the 17 patients treated intraoperatively with Mialgin, 35.3% had iron deficiency anemia ($p = 0.494$), 29.4% adhesion syndrome ($p = 0.053$) and 17.6% uterine fibroids ($p = 0.071$). Of the 30 patients treated intraoperatively with Fentanyl, 36.7% had iron deficiency anemia ($p = 0.359$), 26.7% postoperative adhesion syndrome ($p = 0.019$), 10% hereditary thrombophilia ($p = 0.283$), 6.7% had hypertension ($p = 0.177$) and 3.3% diabetes mellitus ($p = 0.705$). Of the 12 patients treated intraoperatively with Diazepam, 25% had uterine fibroids ($p = 0.027$), 16.7% iron deficiency anemia ($p=0.184$), 16.7% adhesion syndrome ($p = 0.506$) and 8.3% had hereditary thrombophilia ($p = 0.562$).

Hospitalization period, according to OG surgical interventions performed

Treatment with analgesics, depending on the surgical intervention, highlights the following aspects (fig. 1):

- 8.6% patients with cesarean section (CST), were treated intraoperatively with Fentanyl (p = 0.001);
- 62.5% of patients with total hysterectomy with bilateral adnexectomy (HT+AB) were treated intraoperatively with Fentanyl (p=0.001);
- 40% patients with classical or laparoscopic unilateral ovarian cystectomy were treated intraoperatively with Fentanyl and 20% with Diazepam (p=0.001);
- 33.3% of patients with interventional hysteroscopy were treated intraoperatively with Mialgin and 33.3% with Fentanyl (p = 0.001);
- among patients with classical or laparoscopic uterine myomectomy, 50% were treated intraoperatively with Mialgin, 50% with Fentanyl and 50% with Diazepam (p = 0.001);
- among patients with Madden-type radical mastectomy, 50% were treated intraoperatively with Mialgin, all patients with Fentanyl and 50% with Diazepam (p = 0.001).

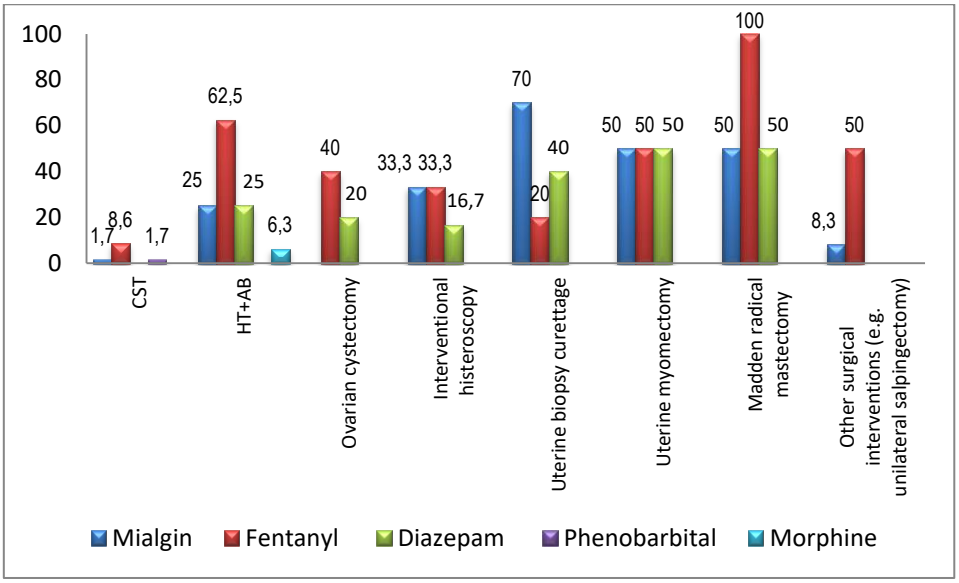

Fig. 1. Correlation of medication with type of surgical intervention

Intraoperative drug treatment

Ibuprofen, dosage 400 mg injectable solution / 100 mL was administered intraoperatively in 1 to 3 doses to 25.9% of patients with CST and 17% of those with other types (p=0.258) .80% of the patients with CST, 33.3% of those with ovarian cystectomy and all with HT+AB received a single dose of Ibuprofen (p=0.329). Paracetamol, 10 mg/mL 100 mL was administered intraoperatively in 1-4 doses to 89.7% of the patients with CST and 79.3% of those with other types (p = 0.130). A total of 51.9% of the patients with CST, 66.7% of those with hysteroscopy or biopsy uterine curettage received a single dose of Parace-

tamol, while 38.5% of the patients with CST, 50% of those with myomectomy or mastectomy received two doses of Paracetamol. Three doses were received by 53.3% of patients with HT+AB, 50% of those with myomectomy or mastectomy and all with ovarian cystectomy (p=0.001).

Mabron, dosage 50 mg/mL 2 mL was administered intraoperatively in 1-3 doses to 93.1% of patients with CST and 49.1% of those with other types (p = 0.001). 70.9% of patients with CST and all with mastectomy received a single dose of Mabron. Two doses were received by all with ovarian cystectomy, 61.5% of patients with HT+AB, 50% of those with myomectomy, 27.5% of patients with CST (p = 0.004).

Algocamin, dosage 1 g/2 mL was administered intraoperatively in 1-4 doses to 89.7% of patients with CST and 64.2% of those with other types (p = 0.001). For 51.9% of patients with CST, 50% with ovarian cystectomy or myomectomy, 33.3% with hysteroscopy or biopsy curettage they received a single dose of Algocamin. All patients with mastectomy, 66.7% of patients with biopsy curettage, 50% of those with HT+AB, 44.2% of patients with CST received two doses (p = 0.041). Only 14.3% of patients with HT+AB received four doses.

Acupan, dosage 20 mg/2 mL was administered intraoperatively in 1-3 doses to 65.5% of patients with CST and 37.7% of those with other types (p=0.004). 86.8% of patients with CST, all with hysteroscopy, biopsy curettage, myomectomy, or mastectomy received a single dose of Acupan. All patients with ovarian cystectomy received two doses, 50% of patients with HT+AB, 13.2% of patients with CST (p = 0.049). Only 12.5% of patients with HT+AB received three doses.

Refen, dosage 25 mg/3 mL was administered intraoperatively in 1-4 doses to 82.8% of patients with CST and 73.6% of those with other types (p = 0.243). 83.8% of patients with uterine biopsy curettage, 72.9% of patients with CST, 66.7% with hysteroscopy and 15.4% with HT+AB received a single dose of Refen. All patients with ovarian cystectomy or mastectomy, 50% of patients with myomectomy, 30.8% of those with HT+AB and 25% of patients with CST received two doses (p = 0.005). Four doses were received by only 7.7% of the patients with HT+AB.

Postoperative drug treatment

Postoperative treatment with Clexane was received by 76.6% of the patients in the present study group, all patients with myomectomy, 93.1% of the patients with CST, 81.3% of the patients with HT+AB, 60% with ovarian cystectomy, 50% with mastectomy, 33.3% with hysteroscopy and 30% with biopsy curettage (p = 0.001).

Postoperative treatment with Cefotax was received by 17.1% of the patients in the present study group, including all patients with myomectomy, 50% of the patients with HT+AB, 40% with ovarian cystectomy and 33.3% with hysteroscopy (p = 0.001). Refen, 25 mg/3 mL was administered postoperatively in 1-22 doses to 82.8% of patients with CST and 60.4% of those with other types (p = 0.004). 50% of patients with myomectomy, 33.3% of patients with biopsy curettage, Algocamin, dosage 1 g/2 mL was administered postoperatively in 1-16 doses to 74.1% of patients with CST and 52.8% of those with other types (p=0.020). 50% of patients with mastectomy, 33.3% of patients with HT+AB and 20.5% of those with CST received postoperatively 1-2 doses of Algocamin. All patients with myomectomy, 58.2% of patients with CST, 33.3% of patients with

ovarian cystectomy and 8.3% of those with HT+AB received 3-5 doses of Algocamin postoperatively. More than 5 doses of Algocamin were received by all patients with hysteroscopy, 66.7% of patients with ovarian cystectomy, 58.4% with HT+AB, 50% of those with mastectomy and 20.5% of patients with CST ($p = 0.005$). The morphine receptor mutation (OPRM1) was more frequently present in patients with biopsy curettage (60%), myomectomy (50%), mastectomy (50%), hysteroscopy (33.3%), CST (20.7%) and ovarian cystectomy (20%) ($p = 0.048$). There were no statistically significant differences by age group ($p = 0.785$).

DISCUSSION

Cesarean rates in Romania are increasing at a total of 60% of births. Our results show that caesarean sections were performed in 79.3% of cases in patients younger than 36 years of age. Pain management with opioids and anxiolytics during and after this type of surgery was managed with Mialgin in 17 patients, Diazepam in 12 patients, Fentanyl in 8.6% of cases.

Opioids bind to and stimulate opioid receptors (mu, kappa, and delta) that are found in the brain, spinal cord, peripheral nervous system, and gastrointestinal tract. Opioid receptors are a type of G protein-coupled receptor and are found in the central and peripheral nervous systems and are involved in many aspects of nervous system function, including sympathetic and parasympathetic activity and pain modulation. (18, 19). Because exogenous opioids, such as morphine and fentanyl, perform the same action as enkephalins at the receptor level, they can have an effect anywhere, especially in the midbrain, pons, medulla, and spinal cord (7).

In terms of pain modulation, studies describe that some signals from the sensory cortex or from afferent fibers travel along the spinothalamic tract ending in the periaqueductal gray area of the midbrain. Endogenous opioids are released, enkephalins, which stimulate neurons with fibers that transmit information to the locus coeruleus within the brainstem pons and the locus raphe magnus in the medulla. (10, 21). Our study followed the usage of dexamethasone as a co-administration during the induction phase of the regional analgesia, before blockage pain installment, because it can reduce pain by blocking the intracellular conversion of phospholipids to arachidonic acid, and our study found it beneficial for pain management (16, 17).

In our study intraoperative drug treatments consisted of 1-3 doses of Ibuprofen in 25.9% of caesarean surgeries, 33.3% of ovarian cystectomies and at least one dose in all cases of hysterectomies. More than half of patients received a dose of Paracetamol, while larger surgeries such as mastectomies and myomectomies received 2 doses. Analgesics, such as, non-steroid anti-inflammatory pills (NSAIDs), as ibuprofen, block the synthesis of all prostaglandins, including PGI2 (prostacyclin), thereby slowing or stopping inflammation (13). The inflammatory process and pain reduction occur by blocking the formation of PGE2. This is achieved by blocking COX-1 and COX-2. COX-1 is found in low concentrations in a balanced proportion throughout the body, and COX-2 is the inducible form, which is dramatically increased during inflammatory states, such as immediate post operatory phase (14, 15).

Mabron, tramadol, an opioid analgesic, that relieves pain by acting on specific nerve cells in the spinal cord and brain was administered in 93% of caesarean surgeries

and in 49.1% of total surgeries described.

Algocalmin an analgesic and antipyretic with indications that include severe post-injury or postoperative pain, was prescribed in up to 4 doses in 89.7% of caesarean surgeries and more than half of all other surgeries.

Acupan, an analgesic symptomatic treatment of short-term painful conditions, in particular post-operative pain was administered in 65.5% of caesarean sections and 37.7% of all other surgeries, during the intraoperative period and did not require multiple doses except for 15% of total hysterectomies.

Refen is a non-steroidal anti inflammatory drug that has the active substance diclofenac sodium. Refen is presented as an injection/infusion solution and is used to treat acute pain.

Our study described its utility in more than 72% of caesarean sections, half of uterine gynecological surgeries, with up to 4 doses in 7.7% of cases during the intraoperative period.

Multimodal pain management offers a comprehensive strategy to improve function and quality of life while minimizing opioid use. Increasing access to interventional pain techniques through collaboration between surgeon and anesthesiologist can make an important difference between poorly controlled pain and a stable, comfortable postoperative patient.

Minimizing pain enhances long and short - term quality of life, reduces complications in the immediate and late postoperative phase and increases confidence in the surgical act and in the postoperative recovery time.

CONCLUSIONS

Multimodal analgesia is used to characterize the use of combinations of drugs with different modes of action. In this way, it is possible to use lower doses of drugs, thus minimizing unwanted side effects.

By using a combination of NSAIDs and opioids for postoperative pain, the amount of opioid required is much lower and opioid-related side effects become less significant. Pain is both a sensory and emotional experience, and patients' previous experiences, fears and anxieties can play an important role. Pain transmission is the result of complex peripheral and central processes. These processes can be modulated at different levels, and pain perception is the result of the balance between facilitatory and inhibitory interactions.

Current areas of interest in pain research include investigating the effect of mood on pain processing in the brain and the search for new drugs to block the channels involved in pain transmission.

Thus, in the pathophysiology of pain it is important to understand that: the pain experienced by patients is the result of the interaction between sensory and emotional experiences. Aδ fibers transmit fast, sharp, localized pain. 3 C fibers transmit slow, diffuse, dull pain.

Pain transmission can be modulated at several levels, including the dorsal horn of the spinal cord and by descending inhibitory pathways. The spinothalamic and spinoreticular tracts are important ascending pain transmission pathways. Neuropathic pain can be spontaneous and is often described as a burning sensation or can be felt very strongly by the patient.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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INDEPENDENT PREDICTORS OF RESILIENCE IN OLDER ADULTS: INSIGHTS FROM A PROSPECTIVE CLINICAL STUDY

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INDEPENDENT PREDICTORS OF RESILIENCE IN OLDER ADULTS: INSIGHTS FROM A PROSPECTIVE CLINICAL STUDY (Abstract): **Aims:** To evaluate the four types of resilience (physical, mental, emotional, and social) and their associations with functional capacity, physical performance, nutritional and cognitive status, muscle strength, and depressive symptoms in older adults. **Materials and methods:** This prospective study included 110 patients aged ≥ 50 years admitted to Dr. C.I. Parhon Clinical Hospital, Iași, with acute conditions or acute exacerbations of chronic diseases who underwent comprehensive geriatric assessment and were evaluated with the Physical Resilience Scale. Data were analyzed using nonparametric tests, Spearman correlations, and univariate/multivariate generalized linear models; significance was set at $p<0.05$. **Results:** Median resilience was 10 (IQR 8–13). Resilience showed significant positive correlations with ADL (R=0.218), IADL (R=0.397), SPPB (R=0.458), MNA (R=0.377), MMSE (R=0.322), and upper-limb muscle strength (R≈0.33). Negative correlations were observed with GDS (R=-0.676), Fried phenotype (R=-0.462), and fall risk (R=-0.331). Multivariable analysis identified IADL (IRR=1.032, p=0.043) and GDS (IRR=0.947, $p<0.001$) as independent predictors of resilience. Resilience did not differ according to heart failure, urinary tract infection, or osteoarticular disease, but was significantly lower in patients with reduced muscle strength. **Conclusions:** Resilience in older adults is a multidimensional construct closely associated with functional independence and mental health. IADL and depressive symptoms are independent determinants of resilience, underscoring the importance of maintaining autonomy and addressing depression. Multidomain interventions targeting these factors may enhance resilience and reduce vulnerability to adverse outcomes in aging populations. Longitudinal studies are warranted to clarify causal relationships and evaluate the efficacy of resilience-promoting strategies. **Keywords**: RESILIENCE, INTRINSIC CAPACITY, OLD PATIENTS, FRAILTY, RECOVERY.

INTRODUCTION

Aging is accompanied by a progressive loss of physiological reserves and an increased vulnerability to disease, functional decline, and disability, which needs a shift from a care model focused exclusively on disease to one oriented toward preserving functional capacity and quality of life in older adults (1). In this context, the concepts of resilience (particularly physical resilience) and intrinsic capacity (IC) have emerged as complementary frameworks

that can guide proactive interventions for "successful aging" (2,3). Resilience is defined as a multidimensional dynamic process that enables a person to withstand, adapt to, and recover from acute or chronic stressors. Resilience has four major components: physical, mental, emotional, and social resilience. Physical resilience specifically reflects the ability to avoid or recover from functional decline following an acute adverse health event. Psychological resilience reflects the capacity to maintain or regain stable mental health and to adapt well in the face of adversity, and to sustain functioning without long-term psychological disruption. Emotional resilience is the ability to recognize, manage, and recover from emotional stressors and and to regulate emotional responses effectively. Social resilience stems from social connection and involves teamwork, network-building, and community engagement (4). Intrinsic capacity, promoted by the World Health Organization through the ICOPE framework, brings together an individual's mental and physical reserves across five interconnected domains: locomotor, vitality, cognitive, sensory, and psychological; the composite IC score is a strong predictor of progression toward frailty, functional decline, and mortality (5, 6).

The Comprehensive Geriatric Assessment (CGA) integrates the measurement of geriatric functions and syndromes and gains additional prognostic and interventional value when it also includes the assessment of resilience and IC. Recent data show that implementing the CGA reduces unplanned hospitalizations, institutionalization, and deterioration in the ability to perform activities of daily living (8, 9). In practice, the short, validated instruments frequently used in geriatric assessment: activities of daily living (ADL), instrumental activities of daily living (IADL), Mini - Mental State Examination (MMSE), Geriatric Depression Scale (GDS), Mini Nutritional Assessment (MNA), Short Physical Performance Battery (SPPB), handgrip strength test, and fall risk scores (e.g., the Downton Scale), and the Fried frailty phenotype correspond, in most studies, to the IC domains and physiological components of resilience and have predictive value for clinical outcomes (falls, functional decline, hospitalization, mortality)(10, 11, 12). For example, the SPPB and walking speed are recognized as strong predictors of disability and morbidity, and the "vitality" domain (MNA, grip strength) frequently emerges as a major determinant of physical resilience and frailty risk (13, 14). The Physical Resilience Scale (PRS), validated in studies of older adults (e.g., Resnick *et al.*), provides a specific measure of post-stressor recovery capacity through self-reported assessment of reactions to health events, complementing functional tests and providing prognostic information regarding the trajectory of recovery and the need for rehabilitation interventions (15, 16).

Recent literature highlights the interdependence between IC and resilience: IC represents the necessary baseline reserves, while resilience reflects the dynamic mobilization of these reserves in the face of stressors; thus, interventions that enhance key domains of CI (e.g., multicomponent exercise programs, nutritional optimization, psychosocial support) also enhance resilience and reduce the risk of progression toward frailty and disability (17, 18, 19). Recent meta-analyses and randomized trials show that multidomain interventions (exercise + nutrition + cognitive stimulation) improve functional performance,

cognitive and psych affective status, and reduce the prevalence of frailty and loss of independence over follow-up periods (20, 21). Consequently, the systematic inclusion of measures such as ADL/IADL, MMSE, GDS, MNA, SPPB, the Downton scale, and the Fried phenotype assessment in geriatric patients not only provides a useful functional description but can also guide targeted interventions to increase physiological reserve and recovery capacity in older adults (22, 23).

Standard CGA assessments remain essential, as they correlate with relevant components of resilience: locomotor and nutritional reserve, cognitive status, and psychological status, all of which determine recovery potential. However, resilience, as assessed by the physical resilience scale, complements these static measures by highlighting the speed and trajectory of recovery, key indicators for therapeutic decisions and rehabilitation planning (2, 22).

MATERIALS AND METHODS

This prospective study was initiated in March 2025, and presents preliminary data collected between March - September 2025 on 110 patients aged > 50 years admitted to the Dr. C.I. Parhon Clinical Hospital in Iași for acute medical events such as acute decompensated heart failure, urinary sepsis, and functional decompensation of chronic osteoarticular diseases. The exclusion criteria were as follows: age under 50 years, lack of informed consent, inability to obtain clinical and biochemical data, impaired cognitive status with an MMSE < 15, and inability to apply the resilience assessment scale. The study was approved by the Ethics Committee of the hospital and by the Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy, Iasi (Registration No: 8357/17.09.2024; 490/ 02.11.2024).

All patients included in the study underwent an assessment of intrinsic capacity using the following scales: MMSE, MNA, GDS, functional status - ADL, IADL, SPPB, fall risk- Downton scale, and the Frailty Phenotype by Freid (24). At the same time, we measured muscle strength in both upper limbs using a dynamometer, assessed pain using a visual analog scale, and collected data on the presence of heart failure. Additionally, to assess resilience, we applied the Physical Resilience Scale (16). Muscle strength was assessed using a dynamometer, and the values were interpreted according to sex and age, with specific thresholds for men and women, defining normal, low, or high values. For men aged 65 to 69, normal muscle strength ranged from 28.2 kg to 44.0 kg, and for those aged 70 to 99, from 21.3 kg to 35.1 kg. For women aged 65 to 69, normal muscle strength ranged from 15.4 kg to 27.2 kg, and for those aged 70 to 99, from 14.7 kg to 24.5 kg. Values below the lower limit were considered low, and those above the upper limit were considered high (25). Cognitive status was assessed using the MMSE scale (0–30 points), with normal cognitive function ranging from 25 to 30, mild impairment from 20 to 24, moderate impairment from 10 to 19, and severe impairment below 10 points (26). Nutritional assessment was based on the MNA questionnaire (24-30 indicates normal nutrition, 17-23.5 indicates a risk of malnutrition, <17 indicates malnutrition) (27).

The presence of a urinary tract infection was confirmed by a positive urine culture. Heart failure was confirmed by the presence of specific signs and symptoms and by ejection fraction (EF ≥ 50% - preserved

EF; EF < 50% - reduced EF), and the presence of osteoarticular pathology was evident from the patients' previous medical records. Emotional status was assessed using the 15-item Geriatric Depression Scale (GDS), with scores below 5 considered normal, 5–8 as mild depression, 9-11 as moderate depression, and above 11 as severe depression (28). The adapted Physical Resilience Scale was interpreted as follows: low resilience (score 1-5), moderate resilience (score 6-10), and high resilience (score 11-15) (16). The questionnaires were completed after obtaining the patient's informed consent and were administered by the same trained medical team.

Data were collected by a geriatric team and analyzed using SPSS Statistics 25 and Microsoft Office Excel/Word 2024. The Shapiro-Wilk test, the Mann-Whitney U/Kruskal-Wallis H test (with Dunn-Bonferroni post-hoc tests), Spearman's rho correlation coefficient, and univariate and multivariate generalized linear models using the Poisson method and the forward step-wise Wald method were performed. Statistical significance was defined at a 95% confidence interval ($p < 0.05$).

RESULTS

The study included 110 patients, with the majority being women (63.6%). The mean age was 75.94 ± 6.87 years (median=76), with 47 patients (42.7%) under the age of 75 (younger adults) and 50 patients (45.5%) between the ages of 75 and 84 (middle-aged adults), and 13 patients (11.8%) (older adults) (tab. I).

Most patients had no risk of malnutrition (median MNA 23 (20.5-25.3)), normal cognitive status (median MMSE 27 (24-29)), somewhat depressed status (median GDS 5 (3-8)), and independence in performing simple activities of daily living (ADL, median 5 (5-6)) and instrumental activities of daily living (IADL, median 7 (5-8)).

Furthermore, the study group exhibits frailty (Freid Phenotype median 3 (2-4)) (tab. I). In terms of resilience, the study group has a median of 10 (8-13).

Furthermore, in the study group, normal muscle strength values were observed in the right arm relative to the left arm, and there were also fewer low values in the right arm compared to the left, which are interpreted in the context of the dominant upper limb (see Table I).

Of the three conditions studied, the highest proportion of patients had osteoarticular disease (69.1%), followed by decompensated heart failure (62.7%). Only 13.6% of the enrolled patients had a urinary tract infection (tab. I).

Moreover, data from our study shows significant and positive correlations of low/moderate power between the resilience scale and ADL ($p=0.022$, $R=0.218$), IADL ($p<0.001$, $R=0.397$), SPPB ($p<0.001$, $R=0.458$), MNA ($p<0.001$, $R=0.377$), MMSE ($p=0.001$, $R=0.322$), left arm muscle force ($p<0.001$, $R=0.335$) or right arm muscle force ($p=0.001$, $R=0.332$), showing that patients with higher values for these parameters were significantly associated with higher values for the resilience scale and vice versa (tab. II). Also, there are significant and negative correlations of low/moderate/high power between the resilience scale and falling risk ($p<0.001$, $R= -0.331$), GDS ($p<0.001$, $R= -0.676$) or Fried phenotype score ($p<0.001$, $R= -0.462$), showing that patients with higher values for these parameters were significantly associated with lower values for the resilience scale and vice versa (tab. II).

TABLE I.
Characteristics of the analyzed patients

Parameter	Value
Age (Mean ± SD, Median (IQR))	75.94 ± 6.87, 76 (71.75-80)
Sex (No, %)	
Female	70 (63.6%)
< 75 years	31 (44.3%)
75-84 years	32 (45.7%)
≥ 85 years	7 (10%)
Male	40 (36.4%)
< 75 years	16 (40%)
75-84 years	18 (45%)
≥ 85 years	6 (15%)
MNA (Median (IQR))	23 (20.5-25.3)
MMSE (Median (IQR))	27 (24-29)
GDS (Median (IQR))	5 (3-8)
ADL (Median (IQR))	6 (5-6)
IADL (Median (IQR))	7 (5-8)
Fried phenotype (Median (IQR))	3 (2-4)
Resilience (Median (IQR))	10 (8-13)
SPPB (Median (IQR))	5 (3-7)
Pain score (Median (IQR))	5 (3-7)
Falling risk (Median (IQR))	3 (2-4)
CHF (No, %)	69 (62.7%)
CHF maintained (No, %)	30 (27.3%)
CHF reduced (No, %)	39 (35.5%)
UTI (No, %)	15 (13.6%)
Osteoarticular disease (No., %)	76 (69.1%)
Muscle force – left arm (Mean ± SD, Median (IQR))	19.23 ± 7.92, 17.5 (13.8-24.08)
Muscle force – left arm (No., %)	
Normal	55 (50%)
Higher	10 (9.1%)
Lower	45 (40.9%)
Muscle force – right arm (Mean ± SD, Median (IQR))	20.39 ± 7.68, 18.85 (15.2-24.9)
Muscle force – right arm (No., %)	
Normal	70 (63.6%)
Higher	8 (7.3%)
Lower	32 (29.1%)

TABLE II.

Correlations between resilience scale and other analyzed parameters

Correlation	Value
Resilience x ADL	0.022, R=0.218
Resilience x IADL	<0.001, R=0.397
Resilience x SPPB	<0.001, R=0.458
Resilience x Falling risk	<0.001, R= -0.331
Resilience x MNA	<0.001, R=0.377
Resilience x MMSE	0.001, R=0.322
Resilience x GDS	<0.001, R= -0.676
Resilience x Fried phenotype	<0.001, R= -0.462
Resilience x MF-LA	<0.001, R=0.335
Resilience x MF-RA	0.001, R=0.332
Resilience x BMI	0.352, R=0.090

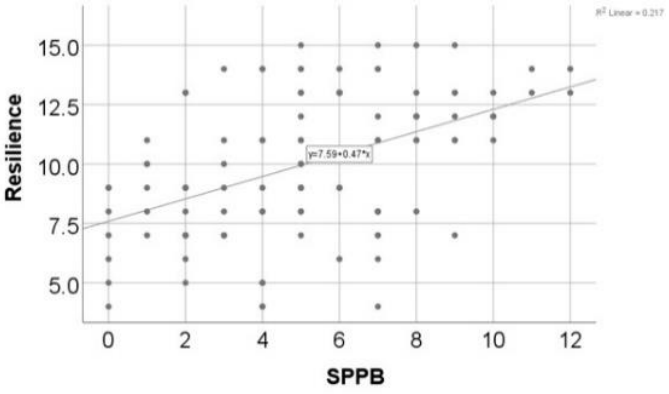

Fig. 1. Correlation between resilience scale and SPPB

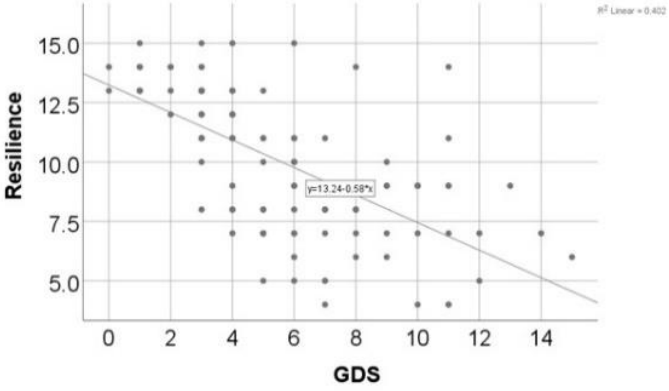

Fig. 2. Correlation between resilience scale and GDS

Table III and figures 3 and 4 present a comparison of the resilience scale in relation to the presence of heart failure, osteo-articular disorders, infection, and muscle strength in the study patients. The results showed that resilience did not differ in relation to these conditions: heart failure (p=0.171), urinary tract infection (p=0.517), and osteo-articular conditions (p=0.120). Patients with lower muscle force had significantly lower values for the resilience scale than patients with normal muscle force (p = 0.002 / p = 0.005). Furthermore, resilience differed significantly in relation to muscle strength: resilience (median 10 (8-13)).

TABLE III.

Comparison of resilience scale according to other parameters investigated

CHF	Mean ± SD	Median (IQR)	Mean rank	p*
Absent	10.46 ± 3.03	11 (8-13)	60.87	0.171
Present	9.67 ± 2.95	9 (7-13)	52.31	
CHF preserved	Mean ± SD	Median (IQR)	Mean rank	p*
Absent	10.05 ± 3.15	11 (7.25-13)	56.68	0.523
Present	9.73 ± 2.56	9 (7.75-12.25)	52.35	
CHF reduced	Mean ± SD	Median (IQR)	Mean rank	p*
Absent	10.15 ± 2.84	11 (8-13)	57.27	0.430
Present	9.62 ± 3.25	9 (7-13)	52.28	
UTI	Mean ± SD	Median (IQR)	Mean rank	p*
Absent	9.89 ± 2.93	10 (8-13)	54.72	0.517
Present	10.4 ± 3.44	11 (7-14)	60.43	
Osteoarticular disease	Mean ± SD	Median (IQR)	Mean rank	p*
Absent	9.26 ± 3.23	9 (7-13)	48.47	0.120
Present	10.28 ± 2.85	11 (8-13)	58.64	
MF-LA	Mean ± SD	Median (IQR)	Mean rank	p**
Normal	10.89 ± 2.61	11 (9-13)	65.21	0.002
Higher	10.2 ± 3.61	10.5 (6.75-13.25)	59.00	
Lower	8.78 ± 2.93	8 (7-11)	43.08	
MF-RA	Mean ± SD	Median (IQR)	Mean rank	p**
Normal	10.53 ± 2.89	11 (8-13)	61.40	0.004
Higher	11 ± 3.11	11.5 (8.25-13.75)	66.19	
Lower	8.47 ± 2.72	8.5 (7-10.75)	39.92	

*Mann-Whitney U Test, **Kruskal-Wallis H Test, MF-LA = Muscle force – left arm, MF-RA = Muscle force – right arm; CHR – chronic heart failure; UTI – urinary tract infection

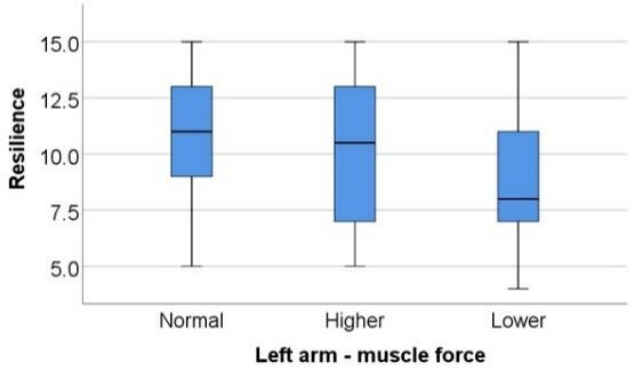

Fig. 3. Comparison of resilience scale according to the muscle force at the left arm

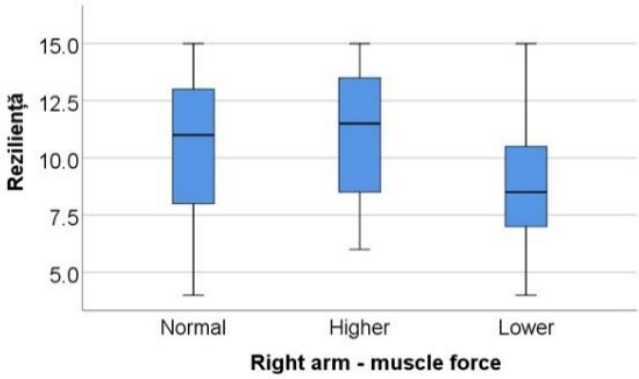

Fig. 4. Comparison of resilience scale according to the muscle force at the right arm

The univariable and multivariable generalized linear models used in the prediction of resilience scale are shown in table IV. According to the data from the multivariable model, the results show the following:

- The increase of one point for the IADL score increases the resilience scale by 3.2% (IRR = 1.032, 95% C.I. = 1.001-1.064, p=0.043).
- The increase of one point for the GDS score decreases the resilience scale by 5.7% (IRR = 0.947, 95% C.I. = 0.927-0.966, p<0.001) (tab. IV).

Physical resilience was positively associated with functional and nutritional status, including ADL, IADL, SPPB, upper-limb muscle strength, and MNA, with significantly lower resilience observed in patients with reduced muscle force. Psychological resilience correlated positively with cognitive status as measured by MMSE. Emotional resilience demonstrated a strong negative association with depressive symptoms (GDS), indicating that higher depression scores are linked to markedly lower resilience. Social resilience appeared indirectly related to functional autonomy (particularly IADL) and thus to social participation and network engagement, with IADL emerging as an independent predictor of resilience.

TABLE IV.
Univariable and multivariable generalized linear models used in the prediction of resilience scale

Parameter	Univariable		Multivariable*	
	IRR (95% C.I.)	p	IRR (95% C.I.)	p
ADL	1.096 (1.008-1.191)	0.033	-	-
IADL	1.060 (1.030-1.092)	<0.001	1.032 (1.001-1.064)	0.043
SPPB	1.048 (1.027-1.069)	<0.001	-	-
Falling risk	0.929 (0.887-0.972)	0.002	-	-
MNA	1.027 (1.011-1.043)	0.001	-	-
MMSE	1.027 (1.009-1.046)	0.003	-	-
GDS	0.940 (0.922-0.958)	<0.001	0.947 (0.927-0.966)	<0.001
Fried phenotype	0.894 (0.853-0.937)	<0.001	-	-
FM-BS	1.008 (1.001-1.016)	0.024	-	-
FM-BD	1.009 (1.002-1.017)	0.016	-	-

DISCUSSION

The present study demonstrates that resilience in older adults is closely linked to multiple domains of geriatric assessment, including functional status, physical performance, nutritional state, cognitive function, and muscle strength. At the same time, resilience is inversely associated with depressive symptoms, fall risk, and frailty. These findings reinforce the conceptualization of resilience as a multidimensional construct that integrates physical, psychological, and functional components.

The strong positive association between functional independence (ADL/IADL) and resilience is consistent with current research emphasizing the central role of autonomy in successful aging. Recent studies have shown that preservation of IADL is a key determinant of psychological resilience, as it reflects not only physical capacity but also executive function and social participation (29, 30). Loss of independence has been associated with diminished self-efficacy and reduced coping resources, thereby negatively impacting resilience trajectories in older populations.

The observed relationships between physical performance (SPPB), muscle strength, and resilience are also supported by emerging evidence linking sarcopenia and mobility limitations to reduced adaptive capacity. An increasing number of studies indicates that physical frailty and sarcopenia are not only biological conditions but also predictors of psychological vulnerability (31, 32, 33). In this context, better physical performance may contribute to resilience by preserving autonomy, reducing disability risk, and maintaining engagement in daily and social activities.

Similarly, the positive associations between nutritional status and cognitive function with resilience align with recent findings highlighting the interplay between metabolic, neurological, and psychological

health. Malnutrition and cognitive impairment have both been independently associated with decreased resilience and increased vulnerability to stressors (34, 35). Adequate nutrition supports brain function and energy balance, while preserved cognition enhances problem-solving abilities and adaptive coping strategies.

One of the most reliable findings of this study is the strong inverse relationship between depressive symptoms and resilience. This is consistent with current literature demonstrating a bidirectional association between depression and resilience in older adults (36). Depressive symptoms undermine coping mechanisms, reduce motivation, and impair social functioning, all of which are critical components of resilience. Importantly, our multivariable analysis confirms that depressive symptoms remain an independent predictor, underscoring their central role in resilience frameworks.

The negative association between frailty, fall risk, and resilience further supports the notion that resilience is deeply embedded within the broader frailty concept. Previous studies suggest that resilience may act as a protective factor mitigating the progression of frailty and its adverse outcomes (29, 31).

In contrast, frailty contributes to a cycle of decline characterized by reduced physical reserve, increased dependency, and heightened psychological distress.

From a clinical standpoint, these findings highlight the importance of adopting a comprehensive, multidimensional approach to geriatric care. Interventions targeting physical rehabilitation, nutritional support, cognitive stimulation, and mental health particularly depression screening and management may have synergistic effects on both resilience and overall health outcomes. Integrated care models have been shown to improve functional status and quality of life while reducing hospitalization and institutionalization rates (1, 37).

Notably, the identification of IADL and depressive symptoms as independent predictors of resilience suggests that these domains may represent key therapeutic targets. Programs aimed at maintaining or restoring functional independence, alongside early detection, and treatment of depression, could play an essential role in enhancing resilience and promoting healthy aging.

CONCLUSIONS

This study underscores that resilience in older adults is a complex, multidimensional construct, intimately linked to functional autonomy, physical performance, cognitive and nutritional status, and muscle strength, while inversely associated with depressive symptoms, frailty, and fall risk. Functional status and depressive symptomatology emerged as independent and robust determinants, highlighting that resilience is strongly shaped by both functional independence and mental health. These findings emphasize resilience not merely as a psychological trait but as a central factor influencing adaptive capacity and overall health in aging populations. Multidomain interventions aimed at preserving functional abilities and addressing depression may therefore substantially enhance resilience and mitigate vulnerability to adverse outcomes. Longitudinal studies are needed to clarify causal pathways and assess the effectiveness of strategies designed to strengthen resilience in elderly.

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We used AI tools, in a limited way, to verify the accuracy of proper English language usage for some paragraphs, since we are not native English speakers.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no funding regarding this scientific research.

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REVERSE LEFT ATRIAL REMODELING ASSESSMENT AFTER PAROXYSMAL ATRIAL FIBRILLATION ABLATION: OUR FIRST EXPERIENCE

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REVERSE LEFT ATRIAL REMODELING ASSESSMENT AFTER PAROXYSMAL ATRIAL FIBRILLATION ABLATION: OUR FIRST EXPERIENCE (Abstract) We aimed to assess reverse left atrial (LA) remodeling at 6 months after ablation of paroxysmal atrial fibrillation (AF), and to identify the most relevant parameter of reverse LA remodeling. **Material and methods**: Left atrial size was assessed by echocardiography: antero-posterior (AP) diameter, LA area in apical 4 and 2 chambers (A4c, A2c), LA volume by biplane formula (LAV1), and by computed-tomography (LAV2). **Results:** Seventy-eight consecutive patients with mean age of 57.41 ± 8.05 years were prospectively included; 60 (76.9%) were with paroxysmal AF less than 24 hours and 18 (23.1%) less than 7 days duration. All echocardiographic and computed-tomographic evaluations were made within 4 weeks before ablation and at 6 months after ablation procedure by the same operator. Circumferential or segmental pulmonary vein isolation was performed by radiofrequency in 58 (74.4%) patients, and by cryotherapy in 18 (23.1%) patients. At 6 months 53 (67.9%) patients were in stable sinus rhythm and 18 (23%) out of those on antiarrhythmic drugs. At 6 months after ablation all parameters of LA size decreased statistically significant. **Conclusions:** In our first experience, endocardial catheter ablation results in significant conversion in sinus rhythm in patients with paroxysmal AF. The maintenance of stable sinus rhythm after ablation procedure determined a significant reverse LA remodeling, irrespective of parameter used for LA size assessment. The most relevant parameter for assessing reverse LA remodeling in patients with endocardial ablation of paroxysmal AF was LA volume by echocardiography. **Keywords:** ATRIAL FIBRILLATION, LEFT ATRIUM, VOLUME, REMODELING.

Discovering of the pulmonary vein role as triggers in atrial fibrillation (AF) initiation and maintenance was fundamental for its therapy (1). Pulmonary vein isolation by catheter ablation is nowadays a well-known curative technique in selected patients (2, 3). After AF onset, structural, electrical and mechanical remodeling of the left atrium (LA) facilitates its own perpetuation ("AF begets AF") (4). LA size is an important marker of structural remodeling, recommended by current guidelines (5). LA size can be assessed by anterior-posterior diameter, area or volume; the current echocardi-

ographic recommendation being area or volume as parameters of LA remodeling assessment (6). It is well-known that echocardiography underestimates and computed-tomography (CT) overestimates LA volume (5).

It is well-known that stable sinus rhythm maintenance after AF ablation means reverse structural LA remodeling occurrence (decreasing LA size). It is associated with better outcomes in patients underwent AF ablation.

We aimed to assess reverse left atrial (LA) remodeling at 6 months after paroxysmal AF ablation and to identify the most relevant parameter of reverse LA remodeling.

MATERIAL AND METHODS

In this single center study, patients with paroxysmal or persistent AF were selected to undergo pulmonary vein isolation using first generation cryoballoon or by means of radiofrequency. Patients selection and inclusion in the study was made according with the current guidelines (2,3). The ablation procedure consisted of endocardial ostial isolation of each PV antrum by radiofrequency energy using a Lasso™ catheter (BiosenseWebster, CA, USA) and an irrigated tip Celsius™ Thermo Cool (Biosense Webster, CA, USA) or cryoablation (ICE® Cryoablation System, Medtronic, USA). Both procedures were done using local anesthesia by femoral access and deep sedation with Morphine fractionated i. v. during application of energy.

Left atrial size was assessed by echocardiography: antero-posterior diameter (AP), LA area in apical 4 and 2 chambers (A4c, A2c), LA volume by biplane formula (LAV1), and by computed-tomography (LAV2). All echocardiographic and CT measurements were made within 4 weeks before ablation and at 6 months after ablation procedure by the same operator.

Echocardiographic evaluation of left atrium size

Transthoracic echocardiography is the recommended approach for assessing LA size. Left atrial AP diameter (fig. 1), LA area in 4 and 2 apical chamber view and LA volume by biplane area-length formula (fig. 2) measurements were done using two-dimensional (2D) transthoracic echocardiography with an IE33 machine (IE 33 Echocardiography System, Philips Healthcare, The Netherlands). All echocardiographic measurements were performed at end-ventricular systole, at maximal LA size (for maximal LA volume assessment).

As currently recommended, we optimized each view to avoid: an underestimation of LA volume by foreshortening of the major length of the LA, inaccurate assumption of the mitral annulus boundary, loss of lateral resolution of the LA wall in the apical view or dropout of the interatrial septum or anterior wall (5). All measurements by echocardiography were performed by the same operator, before and at 6 months after ostial endocardial AF ablation.

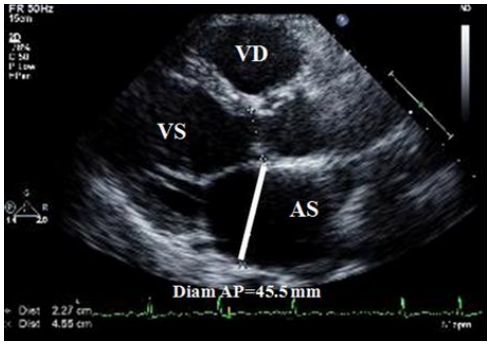

Fig. 1. Left atrium anterior-posterior diameter measurement in parasternal long-axis view by bi-dimensional transthoracic echocardiography

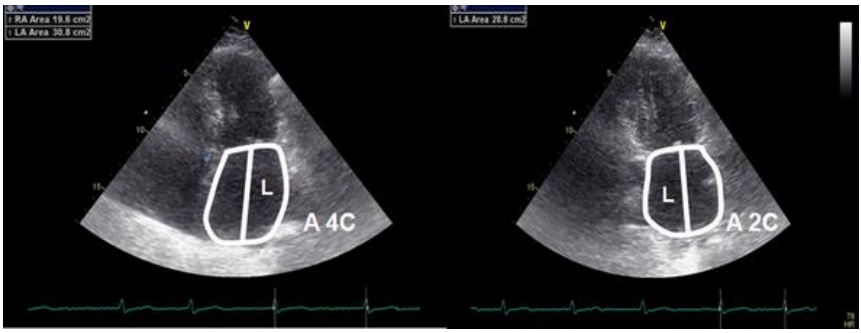

Fig. 2. Left atrium area and volume measurement in 4 and 2 chambers view by bi-dimensional transthoracic echocardiography *(A4C: apical four chamber views; A2C: apical two chamber views; L: length)*

Computed tomographic evaluation of left atrium volume

All patients underwent cardiac contrast enhanced CT (Siemens Somatom Senzation 128-Slice Configuration, Siemens AG, Germany) for evaluation of the morphology of the left atrium, before AF ablation and at ± 6 months after this procedure. For maximal LA volume assessment, all CT images were ECG-gated. We applied the ellipsoid biplane area-length formula, after we obtained an optimal contour in transverse axis by tracing the endocardial border cavity (fig. 3). Each of the CT slices was performed at 1 mm so L dimension was equal with the totally number of the slices.

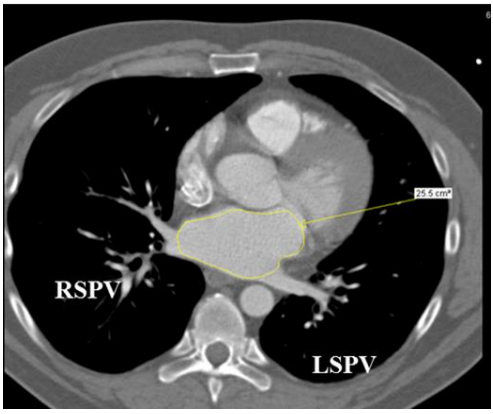

Fig. 3. Computed tomography image in axial view of left atrial volume measurement (*RSPV - right superior pulmonary vein, LSPV- left superior pulmonary vein*).

We performed the measurements avoiding an overestimation of LA volume on CT, by the following: the inclusion of part of LA appendage volume, the incorporation of the PV or overlap and the duplicate measurements of the multiple sequence scans. All measurements on CT were performed by the same operator, before and at 3 months after ostial endocardial AF ablation.

Statistical analysis

Data are presented as frequency distributions and simple percentages. Continuous variables are expressed as mean ± standard deviation. Statistical analysis was performed using *SPSS 10* for Windows (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered significant.

RESULTS

Seventy-eight consecutive patients undergoing non-valvular AF catheter ablation were prospectively and consecutively included in this study; 60 (76.9%) out of these patients were with paroxysmal AF less than 24 hours and 18 (23.1%) with paroxysmal AF less than 7 days. Mean age

was 57.41 ± 8.05 years; 67% were men; 74% had have arterial hypertension, 33% coronary heart disease and 19% diabetes mellitus. Mean paroxysmal AF duration was 4.7±1.7 months.

Circumferential or segmental pulmonary vein isolation was performed by radiofrequency energy in 58 (74.4%) patients and by cryotherapy in 18 (23.1%) patients. Antral pulmonary vein isolation was obtained at 35W (by radiofrequency energy) and minus 40°C (by cryotherapy). In patients with paroxysmal AF longer than 24 hours we used exclusively radiofrequency energy and ablation procedure was completed by complex fractionated atrial electrogram and cavo-tricuspid istmus ablation (if common atrial flutter was confirmed before or during procedure).

At 6 months 53 (67.9%) patients were in stable sinus rhythm, 18 out of these patients being on antiarrhythmic drugs. A redo (a second procedure) procedure was made in 23 (29.5%) patients and 18 (78.3%) of these patients were converted in stable sinus rhythm until 6 months. At 6 months, all parameters for LA size decreased statistically significant (tab. I).

TABLE I.

Mean values of left atrial parameters assessing left atrial size at baseline and after 6 months post-ablation

PARAMETERS	BASELINE	AFTER 6 MONTHS	p
Anteroposterior diameter (AP); mm	42±7	40±8	**0.050**
Apical 4 chamber area (A4c); cm^2/m^2	26±7	23±6	**0.003**
Apical 2 chamber area (A2c); cm^2/m^2	26±8	24±6	**0.011**
Indexed left atrial volume by biplan formula (LAV1); ml/m^2	44±16	37±10	**0.010**
Indexed left atrial volume by computed-tomography (LAV2); ml/m^2	49±23	44±17	**0.026**

DISCUSSION

Catheter ablation is now an accepted procedure for the treatment of AF (2, 3). In AF patients LA dilation is a marker of LA structural remodeling. This could result in a changed LA morphology and shape with a larger LA area and volume. LA volume and area and shape are the best parameters for LA size assessment as marker of LA structural remodeling.

The atrium dilation has different consequences over the LA anatomic segments (5). The mitral annulus, the mitral-aortic junction and the interatrial septum are less susceptible to dilatation because they are relatively fixed. Because of lack of the fibrous component, the pulmonary vein antrum could modify their thickness and geometry in structural remodeled atria (7,8). Progressive LA dilation is associated with asymmetrical structural remodeling and shape changes (5,8). Recently it was shown that a remodeled LA during dilatation evolved from ellipsoidal shape through a spheroidal one. This spheroidal shape could be an independent predictor of recurrence after AF ablation and may be useful in selecting the best candidates for AF ablation (9).

The most widely used parameter for LA size assessment, AP linear dimension, should not be used as the sole measure of LA size (5). If is still used, 2D echocardiog-

raphy in parasternal long-axis view is preferred over M-mode echocardiography (5).

When assessing the LA size and remodeling, the measurement of LA volume is recommended, because it considers alterations in LA chamber size in all directions. Two-dimensional echocardiographic LA volumes are typically smaller than those reported from CT or magnetic resonance imaging (5). Different methods exist for measuring LA volumes. Although the three linear measurements have been used to calculate LA volume using an ellipsoid model, the relative inaccuracy of these linear measurements limits this method. LA volume should be measured using the disk summation algorithm.

The LA endocardial borders should be traced in both the apical four- and two-chamber views. A single-plane approach can also be used, but this method is based on the geometric assumption that the LA is circular in the short-axis cut plane, which may not be always accurate. Although not recommended for routine use, this approach could be used in cases when planimetry in both views is difficult. Single-plane apical four-chamber indexed LA volumes are typically 1 to 2 mL/m2 smaller than apical two-chamber volumes (5). Alternatively, a bi-plane calculation could also be performed using the LA areas and lengths measured from both the apical four- and two-chamber views. Although the area-length method still assumes an ellipsoidal LA shape, it has the advantage of reducing linear dimensions to only two measurements of atrial length, of which the shorter one is selected.

All parameters used to assess LA size decreased statistically significant after 6 months; it means that LA suffered a reverse remodeling after catheter ablation of AF. The most significant reducing was unregistered of LAV1 (16%) comparing with 12% for A4C, 11% for LAV2, 10% for AP diameter and 8% for A2C. Therefore, we consider LAV1 or LA volume by echocardiography (bi-plane area-length formula) the most relevant parameter for assessing reverse LA remodeling in patients with endocardial ablation of paroxysmal AF.

Mean LA volume by echocardiography was smaller than by CT; it confirms that echocardiography might underestimates and CT might overestimate LA volume.

Echocardiography with the ellipsoid bi-plane dimension-length formula, is one of the recommended methods by both echocardiographic recommendations (5,6) although implying a certain degree of volume underestimation. Usually all patients selected for AF catheter ablation underwent a CT exam because of pre-procedural and intra-procedural utility.

However, echocardiography comparing with CT is cost efficient avoids irradiation exposure and is easier to use in daily practice.

Lower LA appendage contraction velocity could be a non-invasive pre-procedural predictor of persistent AF recurrences after ablation, indicating the possibility of reduced reverse remodeling of the LA (10).

Reverse structural remodeling takes longer and is still present 2 to 4 months after restoration of sinus rhythm (11). Pre-procedural delayed enhancement magnetic resonance imaging is also able to determine the degree of atrial fibrosis and is another tool to predict the reverse remodeling after ablation.

Study limits

This study was mainly designed to assess LA reverse structural remodeling in patients with non-valvular underwent AF

catheter ablation. In this study, we did not use any software for CT volume measurements. By performing single operator evaluations, we avoided inter- but not intraoperatory variability. Left atrial volume, as marker of structural remodeling, was assessed before and 3 months after AF ablation. It is difficult to discuss the influence of the type of ablation energy during AF ablation technique on LA volume, because the reduced number of patients; in addition, the follow-up was only 6 months.

CONCLUSIONS

Endocardial catheter ablation results in significant conversion on sinus rhythm in patients with paroxysmal AF. The maintenance of stable sinus rhythm after ablation procedure determined a significant reverse LA remodeling, irrespective of parameter used for LA size assessment. The most relevant parameter for assessing reverse LA remodeling in patients with endocardial ablation of paroxysmal AF was LA volume by echocardiography.

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BREAST CANCER AND BASAL CELL CARCINOMA IN THE SAME PATIENT - IS IT RANDOM? (CASE SERIES REPORTS)

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BREAST CANCER AND BASAL CELL CARCINOMA IN THE SAME PATIENT - IS IT RANDOM? (CASE SERIES REPORTS) (Abstract): Non-melanoma skin cancer, including basal cell carcinomas and squamous cell carcinomas, is the most common cancer affecting white-skinned individuals and its incidence is increasing worldwide. Several studies have reported that individuals diagnosed with non-melanoma skin cancer are at higher risk of subsequent or prior diagnoses of second primary malignancies. We report a series of five cases with concomitant basal cell carcinoma and breast cancer; in two cases the basal cell carcinoma was diagnosed after the breast cancer and in three cases it preceded it. Considering the high frequency and low mortality of basal cell carcinomas, they offer an excellent opportunity to study the factors that put some individuals at increased risk of multiple malignancies.
Keywords: BREAST CANCER, BASAL CELL CARCINOMA, NON-MELANOMA SKIN CANCER, MULTIPLE MALIGNANCIES.

Non-melanoma skin cancer (NMSC) is the most common cancer affecting white-skinned individuals and its incidence is increasing worldwide (1). The two major types of NMSC, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), have a relatively small impact on mortality but their public health impact is considerable. Although they share many similarities, they have different incidence rates and important etiological differences (2, 3).

In comparison with other malignancies, information regarding the incidence of NMSC and BCC is limited due to poor registration practice in most of countries, including Romania (4). The incidence rate appears to be higher compared with other malignancies and it is increasing. Worldwide the incidence of NMSC varies widely with the highest rates in Australia (>1,000/100,000 person-years for BCC) and the lowest rates in parts of Africa (<1/100,000 person-years for BCC) (1). Although the reason for this is unclear, in some countries it may be linked to increased sun-seeking behaviors and improved registration procedures (1).

Several studies have reported that indi-

viduals diagnosed with BCC are at higher risk of subsequent or prior diagnoses of second primary malignancy by about 20-60% (5).

In 2012, the estimated age-adjusted annual incidence of breast cancer in 40 European countries was 94.2/100,000 and the mortality 23.1/100,000, with rather decreased values for Romania: significantly lower incidence of 66.2/100,000 and similar mortality of 21.6/100,000 (6), possibly due to delay in or avoidance of seeking medical attention and advanced stage at diagnosis. Since breast cancer is the most frequently diagnosed non-cutaneous cancer among women, it is not surprising that many individuals with breast cancer will associate BCC.

During a prospective study on breast cancer comprising of 65 patients, we identified 8 patients that associated multiple malignancies, of which 5 (7.7% of all cases) associated basal cell carcinomas. We present the particularities of these 5 patients.

CASES REPORTS

Case 1. Female patient, aged 77, was diagnosed with malignant tumor of the left mammary gland in September 2013, which was surgically removed (superior-external quadrantectomy with lymphadenectomy - 13 lymph nodes) and morphopathological examination revealed an invasive carcinoma of the mammary gland with mucinous areas, stage pT2N0G1 (tab. I). The patient received subsequent radiotherapy and is now continuing treatment with hormone therapy (Tamoxifen).

In July 2015, the patient underwent surgical removal of a white nuanced frontal lesion with central ulceration, which appeared one year before surgery. The anatomo-pathological examination revealed that the lesion measuring 0.7/0.6/0.1 cm was a superficial multicentric basal cell carcinoma and confirmed the excision was in the healthy tissue in all plans (tab. II).

Case 2. Female patient, aged 69, was diagnosed with malignant tumor of the right mammary gland in December 2004. Subsequent surgery (Madden modified radical mastectomy, including lymphadenectomy of 8 lymph nodes) followed by the morpho pathological examination identified NOS invasive ductal carcinoma, stage pT1cN2aMxG3 (7 lymph nodes with metastases, 2 lymph nodes > 2cm) (tab. I). The treatment included chemotherapy and radiotherapy, followed by hormone therapy (3 years with Tamoxifen and 2 years of Letrozole).

In September 2015 the patient underwent surgery for a white skin lesion in the nasal region, which appeared 3 years earlier and in the absence of clinical improvement the patient sought medical attention. Surgery was performed and morpho pathological examination revealed a lesion measuring 0.7/0.6 cm corresponding to a superficial multicentric basal cell carcinoma. The examination also confirmed the excision was made in healthy tissue in all the plans (tab. II).

Case 3. Female patient, aged 77, was diagnosed with malignant tumor of the right mammary gland in December 2009 when surgery was performed (Madden modified radical mastectomy, including lymphadenectomy of 7 lymph nodes). The morphopathological examination identified NOS invasive ductal carcinoma, stage pT1bN0MxG2 (tab. I). The treatment included chemotherapy followed by hormone therapy (5 years of Letrozole).

In this case, the history of skin lesions preceded the diagnosis of mammary gland tumor. From patient's data, the first skin lesion appeared around the age of 22

(1968), after a severe sunburn, under the form of a pigmented asymmetrical lesion localized in the left suprascapular region that began to ulcerate and was surgical removed in 2004. The morphopathological examination confirmed a basal cell carcinoma. Four years later, in the proximity of the first lesion, in the posterior cervical region, another lesion appeared and was rapidly surgically removed. The morphopathological examination revealed another lesion of basal cell carcinoma, and confirmed the surgery was performed within oncological safety limits (tab. II).

TABLE I

Details on the onset and morphopathological examination of the mammary gland tumor

	Mammary gland tumor						
	Year of diagnosis	**Estrogen Receptor**	**Progesterone Receptor**	**HER 2-neu**	**Ki67**	**Ellis-Elston score**	**Stage**
Case 1	2013	95%	<1%	2+	5%	4	pT2 N0 Mx-G1
Case 2	2004	NE	NE	NE	9%	3	pT1 cN2a Mx-G3
Case 3	2009	90%	1-2%	2+	11%	2	pT1b N0 Mx-G2
Case 4	2006	25%	35%	NC	NE	1	pT2 N2a Mx-G1
Case 5	2013	80%	80%	1+	20%	6	pT2m N2a Mx-G2

(NE - not evaluated, NC -inconclusive)

TABLE II

Details on the onset and morphopathological examination of the skin lesion

	Basal cell carcinoma				
	Year of clinical onset*	**Year of surgical intervention**	**Size**	**Characteristics**	**Localization**
Case 1	2014	2015	0.7/0.6/0.1 cm	superficial, multicentric	Face (nasal region)
Case 2	2012	2015	0.7/0.6 cm	superficial, multicentric	Face (frontal region)
Case 3	1968 (?)	2004	0.7/0.5 cm	superficial, multicentric	Left suprascapular region
	2008	2008	0.5/0.5 cm	superficial, multicentric	Posterior cervical region
Case 4	2003	2015	0.9/0.9/0.4 cm	solid, with pigmented areas	Posterior cervical region
Case 5	2001	2013	1/0.8 cm	solid, with ulcerative areas	Face (temporal region)

(*approximately)

TABLE III

Time between the diagnosis of breast cancer and onset of BCC

	Date of birth	**Year of breast cancer diagnosis**	**Onset of BCC***	**Radiotherapy for breast cancer**	**BCC *vs.* breast cancer**
Case 1	1938	2013	2014	Yes	followed
Case 2	1946	2004	2012	Yes	followed
Case 3	1938	2009	1968	No	preceded
			2008		
Case 4	1953	2006	2003	No	followed
Case 5	1950	2013	2001	Yes	preceded

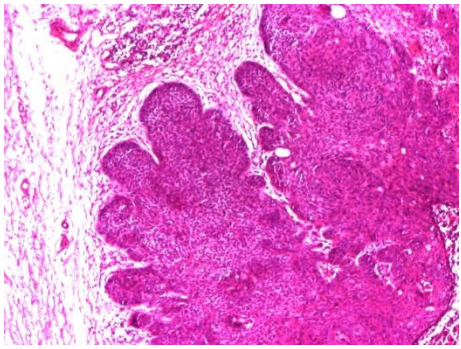
* presumed

Case 4. Female patient, aged 62, known with malignant tumor of the left mammary gland that was surgically removed in August 2006 (Madden modified radical mastectomy, including lymphadenectomy of 19 lymph nodes) followed by the morphopathological examination that identified invasive tubular carcinoma, stage pT2N2aMx-G1 (7 lymph nodes with metastases) (tab .I), underwent chemotherapy and afterwards hormone therapy (3 years Letrozole, 1 year Tamoxifen and is currently on 6-month treatment with Exemestane).

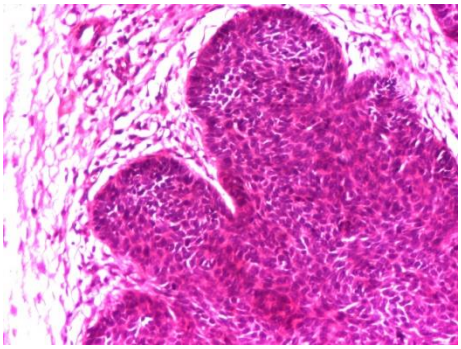
In November 2015 the patients underwent surgery for a skin lesion localized in the nasal region that appeared approximately 12 years before. The morphopathological examination revealed a lesion corresponding to a solid ulcerative basal cell carcinoma, with pigmented areas (fig. 1). The examination also confirmed the excision was made in healthy tissue in all plans (tab. II).

Case 5. Female patient, aged 65, diagnosed with malignant tumor of the left mammary gland and operated in January 2013 (Madden modified radical mastectomy, including lymphadenectomy of 33 lymph nodes) and the morphopathological examination identified NOS invasive ductal carcinoma, stage pT2mN2aMxG2 (tab. I). The treatment included chemotherapy and radiation therapy followed by hormone therapy (Letrozole).

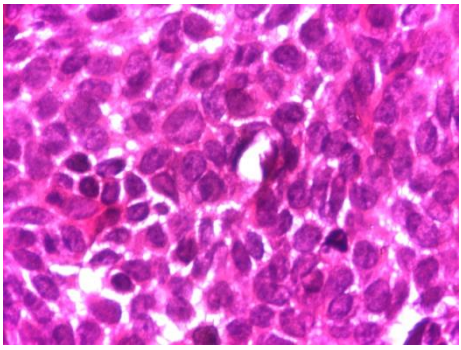
The patient reported in 2001 a pathological skin lesion located on the face (left temporal region) that was surgically removed simultaneously with the breast lesions. The morphopathological examination confirmed a solid basal cell carcinoma with an ulcerative area (tab. II).



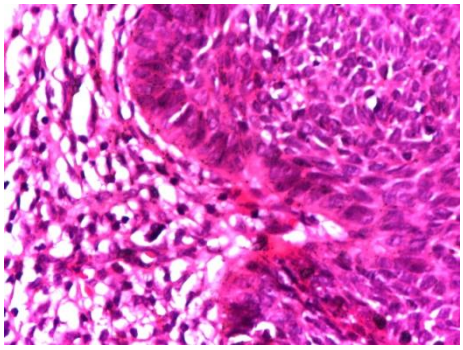
a) Basal cell carcinoma (x4)



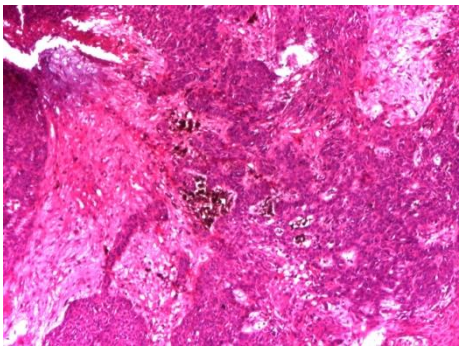
b) Basal cell carcinoma (x10)



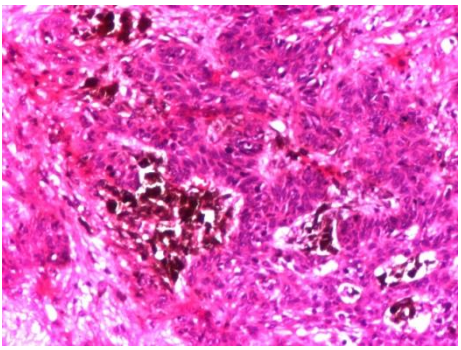
c) Abnormal Mitosis (x40)



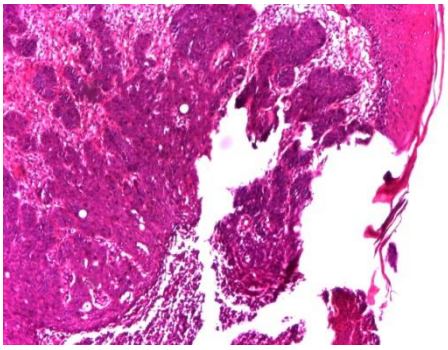
d) Peripheral palisade (x20)



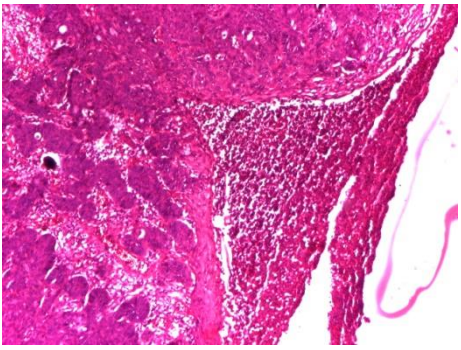
e) Pigmented areas (x4)



f) Pigmented areas (x10)



g) Ulcerative area - 1 (x4)



h) Ulcerative area - 2 (x4)

Fig. 1. Patient 4: Morphopathological appearance of a basal cell carcinoma (Delia Ciobanu, M.D.)

DISCUSSION

We presented five cases associating two primary malignancies: BCC and breast cancer. Considering that our ongoing research included 68 patients with breast cancer, this association was met in 7.4% of the patients. This raised the question of possible underlying mechanisms that lead to this condition.

BCC seems to form in the epidermal stem cells of the outer root sheet of the hair follicle, but the precise origin of BCC it is still unknown. The lesions are classified into five types: nodular-ulcerative, pigmented, sclerodeiform or fibrosing, superficial and fibroepithelioma. Apart from the environmental exposure and immunosuppressive therapy, people with a fair skin type-I complexion (red or blonde hair, light colored eyes, freckles), and those with a history of intermittent sun exposure, especially ultraviolet (UV) - B radiation and severe sunburn during childhood, are at highest risk (7). The treatment options are focused on local control and include mainly surgical techniques. Nonsurgical approaches comprise of radiotherapy, topical injection therapy and photodynamic therapy (8, 9).

Regarding breast cancer, this is a heterogeneous disease with histological differences within tumors and between patients. The cancer development in the normal mammary gland depends on stem cells,

neighboring cells including fat cells and fibroblasts that play distinct roles through specific signaling pathways (10). Endogenous hormones (sex hormones, growth hormone axis), reproductive factors (lower parity, older age at first giving birth, younger age at menarche, older age at menopause), oral contraceptives and hormone replacement therapy, family history and genetic factors (BRCA1 and BRCA2 mutations), alcohol and smoking, diet, overweight and obesity are known to be risk factors in breast cancer (11). The treatment of breast cancer includes surgery, radiation therapy, chemotherapy, and hormone therapy and the prognosis and selection of therapy may be influenced by clinical and pathological features.

It is likely that the association of BCC and other cancers may have an etiologic link. The deficiencies of pathways responsible for protecting against cellular transformation in multiple tissues, such as DNA repair (e.g.: nucleotide excision repair - NER - pathway) (12,13,14) or immune responses (e.g.: UV-induced immunosuppression) (15,16), may play a role in cutaneous and internal carcinogenesis.

The major environmental cause of BCC is exposure to solar UV radiation (17, 18). The individual risk appears to be determined largely by cumulative exposure to solar radiation, in combination with individual susceptibility. Thus, it is important to realize that BCC incidence is based on a lifetime of exposure to risk factors, and the older population that is currently being diagnosed used very little sun protection and received very little education regarding the effects of ultraviolet radiation (UV) (19). On the other hand, the exposure to UV-B radiation is necessary to provide sufficient vitamin D, known to reduce the risk of cancer incidence, but data considering this issue are conflicting (19,20).

Breast cancer after BCC

In addition to having an increased risk for new skin cancer, patients with basal-cell carcinoma are at increased risk for non-cutaneous cancer at various sites.

In one of the largest prospective cohort study on topic (18) (performed in U.S.A.; included 107339 women, 46237 men), the researchers found that among patients with a personal history of NMSC (without specification to BCC or SCC) there was an increased risk of subsequent malignancies, specifically breast (RR = 1.19; 95% CI 1.11-1.28, $p<0.0001$; AR = 87 per 100,000 person-years) and lung (RR=1.32, AR=22 per 100,000 person-years) cancer in women and melanoma in both men (RR=1.11; 95% CI 1.05-1.18) and women (RR=1.20; 95% CI 1.15-1.25). They also showed that patients with medical history of NMSC were more likely to be older and tended to burn and have more severe sunburns.

In population-based case-control study on NMSC (U.S.A) (5) (3584 participants: 1543 individuals with BCC, 1170 with SCC, 1416 age- and sex-matched controls) a significantly increased cancer risk after BCC was identified, that could not be explained by a variety of environmental, nutritional or behavioral risk factors, or by family history of cancer (adjusted HR=1.4, 95% CI 1.15, 1.71). In the site-specific analysis, BCC was associated with an increased risk of subsequent breast cancer (adjusted HR=1.13, 95% CI 0.62, 2.06).

A population-based cohort study (21) (Denmark; it included 37,674 patients followed for a maximum of 14 years after a first diagnosis of basal-cell carcinoma) showed that patients diagnosed with basal-

cell carcinoma before 60 years of age (SIR=1.26) had a statistically higher risk for developing new cancer (P < 0.01) than did those diagnosed at 60 years of age or older (SIR=1.11). Also, the study revealed that patients with BCC had an increased risk of developing breast cancer, and that this risk was also significantly affected with age (SIR=1.37 in patients < 60 years of age compared with 1.05 in those > or = 60 years of age).

Another study (22) (Canada, 15,586 cases of BCC and SCC) reported a greater risk of female breast cancer following a BCC. Overall, the increased risk of developing a subsequent cancer was observed only in the first 4 years following a NMSC, although it remained increased for specific cancer sites and that the risk remained higher in all age groups up to 75 years of age. For breast cancer, the standard incidence ratios for developing the second primary cancer varied by time since diagnosis: SIR=1.47 for <1 year from BCC, SIR=1.26 for 1-4 years and SIR=1.22, for >5 years; the total SIR was 1.22 (95% CI 1.11-1.34).

BCC after breast cancer

Besides an elevated risk of contralateral breast cancer, women with a primary breast cancer are at increased risk of developing a subsequent non-breast cancer (attributed to genetic and other risk factors, and treatment: radiotherapy, chemotherapy and hormonal therapy).

Data concerning the incidence of BCC in breast cancer patients is scarce, due to mostly various registrations (regarding NMSC some registries include all types, others include only SCC, while others have no data on NMSC).

A cohort study (23) (13 population-based cancer registries in Europe, Canada, Australia and Singapore; included 525.527 women with primary breast cancer) identified an increased risk of NMSC (SIR=1.58). The variations on the time since first primary tumor and the age at breast cancer diagnosis are presented in table IV.

TABLE IV

SIRs for NMSC among 535.527 women with a first primary breast cancer by time since first primary breast cancer and by age at breast cancer diagnosis (23)

	<1 year after breast cancer	1-9 years after breast cancer	10+ years after breast cancer	Pre-menopausal breast cancer (45 years)	Peri-menopausal breast cancer (46-55 years)	Post-menopausal breast cancer (561 years)
NMSC	1.23, 95 % CI 1.10-1.39	1.55, 95 % CI 1.48-1.62	**1.77**, 95 % CI 1.67-1.87	**2.08**, 95 % CI 1.87-2.31	1.68, 95 % CI 1.55-1.82	1.51, 95 % CI 1.45-1.57

Recent studies focus more on the incidence of melanoma developing after a breast cancer diagnosis (SIRs ranging from 1.16 to 2.74) (24, 25, 26) and this risk was attributed especially to external radiation therapy (even at non-irradiated sites) (27).

In 2 cases of our 5 patients, which were diagnosed with BCC after breast cancer, the lesions were localized on the face (sun-exposed regions) and patients underwent radiotherapy as part of breast cancer management before the diagnosis of BCC.

Future knowledge about the impact of

risk factors and effects of treatment in breast cancer may add further explanations on the association of BCC, in addition to potential influences from increased surveillance and general cancer susceptibility.

CONCLUSIONS

In our study regarding breast cancer patients, 5 associated BCC. Unfortunately, extensive data on the incidence of BCC is unavailable in Romania. Due to their high frequency and low mortality, BCCs offer an excellent opportunity to study factors that put some individuals at increased risk of multiple malignancies. Thus understanding the shared risk factors that contribute to multiple malignancies may lead to new etiologic insights.

Also, knowing the risk of developing new primary cancers after breast cancer is important not only in relation to potential side effects of their cancer treatment, but also in relation to the possibility of shared etiology with other types of cancer.

CONSENT. This study was approved by the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania, and written informed consent was obtained from the patients for the research and publication of this study and any accompanying images.

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PRIMARY HYPERPARATHYROIDISM: HORMONAL PROFILE AND RISK OF COMPLICATIONS

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PRIMARY HYPERPARATHYROIDISM: HORMONAL PROFILE AND RISK OF COMPLICATIONS (Abstract): **Aim**: to assess the role of vitamin D status on the metabolic profile and spectrum of complications at patients with primary hyperparathyroidism function of the vitamin D repletion status. **Material and methods**: transversal study involving 42 patients with primary hyperparathyroidism submitted to parathyroidectomy. We evaluated serum calcium, phosphate, serum PTH and $25OHD_3$, bone mineral density by dual-energy X-ray absorptiometry (DXA-Hologic) and history of bone fractures and renal lithiasis. Fifteen (35.7%) patients had episodes of kidney lithiasis, whereas 27 (64.26%) patients did not develop this complication. Twenty (47.6%) patients were diagnosed with osteoporosis whereas 22 (52.36%) patients had normal bone mass or osteopenia. Data between groups were compared using the t test and were considered significant at p values < 0.05. **Results**: Twenty-nine (62.5%) of the 42 patients had $25OHD_3$ in the range of deficiency (lower than 20 ng/mL) and 9 (28.12%) had severe $25OHD_3$ deficiency (lower than 10 ng/mL). Serum calcium and phosphate were similar irrespective of the presence or absence of complications. No differences were found between mean PTH and $25OHD_3$ levels of patients with or without osteoporosis. Patients with kidney lithiasis had, however, higher PTH (390 +/- 90 pg/mL *vs.* 209 +/- 64 pg/mL, p< 0.001) and lower $25OHD_3$ levels (12.8 +/- 6 ng/mL *vs.* 19.2 +/- 7.8 ng/mL, p < 0.05) than patients without kidney lithiasis. **Conclusions**: D hypovitaminosis accompanies frequently primary hyperparathyroidism. Patients with higher risk of kidney lithiasis seem to have higher PTH and lower $25OHD_3$ levels. **Keywords:** PRIMARY HYPERPARATHYROIDISM, HYPOVITAMINOSIS D, OSTEOPOROSIS

Primary hyperparathyroidism (pHPTH) is the result of increased secretion of parathyroid hormone (PTH). Almost 85% of cases of pHPTH are caused by a single adenoma (1). pHPTH is usually accompanied by high levels of the active form of vitamin D ($1,25(OH)_2D_3$), due to excessive stimulation of 1-α hydroxylase by PTH. Vitamin D deficiency has a high frequency in the general population, and in patients with pHPTH (2, 3). This coexistence may mask certain biochemical modifications in pHPTH, such as hypercalcemia, but does not diminish the risk of bone or kidney

stone complications caused by the overproduction of PTH (4).

The co-existence of hypovitaminosis D with pHPTH was moreover associated with an increased circulating PTH, larger parathyroid glands, accelerated bone turnover and higher risk of fracture (2,5-7). Patients submitted to parathyroidectomy despite low vitamin D levels prior to surgery seem to have a higher risk of hungry bone syndrome (5, 8, 9). Moreover, severe long-standing vitamin D deficiency leads per se to disorders of bone mineralization, causing skeletal deformations and poor bone quality, called osteomalacia (10). These modifications may be added to the direct impact of PTH excess on bone (11, 12). The consequences of vitamin D deficiency in patients with pHPTH and their appropriate management are presently not completely clear. Although vitamin D repletion may decrease certain risks such as the appearance of hungry bone syndrome after surgery, there is a fear among clinicians for aggravating hypercalcemia or hypercalciuric by vitamin D supplementation and even to worsen or mask bone damage in patients with pHPTH (9). The D repletion – related risks are however inconsistently proven.

We wanted to investigate the impact of vitamin D status on the metabolic profile, severity and risk of complications in patients with primary hyperparathyroidism.

MATERIAL AND METHODS

We performed a transversal study involving 41 female patients aged 30-80 years and 1 man, 57 years, diagnosed with pHPTH in the Endocrinology Department of "St. Spiridon" Hospital between January 2014-December 2015, and submitted to parathyroidectomy. Prior to enrolment all patients signed an informed consent, approved by the Ethical Committee of "Grigore T. Popa" University of Medicine and Pharmacy Iasi.

Patients underwent anamnesis (personal history of fragility fractures or urolithiasis) and clinical examination. A morning fasting blood sample was taken for the dosage of Calcium (Ca), phosphate (P) (spectrophotometric technique, with Roche commercial kits compatible with the automatic analyzer Cobas 6000, Roche), PTH and 25-OH-D_3 (chemiluminescence using the automatic analyzer Cobas e 411, Roche BV). 24-hour urine sample was used for the measurement of Ca and P excretion. Abdominal ultrasound was performed to observe the presence of renal lithiasis. BMD at lumbar (L1-L4) and hip regions was assessed by dual-energy X-ray absorptiometry technique (DXA, Hologic Delphi W), by a certified technician.

All results were expressed as mean +/- SD. Statistical analysis was performed with SPSS 18.0. For the statistical analysis were used: Paired Sample T-Test / F_{ANOVA}, χ^2 / Kruskal-Wallis test, Pearson' s simple correlations. Differences were considered significant for $P < 0.05$.

RESULTS

This study included 41 women (23-82 years) and 1 man (57-years-old).

PTH was higher than the reference ranges in all patients (median value of 406.92 pg/mL). PTH was higher in the 28 patients under 60 years old than in the 14 patients over 60 (481.05 *vs.* 252.94 pg/mL; p= 0.044).

29 (62.5%) patients had $25OHD_3$ in the range of deficiency (lower than 20 ng/mL) and 9 (28.12%) had severe vitamin D deficiency (lower than 10 ng/mL). Other 9 (28.12%) patients had low (insufficient, i.e.

between 20 and 30 ng/mL) levels of $25OHD_3$. Only four (9.37%) patients had $25OHD_3$ in the range of repletion (> 30 ng/mL) (fig.1).

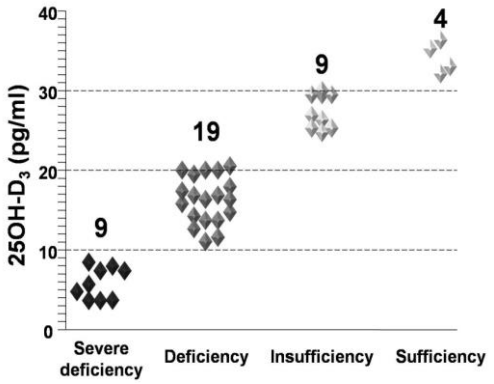

Fig.1. Vitamin D levels at patients with pHPTH

Low levels of vitamin D were associated with higher PTH in 65% of pHPTH patients (r=-0.65; p=0.009).

Serum calcium levels varied between 10.01–19.62 mg/dl (mean value 11.93 mg/dl) and was not significantly correlated with vitamin D levels (r= -0,194; p=0,288).

Serum phosphate was below reference ranges in 47.6% of patients, varying between 1.38-3.64 mg/dl (mean phosphate 2.40 mg/dl). Vitamin D was in direct correlation with phosphate levels, 47.1% of patients associating higher levels of phosphate with higher values of vitamin D (r= +0.471; p=0.008).

Median lumbar, femoral and forearm BMD were in the osteopenia/osteoporosis range for T-score and had no further loss one year after surgery (fig. 2a, b, c).

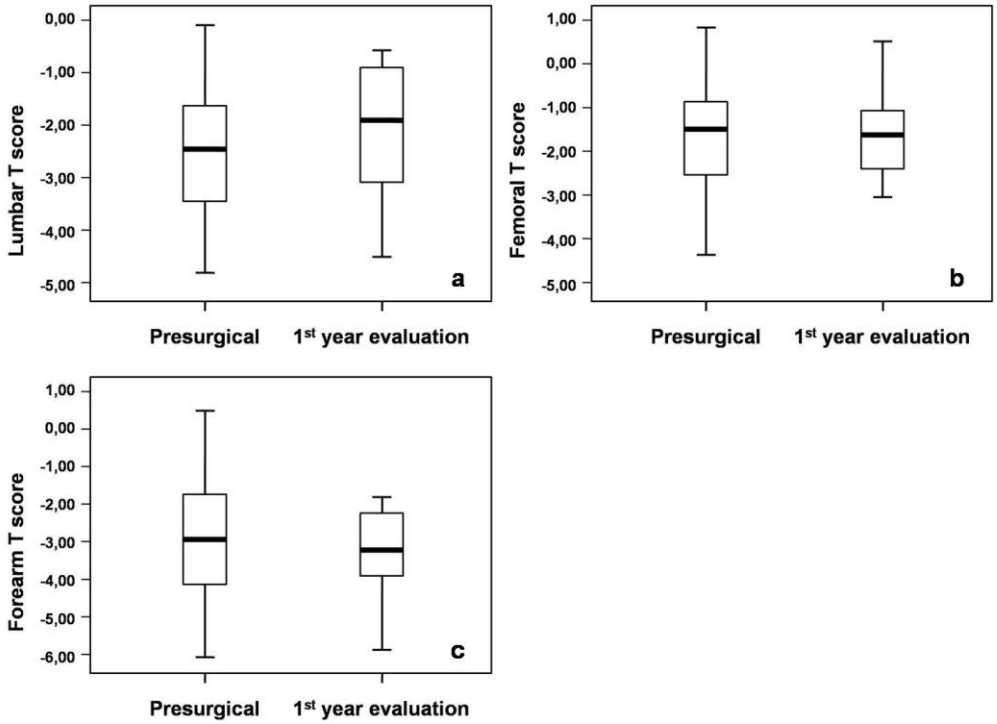

Fig.2. BMD expressed as T score before and one year after parathyroidectomy at lumbar (L1-L4) (**a**), femoral (**b**) and forearm level (**c**)

20 (47.6%) patients were diagnosed with osteoporosis, whereas 22 (52.36%) patients had bone mass in the range of normal or osteopenia.

PTH levels decreased drastically after surgery, suggesting cure. There were no significant differences between median values of pre- or postsurgical PTH from patients with or without osteoporosis (fig. 3a). Patients with hypovitaminosis D received vitamin D supplementation (1000 IU $25OHD_3$ per day), therefore median vitamin D increased significantly after one year ($p < 0.05$). We found no differences between median $25OHD_3$ levels from patients with or without osteoporosis before or one year after surgery (fig. 3b). Only 5 (11.9%) patients had a history of fragility fractures. Again, no differences in presurgical PTH or $25OHD_3$ levels were observed between patients with or without fragility fractures (fig. 3c, d).

Anamnesis revealed that 15 (35.7 %) patients had a history of renal lithiasis, whereas 27 (64.26%) patients did not develop this complication. Patients with renal lithiasis had higher PTH (390 +/- 90 pg/mL *vs.* 209 +/- 64 pg/mL, $p < 0.001$) and lower $25OHD_3$ levels (12.8 +/- 6 ng/mL *vs.* 19.2 +/- 7.8 ng/mL, $p < 0.05$) than patients without renal lithiasis (fig. 4a, b).

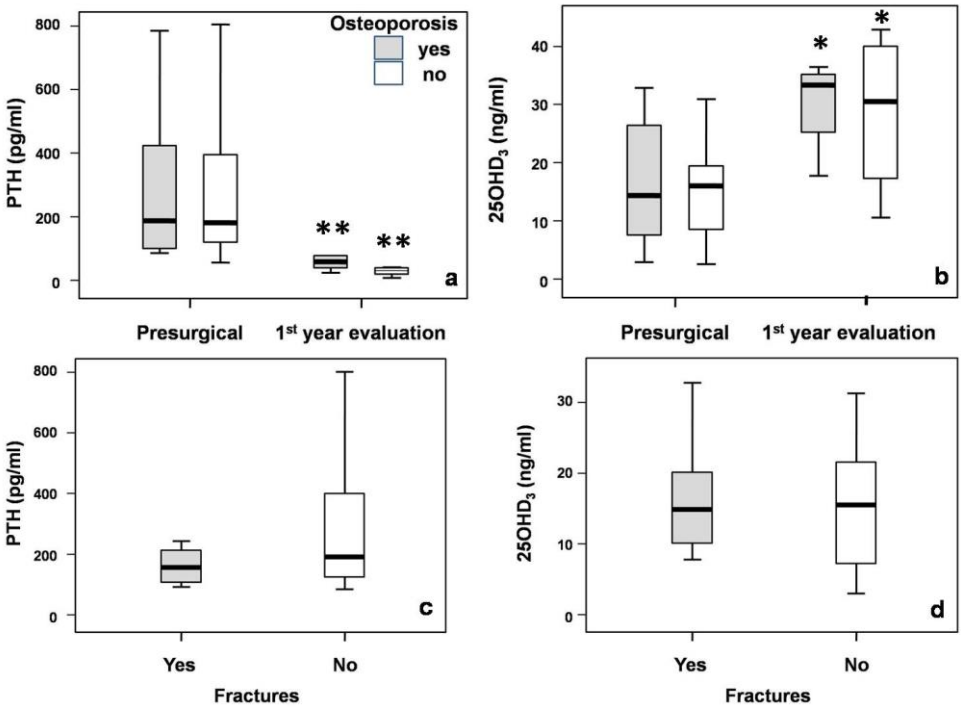

Fig. 3. a, b. Serum PTH and $25OHD_3$ before and one year after parathyroidectomy at patients without (white) or with (gray) osteoporosis (defined as having a T score lower than – 2.5 at one or more situses by DXA). * $p < 0.05$ and ** $p < 0.001$ when compared to presurgical values. **c, d.** Presurgical PTH and $25OHD_3$ at patients without (white) or with (gray) frailty fractures.

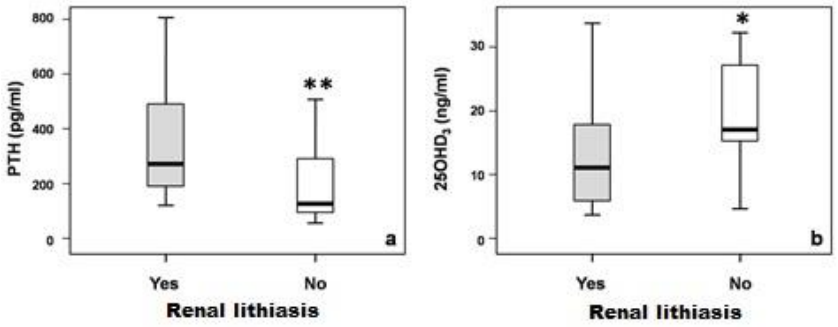

Fig. 4. Mean PTH (**a**) and mean $25OHD_3$ (**b**) in patients with pHPTH with (gray bars) or without (white bars) kidney lithiasis. * $p < 0.05$ and ** $p < 0.001$ when compared with values from patients with lithiasis.

DISCUSSION

We assessed the impact of vitamin D status on the metabolic profile, severity and risk of complications in patients with pHPTH.

We found higher PTH levels in patients younger than 60. Whereas disease incidence is higher at older age, earlier onset may imply increase disease severity, thereby easing a quick diagnosis.

The high incidence of D hypovitaminosis in our patients with pHPTH is striking and may be due to less sunlight exposure and to lack of vitamin D intake (3). After rigorous supplementation, vitamin D levels entered the normal range within one year after surgery. Before surgery, lower vitamin D were associated with higher PTH (p=0.009), fact described by other authors (2,5) and suggesting that hypovitaminosis D may amplify PTH secretion, possibly due to low negative feedback, but could also mask the diagnosis by lowering serum calcium levels (13).

Vitamin D administration in deficient patients is important, but some doctors avoid it in pHPTH before surgery, fearing for an aggravation of hypercalcemia or hypercalciuria. Certain studies (5,8,9) concluded that supplementation of D depleted pHPTH patients before surgery may lower the risk of postsurgical complications, such as hungry bone syndrome. In a 15-year retrospective study Randle et al. (14) suggested that waiting for vitamin D normalization may increase the risk of multiglandular disease and that recurrence, persistence or hypocalcemia were not influenced by the presence or absence of presurgical D hypovitaminosis. Like the previous study, although many of our patients were vitamin D depleted and not substituted before surgery, they did not experience significant hungry bones syndrome after the resection of parathyroid adenomas.

There are also conflicting data regarding bone damage in patients with pHPTH associated with hypovitaminosis D. Whereas several studies (13, 14) described a lower bone mass when hypovitaminosis D coexisted with pHPTH, others (16) found no differences in bone density or microarchitecture despite higher PTH levels.

The patients of our group had overall decreased BMD at all sites before surgery. One year after surgery BMD did not suffer further decrease, and had even a trend to increase at the lumbar spine, as found by others (17), suggesting that the process of bone loss was stopped by the healing of pHPTH. We did not find any differences in

the PTH or vitamin D levels from osteoporotic patients or patients with frailty fractures when compared to patients without osteoporosis or without fractures. Most of our patients had, however, vitamin D levels in a close range of deficiency, severe deficiency or insufficiency; therefore, it would be hazardous to jump to conclusions regarding the impact of low vitamin D levels on bone density in patients with pHPTH.

Renal lithiasis, a classical complication of pHPTH (18), was present in 35.7% of the patients enrolled in the study. The patients with renal lithiasis had significantly higher PTH levels and a significantly more severe D-hypovitaminosis when compared to the patients with pHPTH but no renal lithiasis. To our knowledge, it is the first time when PTH and $25OHD_3$ levels are related to the risk of renal lithiasis in pHPTH. Low $25OHD_3$ may influence lithiasis risk indirectly by favoring the increase of higher PTH levels in pHPTH, as it was recently described (19) in young male patients with relapsing lithiasis and idiopathic hypercalciuria.

CONCLUSIONS

Our study underlined the frequent coexistence between primary hyperparathyroidism and hypovitaminosis D, and how this association may modulate the spectrum of metabolic parameters, thereby influencing disease diagnosis and evolution. Assessment of $25OHD_3$ levels is therefore mandatory in pHPTH. Severe D hypovitaminosis seems to be correlated with larger parathyroid adenomas and higher PTH, possibly influencing the risk of kidney stone formation. It is currently not clear whether D hypovitaminosis contributes to bone frailty or if it increases the risk of postsurgical hungry bones syndrome. Another unanswered question is whether vitamin D reserves should be best repleted before or immediately after surgery.

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NEWS

2-AMINOBENZIMIDAZOLE DERIVATIVES SUPPRESS B-LACTAM RESISTANCE IN *MYCOBACTERIUM SMEGMATIS* AND *M. TUBERCULOSIS*

In a study by Nguyen *et al*, 2-aminobenzimidazole derivatives were tested for the ability to suppress β-lactam resistance in *Mycobacterium smegmatis*. The compounds proved to reverse intrinsic resistance to β-lactam antibiotics of this microorganism. The mechanism of action was different from that of traditional β-lactamase inhibitors. This activity extends to *M. tuberculosis* strains, with suppression of carbenicillin resistance, including in multidrug-resistant strains (Nguyen TV, Blackledge MS, Lindsey EA, Minrovic BM, Ackart DF, Jeon AB, et al. The discovery of 2-aminobenzimidazoles that sensitize *Mycobacterium smegmatis* and *M. tuberculosis* to β-lactam antibiotics in a pattern distinct from β-lactamase inhibitors. Angew Chem Int Ed Engl. 2017 Mar 1. doi: 10.1002/anie.201612006).

Teodora Vremera

CORONARY CIRCULATION AND CARDIAC HEMODYNAMICS – MORPHOFUNCTIONAL AND IMMUNOHISTOCHEMICAL PARTICULARITIES IN PATIENTS WITH ISCHEMIC HEART DISEASE

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CORONARY CIRCULATION AND CARDIAC HEMODYNAMICS – MORPHOFUNCTIONAL AND IMMUNOHISTOCHEMICAL PARTICULARITIES IN PATIENTS WITH ISCHEMIC HEART DISEASE (Abstract): **Aim**: To determine the structural changes and the degree of cardiomyocyte degeneration in relation to coronary circulation and intracardiac hemodynamic indices in patients with ischemic cardiomyopathy (ICM) and ischemic heart disease (IHD). **Material and methods**: 134 patients with circulatory insufficiency NYHA Class I-IV were investigated: 71 with dilated cardiomyopathy (DCM) (mean age 35.6 ± 1.3 years), 17 with ICM (mean age 45 ± 7 years), and 46 with IHD (mean age 54 ± 12 years). Myocardial scintigraphy, CT coronary angiography, ventriculography and morphological and immunohistochemical analysis of sub endomyocardial biopsy were performed. **Results and discussion**: Coronary lesions in patients with ICM/ IHD: 1. circumflex branch - in 86%: grade III stenosis - 31.6%, obstruction in 4.7%. 2. diagonal branch - in 28.0%: grade III stenosis - 100%. LMA - in 62.3%: grade III stenosis - 100%. RCA - in 90.5%: grade III stenosis - 59.2%, obstruction - 40.8%. one-vessel disease - 0.0%; two-vessel disease - 22.7%; three-vessel disease - 28.1%; four-vessel disease - 49.2%. Coincidence of compromised myocardial contractility areas with perfusion defects by combining VRE and rest ^{201}Tl-myocardial scintigraphy was noted in 80% of patients with DCM and in 84.6% of those with ICM / IHD. Hemodynamic indices in patients with ICM and DCM were identical, and in those with IHD, PAP and PTDVS were lower and VTD and IB of VD - higher. Morphologically, cardiomyocytes in a state of apoptotic degeneration were found in all study groups. Immunohistochemically, the apoptotic index (AI) was 13.82 ± 1.66% in the fibrous tissue surrounding area and 2.25 ± 0.86% in areas far from the fibrous portions of the myocardium. **Conclusions**: The severity of cardiac hemodynamic disorders, coronary and intramyocardial circulation in patients with ICM/IHD and DCM directly depends on the nature of morphological and immunohistochemical changes in the myocardium; persistence and similarity of apoptotic process in ICM/IHD and DCM show that apoptosis is the main mechanism of cardiomyocyte death that contributes to the development and progression of heart failure in patients with the above-mentioned diseases. **Keywords**: CARDIOMYOPATHY, APOPTOSIS, CARDIOMYOCYTE, CARDIAC FAILURE.

Mechanisms of development and progression of heart failure depend largely on the ratio of cardiomyocyte death and regeneration. Loss of heart cells occurs main-

ly through apoptosis and necrosis. Involved in cardiomyocyte death are multiple factors, such as Ca^{2+} homeostasis disorder, proteins regulating mitochondrial morphology, ischemia, etc. that have been highlighted in many cardiovascular diseases such as those secondary to ischemia - ICM/IHD and primary myocardial diseases - DCM. In primary myocardial disease, coronary arteries usually show no changes, lumen stenosis and atherosclerotic changes being absent.

In elderly patients, can be detected hemodynamically insignificant atherosclerotic changes of the vessels, and their degree does not reflect the severity of morphological changes of the myocardium.

The small intramyocardial arteries are normal in most cases, but intimal thickening particularly in the areas of cardiosclerosis, increased coronary artery tone, decreased coronary blood flow, reduced degree of coronary artery dilation and therefore decreased coronary reserve can be detected (1). These changes can be related to either to elevated left ventricular end-diastolic pressure (LVEDP) and increased sub endomyocardic and perfusion pressure, or to structural and/or functional changes in the myocardial microvascular bed.

The results with information role in the study of myocardial coronary reserve are obtained by ^{201}TI-scintigraphy using a vasodilator (2). Detection of stable perfusion defects before and after vasodilator administration reveals the presence in those segments of myocardial sclerotic changes or scarring, and their dynamics in one or another direction indicates the perfusion state in the viable myocardium. Based on literature reviews on this topic (3) it can be concluded that the majority of patients with intrinsic myocardial disease present coronary and/or intramyocardial circulation disorders.

Of interest is the comparative study of the possible features of myocardial vascularization, especially at microcirculatory level, intracardiac hemodynamics and cardiomyocyte degeneration in patients with secondary - ICM/IHD and primary myocardial diseases - DCM, of complications of heart failure (HF).

MATERIAL AND METHODS

Our study included 134 patients with circulatory failure NYHA class I-IV: 71 patients with DCM (mean age 35.6 ± 1.3 years), 63 (88.8%) males and 8 (11.2%) females; 17 male patients with ICM (mean age 45 ± 7 years); and 46 male patients with ischemic heart disease (IHD), (mean age 54 ± 12 years).

The used investigation methods were: echocardiography, cardiac catheterization, multi-position coronary angiography, left and right balanced radionuclide ventriculography, sub endomyocardial biopsy with morpho-logical, morphometric and immunohistochemical analysis (TUNEL test).

RESULTS

According to segmentally computerized scintigraphy data, LV myocardial perfusion defects were found in similar frequency in patients with ICM/IHDC (82.5%) and DCM (81.1%), the incidence of perfusion defects occupying over 20% of the left ventricular surface, 45.5% in ICM/IHD and 35.2% in DCM. By combining VRE and rest ^{201}Tl-myocardial scintigraphy, coincidence of compromised myocardial contractility areas with perfusion defects was noted in 84.6% of patients with ICM/IHD and 80% of patients with DCM. Most commonly, in 75% of ICM/IHD cases perfusion

defects coincided with the location of myocardial scarring according to instrumental investigation data (ECG, ECOCG, coronaro ventriculography); in DCM patients, this concordance was found in only 31.8%. Perfusion defect size did not practically differ between patients with ICM/IHD and DCM (tab. I).

TABLE I

Comparison of left ventricular myocardial perfusion defect sizes in patients with ICM/IHD and DCM according to rest ^{201}Tl-myocardial scintigraphy data

	Perfusion defects (% of LV surface)			
	Total per group	**HF I-II (NYHA)**	**HF III-IV (NYHA)**	**p**
DCM	n = 71 13.7 ± 1.3	n = 49 13.1 ± 1.6	n = 22 15.1 ± 2.3	NS
ICM/IHD	n = 63 14.2 ± 1.7	n = 28 9.5 ± 2.0	n = 35 17.7 ± 2.4	< 0.02
Accuracy of p differences	1-3 < 0.002 2-3 < 0.001 1-2 – NS	1-3 < 0.01 2-3 < 0.002 1-2 – NS	NS	

Coronary artery lesions according to coronary angiography data in patients with ICM/IHD consisted in: 1. circumflex branch - in 86%: grade III stenosis - 31.6%, obstruction - 4.7%. 2. diagonal branch - in 28.0%: grade III stenosis - 100%. LMA - in 62.3%: grade III stenosis - 100%. RCA - in 90.5%: grade III stenosis - 59.2%, obstruction - 40.8%. One-vessel disease - 0.0%; two-vessel disease - 22.7%; three-vessel disease - 28.1%; four-vessel disease - 49.2%. All patients with ICM presented right and left ventricular dilatation up to cardiomegaly without cardiac aneurysm formation and left ventricular wall thickening. Optical microscopy revealed in virtually all cases marked endocardial sclerosis progressing to interstitial sclerosis. Also, frequently detected was diffuse-focal myocardial interstitial macro sclerosis. The number of capillaries per surface area unit was greatly reduced. Some cardiomyocytes were vacuolated, and their nuclei were pyknotic. Electron microscopy revealed a significant number of cardiomyocytes in the state of apoptosis. Identification of apoptosis in myocardial biopsy specimens by TUNEL method revealed the presence of immunoreactivity nuclei in all studied patients with DCM, ICM/IHD. The apoptotic index (AI) ranged from 1% to 3% in patients with DCM, and from 1% up to 16% in those with ICM/IHD. In myocardial samples of patients with ICM and high AI (AI = 13.82 ± 1.66%), most apoptotic cells were situated at the periphery of fibrous foci, while in the myocardial segments more distant to these the percentage of apoptotic cells decreased substantially (2.25 ± 0.86%), being identical to the percentage in DCM (2.19 ± 0.82%).

Comparative analysis of hemodynamic parameters and AI% by study group (DCM and ICM/IHD) was made globally in all study patients, taking into consideration only the severity of circulatory insufficiency (tab. II).

When comparing the intracardiac hemodynamic parameters in patients with DCM and ICM with the persistence of

apoptosis process (IA) we have found a direct correlation with left and right ventricle PTD and VTD and an inverse dependence with ejection fraction.

TABLE II

Interaction between intracardiac hemodynamic indices and AI (%) in patients with DMC and ICM

		EDV		EF		EDP		IA (%)
		LV	RV	LV	RV	LV	RV	
DCM	M	192.1	157.7	0.20	0.25	22.1	11.3	2.19
	SD	56.50	46.41	0.05	0.05	6.77	3.61	0.82
	Corr	0.714	0.690	0.595	-0.550	0.525	0.307	
	P	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.005	
ICM	M	183	138.82	0.22	0.36	22.76	10.29	2.25
	SD	44.24	40.76	0.06	0.05	6.47	2.87	0.86
	Corr	0.634	0.641	0.480	-0.534	0.700		
	P	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

EDV=end-diastolic volume, EF=ejection fraction, EDP=end-diastolic pressure, LV=left ventricle, RV=right ventricle

DISCUSSION

Most patients with primary myocardial disease present coronary and/or intramyocardial circulation disorders (3). According to our data, changes in myocardial vascularization at microcirculatory level, intracardiac hemodynamics and immunomorphological degeneration in patients with myocardial diseases secondary to ischemia - ICM/IHD and primary - DCM, and complications of heart failure (HF), are closely related to intimal thickening of small intramyocardial arteries and increased coronary tone, but also to the decrease in coronary blood flow (extraction of ^{201}Tl is increased) and decrease in the degree of coronary artery dilation with reduction of coronary reserve. The above-mentioned changes may be a result of either increased left ventricular end-diastolic pressure (LVEDP) and increased sub endomyocardial and perfusion pressure or of structural and/or functional changes in the myocardial microvascular bed (1). Mechanisms of development and progression of heart failure depend largely on the ratio of cardiomyocyte death and regeneration. Loss of heart cells occurs mainly through apoptosis and necrosis (4, 5, 6). In cardiomyocyte death are involving multiple factors such as interstitial or substitutional fibrosis, metabolic acidosis, Ca^{2+} homeostasis disorder, proteins with a potential of regulating mitochondrial morphology, ischemia etc. and have been highlighted in many cardiovascular diseases such as in those secondary to myocardial ischemia and those associated with primary myocardial disorders (7, 8, 9, 10). The data reported by our study clearly demonstrate both the morphological and functional changes in myocardial vascularization, at all levels, in patients with ICM/DCM, thus confirming the presence in these patients of chronic myocardial hypoxia (ischemia) and its role in primary and/or secondary cardiomyocyte lesions. The immunohistochemical method revealed the presence of immunoreactivity nuclei in

all myocardial biopsy specimens from patients with ICM/IHD - 2.25 ± 0.86% and DCM - 2.19 ± 0.82%. AI index showed a direct correlation with EDP and EDV of the left and right ventricles, and inverse dependence with ejection fraction ($p<0.001$). This demonstrates that excessive myocardial dilation is related to activation of the apoptotic process and desynchronization of myocytes, leading to reduced myocardial contractility and progression of heart failure in patients with ICM/IHD and DCM, thus expanding our knowledge of the pathogenesis of chronic heart failure, and stimulates research and development of measures to prevent circulatory insufficiency in patients with these diseases.

CONCLUSIONS

Our study reveals that the severity of disturbances in cardiac hemodynamics and coronary and intramyocardial circulation in patients with ICM/IHD and DCM is directly dependent on the nature of morphological and immunohistochemical changes of the myocardium; persistence and similarity of apoptotic process in ICM/IHD and DCM show that apoptosis is the main mechanism of cardiomyocyte death that contributes to the development and progression of heart failure in patients with the above mentioned diseases.

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MODERN PARACLINICAL EXPLORATION OF RENOVASCULAR ARTERIAL HYPERTENSION

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MODERN PARACLINICAL EXPLORATION OF RENOVASCULAR ARTERIAL HYPERTENSION (Abstract): Renovascular arterial hypertension (RVAHT) is a type of secondary arterial hypertension (considered the second one in terms of frequency in secondary AHT in adults), which is in a causality relation with a uni- or bilateral stenosis of the renal artery. The concept of RVAHT appeared with Goldblat's experiments (1934), RVAHT being considered a clinical consequence of activation of the renin-angiotensin-aldosterone system. The prevalence of renal artery stenosis (RAS) increases with age, being seen in 7% of the individuals who are over 65 years old. Paraclinical investigations have the role to confirm the clinical suspicion (by objectifying the morphological lesion) and estimate the functional consequences of this lesion. Currently, many of these methods have maintained only their historical value, while in the forefront there are the non-invasive methods which allow a rapid and highly accurate diagnosis. **Keywords:** RENOVASCULAR ARTERIAL HYPERTENSION, RENAL ARTERY STENOSIS, RENIN.

Renovascular arterial hypertension (RVAHT) is a type of secondary arterial hypertension (considered the second one in terms of frequency in secondary AHT in adults), which is in a causality relation with a uni- or bilateral stenosis of the renal artery. The concept of RVAHT appeared with Goldblat's experiments (1934), RVAHT being considered a clinical consequence of activation of the renin-angiotensin-aldosterone system (1-5).

The prevalence of renal artery stenosis (RAS) increases with age, being seen in 7% of the individuals who are over 65 years old. Renal artery stenosis is seen in 20% of the patients with coronary artery disease, 30% of those with aortoiliac disease and 50% of those with peripheral vascular disease. Classically, the accepted pathological criterion is that of "hemodynamically significant stenosis" (1, 2). The positive criterion is the reduction of the diameter by more than 60%, which corresponds to a reduction of the lumen area by about 85%. It is believed that a hemodynamically significant RAS –frequently contributes to the two major complications of the disease: causing renovascular arterial hypertension (RVAHT) and/or altering the renal parenchyma, structurally and functionally, with developing chronic renal failure. It must be remembered that a RAS of over 60% only

sometimes causes RVAHT and more commonly, but not necessarily, it causes renal ischemia with morpho-functional alteration (1, 7).

The causes of RAS can be systematized in the following manner:

1. RENAL ARTERY ATHEROSCLEROSIS (Renal artery stenosis through atheromatous plaque) – is the most common cause, it affects males, it appears in late ages; in this case, the stenosis is in the proximal third of the renal artery, sometimes associated with post-stenotic dilation; it can coexist with other stenosed vascular lesions (carotid lesions, lesions of the lower limb arteries).

2. RENAL ARTERY FIBRODYSPLASIA: it can present itself as medial fibro dysplasia, perimedial fibro dysplasia, intimal fibro dysplasia (more common in children), and subadventitial fibro dysplasia. Fibro dysplasia is more common in early ages, especially in women, and it usually affects 2/3 of the distal area of the renal arteries. They can also be seen in the context of collagen diseases (e.g., Marfan syndrome).

3. RARE CAUSES: renal artery thrombosis and embolism, arterial aneurysm, extrinsic compressions of the renal artery through tumors, arteriovenous fistula, abdominal aorta coarctation, Takayasu's aortitis, renal artery hypoplasia, systemic vasculitis, neurofibromatosis, primary antiphospholipid syndrome, post-irradiation renal artery stenosis, cholesterol crystal micro embolism (1, 3-6, 11, 12).

The pathogenic mechanism of renovascular AHT mainly recognizes the activation of the renin-angiotensin-aldosterone system (experimentally described ever since 1934 by Goldblat), with particularities depending on the uni- or bilateral impairment of the renal artery (and, implicitly from the function of the contralateral kidney) (1, 5, 10).

The diagnosis of renovascular AHT obviously starts from a clinical suspicion which is based on the existence of some suggestive anamnestic or clinical elements. Starting from the criteria of Zanchetti issued in 2001, there are currently several clinical elements which, from a practical point of view, are indicating to the diagnosis of renovascular AHT (1, 11, 12, 14).

They can be represented by one or several of the following elements of clinical suspicion:

- Severe AHT in a child or young adult with no risk factors or after the age of 50 years;
- Severe AHT with onset at any age; onset of grade II severe hypertension (>160/100 mmHg in patients over 55 years old);
- Epigastric, subcostal or flank systolic/diastolic murmur;
- Short-time accelerated evolution of an AHT initially considered essential;
- AHT and inexplicable impairment of renal function;
- Relatively sudden deterioration of renal function in a hypertensive person;
- Refractory AHT in triple drug association;
- Deterioration of renal function as a response to treatment with ACE inhibitor;
- Extensive atherosclerotic lesions of the coronary, cerebral and peripheral arteries in a hypertensive person;
- Recurrent acute pulmonary edema apparently with no obvious cause;
- Lumbar pain with acute onset in the absence of urethral obstruction (1, 4, 5, 7, 9, 11, 12, 14).

Other elements which can orient the clinical suspicion are: the coexistence of

other cardiovascular risk factors: smoking, dyslipidemia, diabetes, worsening of renal failure under antihypertensive treatment, occurrence or worsening of renal failure after arterial catheterization, negative family history for hypertension, severe changes of fundus of eye in a hypertensive person.

There are also biological data which raise the suspicion of renovascular AHT:

- Hypokalemia;
- Hyponatremia;
- Increase of serum creatinine;
- High RA (renin-angiotensin);
- Moderate proteinuria;
- Increase of plasma renin.

Sometimes, a difference of more than 1.5 cm between the longitudinal diameters of the two kidneys in the echography examination, in the context of high BP values, can raise the suspicion of its renovascular etiology (imaging suspicion) (1, 4, 5, 7, 9, 11, 12, 14).

PARACLINICAL INVESTIGATIONS have the role to confirm the clinical suspicion (by objectifying the morphological lesion) and estimate the functional consequences of this lesion.

Currently, many of these methods have maintained only their historical value, while in the forefront there are the non-invasive methods which allow a rapid and highly accurate diagnosis.

Doppler examination of renal arteries combined with renal echography is a non-invasive, cheap method with high sensitivity and specificity, which can reveal the presence of renal artery stenosis (defined as the decrease of more than 60% of the renal artery diameter in the vascular duplex ultrasound) (7, 15 - 17). The method allows the anatomical and functional assessment of the renal arteries. Direct visualization of the main arteries is combined with measurement of the intrarenal pressure and velocity. The main drawback of this procedure is the lack of arterial visualization due to intestinal gases in 50-90% of the cases. At the same time, it allows to determine the resistive index at the level of the renal arteries which is usually high in the hypertensive patient in whom lesions of hypertensive nephron angiosclerosis occurred. The increase of maximum systolic velocity in the renal arteries >180 cm/s and a renal/aortic systolic velocity ratio >3.5 are suggestive of renal artery stenosis. A translesional systolic pressure gradient >15% may represent an indication for angioplasty (15-17). Duplex ultrasound also has a predictive value for the success of renal angioplasty; thus, if the resistive index in intrarenal circulation is higher than 0.8, it is considered that blood pressure will not return to normal after angioplasty in 97% of the patient, and in 80% of the subjects the renal function will not improve (18). However, Zeller et al. (19) have proven in a study on diabetic patients with severe stenosis of the renal arteries at the ostial level that favorable post-angioplasty results can be obtained even if this index exceeds 0.8. The main drawback of this procedure is sometimes the difficult visualization of the arteries due to intestinal gases in 50-90% of the cases.

Renal angiography by nuclear magnetic resonance (with gadolinium) allows the visualization of the renal arteries and accurately tracking renal artery stenosis, it has a high sensitivity and specificity, is non-invasive and potentially non-nephrotoxic. Its drawbacks are related to the need for the patient to maintain apnea during examination and the potential worsening of the results in the case of presence of intracor-

poral metallic bodies (stents, vascular clips, pacemakers) (fig. 1) (20-22).

Spiral computed tomography with CT angiography–entails contrast agent and irradiation of the patient. It has a good sensitivity and specificity for the diagnosis of renal artery stenosis, but it must be avoided in patients with severe chronic renal failure (7, 11, 12).

Selective renal arteriography is the gold standard to confirm the diagnosis. It is currently used in patients with high suspicion of renal artery stenosis in whom non-invasive explorations have been inconclusive. It also allows tracking of accessory renal arteries. The method helps establish the therapeutic indication: thus, concentric, unique critical stenosed (more than 75%) have indication of interventional treatment, while eccentric or multiple stenoses are directed to surgical approach. It has two important risks: nephrotoxicity of the contrast agent and the potential risk of renal atheroembolism. The limits are related to the lack of information regarding renal ischemia and the interdiction of use in significant renal failure (5, 7, 11, 12).

Analyzing 43 clinical cases explored by angiography in the Cardiology Department of " Sf. Spiridon" Hospital, result some practical conclusions. First, renovascular hypertension should be excluded before refractory hypertension is affirmed; 7 patients (16.2%) had this type of blood pressure profile in our analysis. Secondly, secondary hypertension is more frequent in young patients, but the atherosclerotic renovascular hypertension should be taken in account in patients older than 55 years (29 patients in our analysis – 67.4%) with acceleration of a pre-existing essential hypertension (8 patients – 18.6%). Thirdly, although duplex ultrasonography is recommended as the first-line imaging test to establish the diagnosis, the accuracy is operator dependent. When the clinical presentation is highly suggestive, digital subtractions angiography is the key method for both final diagnosis and endovascular treatment. If measuring the translesional pressure gradient to detect a functional artery stenosis is not available, selective bilateral renal vein renin measurement can be useful (19 patients had a significant ratio of 1.8-2) to decide revascularization. 31 of our patients had a successful endovascular revascularization (27 atherosclerotic lesions, 4 fibromuscular dysplasia lesions).

Fig.1. Right renal artery stenosis (collection from the Clinic of Cardiology)

Other methods: Renal isotopic scintigraphy, minuted intravenous urography – are rarely used in present day. Renal scintigraphy (infusion test after captopril) and other radio isotopic methods do not offer anatomic data. On the other hand, by clearly highlighting the infusion asymmetry, scintigram offers predictability data of curing AHT after revascularization, with a sensitivity of 75% and a specificity of 90%.

Scintigram cannot be interpreted in case of bilateral renal artery stenoses and cannot be applied in renal failure. Of the radio isotopic assessment methods of renal flow, those that use technetium-mertiotide or technetium-pentetic acid (DTPA) can determine, quite accurately, the fractional flow for each kidney. This very clearly suggests the possible ischemia, even in conditions of bilateral RAS. In the absence of any morphological information, radio isotopic explorations have not entered the routine practice (7).

The imaging methods will be completed by laboratory data:

- plasma renin activity (PRA) is increased by 50-80%;
- renin-captopril test has a sensitivity and specificity of 75-100%;
- determining plasma renin activity and especially the PRA ratio from the renal vain from the stenotic part and the PRA from the contralateral vein, comparing renin discharge from each kidney; it is useful to predict the success of a surgical revascularization. The increase of renin discharge from the ischemic kidney compared to the health kidney by 1.5 times is considered a positive test (1, 5, 7, 12).

CONCLUSIONS

We confirm that renovascular arterial hypertension continues to remain a challenge for the practitioner physician. The diagnosis is always based on the clinical suspicion and the combination of paraclinical investigations increases the sensitivity and specificity of the diagnosis. Of all methods, selective renal arteriography remains the gold standard of all paraclinical methods to confirm the diagnosis of renal artery stenosis.

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NEWS

FATAL TOXIC MYOCARDITIS INDUCED BY PARAPHENYLENE DIAMINE. A CASE REPORT

Para phenyl diamine (PPD) is an aromatic amine derivative of Aniline that is widely used as a cosmetic product, particularly in hair dye preparations. PPD acute poisoning leads to respiratory distress, rhabdomyolysis, muscle necrosis and renal failure. Cardiac complications are rarely reported in the literature. We will present a case of PPD poisoning presenting as myocarditis, associated to rhabdomyolysis with a fatal outcome. The study presented the case of a 22-year-old man who was hospitalized for ingestion of a hair dye. At admission it had a severe edema of the face and neck. Laboratory exams showed renal failure, increased serum lactate deshydrogenase, creatine phosphokinase and troponine T. Toxicological screening showed the presence of Paraphenyl Diamine in blood and stomach. The patient died in the tenth day due to a cardiogenic shock. A forensic autopsy was performed. The pericardium was spotted by multiple hemorrhagic petechiae. The myocardium and the epicardium had multiple hemorrhagic areas. Histological examinations revealed the presence of acute inflammation of the myocardium and epicardium associated with severe ulceration of the endocardium. Inflammatory infiltrate consisted mainly of neutrophils with frequent micro abscesses in the myocardium and epicardium. Death was attributed to Paraphenyl Diamine poisoning complicated with rhabdomyolysis and acute myocarditis. In conclusion, PPD is often used as for suicidal attempt having acute toxic effects such as rhabdomyolysis with renal failure and respiratory distress. There is no specific antidote available in the management of the PPD poisining (Jedidi M, Hadj MD, Masmoudi T, et al. Fatal toxic myocarditis induced by Paraphenylene Diamine. A case report. *Rom J Leg Med* 2016, 24(1): 17-20).

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PATHOGENIC FACTORS IN POSTINFECTIOUS IRRITABLE BOWEL SYNDROME - AN UPDATE

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PATHOGENIC FACTORS IN POSTINFECTIOUS IRRITABLE BOWEL SYNDROME-AN UPDATE (Abstract): After acute infectious gastroenteritis, up to thirty percent of patients present prolonged gastrointestinal symptoms and a part of those affected patients can have the diagnostic criteria for postinfectious irritable bowel syndrome. The main diagnosis of a patient with postinfectious irritable bowel syndrome was till this summer, clinically based on Roma III criteria. The Rome IV criteria brought some changes that involve also the postinfectious irritable bowel syndrome, recognizing further the postinfectious IBS as a specific entity according to the multidimensional clinical chronic mucosal inflammation triggered by enteric infection, may underlie persistent bowel symptoms in patients who develop postinfectious irritable bowel syndrome. **Keywords:** GASTROENTERITIS, INTESTINAL INFECTION, POSTINFECTIOUS IRRITABLE BOWEL SYNDROME.

The irritable bowel syndrome (IBS) is an association of chronic and recurrent symptoms such as constipation, diarrhea, bloating and/or abdominal pain, having not any abnormalities (biochemical or structural) detectable by conventional laboratory methods. The irritable bowel syndrome affects 9-13% of the normal population at any particular period in time (1).

Irritable bowel syndrome is defined as recurrent abdominal pain on average at least 1 day a week in the last 3 months associated with two or more of the following: related to defecation, associated with a change in a frequency of stool, associated with a change in form (consistency) of stool. Symptoms must have started at least 6 months ago, according to Rome IV criteria.

The earliest description of postinfectious irritable bowel syndrome (PI-IBS) was made in 1962; Chaudhary and Truelove reported that one third of their patients with a history of gastroenteritis went on to develop irritable bowel syndrome type symptoms (2). Since then, other authors have suggested that patients with an episode of infectious diarrhea have a high incidence of developing irritable bowel syndrome in the following months, with estimates ranging from 4% to 32%. How-

ever, these studies have used varying methodologies, have studied different populations, and a control group was most lacked (3,4,5,6). There are numerous reports, indicating that up to a third of people suffering from bacterial enteritis report persisting ongoing symptoms compatible with irritable bowel syndrome between six months and one year afterwards. Furthermore, though some will recover spontaneous, even at six years, nearly two thirds remain symptomatic (7).

The current conceptual framework regarding the pathogenic mechanisms for postinfectious irritable bowel syndrome suggests that postinfectious irritable bowel syndrome is associated with alteration of motility, increased intestinal permeability, an increased number of enterochromaffin cells and the intestinal inflammation persisted. This was characterized by an increased number of T-lymphocytes and also mast cells and an increased expression of proinflammatory cytokines. This suggests that an exposure to pathogenic organisms that disrupts the intestinal barrier function and alters the neuromuscular function and triggers chronic inflammation, which sustains irritable bowel syndrome symptoms (8).

The postinfectious irritable bowel syndrome is characterized by the sudden onset of symptoms mentioned in the diagnostic criteria for irritable bowel syndrome (with Rome IV criteria being the most recently defined). They appear following an episode of acute infectious gastroenteritis characterized by two or more of the following symptoms and findings: diarrhea, vomiting, fever and a positive stool culture result (9).

Epidemiology. Some studies (10) involving patients ill enough to be hospitalized in an infectious diseases unit, indicate that around 1/3 develop postinfectious irritable bowel syndrome, while other studies dealing with patients in the community have found a lower incidence of around 7% (5) and 9% (11). The net result is that approximately 17% of patients attending general practitioners in the UK attribute their irritable bowel syndrome symptoms to an episode of gastroenteritis (12). The incidence in the USA is much lower, around 6%, though whether this reflects differing referral practice is not certain. Infection is much commoner in the tropics where postinfectious irritable bowel syndrome might be expected to be more frequent though this has not been specifically examined. Certainly, the commonest sub-types of irritable bowel syndrome in Southern India appear to be diarrhea predominant (D-irritable bowel syndrome) (13), while in China D-irritable bowel syndrome also predominates with 65% of irritable bowel syndrome patients fitting this categorization (14). This parallels the findings in a UK study (6).

One of the earliest reports of postinfectious irritable bowel syndrome relates to unexplained diarrhea and abdominal discomfort seen in British troops returning from the World War II North Africa campaign after amebic dysentery (15). A study from Sheffield examined 75 individuals admitted to an infectious disease unit with gastroenteritis, 25% of whom developed new irritable bowel syndrome when assessed 6 months after infection (10).

A further community survey in Nottingham, restricted to just *Campylobacter* gastroenteritis, confirmed a percentage with 9% in a new cohort of 189 infected individuals (11). More recently, there has been a case-control study from Newcastle using Rome II criteria for irritable bowel syndrome, indicating an incidence of new postinfectious irritable bowel syndrome over 6 months of 17% compared with just

1,9% of controls, odds ratio 10 (16). With the exception of the study by Parry *et al* (16) and Ji *at al*.(17), the previous studies lacked a control group to define the normal incidence of new irritable bowel syndrome in the absence of infection. This was corrected in a large study of over 550 000 patients whose records form part of a large general practice research database in the United Kingdom. This database records all interviews with the general practitioner, all prescriptions and all laboratory results. They found 318 patients with documented bacterial enteritis (54% *Campylobacter*, 37% *Salmonella*), 12 of whom had new diagnosis of irritable bowel syndrome over the next 12 months, an incidence of 40 (22-70) (mean 95% confidence intervals)/1000 patients years giving a relative risk of 11.9 when compared with uninfected controls with an incidence of just 3.5/1000 patient years. During a one year follow up, the diagnosis rate of irritable bowel syndrome was 4.4% in patients after an episode of bacterial gastroenteritis (14 subjects), compared with a rate of 0,3% in the general population cohort (2027 subjects). The weakness of this study was that stool cultures are only obtained in around 1 in 10 of all cases of gastroenteritis in the United Kingdom (6). In another cohort study of 295 patients who had recovered from bacillary dysentery (BD) and 243 control subjects (consisting of patient siblings or spouses who had not been infected with BD) is investigated the incidence of irritable bowel syndrome and FBD (functional bowel disorders) in a Chinese patient population (in Beijing). All subjects were followed up using questionnaires for 1-2 years to explore the incidence of FBD and irritable bowel syndrome, as defined by the Rome II criteria. In the BD infected cohort, the incidences of FBD and irritable bowel syndrome were 22.4% and 8.1% (in total)-10.2% (among those in who *Shigella* were identified) respectively, which were significantly higher ($p< 0.01$) than the incidences of FBD (7.4%) and irritable bowel syndrome (0.8%) in the control cohort (18). While irritable bowel syndrome is common in western societies such as the USA or UK, early studies suggest that in developing countries, the prevalence is low (1). Irritable bowel syndrome was considered by Henry Bockus to be the most valid example of a civilization disorder (19). It is speculated that low prevalence of irritable bowel syndrome in underdeveloped countries is determined by an increased exposure to infection during the child age, rather than to fiber reach meals. The colonization of the intestine by micro flora and also the development of broad immune tolerance can result from the exposure to a variety of microorganisms early in life and enables the intestinal epithelium to respond more efficiently to antigenic challenge, during an episode of gastroenteritis. This leads to symptoms that resolve more quickly. In area with better hygienic conditions, the immune system of the gut is preserved naïve to exposures. Having episodes of gastroenteritis in later life can cause inflammatory disturbances. This, but also behavioral and psychological factors, predisposes to the persistence of symptoms suggestive for irritable bowel syndrome (1).

Gastroenteritis due to viral etiology is mostly associated with acute episodes of diarrhea and little residual injury. This might be associated with a lower incidence of postinfectious irritable bowel syndrome, in comparison with infection due to bacterial pathogens. In an outbreak of presumed viral gastroenteritis, one quarter of the patients developing gastroenteritis reported symptoms of postinfectious irritable bowel syn-

drome at 3 months after the outbreak (8). Consistent among these studies is the suggestion that postinfectious irritable bowel syndrome is different for any ethnic group or environment and became a global phenomenon. The prevalence or incidence of postinfectious irritable bowel syndrome varies in part because of differences in study methodology, including the criteria used to define irritable bowel syndrome. In general, Rome II criteria generate lower estimates than Rome I or III. Postinfectious irritable bowel syndrome has a defined moment of onset in comparison with sporadic irritable bowel syndrome. Features of the inciting infectious illness such as diarrhea, abdominal cramps, increased stool frequency, bloody or mucous stools, and positive stool culture and weight loss are potent predictors of long term outcome. The risk of postinfectious irritable bowel syndrome appears to correlate with the severity of the acute enteric infection, increasing at least two-fold if diarrhea lasts more than 1 week and over threefold if diarrhea lasts more than 3 weeks. Bloody stools, abdominal cramps and weight loss are also associated with high risk, with abdominal cramps increasing the risk fourfold (8).

Pathogenic mechanisms. Prospective studies provide strong evidence that the development of postinfectious irritable bowel syndrome involves an interactive multifactorial etiopathogenic process. Psychological factors: like hypochondriasis, an important life event stress preceding the infectious episode were strong predictors for the developing of irritable bowel syndrome. Increased expression of interleukin 1 β m RNA present in rectal biopsies and increased numbers of intraepithelial lymphocytes and T- lymphocytes in rectosigmoid biopsies suggest that the pathogenesis of postinfectious irritable bowel syndrome is also sustained by an inflammatory process. Possible other mediators include TNF-α and interferon-γ. Inflammation is usually associated with activation of cyclo-oxygenase 2 (COX-2) enzymes and an associated rise in prostaglandins, which sensitive visceral afferents and stimulate secretion and intestinal propulsion (20). The other striking abnormality was an increase in entero-endocrine cells which may explain an increased serotonin release by test meal which others have reported (21).

In animal models inflammation can induce entero-endocrine cell hyperplasia and increase serotonin release but the effects on bowel motility are unpredictable since in this model there was receptor desensitization and impaired peristalsis (22).

A study from China regarding postinfectious irritable bowel syndrome observed a similar increase in the inflammatory markers above mentioned. They also discovered the numbers of mast cells in terminal ileum biopsies of both postinfectious irritable bowel syndrome as well as non-postinfectious irritable bowel syndrome patients were increased compared with controls. Another study from New Zealand discovered high levels of mast cells in colonic biopsies taken from patients with irritable bowel syndrome with non-specific microscopic inflammation on conventional histology. The particularity in this study was the finding of immune activation in a group of irritable bowel syndrome patients whose presentation was atypical for postinfectious irritable bowel syndrome. These patients had more chronic irritable bowel syndrome symptoms, with an insidious onset and their colonic biopsies were judged to be normal on conventional histology.

Role of inflammation in irritable bowel syndrome. Epidemiological studies sug-

gest an association between inflammation following prior infection and the persistence of irritable bowel syndrome symptoms. The interdependence of prior infection and psychological status has also been consistently documented (22), and this may question the role of persistent inflammation as an independent risk factor. The data are interpreted as evidence of the importance of psychoneuroimmune interactions; it is unclear whether the inflammation is a key common final pathway to non-specific psychological influence on intestinal function, and the prior infection potentially unrelated other than as an initiator of the cascade of events. A summary of the epidemiology data also shows that the prevalence of PI- irritable bowel syndrome is no more prevalent than irritable bowel syndrome in the general population. A second approach is to review the quantitative differences in the inflammation and their association with symptoms. Many studies have documented increased numbers of CD3, CD25 or mast cells in ileal, colonic or rectal biopsies, but the differences for lymphocyte counts between controls and irritable bowel syndrome are small, with predominant overlaps in the counts between the two conditions (24). The third line of evidence to be examined is the response to anti-inflammatory treatment of irritable bowel syndrome. A controlled trial has shown that, among carefully selected patients with postinfectious irritable bowel syndrome, prednisone 30 mg b.i.d. was not significantly better than placebo and while placebo resulted in the customary, no change in score was noted in the prednisone treatment group, significant 30% change in median symptom score relative to baseline (25).

Prognosis. Like other types of irritable bowel syndrome, symptoms in postinfectious irritable bowel syndrome fluctuate but are generally chronic. Spiller found only 43% had recovered at six years which was not significantly different from the 31% of those with pre-existing irritable bowel syndrome (26).

Hypothesis on the cause of increasing irritable bowel syndrome prevalence in developing countries. It is speculated that low prevalence of irritable bowel syndrome in underdeveloped countries is determined by a high childhood exposure to infection, rather than high residue diet. The microflora colonization of the intestine and the development of broad immune tolerance can result from the exposure to a variety of microorganisms early in life and enables the intestinal epithelium to respond more efficiently to antigenic stimulation leading to symptoms that resolve more quickly, during an episode of gastroenteritis. On the other hand, the intestinal immune system remains relatively naive in the more hygienic environment associated with improved socioeconomic conditions. Acquiring acute gastroenteritis later in the life results in inflammatory changes. This factor together other factors, i.e. with psychological and behavioral factors, predisposes to the persistence of symptoms manifesting as irritable bowel syndrome (1).

Postinfectious IBS in Rome IV era. The Rome IV criteria bring (27) some new approaches in the field of functional gastrointestinal disorders and specifically in IBS (28). The pain remains the main feature of IBS diagnosis and frequency of pathological stools have major importance in subtyping IBS following the presentation of the stools according to the Bristol scale. The postinfectious entity is further acknowledged. Its definition changed together with the definition of the IBS as a category, not specifically because of the infectious trigger. However the infectious trigger has to

be mentioned according to the multidimensional clinical (29).

CONCLUSIONS

Our conclusion is that Rome IV recognize further the postinfectious IBS as a specific entity, according to the multidimensional clinical chronic mucosal inflammation triggered by enteric infection may underlie persistent bowel symptoms in patients who develop postinfectious irritable bowel syndrome.

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NEWS

HYDROGEN PEROXIDE VASORELAXATION INVOLVES S-GLUTATHIONYLATION OF VOLTAGE-DEPENDENT K CHANNELS

H_2O_2 is an endothelium-derived hyperpolarizing factor (EDHF), but H_2O_2 effects vary with the vascular bed and experimental conditions. If H_2O_2 relaxes rat mesenteric artery, what is the mechanism? Methods: myography of isolated resistance arteries, patch clamp of mesenteric arterial smooth muscle cells (MASMCs), streptavidin pull-down assays with biotinylated glutathione ethyl ester. Relaxation of precontracted arteries by H_2O_2 was reversed by dithiothreitol, reduced by 4-aminopyridine (4-AP), blocker of voltage-dependent K channels (K_v) channels, but not by blockers of big-conductance Ca^{2+}-activated K^+ channels or of inward rectifier K^+ channels. H_2O_2 increased K_v currents; this was prevented by glutathione reductase; K_v incorporates glutathione (GSH) in the presence of H_2O_2. Thus, H_2O_2 activates Kv channels (maybe via S-glutathionylation) with artery relaxation, while the basal redox status of MASMCs determines K_v response to H_2O_2. (Park SW, Noh HJ, Sung DJ et al. Hydrogen peroxide induces vasorelaxation by enhancing 4-aminopyridine-sensitive Kv currents through S-glutathionylation. *Pflugers Arch* 2015; 467(2), 285-297).

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POTENTIAL NEW ROLES OF LEPTIN IN HEALTH AND DISEASE

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POTENTIAL NEW ROLES OF LEPTIN IN HEALTH AND DISEASE (Abstract). Leptin is an adipose-tissue specific cytokine described 20 years ago and classically thought to be involved in regulation of food intake and energy homeostasis. More recently, leptin was proven to exert other multiple roles in carbohydrate and lipid metabolism, reproductive system, and inflammatory and immune reactions. Emerging data propose leptin to be a modulator of bone mass. In some particular circumstances leptin synthesis follow specific patterns in inflammation and sepsis, not being proportional with fat mass. Therefore, it may be used as diagnostic or prognostic marker for critical illness. **Keywords:** LEPTIN, ADIPOSE TISSUE, BONE MASS, CRITICAL ILLNESS.

The adipose tissue has the capacity to respond to afferent signals and synthesizes efferent signals, contributing to cross-communication between organs and tissues (1,2). Certain molecules synthesized by adipose cells play the role of hormones, being called adipokines, and are known to act locally and systemically on specific receptors (1-3). Both adipose tissue excess and severe deficiency alter the adipokine profile (1). Excessive fat may have a deleterious metabolic impact, contributing to harmful consequences, such as hyperglycemia, insulin resistance, dyslipidemia, and the increased cardiovascular risk that accompany obesity (4). Leptin (name derived from the greek *leptos*, meaning thin) is a hormone mainly synthesized by subcutaneous white adipose cells (2), firstly described in mice more than 20 years ago (5). Defects in leptin synthesis were associated with extreme obesity in ob/ob mice, indicating the hormone as being an important regulator of appetite and fat mass accumulation (6). The human leptin equivalent is a 16 kDa protein (7), acting on target cells through at least six isoforms of plasma membrane receptors and signaling predominantly via the Jak-STAT pathway (6-8). Many leptin receptors are expressed in the brain, especially in the hypothalamus (6), suggesting that the hormone may act on the inhibition of appetite via central effects (2,6.8). Leptin was proven in animal models to regulate satiety, energy homeostasis and body weight through three leptin sensitive neurons found within the hypothalamic arcuate nucleus: neuropeptide Y (NPY), gamma aminobutiric acid (GABA) and proopiomelanocortin (POMC) neurons, the

first two, with an orexigenic role, being inhibited and the third, anorexigenic, being stimulated by the hormone (2). Leptin also affects adipose tissue through autocrine effects on adipocytes, directly increasing energy expenditure (2,9). Initial hopes to find an ideal "slimming hormone" were deceiving: leptin administration has no effects on weight in the vast majority of obese patients (2,10,11). Newer studies used combinations of leptin with other agents that increase the responsiveness to leptin, such as amylin analogues, and showed initially promising weight loss, but the subsequent development of autoantibodies, impeded further beneficial effects (12). The reason why leptin therapy is inefficient in the overwhelming majority of obese patients is that obesity represents most frequently a chronic state of energy excess (13). Obese individuals usually have elevated adipose leptin expression and plasma leptin levels, proportional to fat mass, but these high leptin levels fail to reduce hyperphagia and adiposity excess, indicating a state of resistance to both endogenous and exogenous leptin (14). On the basis of studies conducted mainly in rodent models, leptin resistance has been attributed to multiple factors, including impaired leptin transport across the blood-brain barrier, and a disruption of leptin signaling in target cells (15). An absolute minority of extremely obese patients has a proven defect in leptin synthesis or receptivity and the rare leptin deficient obese patients do benefit from leptin therapy (16). These particular conditions are direct proof of the important leptin involvement in fat tissue homeostasis. It is known that obese children are taller, but their puberty starts at an earlier age. The reach of a certain critical mass of adipose tissue may be an important puberty trigger, and leptin secretion may also play a role in puberty onset (17). Leptin, as well as other adipokines, have other metabolic effects including lipid oxidation, energy utilization, modulation of insulin activity, effects on bone metabolism and an inflammatory role (2-4).

LEPTIN AND BONE

Leptin is presently considered to be a mediator between adipocyte and bone metabolism (18). Leptin receptors were described on osteoblasts, in vitro and in vivo effects of leptin on bone metabolism are multiple and frequently antagonistic (18,19). It is shown that leptin stimulates bone formation through a direct mechanism, but inhibits the recruitment of osteoclastic precursors, thereby decreasing osteolysis (18). However, its central effect of stimulating the sympathetic nervous system causes bone demineralization in animal models (19). The role of leptin on bone mass acquisition in humans is presently not fully comprehended. Buta, Branisteanu et al. observed that leptin was an independent predictor of bone mass at postmenopausal women (Fig. 1) (20).

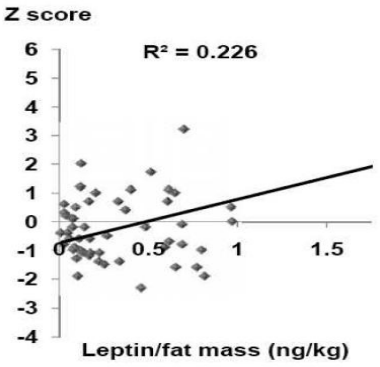

Fig 1. Positive correlation of serum leptin normalized to fat tissue mass with the Z score at postmenopausal women ($p < 0.05$)

LEPTIN AND INFLAMMATION

Leptin has a structural similarity to various cytokines. Moreover, leptin-specific receptors belong to the class I cytokine receptors and are found on immune cells such as monocytes, lymphocytes and neutrophils (2). Leptin may therefore exert significant immune modulating effects. Many obese people present, indeed, a chronic inflammatory condition. This obesity related immune reactivity is attributed to the high leptin levels, which may activate macrophage phagocytosis and T-helper 1 mediated responses, through the synthesis of other proinflammatory cytokines, such as IL-2, IL-6, IFN-gamma or TNF-alpha (21). Taking into consideration the above mentioned data, leptin may be regarded as an adipose cell-synthesized proinflammatory cytokine (22,23).

LEPTIN AND CRITICAL ILNESS

Critical illness represents an extreme stress adaptation caused by a multitude of triggers (24). This adaptation includes important acute immune reactions in the frame of a systemic inflammatory immune response (SIRS), seemingly different in aseptic and septic circumstances (25). The immune system is influenced by the adipose tissue through adipokines such as adiponectin and leptin, therefore SIRS and sepsis are expected to be accompanied by changes of these cytokines (26-28). SIRS or sepsis related leptin level changes may therefore be used as a diagnosis and prognosis marker (29). The role of leptin during critical illness is difficult to be established due to contradicting clinical and experimental data (29-36). These differences may originate in the timing of blood sampling and the different types of critical conditions. Grigoras et al. evaluated for the first time early (first 24 hours) leptin variations in critically ill patients with SIRS or sepsis (29). They observed different leptin dynamics: a steep early leptin increase in SIRS, but a flat answer in sepsis (Fig. 2). The two different early evolution patterns of leptin dynamics may indicate the presence or absence of infection and mandate for the early institution of antibiotherapy, if needed (29).

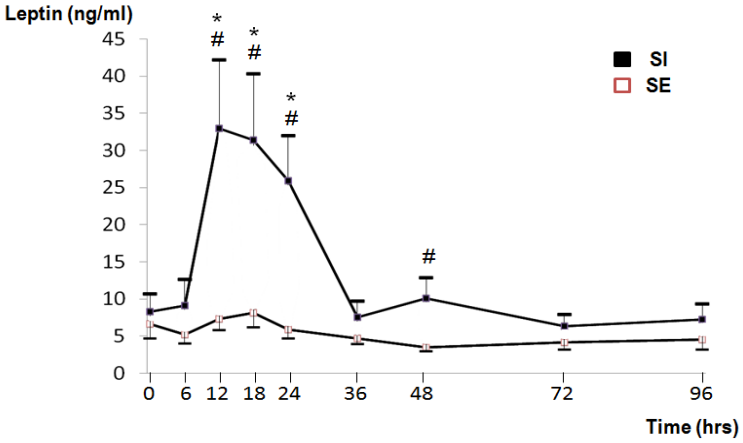

Fig 2. Temporal dynamics of leptin plasma levels in the SIRS group (SI, black rectangles) and in the SEPSIS group (SE, white rectangles). Data are represented as means ± standard deviation. # $p < 0.05$ compared to SE; * $p < 0.05$ compared to initial value.

Moreover, the authors observed that mortality risk of septic patients is higher at low leptin and high IL-6 levels (Fig. 3). Adipose tissue may therefore, via adipokine secretion, play a protective role during sepsis and critical illness in overweight and obese patients (the so called "obesity paradox"), by the capacity to provide energy stores, to spare muscle wasting and to regulate the immune response (29, 37).

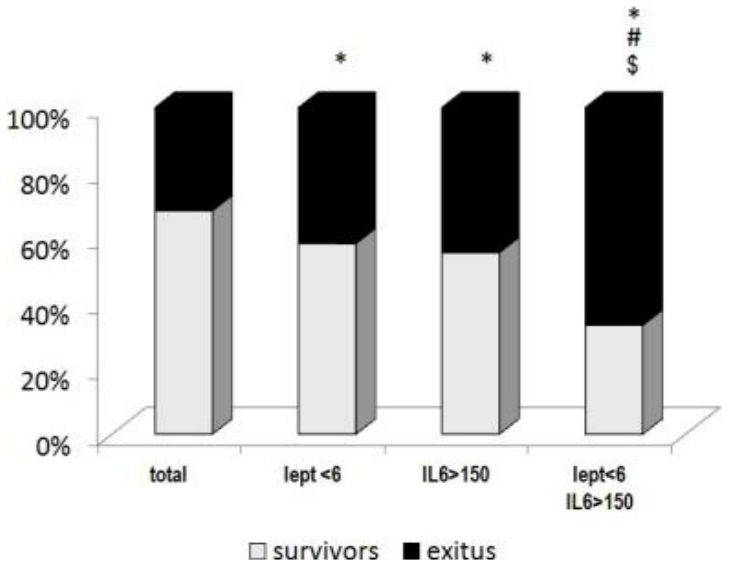

Fig 3. Mortality incidence among patients in critical state with sepsis – whole group (total), patients with leptin < 6 ng/ml, patients with IL6>150 pg/ml and patients with both leptin < 6 ng/ml and IL6>150 pg/ml at the onset of the follow up. Patients with both modified parameters had highest mortality. * $p<0.05$ compared to mortality in the whole group, # compared to mortality in the group with leptin < 6 ng/ml, $ compared to mortality in the group with IL6 > 150 pg/ml (square chi test).

CONCLUSIONS

Leptin, the adipokine originally described as having a crucial role for adipose tissue balance, is presently known to be involved in the interplay between various body compartments. Leptin may play a direct effect on bone mass acquisition and is involved in inflammatory reactions. The critical illness associated inflammation is accompanied by specific leptin level changes, that may be used as diagnosis and prognosis markers in critically ill patients.

ACKNOWLEDGEMENTS

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ATOPIC DERMATITIS - CLINICAL EPIDEMIOLOGY AND IMMUNOLOGICAL CORRELATIONS

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ATOPIC DERMATITIS - CLINICAL EPIDEMIOLOGY AND IMMUNOLOGICAL CORRELATIONS (Abstract) **Aim:** Medical records from 2010-2014 were retrospectively reviewed and analyzed in view of determining the correlations between the clinical severity of atopic dermatitis (AD) and total IgE level, eosinophilia, place of residence, family history of atopy, type of birth, and natural or artificial feeding. **Material and Methods**: Following medical record review, 296 cases diagnosed with AD were included in the study. Statistical analysis was performed using SPSS v20 for determining the frequency and testing the hypotheses, for $p < 0.05$, by t tests and One-Way ANOVA. **Results:** Of the 296 cases, 53% were male children and 47% female children aged 1 month to 16 years. According to total serum IgE level, 49.3% of patients had elevated IgE levels, 20.6% normal levels and in 30.03% of cases it was not determined. According to the SCORAD, children had mild AD in 20% of cases, moderate in 70%, and severe in 10%. The independent samples t tests showed a statistically significant difference between the means demonstrating correlations between IgE level and place of residence ($p < 0.01$), family history of atopy ($p < 0.01$), baby feeding ($p < 0.01$), and by one-way ANOVA for SCORAD ($p < 0.05$). **Conclusions:** Atopy in AD can be influenced by complex factors, both internal and environmental, but this remains a controversial topic. External factors acting on a background genetically predisposed to atopy trigger the manifestation of AD. **Keywords:** ATOPIC DERMATITIS, FOOD ALLERGIES, FAMILY AGGREGATION, ENVIRONMENTAL FACTORS.

Atopic dermatitis (AD) is a chronic inflammatory skin condition characterized by intense itching. In 2003, the European Academy of Allergology and Clinical Immunology revised nomenclature of allergic diseases and proposed the term atopic eczema for the Immunoglobulin E-mediated skin inflammatory disorder occurring in genetically predisposed individuals (1, 2).

Atopic eczema develops in early childhood. About one in five school-age children have atopic eczema. Quality of life is altered as it affects sleep and concentration, school attendance, and self-image. AD starts at ~ 2 months (90% before the age of 5 years). Prevalence is 10-25% in the US, Northwestern Europe, and Australia (3).

In the etiopathogenesis of AD are involved the following factors: genetic predisposition, family aggregation, low expo-

sure to infectious agents and/or commensal microbiota - hygiene hypothesis and rise in living standards - "Western lifestyle" (4).

The genetic risk factor is represented by loss-of-function mutations in the filaggrin gene (FLG), an important epidermal barrier protein and precursor of natural moisturizing factor, involved in epidermal integrity (3,4).

Seventy percent of the patients with AD have a family history of atopy. If both parents are atopic their children have an 81% risk for developing atopy; when one parent is atopic and the other parent is non-atopic the risk is of 56%; frequency of atopic manifestations in other family members (first-degree relatives) is associated with a 28% risk (5).

Among the factors that could favor the onset and progression of AD lesions, the most commonly incriminated are: dietary factors - cow milk, eggs, peanuts, nuts, soy, wheat, fish, seafood, airborne allergens, infectious agents - *Staphylococcus aureus*, *Pytirosporum ovale*, contact factors - cleaning agents, astringent solutions, perfumes, harsh soaps and detergents, tight and abrasive clothing and physical factors - extreme temperatures, emotional stress (6).

MATERIAL AND METHODS

This retrospective study analyzed the data collected from medical records from 2010 - 2014. There were 296 cases of AD of which 53.05% were male and 46.95% female children from all Moldavian regions. Distribution of cases by county of residence was the following: 73.99% Iasi county, 8.44% Neamt county, 4.72% Botosani and Vaslui counties, 4.05% Suceava county, and 0.67% Vrancea and Galati counties.

The diagnosis of AD was made according to Haniffin and Rajka criteria, and the severity of lesions was based on SCORAD score calculation according to the international protocol and classified as mild (<15), moderate (15-40) and severe form > 40 (7).

Inclusion criteria were patients at first presentation to our clinic with or without a personal history of AD. The following data were analyzed: gender, place of residence (rural or urban), family history, personal history (type of delivery and feeding), previous medical history (recurrent allergic reactions and respiratory infections), SCORAD, total IgE and specific IgE levels.

Statistical analysis was performed using SPSS v20 for determining the frequency and testing the hypotheses, for $p < 0.05$.

RESULTS

Of the 296 AD cases 37.5% were recorded in 2014, 19.26% in 2010, 17.22% in 2013, 13.18% in 2011 and 12.84% in 2012 (fig. 1). Fifty-three percent of the children were male and 47% female, aged from 1 month to 16 years. An incidence of 30.3% in the first 6 months of life and of 80.8% in the first 3 years was found (fig. 2), values close to those reported in the literature (7).

These patients were diagnosed according to Haniffin and Rajka criteria corroborated with the clinical and laboratory findings at the first admission (8). Thus, total serum IgE level was elevated in 49.32% of patients, normal in 20.61% of patients, and in 30.07% it had not been determined. Studies report a higher frequency of AD in urban than in rural areas (9, 10, 11). In our study, 52% were urban patients and 48% rural patients, average of 1.52, SD = 0.50 (standard deviation) and variance of 0.250. The independent samples t-test showed that for $t(294) = -0.98$, the p value was greater than 0.01 but F test rejected the null hypothesis, therefore the place of residence influences AD (tab. I).

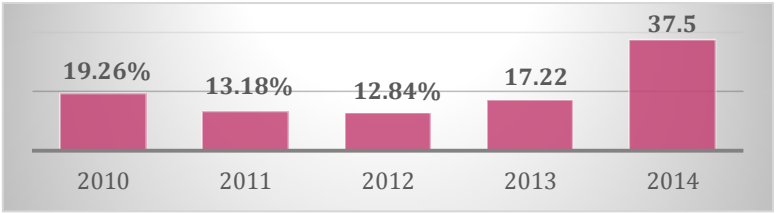

Fig. 1 Distribution of cases by year

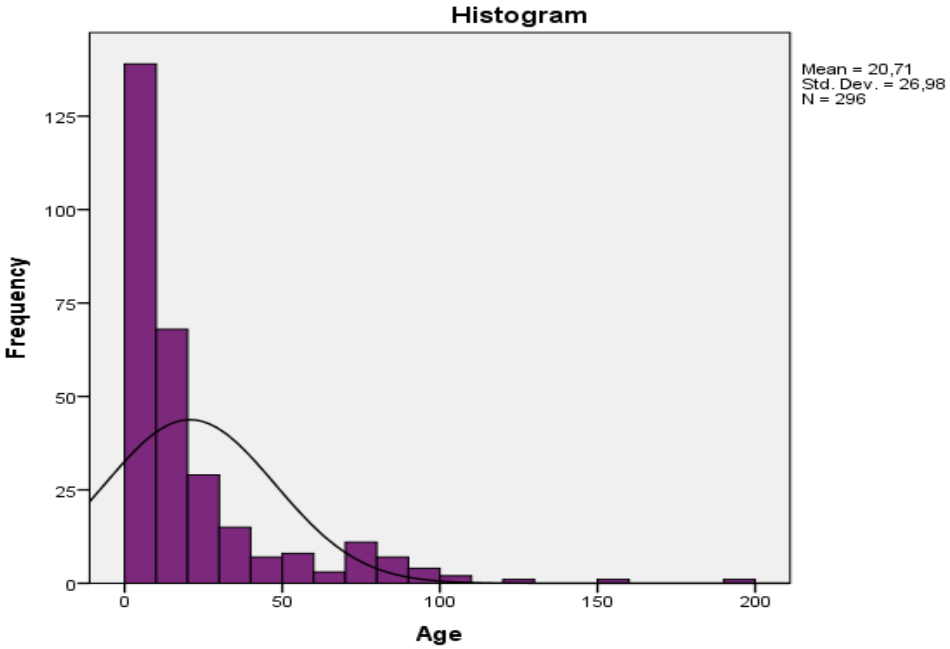

Fig. 2 Distribution by age

TABLE I

The t test for independent samples to compare IgE and the place of residence means

			Immunoglobuline E	
			Equal variances assumed	Equal variances not assumed
Levene's Test for Equality of Variances	F		12,948	
	Sig.		,000	
t-test for Equality of Means	t		-,980	-,975
	df		294	281,978
	Sig. (2-tailed)		,328	,330
	Mean Difference		-,099	-,099
	Std. Error Difference		,101	,102
	95% Confidence Interval of the Difference	Lower	-,299	-,300
		Upper	,100	,101

Babies born by cesarean section are not more likely to develop AD compared to those born naturally (9, 10, 11, 12, 13). In our study, 65.9% of children were born naturally and 34.1% by caesarean section, average of 1.34 and SD = 0.47. T test, bivariate correlation it-s not statistical significant to support the hypothesis that cesarean delivery increases the risk for developing atopy for t (294) = - 0.780, p> 0.05, and F test did not reject the null hypothesis.

The frequency of food-allergy among infants and young children, which contributes to worsening of AD lesions is of approximately 20-40%. Early feeding with powdered or cow milk, early introduction of allergenic foods such as fish, egg, peanuts, in the diet of both breastfeeding mother and infant can aggravate AD lesions (6, 14, 15).

In our study group we could see how the introduction of artificial feeding with milk powder or cow milk during infancy causes shortly after the occurrence of AD lesions. Artificially fed were 76% of children and 24% were breastfed, with a mean of 1.76 and SD = 0.42. 52.36% of cases were clinically diagnosed with cow milk protein allergy, children aged 1 month to 3 years. Determination of specific IgEs revealed: 6.08% of cases with positive specific IgE to milk, 2.36% to house dust mites, 1.68% each to egg, peanuts and animal hair and epithelia (dog, cat), 1.01% to nuts, soy, potato, fish, fruits, chocolate, and grasses. Upper gastrointestinal endoscopy was performed in one case and found eosinophilic esophagitis.

To determine if nutrition affects AD t test was used. For t (294) = 1.30 we obtained a p> 0.05 which is not statistically significant, but F test rejected the null hypothesis (tab. II).

TABLE II

Independent samples t test to compare the averages for the type of food and IgE

			Immunoglobuline E	
			Equal variances assumed	Equal variances not assumed
Levene's Test for Equality of Variances	F		10,997	
	Sig.		,001	
t-test for Equality of Means	t		1,302	1,415
	df		294	136,056
	Sig. (2-tailed)		,194	,159
	Mean Difference		,154	,154
	Std. Error Difference		,118	,109
	95% Confidence Interval of the Difference	Lower	-,079	-,061
		Upper	,387	,370

When comparing the feeding and SCORAD means, p was greater than 0.05 and no correlation between them was found.

The estimation of borderline IgE means showed a stronger correlation between artificial feeding and natural delivery, but feeding and natural delivery also influence atopy to a certain degree (fig. 3).

Family aggregation has been studied from the viewpoint of a family history of asthma, allergic rhinitis, atopic dermatitis and urticaria. In 82.77% of our cases we found the absence of allergies; allergies were present in 14.53% in one parent, grandparent or sibling

and in 2.70% in both parents or a sibling and a parent, with an average of 0.20, variance of 0.21 and SD = 0.46.

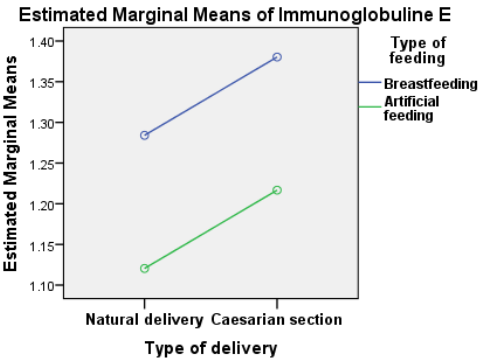

Fig. 3 Correlation between IgE feeding and type of delivery

The use of bivariate analysis showed statistically significant values for IgE, p <0.01, t test for comparing means was statistically insignificant for t (49) = 1.34, p> 0.05, the null hypothesis being rejected by F test which is statistically significant at p <0.01 (tab. III). One-way ANOVA of the means showed difference only between the means for both parents allergic and a brother and one parent allergic without family history of allergies, 1.75 and 1.15, respectively. The difference was not statistically significant because p> 0.05. Multiple comparison revealed no statistically significant differences as p> 0.05, even if the difference between the means was negative.

TABLE III

Independent Samples t Test to compare averages between IgE and familial history of allergy

			Immunoglobuline E	
			Equal variances assumed	Equal variances not assumed
Levene's Test for Equality of Variances	F		10,301	
	Sig.		,002	
t-test for Equality of Means	t		-1,344	-2,019
	df		49	17,803
	Sig. (2-tailed)		,185	,059
	Mean Difference		-,424	-,424
	Std. Error Difference		,316	,210
	95% Confidence Interval of the Difference	Lower	-1,059	-,866
		Upper	,210	,018

According to AD severity score, 19.94% of the children had a mild form of disease, 69.59% moderate and 10.47% severe, with an average of 2.00, SD = 0.54 and variance of 0.29.

Bivariate correlation of the means showed a strong association between IgE level and the degree of AD lesions severity for p <0.01. One-way ANOVA revealed statistically significant means differences between patients with mild and severe disease according to SCORAD index - 0.93 versus 1.35, at multiple comparison the difference of means being significant for p <0.05 (tab. IV). Also the graphical representation of means shows the correlation between SCORAD score and IgE level (fig. 4).

TABLE IV

OneWay Anova to compare means between IgE and SCORAD

Immunoglobuline E					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	5,977	2	2,988	4,016	,019
Within Groups	218,047	293	,744		
Total	224,024	295			

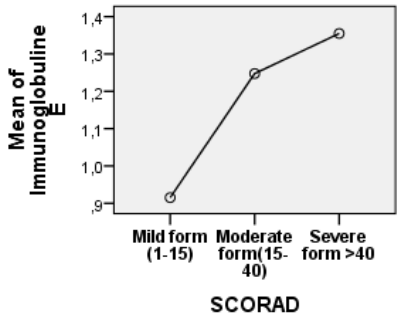

Fig. 4 Graphic representation of IgE and SCORAD means

Skin barrier impairment facilitates superinfections, especially by *Staphylococcus aureus*, *Streptococcus pyogenes*, *Herpes simplex*, *Molluscum contagiosum*. It preserves skin inflammation with further persistence of lesions (10, 17, 18). In our study skin superinfection was found in the severe forms of DA. Thus, 9 of the 31 children with severe SCORAD score presented superinfection by *S. aureus*. Of these children one each associated *Haemophilus influenzae*, *E. coli*, *P. aeruginosa*. Other colonizations: one child by *Streptococcus pyogenes* and one child by *Enterobacter spp* and *Enterococcus faecalis*.

DISCUSSIONS

The epidemiology of AD is important for identifying the risk factors that can be acted upon, it being a dynamic disease influenced by environmental and genetic factors. It is common in infants and small children. This study on AD ethiopathogenesis was in agreement with other similar studies by the incidence close to that in the literature and the onset most frequently occurring in infancy at the age of 2 months (10). Gender distribution was equal.

The place of residence influences the development of AD, with a higher risk in urban areas in developed countries (9, 10, 11). Given the small difference between urban and rural patients in our study, it is more likely that other factors influence the development of AD, such as processed foods or genetic background.

In our study delivery type did not increase the risk of developing AD, but some studies confirmed high birth weight and prematurity by impaired skin barrier function as risk factors (11).

Food allergies are common among patients with AD. Given the age of onset, feeding plays an important role. Breastfeeding is a protective factor against many illnesses. In AD this hypothesis is not supported by studies, as there may be variations in breast milk composition or in other factors that may influence breastfeeding. Thus, in our study the means for IgE, diet type delivery type showed that feeding and natural birth can influence atopy (6, 14, 15). Additional administration of hydrolyzed cow milk formulas and probiotic supplementation may have a beneficial effect in preventing disease development in high-risk children not exclusively breastfed (5, 10, 11, 14, 15).

The well-known risk factors that are strongly correlated with the development of DA are family history of atopy and filaggrin loss-of-function mutations. In our study we found that family history of atopy is statistical significant (11).

As currently there is no specific biological marker that differentiates between AD and other allergic diseases, the diagnosis of DA is clinical, based on history, morphology and distribution of skin lesions, and associated clinical signs (8, 10). The most common laboratory finding in AD is an elevated total IgE and/or specific IgE level. According to IgE level, researchers classified AD into two groups of disease: intrinsic and extrinsic. In our study the extrinsic group type was prevalent. Inflammatory and cytokine profile differentiate between acute onset and chronic forms. These forms are controversial because the individual with AD can later develop elevated IgE levels and specific IgE positivity as a secondary phenomenon. Moreover, although statistically the severity of AD correlates with elevated IgE levels, this does not mean that in all forms of severe AD IgE level is elevated (10, 11, 16, 18).

CONCLUSIONS

The rise in the number of AD cases requires a more thorough analysis of the way environmental factors interact with predisposition of the child to atopy.

In this case feeding and lifestyle influence the child since infancy.

Discovering the cause of this condition to reduce disease severity is difficult in many cases.

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NEWS

HORMONAL CONTRACEPTIVES, GENITAL TRACT INFECTIONS AND HIV RISK

The hormonal contraceptives and genital tract infections are associated with altered cervical immunity. A recent study that evaluated changes in cervical immunity after taking hormonal contraceptives showed that the women who used injectable depot medroxyprogesterone acetate and those who use estrogen-progesterone oral contraceptives had increased levels of proteins that attract HIV host cells. Also, the effect of hormonal contraceptives was exacerbated in presence of disturbed vaginal microbiota or genital tract infections such as gonorrhea, chlamydia, genital herpes, trichomoniasis and candidiasis. The levels and the type of immune mediators, infection status and hormonal contraceptives increase HIV risk by their cumulative action. (Fichorova RN, Chen P-L, Morrison CS, Doncel GF, Mendonca K, Kwok C, Chipato T, Salata R, Mauck C. 2015. The contribution of cervicovaginal infections to the immunomodulatory effects of hormonal contraception. *mBio* 6(5):e00221-15. doi:10.1128/mBio.00221-15).

Cătălina Luncă

CHARACTERISTICS OF HEPATITIS B VIRUS COINFECTION AMONG HIV-INFECTED PREGNANT WOMEN

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CHARACTERISTICS OF HEPATITIS B VIRUS COINFECTION AMONG HIV-INFECTED PREGNANT WOMEN (Abstract)The similar routes of transmission for HIV and HBV place patients with either infection at greater risk for HIV/HBV co-infection.Aim: To determine the particularities of hepatitis B virus (HBV) coinfection in pregnant women infected with human immunodeficiency virus (HIV) and its influence on pregnancy. Methods: Retrospective study of 74 HIV-infected women monitored during pregnancy in the Iasi Regional HIV/AIDS Center (Romania) from 2010 to 2013. The subjects were divided into 2 groups according to the presence or absence of HBV coinfection. Results: Most subjects belonged to age group 20-24 years (90% in the HIV group *versus* 75.5% in the HIV/HBV-coinfected group). HIV infection was most commonly transmitted by parenteral route (65.5 % *vs.* 48.9 %). The majority of patients were married (60 % *vs.* 65 %) and primiparous at the time of enrollment (71.1 % *vs.* 96.6 %). In the HIV/HBV-coinfected group CD4 level was lower and mean plasma viral load higher (1.92 log10 copies/mL *vs*. 2.31 log10 copies/ml). The proportion of cases with severe immunosuppression was similar in both groups (18. 1% *vs.* 17.9%). HBV-HIV coinfection induced a risk of prematurity of 1.51 times, but did not increase the risk of newborns with low birth weight. Conclusions: Advanced stages of HIV disease, age group 21-30 years, low CD4 counts, and low levels of education are significant risk factors for preterm birth and low birth weight. Keywords: HIV/HBV COINFECTION, PREGNANT WOMEN , PLASMA HIV RNA VIRAL LOADS.

The efficacy of antiviral therapy in the era of highly active antiretroviral therapy (HAART) has led to considerable improvement in life expectancy of patients infected with human immunodeficiency virus (HIV). Therefore, in this context, liver disease and in particular HIV/hepatitis viruses coinfection became a key issue in the management of HIV-infected patients, being a primary cause of mortality and morbidity.

Worldwide, approximately 350-400 million people are chronically HBV-infected and 40 million are HIV-infected (1). Since HIV and HBV share the same transmission routes, coinfection is likely and it is estimated that about 10% of HIV-infected individuals have chronic HBV infection (2). In HIV-infected adults, the prevalence of HBV infection rate is up to ten times higher than in the general population.

The association of HIV infection with

HBV infection and pregnancy brings to the fore a number of important issues: the effects of HBV/HIV coinfection on pregnancy, the effects of pregnancy on coinfection, management of antiviral therapy for both HBV and HIV and its effect on pregnancy as well as the prevention of vertical transmission of both viruses (3).

The prevalence of HIV/HBV coinfection in pregnant women ranges from 4% in Europe to 4-10% in Africa, 40% higher than that among the general population (2,4-7).

Several studies have addressed the issue of HBV coinfection in HIV-infected pregnant women (8-10), but the data on the prevalence of HIV/HBV coinfection among this population in Europe is scarce. Understanding the epidemiology and management of HBV and HIV coinfection in pregnant women is important because of the risk of vertical transmission.

The aim of this study was to estimate the prevalence and determine risk factors for HIV/HBV coinfection among HIV-infected pregnant patients, quantification of HBV vertical transmission in infants born to HIV/HBV-coinfected mothers, and to determine the predictors of HIV/HBV infection influence on the risk of preterm birth and low birth weight.

MATERIAL AND METHODS

The Iasi Regional HIV/SIDA Center provides assisted care to 1358 patients, of which 50% are women and 107 of them (39.19%) presenting HBV coinfection.

The study was prospective and included 74 HIV-infected subjects monitored during pregnancy in the Iasi Regional HIV/SIDA Center.

Depending on the presence of HBV coinfection the study group was divided into 2 groups that were compared in terms of epidemiological, clinical, viroimmunologic, and therapeutic features:

- **Group I** - 29 pregnant women diagnosed with HIV/HBV coinfection.

- **Group II** (control) - 45 pregnant women diagnosed with HIV infection without concomitant HBV infection.

The majority of pregnant women enrolled in the study were from the cohort receiving assisted care in the Iasi Regional HIV/AIDS Center (69 patients) to which 3 women who tested positive for HIV during pregnancy and 2 at delivery were added.

The number of prenatal visits varied depending on the time of study entry and mainly included 5 important visits scheduled as follows: at the time of pregnancy diagnosis, 10 ± 2, $24 \pm 2/34 \pm 2$ gestation weeks and immediately after birth.

Data on sociodemographic characteristics, obstetrical history and HIV history were obtained from patient monitoring records. All HIV-positive patients of reproductive age were tested for HBsAg, anti-HCV antibodies, toxoplasma antibodies, cytomegalovirus (CMV) and syphilis serology. Infants were delivered vaginally, by elective cesarean section (recommended at 38 weeks of gestation) or emergency cesarean section. Miscarriage was defined as loss of pregnancy before 24 weeks of gestation and stillbirth as fetal death after 24 weeks of gestation. Low birth weight was defined as birth weight <2500 g and preterm birth as birth before 37 weeks of gestation and after 28 weeks of gestation of an infant weighing less than 1000 grams.

The analyzed laboratory parameters were: viroimmunologic (changes in CD4 count and HIV plasma viral load over pregnancy) and metabolic (transaminase, total cholesterol, and triglyceride levels). CD4 counts were classified as <200, 200-499, and ≥ 500 cells/ml, the last determination of CD count being used in the analysis.

Evolution of HIV viral load was followed during the first, second and third trimesters of pregnancy, and the classification of undetectable HIV viral load (viral suppression) was based on analysis of the lower limit of quantification of the assay.

HBV coinfection was defined based on HBsAg positivity and included chronic infection cases and inactive HBV carrier state.

HAART was defined as a regimen of at least three antiretroviral (ART) drugs, including a combination of two nucleoside reverse transcriptase inhibitor with either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor.

The results were evaluated and interpreted by the use of frequency and structural indicators, processed using SPSS statistical functions using Student t-test, chi-square test, Pearson correlation coefficient, and linear trend. P values were considered significant at <0.05.

RESULTS

The patients enrolled in the study were aged 16 to 38 years. Most subjects, both in the coinfected and control group, belonged to age group 20-29 years age (90% *vs.* 75.5%). The mean age of coinfected patients (24.34 years) was lower than that of monoinfected subjects (25.67 years).

In both groups the majority of pregnant women (67.67% in group I *vs.* 86.65% in group II) were infected during 1989-1990 (of reproductive age at the time of study) in which the transmission route of HIV infection was believed to be nosocomial. Sexual transmission route was incriminated in a smaller proportion of subjects in the HIV/HBV group (34.5%) compared with over 50% in the controls (p=0,244) (table I).

It is worth mentioning that more than half of pregnant women in our study were married (65% in group I versus 60% in group II) (p = 0.816), and there was an unexpectedly high number of discordant couples (79.3% in group I *vs.* 73.3% in group II) (p = 0.758). According to educational level most women in group I and II had a low level (75.9% and 62.2%. respectively; p = 0.473).

TABLE I

Socioepidemiologic and viroimmunologic features by study groups

	HIV/HBV Group (no=29)	Control group (no=45)	p
Marital status			
married	19	27	0.816
discordant couples	23	33	
HIV transmission route			
parenteral	19	22	0.244
sexual	10	23	
Pregnancy			
planned	21	15	0.792
unplanned	8	30	
Mean CD4 count	389.03	253.75	0.001
Mean HIV plasma viral load			
first trimester	50 390	64 421	0.001
second trimester	51 903	20 334	0.001
third trimester	66 683	464 668	0.001

There was no difference between study groups (p = 0.516) in terms of the type of birth; in over 90% of the cases a cesarean section was performed.

Most pregnant women enrolled in the study (85%) were diagnosed before pregnancy, already receiving assisted care in the Iasi Regional HIV/AIDS Center; a small but not negligible number of patients were diagnosed during pregnancy or at the time of delivery. The time from HIV diagnosis to initiation of this study ranged from 1 to 17 years, with a mean slightly higher in the HIV/HBV group (9.93 ± 5.09 years) than in the control group (8. 13 ± 6.50 years) (p = 0.065).

As most patients belonged to age group 20-29, the so-called "long term survivors", most cases were in advanced stages, with a predominance of stage C3 in both groups: 26.1% in group I *vs*.35.6% in group II (p=0,560) (fig.1).

In both study groups predominant were the cases with CD4 cell counts 200 to 99 at/mL (53.6% *vs.* 45.5%) and severe immunosuppression was present in similar percentages (17.8% *vs.* 18.1%). The average CD4 count was significantly lower among controls (253.75 *vs.* 389.03/ml).

Individual HIV plasma viral load ranged from undetectable to a maximum of 2.310.000 copies/ml, with statistically significant differences in mean value between the 2 groups during all trimesters of pregnancy. HIV RNA level in the third trimester of pregnancy was undetectable in similar percentages in the 2 groups (36.6% *vs.* 30. 61%).

At baseline, 29.72% of the cohort had pathological ALAT levels, the proportion being higher in the HIV/HBV group than in monoinfected patients (53.6% *vs.* 15.6%. respectively); statistically significant differences were also found in average transaminases levels.

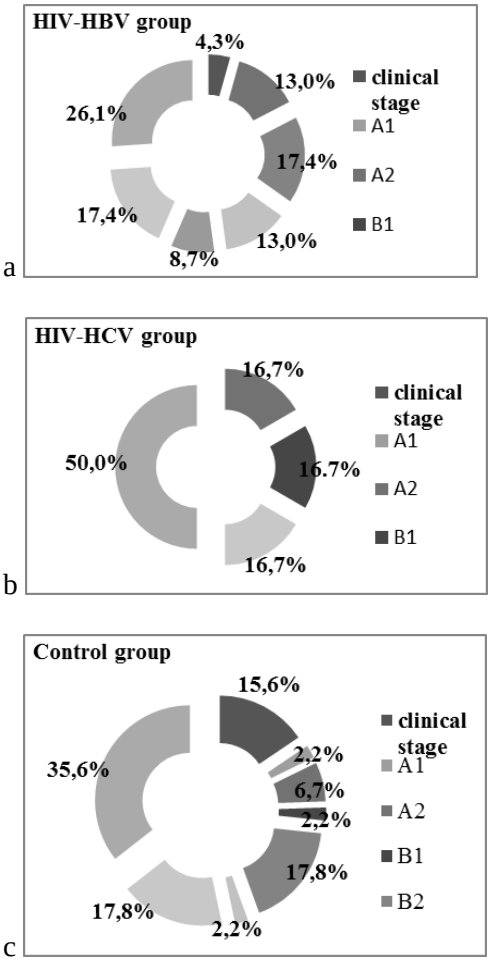

Fig. 1. Structure of study groups by disease stage

In most cases antiretroviral regimen during pregnancy consisted in a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and a protease inhibitor (86.25%), in a small number of cases (5%), "polyexperimented patients", a combination of integrase inhibitors and fusion inhibitors being used as "rescue therapy".

A far from negligible percentage of pregnant women (8.75%) did not receive prophylactic treatment, the diagnosis of HIV infection being made during pregnancy or even at birth.

To determine the influence of HIV/HBV coinfection on pregnancy outcome the risk of preterm birth and low birth weight was assessed.

Gestational age ranged from 34 to 39 weeks, the risk of premature birth in the coinfected group being 1.51 times higher compared with controls; birth weight ranged from 1750-3500 g without significant differences in mean weight between the two study groups. According to the obtained data, the significant risk factors for preterm birth and low birth weight were: advanced AIDS stages, age 21-30 years, CD4 count 200-499 cells/ml, and low level of education. In both groups no association with maternal high viral load was found (table II). Data on other risk factors for preterm delivery, such as previous obstetric history and previous and preterm deliveries were not available .

TABLE II.

Risk factors for treterm birth and low birth weigh

Risk factors	Low birth weight (no=14)	Preterm birth (no=12)	P values for chi2 test	P values for Kruskal-Wallis test
CD4 (cel/ml)				
>500	4 (28.6%)	4 (33.3%)	0.870	0.953
200-499	9 (64.3%)	7 (58.3%)	0.926	
<199	1 (7.1%)	1 (8.3%)	0.532	
VL copies/ml				
undetectable	13 (92.9%)	8 (66.7%)	0.234	
0-10.000 copii/ml	-	2 (16.7%)	0.394	0.108
10.000-100.000 copii/ml	1 (7.1%)	-	0.937	
>100.000 copii/ml	-	2 (16.7%)	0.394	
HIV clinical stage				
A	3 (21.4%)	1 (8.3%)	0.706	
B	2 (14.3%)	2 (16.7%)	0.705	0.600
C	8 (57.1%)	9 (75.0%)	0.589	
Age (years)				
<20	-	-	-	
20-30	13 (92.9%)	12 (100%)	0.937	0.937
>30	1 (7.1%)	-	-	
Level of education				
Low	10 (71.4%)	9 (75.0%)	0.811	0.811
Average	4 (28.6%)	3 (25.0%)	0.811	

The proportion of infants with detectable HIV viral load (repeated at 0,3,6, and 18 months of age) was low in the study groups (3.4% in coinfected group *vs.* 8.9% in control group) due to the fact that specific anti-HIV prophylaxis was successful in both mother and fetus in over 83% of cases. In the group with HIV/HBV coinfection the rate of HBV vertical transmission was 3.44%, as a result by cesarean delivery, breastfeeding avoidance, and successful specific anti-HBV newborn prophylaxis (table III).

TABLE III

Obstetrical and viroimunologic features in the newborns

	HIV/HBV group (n=29)	Control group (n=45)	p	RR
HIV viral load in newborn				
Undetectable	28	41	0.663	-
Detectable	1	4		
Birth weight				
≤ 2500 g	14	20	0.933	-
> 2500 g	15	25		
Preterm birth risk	12 17	13 32	0.306	1.51

DISCUSSIONS

The prevalence of HBV infection in the pregnant women followed up during the study in the Iasi Regional HIV/AIDS Center (36.25%) was much higher than that reported by other studies (11), accounted for by the epidemiological characteristics of HIV and HBV infections in Romania, the common transmission routes often allowing concomitant infection with the two viruses.

Most pregnant women included in our study belonged to the 20-29 age group, in line with the specific feature of HIV epidemic in our country, but in contradiction with the data reported by other studies; therefore, at baseline most of the cases were in advanced stages of disease.

The estimated time from the diagnosis of HIV infection to onset of pregnancy was longer than that reported by other studies (8). A possible explanation could be the particular epidemiological situation in our country, namely the group of "therapeutically polyexperimented patients".

Mean HIV plasma viral load was significantly higher in HIV/HBV coinfected patients. HIV RNA levels in the third trimester of pregnancy were undetectable at a similar rate in the 2 groups. The higher average CD4 cell count as well as the high percentage of undetectable HIV plasma viral load in the patients in our cohort differ from those reported in other articles (12). This can be explained by the fact that the majority of women of reproducible age decided to become pregnant following the indications of the infectious diseases physician of choosing the favorable period in which the viroimmunologic status allowed it.

The gestational age ranged from 34 to 39 weeks without significant differences in the mean values between the groups analyzed (p = 0.991).Compared to controls, the risk of prematurity in HIV-HBV group was 1.51 times higher. In our study a CD4 count of 200-499 cells/ml and age 21-30 years were the most important predictors of low birth

weight and preterm birth, in agreement with other published data. Advanced stages of maternal HIV infection seem to indirectly affect infant survival rate by the risk of low birth weight and preterm birth (3).

The degree of immunosuppression, high viral load, and the rate of maternal HIV disease progression increases the risk of vertical HIV-1 transmission (7, 13). In this analysis the HIV-1 related clinical manifestations were not predictive for the transmission of HBV or HIV-1. Important factors that have positively influenced the low rate of maternal-fetal transmission of these viruses were the administration of antiretroviral drugs with dual anti-VHB and anti-HIV activity, antiretroviral prophylaxis properly administered both to mother and newborn, and avoidance of breastfeeding.

The preventive role of scheduled cesarean section supports the hypothesis according to which the majority of infants are infected with these viruses during delivery (14); in our study HIV transmission to the newborn was noted in all women who delivered naturally (5 cases), without being encountered in cases with cesarean birth (9, 15-18).

Although the group of patients with ongoing pregnancy was representative, several limitations of this study should be mentioned. One limitation was that all we did was HBsAg testing of both mother and infant without having information on HBV infectivity markers and HBV plasma levels determined by PCR (12, 13). The study also had a limited ability to detect all risk factors for preterm birth as the presence of possible genetic abnormalities or obstetric factors could not be identified.

CONCLUSION

The two chronic infections can be transmitted vertically from mother to child. The large number of HIV/HBV-coinfected women of reproducible age and the presence of common risk factors detected in our study suggest that the association of HIV/HBV infections is an important public health issue.

This study provides data on the features of HIV/HBV coinfection in pregnant women, data that reflect the specific features of HIV and HBV infections in Northeastern Romania. The use of the prophylactic measures recommended by the panels of experts in the field made possible the limitation of vertical transmission of both viral infections compared with data reported in other geographical areas.

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ADIPONECTIN IN PATIENTS WITH NON-ALCOHOLIC STEATOHEPATITIS

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ADIPONECTIN IN PATIENTS WITH NON-ALCOHOLIC STEATOHEPATITIS (Abstract)

Aim The purpose of this study was to evaluate plasmatic levels of adiponectin in patients with non-alcoholic steatohepatitis (NASH). **Material and Methods** A cross-sectional study was conducted to evaluate a number of 104 people, 60 with NASH, and 44 healthy individuals. We used the anthropometric measurements (weight, height, BMI (body mass index), waist circumference (WC), body fat percentage (BF%)) for the assessment of the nutritional status. The biochemical analyses included fasting serum lipids, glucose profile (fasting plasma glucose, fasting plasma insulin (FPI), HOMA-IR), liver function tests, adiponectin. Insulin resistance (IR) was determined using Homeostasis model assessment (HOMA-IR). Hepatic steatosis (HS) was determined using ultrasonographic criteria and an algorithm (Hepatic steatosis index - HSI). **Results** There were statistically significant differences between groups for age, BMI, weight, HSI, FPI, HOMA-IR, adiponectin, triglycerides, and HDL-C (all $p<0.001$). In all patients, adiponectin levels were lower in men, in patients with insulin resistance (HOMA-IR>2), with obesity, and with metabolic syndrome (all $p<0.05$). In multivariate regression analysis HOMA-IR was the strongest independent factor for serum adiponectin. **Conclusions** Adiponectin levels were lower in NASH group. In multivariate regression analysis HOMA-IR was the strongest independent factor for serum adiponectin.

Keywords: ADIPONECTIN, NON-ALCOHOLIC FATTY LIVER DISEASE, HEPATIC STEATOSIS INDEX.

The prevalence of non-alcoholic fatty liver disease (NAFLD) has increased dramatically in recent years. NAFLD is the most common cause of liver disease worldwide, with a prevalence of 20%-40% in Western populations (1). Prevalence of NAFLD varies between 20-30% in European countries (2) reaching the level of 58% to 98% in overweight and obese people (3).

Adiponectin is a protein expressed by adipose tissue and hepatocytes (4). Studies have shown that decreased levels of adiponectin are associated with metabolic disorders such obesity, insulin resistance, type 2 diabetes, metabolic syndrome (5). Adiponectin is also negatively correlated

with traditional cardiovascular risk factors, such as high blood pressure, increased levels of low-density lipoprotein cholesterol and triglycerides (6,7), and is positively related to high-density lipoprotein cholesterol levels (8).

The purpose of this study was to evaluate the plasmatic levels of adiponectin in patients with non-alcoholic steatohepatitis (NASH).

MATERIAL AND METHODS

A cross-sectional study was conducted to evaluate a number of 104 people, 60 with NASH (NASH group) and 44 healthy individuals (control group CG).

Participants were recruited from three hospitals in Bucharest, Romania. The study protocol was approved by the Ethics Committee of Cantacuzio Hospital. All the participants have signed an informed written consent.

The inclusion criteria were: age over 35. The exclusion criteria were: patients with other etiology of chronic liver disease, hepatitis B, hepatitis C, autoimmune liver disease, hemochromatosis, steatosis-inducing drug use, patients having an alcohol consumption of more than 20 g/day, history of pancreatitis.

The anthropometric measurements used for the assessment of the nutritional status were: weight, height, BMI (body mass index), waist circumference (WC). Obesity was defined according to World Health Organization (WHO) classification (9). Blood pressure was measured twice with subjects in a sitting position.

Venous blood samples were drawn in the morning before 10:00 AM after a 10-h overnight fast. The biochemical analyses included fasting serum lipids (total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C)), glucose profile (fasting plasma glucose (FPG), fasting plasma insulin (FPI), glycated hemoglobin (HbA1c)), liver function tests (aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT)). Insulin resistance (IR) was determined using Homeostasis model assessment (HOMA-IR) (10). A HOMA-IR index value of more than 2.0 was considered as criteria of IR (10). Plasma adiponectin concentration (ng/ml) was assessed by ELISA. The stages of glucose tolerance were identified according to American Diabetes Association criteria (11). MetS was defined according to the joint harmonized criteria of International Diabetes Federation (IDF) (12).

Hepatic steatosis (HS) was determined using ultrasonographic criteria and using a mathematic models - hepatic steatosis index (HSI) (13). Percentage body fat (BF%) was predicted using an equation developed in Dutch Caucasians (14).

Values were expressed as means ± SD for normally distributed data or median for skewed data. Comparisons among groups were made by use of ANOVA for quantitative variables and the $\chi 2$ test for categorical variables. Relationships between adiponectin levels and other parameters (age, BMI, %BF, waist circumference, FPG, FPI, HOMA-IR, HIS) were analysed by simple correlation and by multiple linear regressions. The values of $p<0.05$ were considered statistically significant.

RESULTS

General characteristics of the study group according to the presence of NASH were summarized in table I. There were statistically significant differences between groups for age, BMI, weight, waist circumference, HSI, AST, ALT, GGT, FPI, HOMA-IR, adiponectin, triglycerides, and

HDL-C (all p<0.001).

Among the 104 patients (26 females in CG and 33 females in NASH group), 42 patients were diagnosed with MetS (n=4, 9.1% in control group and n=38, 63.3% in NASH group) according to the modified IDF criteria. In NASH group, after stratification by gender, 21 men (77.8%) and 17 women (51.5%) had MetS (all p<0.001).

In all patients, adiponectin levels were lower in men (4.2 vs. 5.8 ng/ml, p=0.001), in patients with insulin resistance (HOMA-IR>2) (3.6 vs. 6.4 ng/ml, p<0.001), with obesity (3.42 vs. 6.4 ng/ml, p<0.001), and with metabolic syndrome (3.5 vs. 6.4 ng/ml, p<0.001). Adiponectin levels decreased with increasing BMI (fig. 1). According with the stages of glucose tolerance, adiponectin levels decreased with glucose metabolism impairment, with lowest values in persons with type 2 diabetes (2.4 ng/ml), increasing to a value of 3.2 ng/ml in persons with impaired glucose tolerance (IGT), a value of 3.79 ng/ml in those with impaired fasting glucose, and with the highest value in individuals with normal glucose tolerance (5.4 ng/ml). HSI was 31.7±4.47 in control group and 36.9±6.21 in NASH group (p<0.001). In NASH group HSI increased with age (36.6 in 35-39 years, 36.8 in 40-49 years, 39.3 in 50-59 years, 40.1 in 60-69 years)(p=0.045). The same aspect was observed in the control group (p=0.038).

In univariate analysis adiponectin was negatively correlated with age, weight, BMI, BF(%), WC, HOMA-IR, FPG, FPI, HbA1c, triglycerides, cholesterol, HSI, AST/ALT ratio, GGT (data not shown). It was observed a significant positive correlation between adiponectin and physical activity and HDL-C (data not shown). Multivariate regression analysis revealed that HOMA-IR was the strongest independent factor for serum adiponectin (table II).

TABLE I

Clinical and laboratory parameters

	Control Group (n=44)			NASH Group (n=60)			Total			
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	p*
Age (years)	44.91	7.94	42.00	50.58	7.91	53.00	48.18	8.37	48.50	<0.001
Height (cm)	169.14	7.72	169.50	168.78	8.59	168.00	168.93	8.20	168.00	0.83
Weight (kg)	72.69	14.71	69.65	89.98	14.35	89.55	82.66	16.79	83.35	<0.001
BMI (kg/mp)	25.35	4.68	24.14	31.56	4.57	31.57	28.93	5.54	28.66	<0.001
WC (cm)	80.68	9.18	78.50	97.88	9.67	99.00	90.61	12.72	94.00	<0.001
FPG (mg/dl)	83.05	6.21	82.50	98.38	17.59	94.00	91.89	15.85	90.00	<0.001
FPI (uUI/ml)	5.76	1.68	5.85	11.23	3.33	11.50	8.91	3.86	8.45	<0.001
HOMA-IR	1.20	0.42	1.20	2.79	1.10	2.69	2.12	1.18	1.87	<0.001
HbA1c (%)	5.31	0.36	5.20	5.75	0.73	5.55	5.57	0.63	5.45	<0.001
Adiponectin (ng/ml)	6.90	1.44	7.40	4.14	2.20	3.80	5.30	2.36	4.94	<0.001
LDL-C (mg/dl)	114.00	22.48	109.00	143.10	36.66	139.95	130.79	34.49	127.05	<0.001
TG (mg/dl)	106.55	43.29	106.00	181.68	92.50	153.00	149.89	84.11	128.50	<0.001
Cholesterol (mg/dl)	186.41	22.74	188.00	218.54	44.12	208.00	204.94	39.81	199.00	<0.001
HDL-C (mg/dl)	51.10	10.06	48.75	39.10	7.44	38.00	44.18	10.46	43.15	<0.001
AST (UI/L)	15.06	6.53	12.00	48.79	21.22	45.00	34.52	23.58	27.60	<0.001
ALT (UI/L)	22.88	7.58	22.00	65.61	32.23	59.50	47.53	32.69	36.50	<0.001
GGT (UI/L)	21.74	10.59	19.00	72.63	41.29	64.00	51.10	40.76	40.00	<0.001
Falc (UI/L)	64.66	28.49	62.50	80.85	20.18	82.00	74.00	25.23	69.00	<0.001
Albumin (g/dl)	4.60	0.38	4.70	4.25	0.51	4.28	4.40	0.49	4.43	<0.001
HSI	31.76	4.47	30.31	39.08	5.45	37.94	35.98	6.21	35.65	<0.001
BF (%)	26.54	11.49	26.20	34.00	8.10	33.10	30.84	10.32	31.35	<0.001

* between groups

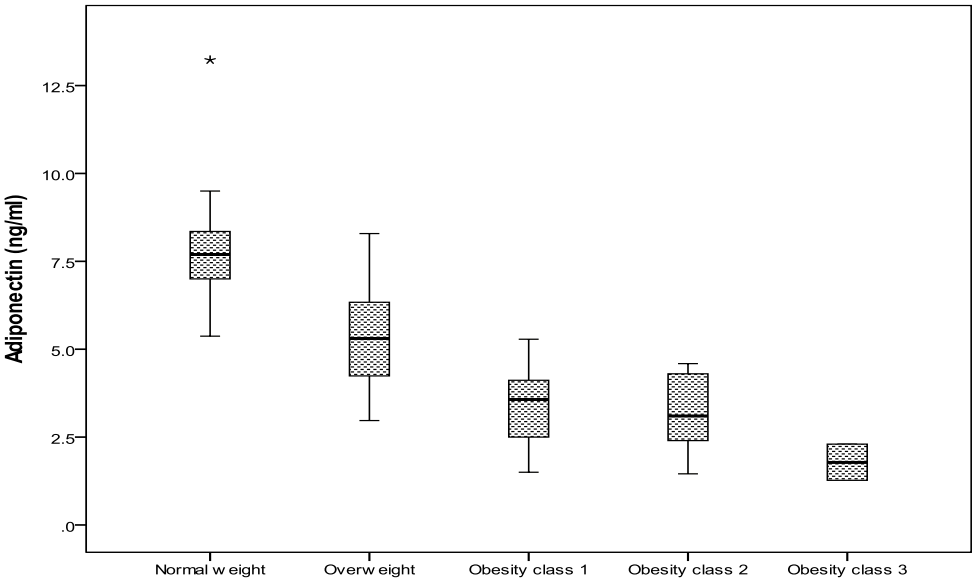

Fig 1. Adiponectin levels and BMI groups

TABLE III

Multivariate linear regression analysis using adiponectin level as the dependent variable

	Unstandardized Coefficients		Standardized Coefficients	t	p
	B	SE	Beta		
(Constant)	5.685	4.958		1.146	0.255
Age (years)	-0.032	0.023	-0.113	-1.365	0.176
Physical activity (min)	0.011	0.007	0.140	1.628	0.107
Weight (kg)	-0.005	0.027	-0.035	-0.183	0.855
BMI (kg/mp)	-0.329	0.223	-0.770	-1.478	0.143
%BF	0.035	0.023	0.152	1.516	0.133
WC (cm)	-0.031	0.033	-0.167	-0.945	0.347
HOMA-IR	-22.077	10.215	-1.473	-2.161	0.033
FPG (mg/dl)	0.062	0.030	0.415	2.088	0.040
FPI (uUI/ml)	14.219	6.298	1.214	2.258	0.027
HbA1c (%)	0.514	0.490	0.138	1.049	0.297
Triglyceride (mg/dl)	0.000	0.003	-0.011	-0.105	0.917
HDL-C (mg/dl)	-0.011	0.022	-0.050	-0.509	0.612
Cgolesterol (mg/dl)	-0.007	0.005	-0.116	-1.305	0.195
HSI	0.089	0.157	0.234	0.567	0.572
AST (UI/L)	-0.035	0.025	-0.351	-1.414	0.161
ALT (UI/L)	0.015	0.015	0.201	0.998	0.321
GGT (UI/L)	-0.001	0.005	-0.024	-0.252	0.802

B – unstandardized coefficient; SE – standard error of unstandardized coefficient; Constant – constant of the regression model

DISCUSSION

In this study adiponectin levels were lower in men, in patients with insulin resistance (HOMA-IR>2), with obesity, and with metabolic syndrome. Adiponectin levels decreased with increasing BMI. Goropashnaya AV et al. found that adiponectin was inversely correlated with adiposity, assessed by either BMI or %BF (15). In other trials central obesity and intra-abdominal fat accumulation was stronger associated with decreased adiponectin levels than subcutaneous fat (16,17). Yamamoto Y. et al.(18) observed the same variation in adiponectin according to sex distribution .

In a study performed in Romania the prevalence of hepatic steatosis was 92.4% in patients with MetS (19). In our study 63.3% from patients with NASH had MetS according to the modified IDF criteria.

Increasing age is accompanied by increased visceral adipose tissue, insulin resistance and decreased adiponectin. In our study HSI raised with increasing age in both groups.

Adiponectin was positive correlated with physical activity and HDL-C. Lifestyle intervention would favorably affect adiponectin levels, also presenting beneficial effects upon the pathogenic processes such as inflammation, hepatic steatosis, fibrogenesis.

In our study multivariate regression analysis revealed that HOMA-IR was the strongest independent factor for serum adiponectin.

There are several limitations of this study: small sample size, assessement of insulin resistance using a mathematical model (HOMA-IR), and hepatic steatosis using a mathematical model respectively ultrasonographic criteria.

CONCLUSIONS

In this study adiponectin levels were lower in men, in patients with insulin resistance (HOMA-IR>2), with obesity, and with metabolic syndrome. Adiponectin levels were lower in NASH group. Adiponectin was negatively correlated with traditional cardiovascular risk factors. Thouth in multivariate regression analysis HOMA-IR was the strongest independent factor for serum adiponectin. Prospective studies should demonstrate that lowering insulin resistance through non-pharmacological and/or pharmacological measures would increase adiponectin levels and reduce cardiovascular risk.

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NONALCOHOLIC FATTY LIVER DISEASE AND ITS COMPLICATIONS - ASSESSING THE POPULATION AT RISK. A SMALL SERIES REPORT AND LITERATURE REVIEW

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NONALCOHOLIC FATTY LIVER DISEASE AND ITS COMPLICATIONS - ASSESSING THE POPULATION AT RISK. A SMALL SERIES REPORT AND LITERATURE REVIEW (Abstract): **The aim** of the study was to highlight correlations between serum biochemical markers and different degrees of liver inflammation or fibrosis revealed by liver biopsy in morbidly obese patients. We also wanted to emphasize that the occurrence of hepatocellular carcinoma (HCC) is increasingly associated with obesity, metabolic syndrome and nonalcoholic fatty liver disease. **Material and methods:** A clinical retrospective study was carried out on a series of 13 patients operated for morbid obesity in our surgical unit. Included in this study were only the obese patients referred for bariatric surgery without other risk factors for liver disease and in whom a liver biopsy was taken during metabolic surgery. **Results:** The pathology report revealed different stages of nonalcoholic fatty liver disease in all 13 patients: pathological features of steatohepatitis (7 patients), hepatic steatosis (5 patients) and lesions specific for evolving cirrhosis (1 patient). Regardless of the pathological changes of the liver, except the patient with evolving cirrhosis, none of these patients showed changes in classical liver function blood tests. **Discussions:** Hepatic alteration in obese patients, ranging from simple steatosis to steatohepatitis or even cirrhosis, is not always correlated with the values of classical biological liver function tests. Literature data suggest the involvement of adipokines in the development and progression of steatosis as the hepatic expression of metabolic and chronic inflammation syndrome occurring in obese patients. Furthermore, these proteins secreted by adipose tissue seem to be related to the HCC occurrence. However, none of these studies show the exact pathway followed by the hepatic cell from simple fatty liver to hepatocellular carcinoma. C**onclusions:** finding and selecting the population at risk for fatty liver disease progression and for HCC development among obese patients is mandatory. **Keywords:** OBESITY, NAFLD, HCC, BARIATRIC SURGERY.

Obesity is defined by the World Health Organization (WHO) as an abnormal or excessive fat accumulation that may impair health, with body mass index (BMI) greater than or equal to 30 kg/m^2 (1). WHO and International Obesity Task Force reported that 312 million adults worldwide are obese. Overall, more than one in 10 of the

world adult population is obese (2). According to International Agency for Research on Cancer (IARC), hepatocellular carcinoma (HCC) is the fifth most common cancer in men and the seventh most common cancer in women, with more than 500,000 new cases per year (3, 4). It is already established that chronic conditions such as viral hepatitis B and C, alcoholic liver disease and hemochromatosis are the main risk factors for developing HCC. Nowadays, due to the growing number of cryptogenic HCC (with no readily identifiable risk factor) along with a dramatic increase in the number of patients with nonalcoholic fatty liver disease (NAFLD) related to obesity and metabolic syndrome, the association of these pathological conditions is becoming a topic increasingly discussed.

NAFLD comprises a spectrum of liver diseases defined by excessive fat accumulation in the form of triglycerides (steatosis) in the liver which cannot be explained by alcohol consumption. It ranges from simple steatosis to non-alcoholic steatohepatitis (NASH) that can have different degrees of fibrosis and can progress to liver cirrhosis and end-stage liver disease. Among patients with NAFLD, one to two in each ten patients will have NASH (5). A recent survey showed that NAFLD-associated cirrhosis is the third indication for liver transplantation in the United States, and it is expected to be the number one indication in 2020 (6).

NAFLD is strongly associated with obesity, insulin resistance (IR) / type 2 diabetes mellitus (T2DM) and the metabolic syndrome. In overweight subjects, the prevalence of steatosis is at least two times more frequent than in lean subjects (7), being directly proportional to the increase of body mass index.

Although cirrhosis precedes the diagnosis of HCC in most individuals, the number of cases in which HCC develops in the absence of cirrhosis is not negligible. A clinical-histopathological review of 804 American patients with HCC confirmed the lack of cirrhosis in as many as 42.6% of all cases (8). A recent review of a United States healthcare claims database covering 18 million lives per year identified 4406 cases of HCC associated with NAFLD as the most common underlying risk factor (59%) followed by diabetes (36%) and chronic HCV infection (22%) (9). Only 46% of all NAFLD-associated HCC cases were reported to have cirrhosis in this study, while 78% of HCC cases associated with chronic hepatitis C occurred in cirrhotic liver (9).

MATERIAL AND METHODS

With the increasing number of bariatric surgeries in our service, we started to take liver biopsy during bariatric surgical intervention as a diagnostic method of liver damage. We retrospectively reviewed the medical records of 13 obese patients receiving sleeve gastrectomy and hepatic biopsy during the past three months in our surgical unit. Inclusion criteria: patients aged over 18 years meeting the NIH criteria for bariatric surgery, irrespective of age, sex or geographical origin. The NIH criteria for bariatric surgery are BMI $\geq$40 kg/m^2 or BMI $\geq$35 kg/m^2 and at least one significant weight-related co morbidity (10). Exclusion criteria: patients diagnosed with viral hepatitis B and C, history of any liver disease, chronic treatment with hepatotoxic drugs or alcohol consumption over 20g per day. Also excluded were the patients in whom liver biopsy was not taken due to

patient refusal or any technical reasons. In all study patients age, clinical parameters (BMI), medical history, pathology reports and biological parameters (AST, ALT, GGT, ALP, PLT, HOMA-IR, Ferritin) were reviewed.

RESULTS

The patients had a median age of 36.25 years and a BMI ranging from 44.19Kg/m^2 to 60 Kg/m^2, with a median BMI of 48.04 Kg/m^2.

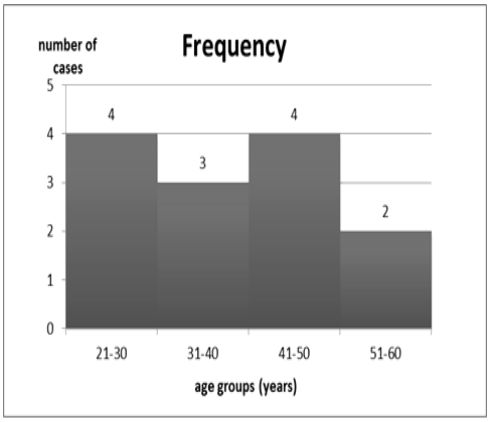

Fig. 1. Age histogram

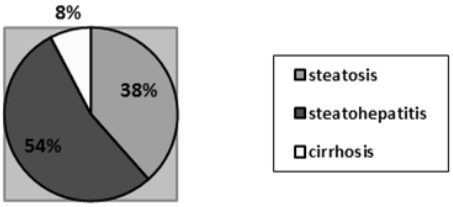

Fig. 2. Hepatic features in obese patients

The pathology reports of all these 13 patients who underwent sleeve gastrectomy and hepatic biopsy showed different stages of nonalcoholic fatty liver disease. The histological changes in the liver ranged from simple steatosis (5 patients) to steatohepatitis (7 patients) expressed by ballooning degeneration of hepatocytes and vacuolation of the nuclei with additional portobiliary fibrosis- in 2 cases; one patient presented obvious features of evolving liver cirrhosis.

Noteworthy, except for the patient diagnosed with liver cirrhosis who presented a slight increase in AST value (AST=49U/L), none of the patients diagnosed with any stage of fatty liver disease had pathologic changes of ALT, AST, GGT or ALP. They also had normal thrombocyte levels and normal coagulation. As to biological markers, we noticed elevated ferritin in 2 of the patients with hepatic steatosis, 3 of the patients with steatohepatitis and 1 patient with evolving cirrhosis; moderately increased cholesterol levels were found in 6 cases of steatohepatitis (range 203mg/dl-271mg/dl). Concerning obesity-related comorbidities, only 2 patients had history of type II diabetes and 1 patient was on antihypertensive therapy. According to HOMA-IR test, moderate insulin resistance was detected in 4 nondiabetic patients (2 of them having steatohepatitis and 2 hepatic steatosis); we considered that in the two study patients with type 2 diabetes abnormal HOMA-IR was a common situation.

DISCUSSION

Although the number of cases included in the study is small, our study shows that, except for the patient with evolving cirrhosis who presented a slight increased AST level, liver disease demonstrated by pathological examination had no impact on liver transaminases, blood count, alkaline phosphatase or coagulation tests. The results are in accordance with recent literature data showing that patients with normal versus elevated ALT had similar severity of

NASH and suggesting that plasma aminotransferase levels are misleading parameters for guiding clinical management (11).

Calculation of APRI (aspartate aminotransferase to platelet ratio index) showed values ranging from 0.135 to 0.297 with a median of 0.232; values higher than 0.5 were found in patient diagnosed with liver cirrhosis (0.572) and in one of the two patients presenting steatohepatitis and liver fibrosis (0.508). These data are partly in concordance with literature data showing that APRI values ≤ 0.3 and ≤ 0.5 rules out significant fibrosis and cirrhosis (12).

In recent years, adipose tissue has been considered an endocrine organ because of its capacity to secrete a variety of proteins named adipokines, with broad biological activities. The adipose tissue expansion may occur in inert subcutaneous tissue but the metabolically active adipose tissue is the visceral fat. The incrimination of the activity of adipokines in the development of associated morbidities makes them among the most studied proteins today. Even so, the exact causal link between obesity, secretion of adipokines and the hepatic manifestation of metabolic syndrome - nonalcoholic liver disease with its possible complications, steatohepatitis, liver cirrhosis and even hepatocellular carcinoma has not been found.

Speaking of the least common but most dangerous morbidity associated to NAFLD, the incriminated mechanisms in hepatic carcinogenesis related to NAFLD are insulin resistance, oxidative stress and lipotoxicity, on a background of low-grade inflammatory status associated with obesity and metabolic syndrome. Another important factor that contributes to the pathogenesis of HCC is hyperinsulinemia, resulting in increased insulin growth factor-1 (IGF-1) which has important proliferative and antiapoptotic effects. IGF-1 promotes angiogenesis through increased vascular endothelial growth factor production, which in turn leads to cancer cell proliferation (13). In our study group, excepting the two diabetic patients, we detected 4 more cases with elevated insulin, 2 patients presenting steatohepatitis and 2 steatosis.

Normally, insulin suppresses lipolysis, hydrolysis of triglycerides to free fatty acids (FFA). In conditions involving insulin resistance, such as fatty liver disease, lipolysis is active, resulting in the release of FFA from the adipose tissue. Insulin resistance (IR) has a major role in the occurrence of hepatic steatosis and in the progressive development of steatohepatitis, liver cirrhosis and even hepatocellular carcinoma. Steatosis itself also promotes IR, endorsing a self-perpetuating vicious cycle (14). The excessive accumulation of fat in adipocytes promotes an increase in oxidative stress, which deregulates adipocytokines production and promotes low grade inflammatory state in the adipose tissue, through the release of interleukin (IL)-6 among others (15). Subsequently, macrophages and lymphocytes are activated, promoting further release of pro inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interferon-γ. The spillover of FFA from the adipose tissue leads to ectopic accumulation of fat in muscles and liver. Noteworthy, in the liver, not all insulin actions are impaired; it preserves its lipogenic actions further inducing steatosis, and its pro-mitogenic actions, which may enhance hepato-carcinogenesis (16). Excepting the two patients with type 2 diabetes in whom abnormal HOMA-IR was a common situation, moderate IR was present in 4 nondiabetic patients (2 with

steatohepatitis and 2 with hepatic steatosis).

Increased serum ferritin levels are present in more than 30% of patients with NAFLD and it has been reported to correlate with IR and the other metabolic abnormalities defining the metabolic syndrome (17, 18). Our data are consistent with the literature data, showing increased ferritin levels in 46.15% of our NAFLD patients, changes not related to a specific degree of liver damage. Other mechanisms involved in hepatic disorders up to HHC development, such as hepatic iron overload, seem to be linked to IR and metabolic syndrome (19). Iron overload has been hypothesized to induce IR by catalyzing oxidative stress in the liver (20). The iron may fuel oxidative stress-driven cell toxicity or activate signaling pathways involved in fibro genesis or carcinogenesis occurring in the metabolic syndrome (18). Metabolic derangement and progression of liver disease are more severe with NASH than with NAFLD and hepatic iron deposition has been shown to increase the risk of malignant transformation in NASH-derived cirrhosis (17, 21). Considering these data, we believe that the assessment of liver iron overload should be part of the diagnosis of NASH.

All these mechanisms have in common the involvement of adipokines in their occurrence and development. Leptin, a protein mainly produced by adipose tissues is best known as a regulator of food intake and energy expenditure via hypothalamic-mediated effects. This adipokine has many additional effects, including angiogenesis - by induction of VEGF activation with an important impact on liver fibrosis, as formation of new blood vessels is a key component of the wound-healing, with important implications in the development of liver fibrosis, cirrhosis and HCC (22). Considering that obesity induces a chronic inflammatory status, it has been demonstrated that leptin also has a regulatory immune function. Leptin levels are acutely increased by, and also may affect the secretion of many acute phase factors such as tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6 (23, 24). Both dietary and genetic obesity promote liver inflammation and tumor genesis by enhancing IL-6 and TNF expression (25). As we can see, hepatic impairment in obese patients, ranging from simple steatosis to steatohepatitis or even cirrhosis, does not always have an impact on classical biological liver function test values. Liver biopsy remains the gold standard for differentiating nonalcoholic steatosis from other stages of fatty liver disease; still, it is an invasive procedure, associated with morbidity, mortality and several limitations (26). The clinical experience shows that most patients refuse invasive methods for assessing the progression of liver pathology associated with obesity. Therefore we believe that finding a noninvasive algorithm for assessing the degree of liver damage and especially the evolution of liver lesions after bariatric surgery is required.

CONCLUSIONS

As our study shows, the number of cases of nonalcoholic steatohepatitis with different degrees of liver fibrosis that do not present any pathological changes in liver function blood tests is not to be neglected. There is increasing evidence that obesity, associated metabolic disorders and mainly NAFLD - the hepatic expression of metabolic syndrome - are involved in the development of HCC. The incidence of

HCC occurrence in patients with NAFLD is low, but the number of obese patients is increasing constantly worldwide. A worrying fact is that cirrhosis is not indispensable for HCC development in patients with NAFLD. For an efficient follow-up, noninvasive algorithms to assess liver damage in patients with morbid obesity as well as selection criteria of patients at risk to develop HCC among patients with obesity and metabolic syndrome are needed.

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NEWS

SURGICAL TREATMENT OF AORTIC VALVE ENDOCARDITIS: A 26-YEAR EXPERIENCE

Microbial infection of the endothelial lining of any part of the heart, infective endocarditis (IE), is an uncommon but life threatening condition. Despite advances in diagnosis, antimicrobial therapy, surgical techniques, and management of complications, the incidence has not decreased in the past 30 years and patients with IE still have high morbidity and mortality rates related to this condition. Its management aims to eradicate the infecting organism as soon as possible mainly with antibioticotherapy but clinical complications and treatment failure suggest surgery up to 60% of the cases. IE effecting aortic valve accounts about 40-67% of all cases and about 60-70% of these cases undergo surgery in the acute phase. In spite of the high mortality and morbidity it carries, surgical therapy is still the mainstay in the treatment of aortic valve IE. Numerous studies have assessed different risk factors for mortality and morbidities in the treatment of IE but risk factors for surgical treatment of aortic valve IE patients need to be clarified. In this study, authors retrospectively assessed the results of the surgical treatment of patients with aortic valve endocarditis over a period of 26 years in an attempt to address these issues (Adademir T, Tuncer EY, Tas S et al. Surgical treatment of aortic valve endocarditis: a 26-year experience. *Rev Bras Cir Cardiovasc* 2014; 29 (1): 16-24).

Doina Butcovan

CHRONIC OCCUPATIONAL STRESS EXPOSURE MAY INCREASE THE VULNERABILITY TO ACOUSTIC TRAUMA IN MILITARY PROFESSIONALS

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CHRONIC OCCUPATIONAL STRESS EXPOSURE MAY INCREASE THE VULNERABILITY TO ACOUSTIC TRAUMA IN MILITARY PROFESSIONALS (Abstract): Today little is known about the connection between chronic stress exposure and hearing loss. These effects cannot be explained by differences in HPA axis response but recent studies saying that chronic stress induced limbic system alterations spread to nonlimbic areas affecting auditory system might be the key. On the other hand we know that subjects exposed to chronic stress may prove hypersensitivity to novel stressors. **The aim** of this study is to confirm that occupational chronic stress (OCS) exposure determines vulnerability to acoustic trauma and to establish a method to identify individuals at risk prior to their exposure to high intensity acoustic stimulus. **Material and methods:** 60 military personnel with known acoustic trauma injury evidentiated through audiograms and occupational chronic stress exposure quantified through validated questionnaires were exposed to mild novel stressor: occupational medicine evaluation and clinically assessed for maladaptive cardiovascular response (MCVR). Employees were split in two groups, group 1 (MCVR) and group 2 (non MCVR). **Results:** We found positive correlation between level of perceived OCS and level of hearing loss on entire group and between groups with values of parameters significantly higher in group 1. Subjects exposed to OCS with hypersensitivity to novel stressor evidentiated through maladaptive cardiovascular stress response may be more vulnerable to high intensity acoustic stimulus and consequently acoustic trauma. **Conclusions:** Establishing methods and biomarkers that help us indentify individuals at risk of developing acoustic trauma might decrease the high burden of hearing loss. **Keywords**: CHRONIC OCCUPATIONAL STRESS, HYPERSENSITIVITY, NOVEL STRESSOR, ACOUSTIC TRAUMA

Hearing is a critical sensor for survivability and lethality in military operations, the ability to accomplish unit's mission being directly proportional to ability to communicate effectively. Besides the roles of hearing in a peace operation: identifying sound source, understanding verbal orders, radio communications, in a combat we have to take in account the chaotic environment, the complexity of problems encountered and the reaction time required, so a mild impairment of hearing in a peace operation might be judged differently in a combat situation (1). Hearing loss (HL) was considered an invisible injury viewed as acceptable by product of military service with little impact on military operations but 55864 veterans compensations cases between 2001- 2006 summing 900 ml $ made HL a primary disability second only to

tinnitus and a huge burden (2). In a combat sound is often the first source of information before direct contact. Information carried by sound comes from all directions, through darkness and over or through visual obstacles. Aggressive action produces sounds that cannot be camouflaged. The ability to hear and recognize combat relevant sounds is a vital component to situational understanding and provides tactical advantage. So noise induced hearing loss is a tactical risk and threatens both individual and unit combat effectiveness and preserving hearing capabilities of an unit is an important challenge for the medical team involved in prevention (1). Any factor that may influence good hearing capability should be thought of and considered in situations, peace and combat.

Recent studies pointed out that chronic stress determines limbic system alterations that spread to non limbic areas affecting specific sensory systems such as auditory system and complex cognitive functions (3). Moderate or high stress levels at the time of acoustic trauma might play a pivotal role in the vulnerability of the cochlea to acoustic damage (4). Chronic stress induces dendritic atrophy in auditory cortex thus influencing complex cognitive behavior with impact on auditory attention and decision making processes and decrease in environmental adaptation. We might so easily imagine a scenario in which a chronic stress exposed military with maladaptive cardiovascular stress response (MCVSR) or white coat effect pattern with subsequently exaggerated alarm reaction is put in front of the wide variety of stressors in an operational theater (5, 6, 7, 8, 10). His impaired processing sound characteristic transforms him in a possible candidate for noise induced hearing loss or worse affecting direct performance and safety of the military operation (1, 2, 11).

That is why best preventive measures must be taken before the units are involved in high-stress, high-risk actions (9).

Following this logic we thought that we can identify individuals at risk of developing hearing loss prior to their exposure to high intensity acoustic stimulus by looking at their cardiovascular stress response pattern.

MATERIAL AND METHODS

Our study is open label randomized type of study done on 60 military personnel, all males, recruited during routine occupational medical evaluations in the occupational medical office from Emergency Military Hospital, Iasi. The selection was based on inclusion and exclusion criteria. The inclusion criteria were bilateral acoustic trauma with hearing loss disability ≥30dB in 4,000 Hz with age correction factor (Romanian occupational health diagnostic standards for acoustic trauma), healthy with no relevant personal or family history, BMI<25, level of perceived work stress > 5 (significant level of perceived occupational stress) on occupational stress evaluation validated questionnaires and exclusion criteria were unilateral acoustic trauma, cardiovascular disease, endocrine disease, neurologic disease, obesity and level of perceived work stress < 5.

We considered the occupational medicine routine evaluation a mild novel stressor and cardiovascular dysfunctions discovered (arterial blood pressure ≥ 140/80 mm Hg and rest heart beat >80 bpm) an exaggerated alarm reaction and anxious behavior of a chronic occupational stressed hypersensitive subject. We divided the entire group in two, group 1 being the MCVSR

group with cardiovascular dysfunctions and group 2 being the non MCVSR group with normal cardiovascular stress response and normal values of cardiovascular parameters. Than we studied the differences between the two groups and the entire group in terms of level of stress, level of exaggerated alarm reaction (values of cardiovascular parameters) and hearing loss levels. We were looking in our research for confirmation or not that chronic occupational stress exposure determines increased vulnerability to acoustic trauma and if the answer was positive to establish a method to identify individuals at risk of developing it prior to their exposure to high intensity acoustic stimulus.

RESULTS AND DISCUSSION

We used descriptive statistic and inferential statistic parameters from *SPSS 11.0*. We found a high level of work stress: 7.1 ±0.89 on entire group with positive significant correlation of medium strength: r-0.455 between hearing loss level and level of work stress on entire group and positive significant correlation of high strength- r-0.695 between hearing loss level cardiovascular parameters modifications on entire group.

TABLE I

Comparative values between the two groups (MCVSR-group1 and non MCVSR-group2) in hearing loss and occupational stress level

Group	N	HLBE	HLWE	MHL	OccSL
MCVSR-group 1	28	45.96±9.98	59.78±9.36	52.87±8.47	7.42±0.74
nonMCVSR-group 2	32	37.03±5.04	42.37±6.52	39.70±5.20	6.81±0.93

HLBE- hearing loss best ear; HLWE- hearing loss worst ear; MHL- medium hearing loss; OccSL- occupational stress level; MCVSR- maladaptive cardiovascular stress response.

The differences found between the two groups in terms of hearing loss and occupational stress levels suggest that military exposed to chronic stress with exaggerated alarm reaction and maladaptive cardiovascular pattern might have a vulnerability to acoustic trauma with higher levels of hearing loss thus confirming the existed data in scientific literature and recent studies. Cardiovascular stress response can be used to early identify individuals at risk of developing noise induced hearing loss prior to their involvement in high-stress and high-risks operations. We need to confirm the relationship with broaden studies and in larger groups.

CONCLUSIONS

We found out that military subjects exposed to high level of work stress that have maladaptive cardiovascular stress pattern may be more vulnerable to high intensity acoustic stimulus and consequently acoustic trauma, this being in accord with recent scientific data. The pattern can be easily recognized and used as biomarker for early identification of individuals at risk of developing hearing problems prior of their deployment.

Establishing methods and biomarkers that help us identify individuals at risk of

developing acoustic trauma might decrease the high burden of hearing loss and preventive interventions as part of military health strategies might decrease the occurrence of stress related hearing problems.

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IMPACT OF HEPATIC STEATOSIS ON DISEASE COURSE IN PATIENTS WITH COMPENSATED HEPATITIS C VIRUS-RELATED CIRRHOSIS RECEIVING INTERFERON-FREE THERAPY (PARITAPREVIR, RITONAVIR, OMBITASVIR DASABUVIR AND RIBAVIRINA)

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IMPACT OF HEPATIC STEATOSIS ON DISEASE COURSE IN PATIENTS WITH COMPENSATED HEPATITIS C VIRUS-RELATED CIRRHOSIS RECEIVING INTERFERON-FREE THERAPY (PARITAPREVIR, RITONAVIR, OMBITASVIR DASABUVIR AND RIBAVIRINA) (Abstract). **Aim**: In Romania, genotype 1 of hepatitis C virus (HCV) is the most common and the aim of this study was to assess comparatively the biochemical, hematological and virological parameters in patients with different degrees of steatosis before the initiation of interferon-free therapy (paritaprevir/ritonavir/ombitasvir/ dasabuvir plus ribavirin) (PrOD-R) and at its completion. M**aterial and methods:** This retrospective study included 113 patients with compensated liver cirrhosis with C-genotype 1b, treated with PrOD-R evaluated and clinically-biologically monitored at the Iasi Hospital of Infectious Diseases between November 2015 and May 2017**. Results**: We found an advanced steatosis (grades ≥2) in 54% of patients. Associated cardiovascular and kidney diseases and diabetes mellitus were more frequent in the group of patients with advanced steatosis compared to the group with incipient steatosis (19.5% *vs*. 8% and 10.6% *vs*. 0.9%, respectively) (p = 0.02 and p = 0.003, respectively). In most patients the hepatic cytolysis syndrome was identified before the initiation of therapy (93%) with a significant difference between patients with advanced versus low grade steatosis (53% *vs*. 40%, respectively, p = 0.014). In patients with steatosis grade ≥2, GGT levels above the normal range were detected in 52.2% cases and in the group with steatosis grade <2 in 31% (p = 0.0003). At the time of treatment initiation, only 3.5% of patients presented various grades of anemia, but at the end of therapy 38% had different grades of anemia, this anomaly being more common in patients with advanced steatosis (23% *vs*. 15%, respectively, p = 0.27). At the end of therapy, only 14.2% still had higher-than-normal ALT levels, most of them in the group with advanced steatosis (11.5% *vs*. 2.6%, respectively, p = 0.018). **Conclusions**: We found a significant correlation between the

advanced grade of hepatic steatosis and the levels of hepatic cytolysis enzymes and GGT both before initiation of therapy and at its completion in patients with advanced steatosis.
Keywords: STEATOSIS, HCV CIRRHOSIS, INTERFERON-FREE TREATMENT.

HCV infection has been reported to be strongly associated with hepatic steatosis. But there are other factors responsible for steatosis, including alcohol consumption, obesity and diabetes.

Experimental animal studies have shown that HCV core protein promotes liver steatosis. Also, when steatosis was studied in connection with HCV genotypes, it was found that although steatosis is induced by all HCV genotypes, it appears to be more evident and more frequent with genotype 3 infection, in these patients being a good correlation between the steatosis grade and HCV replication, the presence of HCV core protein in the liver, respectively (1). Moreover, it has been reported that in patients with genotype 3 infection steatosis regresses with successful antiviral therapy compared to those with other genotypes that remain steatosis (2).

Different mechanisms have been incriminated in the alteration of the lipid metabolism and steatosis progression in HCV-infected patients:

(1) *Abnormal VLDL secretion*;

(2) *Increased de novo free fatty acid synthesis*;

(3) *Abnormal AGL degradation* (3,4,5).

Several viral proteins can occasionally cause cell lesions (by oxidative stress and steatosis), probably in a specific sequence and directly activating the hepatic stellate cells without participating in the inflammatory response (6). These findings may explain why some chronic hepatitis C patients may present significant fibrosis at the histological assessment, despite normal liver enzymes and minimal/ mild inflammation.

It is known that steatosis is a cofactor that influences the progression of fibrosis in chronic hepatitis C. Various studies have directly associated steatosis with HCV genotype 3. Thus, Kumar et al. identified steatosis reduction as a predictor of the virological response in the treatment of chronic hepatitis C in individuals infected with genotype 3 (2).

Our study aimed to evaluate the impact of advanced steatosis grade on virological, hematological and biochemical parameters in patients with HCV cirrhosis treated with Ombitasvir + Paritaprevir + Ritonavir + Dasabuvir and Ribavirin.

The need for such an assessment is justified by the existence of regional particularities regarding comorbidities, diet, age at the time of treatment, previous use of an antiviral drug and the response to it.

MATERIAL AND METHODS

In this retrospective study, we analyzed 113 patients with a diagnosis of compensated liver cirrhosis, with HCV genotype 1b, assessed, treated and clinically-biologically monitored at the Iasi “St. Parascheva" Hospital of Infectious Diseases (section 2) from November 2015 until May 2017.

Patient selection was strictly guided by the inclusion criteria set forth in the therapeutic protocol appropriate to DCI (Ombitasvirum + Paritaprevirum + Ritonavirum + Dasabuvirum, currently in force (7, 8).

Patient analysis required the collection and systematization of personal data of

epidemiological, clinical and laboratory relevance from the medical records and the creation of databases (*Excel, SPSS*) for statistical evaluation and interpretation.

Statistical analysis. The chi-squared test was used for statistical analysis and a value of $p<0.05$ was considered statistically significant.

Ethical statement. The objectives and procedures of the study were approved by the Ethics Committee of the Iasi Infectious Diseases Hospital. No written consent was given by the participants, as data were collected and analyzed anonymously.

RESULTS

In all patients, steatosis was graded using the baseline Fibromax test. We found an incipient steatosis (<2) in 52 (46%) patients and advanced steatosis (≥2) in 61 (54%) patients Extreme steatosis grades, namely S0/S0-1 and S3, were recorded in 19 (18%) patients and 34 (30%) patients, respectively.

Seventy-five (66.37%) patients had been previously treated with peginterferon and ribavirin; of them 54 (47.8%) were considered non-responders to previous treatment and 21 (18.6%) patients relapsed. Thirty-eight (33.62%) patients did not receive previous interferon-based regimen.

Efficacy and safety of therapy were assessed in patients with advanced steatosis (≥2) and compared with data from patients with incipient steatosis (< 2) (tab. I).

TABLE I

Response to previous treatment with interferon + ribavirin and the presence of comorbidities by steatosis grade

	Steatosis<2 (no=52)		Steatosis≥2 (no=61)		Total (no=113)		p
	No	%	No	%	No	%	
Previous interferon-based regimen. (no=75)							
- Non-responder	27	36	27	36	54	47.8	0.85
- Relapse	11	14.7	10	13.3	21	18.6	
Treatment naïve (no=38)	14	12.4	24	21.3	38	33.6	0.16
With cardiovascular or kidney disease (no=31)	9	8%	22	19.5	31	27.4	**0.02**
With diabetes mellitus (no=13)	1	0.89	12	10.6	13	11.5	**0.003**

A higher rate of the patients with cardiovascular, kidney and metabolic (diabetes mellitus) were in the advanced steatosis group compared with the early steatosis group (19.5% versus 8% and 10.6% *vs*. 0.9%, respectively) ($p = 0.02$ and $p = 0.003$, respectively).

Nearly half of the patients had thrombocytopenia (<150,000 / mm^3) at baseline and was identified in a slightly higher rate in the patients with steatosis ≥2 compared to those with steatosis <2 (28% *vs*. 22%, $p = 0.64$).

The hepatic cytolysis syndrome was identified before the initiation of treatment in most patients (93%) with a significant difference between those with advanced steatosis versus those with low-grade steatosis (53% *vs* 40%, respectively, $p = 0.014$).

TABLE II

Hematologic and biochemical parameters at the initiation of therapy PrOD- R by steatosis grade

Parameters at the initiation of therapy	Steatosis <2 (no=52)		Steatosis ≥2 (no=61)		Total (no=113)		p
	No	%	No	%	No	%	
Anemia (Hb<11g/dL)	1	0.9	3	2.7	4	3.5	0.39
Thrombocytopenia (<150,000/mmc)	25	22	32	28.3	57	50.4	0.64
Increased ALT level*	45	39.8	60	53.1	105	92.9	**0.014**
Increased bilirubin level (>1.2mg/dL)	11	9.7	9	8	20	17.7	0.11
Increased GGT level (>65UI/L)	35	31	59	52.2	94	83.2	**0.0003**
Increased AFP level >20 UI	11	9.7	14	11.5	25	22.1	0.81

*> 30 UI in women and > 40 UI in men

In the study patients, not only the level of cytolytic enzymes was directly influenced by the steatosis grade but also the level of cholestasis enzymes. Thus, in patients with steatosis ≥2, elevated GGT levels were detected in 59 cases (52.2%), and in the group with steatosis<2, 35 patients (31%) showed levels above the normal (> 65UI /L) (p = 0.0003) (tab. II).

Approximately 18% of patients had initially mild hyperbilirubinemia, with no significant differences between the two study groups.

More clear-cut was the comparison of the extreme steatosis grades (S0 *vs.* S3) with respect to mean levels of viral load, ALT, GGT and AFP at baseline. Statistical analysis revealed significant differences between the obviously higher values in those with significantly advanced steatosis compared to those without steatosis.

Comparing the mean ALT levels in patients with steatosis S0 (71 IU) and steatosis S3 (172 IU), we noticed the significant influence of steatosis grade on the level of this enzyme (p = 0.002) (tab. III).

TABLE III

Mean levels of viral load, ALT, GGT and AFP at baseline by steatosis grade

Mean levels	Steatosis: S0 (n=19)	Steatosis: S3 (n=34)	p
Mean viral load (UI/mL)	582,450	1,613,050	**0.048**
Mean ALT	71 UI	172 UI	**0.002**
Mean GGT	42.3 UI	162.4 UI	**0.0003**
Mean AFP	5.37 UI/mL	30.82 UI/mL	**0.018**

Alpha-fetoprotein (AFP) is a very important parameter in the initial assessment being a predictor of hepatocellular carcinoma. In our study, we found a clear relationship between steatosis grade and AFP level. Thus, in S0 patients, mean AFP was 5.37 IU/mL compared to 30.82 IU/mL in S3 patients (p = 0.018).

If only 4 (3.5%) patients had anemia at baseline (Hb <11g/dL), at the end of therapy, 38% had different degrees of anemia, this hematological abnormality being more common in advanced steatosis cases (23% *vs.* 15%, respectively, p = 0.27) (tab. IV).

We also noticed a significant reduction in the number of thrombocytopenic patients

at completion of antiviral therapy from 50% at baseline to 34%, in contrast to the elevation trend of bilirubin (from 17% at baseline to 31% at the end therapy).

TABLE IV

Hematological and biochemical parameters at the end of PrOD- R therapy by steatosis grade

Parameters at the end of therapy	Steatosis <2 (no=52)		Steatosis ≥2 (no=61)		Total(no=113)		p
	No	%	No	%	No	%	
Anemia (<12 g%)	17	15	26	23	43	38	0.27
Thrombocytopenia (<150,000/mm^3)	19	16.8	19	16.8	38	33.6	0.54
Increased ALT level	3	2.7	13	11.5	16	14.2	**0.018**
Increased bilirubin level (>1.2mg/dl)	20	17.7	15	13.3	35	31	0.11
Increased GGT level (>65UI/l)	4	3.5	15	13.3	19	16.8	**0.016**
Increased INR ↑ level (>1.2)	44	38.9	53	46.9	97	85.8	0.73

Most of the treated patients responded by the return to normal of liver cytolytic enzymes, only 14.2% continuing to show ALT above the normal level at the end of therapy, their proportion being significantly higher in those with advanced steatosis (11.5% *vs.* 2.6%, respectively, p = 0.018).

A favorable evolution was also recorded for GGT; thus, only 16.8% of the treated patients had higher-than-normal levels, with significant differences between those with steatosis ≥2 *vs.* those with S <2 (13.3% *vs.* 3.5 %, respectively, p = 0.016).

A sustained virological response was recorded in all cases (at 12 weeks after completion of therapy) except for one person who discontinued medication for 4 days because of a death in the family.

DISCUSSION

The severity of hepatic steatosis in chronic HCV infection is due to the combination of several host factors (obesity, medication, alcoholism) and viral factors (genotype, viral load, mutations) that interfere with the hepatocyte lipid metabolism. Thus, steatosis occurs in chronic HCV infection more frequently than in any other chronic hepatitis and, more importantly, in patients with no other causes of fatty liver, indicating that hepatic steatosis is a direct cytopathic effect of the virus. That is why we considered extremely important to evaluate several aspects related to the presence and severity of steatosis in the study patients (2).

Of the total of 113 patients, 91.2% (103 patients) had various grades of steatosis, with a moderate-severe steatosis (grade ≥2) in over half of them. These results reveal a higher prevalence of steatosis in the study group compared to literature data reporting a prevalence of steatosis in patients with chronic HCV infection of 40-86% (9) or, in another case, of only 55% (10).

Another important aspect is the relationship between the viral genotype and the prevalence of steatosis. As already men-

tioned, all patients included in the study had HCV genotype 1b infection. It was determined (by Fibromax) that 90% of patients had various grades of steatosis, value significantly higher than those reported by other studies which showed a prevalence of steatosis in patients with HCV genotype 1 infection of about 50% (10). Also, most studies suggest that genotype 3 is the most steatogenic, with a prevalence of steatosis of >70%, associating it with the lowest rate of response to classical interferon-based therapies (10).

The introduction of new antiviral agents with direct mechanism has greatly improved the response rate of the patients with steatosis included, this parameter not being so important in predicting the response to treatment, but rather a prognostic factor for other comorbidities: accelerated atherosclerosis, cirrhosis or hepatocellular carcinoma (11, 12).

Thus, referring to the long-term negative potential of steatosis, we considered it necessary to evaluate its severity in our study patients. Over half of the patients enrolled in the study had moderate-severe steatosis grade S $\geq$2 (54% of the total).

The group of patients with steatosis grade S3 was the most numerous (25.7%), followed by grade S2 (23.9%), the smallest number of patients presenting steatosis grade S2-3 (4.4%). Only 10 of the total 113 patients showed no evidence of steatosis by Fibromax (S0), while 9 had steatosis grade S0-1, suggesting an incipient steatosis. Thus, there is an increased prevalence of moderate-severe steatosis in HCV genotype 1b patients, as opposed to other studies that rather highlight the involvement of genotype 3 in these advanced steatosis forms (moderate-severe in 40-50% of cases), and less of genotypes 1 or 4, with the presence of moderate-severe steatosis in only 10-15% of cases (11).

Another important aspect was the interpretation of the correlation between the initial viral load and steatosis grade. A significant correlation ($p = 0.048$) was found between steatosis grade and viral load, so that patients with steatosis S0 had a mean viral load of 582,450 IU/mL, significantly lower than that of patients with steatosis S3, with a viral load of 1,613,050 IU/mL. Considering that all our patients had HCV genotype 1b, these data are somewhat contradictory to those obtained by other international studies that have demonstrated the direct correlation between steatosis grade and baseline viral load in case of genotype 3 rather than in genotype 1 (13). Thus, Jackel-Cram investigated the effects of HCV 3a core protein compared to HCV 1b core on fatty acid synthase (involved in de novo synthesis of AG) and revealed a higher prevalence as well as an increased severity of steatosis in the case of genotype 3a compared to 1b (13).

However, a study by Kara (14) also highlights the direct relationship between HCV genotype 1 infection and the presence of steatosis in 82% of patients in the absence of any additional risk factors for this histopathological change. This study also demonstrated a significant link between initial viral load and steatosis grade, similar in relevance to the results of our study ($p = 0.02$ *vs.* $p = 0.048$)

Another interesting aspect in our study was the significant correlation between the high AFP level and grade of steatosis in patients with chronic HCV infection. Thus, in patients without steatosis, mean AFP level was 5.37 IU/mL, compared to 30.82 IU/mL in patients with steatosis S3 ($p = 0.018$). Similar results have been reported

by many other studies, such as that conducted by Mousa et al. (15), which compared two groups of patients (50 patients with steatosis, 50 without steatosis) and showed a significantly higher AFP level in patients with steatosis ($p < 0.01$) In other studies are highlighted the clear association ($p < 0.05$) of elevated AFP levels not only with hepatic steatosis, but also with elevated levels of uric acid, fasting blood glucose, vitamin D deficit and AST (16, 17, 18).

CONCLUSIONS

In North-eastern Romania, we found an increased prevalence of advanced steatosis in HCV genotype 1b infected cirrhotic patients, correlated with the presence of cardiovascular, renal and metabolic comorbidities in over 30% of cases. However, the sustained virologic response was very good (approximately 100%), with the normalization of cytolytic enzymes and platelets in significant percentages. We have found a significant correlation between the advanced grade of hepatic steatosis and the level of hepatic cytolytic enzymes and GGT both before the initiation of therapy and at its completion. This finding pleads for the need to reduce the steatosis grade prior to antiviral therapy to limit the level of hepatic cytolysis that would predispose to a more rapid progression to complications even in those who attained sustained virological response.

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NEWS

MAASTRICHT V: BEST EVIDENCE AND RELEVANCE TO THE MANAGEMENT OF HELICOBACTER PYLORI INFECTIONS

The Maastricht conference first took the initiative in 1996 and since then has been repeated at intervals of 4–5 years. Experts invited were chosen for their expertise and contribution to *H pylori* research and/or guideline methodology to review and discuss all relevant clinical data to arrive at recommendations for the clinical management of *H pylori* infection. It has been made important progress in the management of *Helicobacter pylori* infection in this fifth edition of the Maastricht Consensus Report. Key aspects related to the clinical role of *H. pylori*, to the increasing H. pylori resistance to previously efficacious antibiotic regimens, to the feasibility and efficacy of gastric cancer prevention, to the recommendation of treatment of all *H. pylori* infected subjects, to the perspective of potential interactions with other microbiota were re-evaluated. The aim of this report is to serve as a state-of-the-art guide for the management of *H. pylori* infection (Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, Bazzoli F,Gasbarrini A , Atherton J, Graham DY, Hunt R, Moayyedi P, Rokkas T, Rugge M, Selgrad M, Suerbaum S, Sugano K, El-Omar EM , European Helicobacter and Microbiota Study Group and Consensus panel. Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. *Gut* 2017; 66(1): 6-30).

Elena Luminita Popovici

ACUTE GOUT ATTACK WITH NORMAL SERUM URIC ACID LEVELS

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ACUTE GOUT ATTACK WITH NORMAL SERUM URIC ACID LEVELS (Abstract): **Aim:** The paper aims to determine the prevalence of clinical cases with acute gout attacks and normal serum uric acid levels. **Material and methods:** This retrospective study was conducted between October, 2013 and June, 2014 in 30 patients diagnosed with acute gout attack admitted to the Ist Clinic of Rheumatology of Iaşi. The diagnosis was based on clinical manifestations and positive response to therapeutic test with colchicine. **Results:** Normal serum uric acid levels were found in 63.3% of the patients with acute gout attack. The inflammatory syndrome was detected in 76.6% of patients with high erythrocyte sedimentation rate (ESR) levels and in 30% of patients who had elevated C-reactive protein levels. The urinary excretion of uric acid was measured in 11 patients and it was found increased in 55% of them. **Conclusions:** The diagnosis of acute gout attack should not be excluded in the presence of normal serum uric acid levels if the clinical manifestations or the positive response at colchicine are suggestive of gout. The low uric acid level is correlated with increased inflammatory factors and urinary excretion of uric acid. **Keywords:** ATTACK, GOUT, URIC ACID, PCR, COLCHICINE

Described since antiquity and named by Hippocrates the "disease of kings and queen of diseases", emphasizing the importance of overeating and heredity in the pathogenesis of this disease, gout is today known as an inflammatory arthritis caused by an abnormality of purine metabolism. The prevalence of this disease is 0.16 to 1.36%, with higher rates among men (male: female ratio = 2-7: 1) and a mean age of onset of 50 years. The natural history of this inflammatory arthritis has four stages: asymptomatic hyperuricemia, acute gouty arthritis, intercritical phase and chronic tophaceous gout. In asymptomatic hyperuricemia high blood uric acid levels are not accompanied by clinical manifestations, which occur during acute attacks of gout. Intercritical phase is by definition asymptomatic with a variable time between gout attacks and chronic tophaceous gout is the advanced stage of this disease characterized by accumulation of urate crystals in tissues and formation of tophi (1, 2, 3).

Clinical expression of gout is the appearance of inflammatory symptoms predominantly in the metatarsophalangeal joint of the big toe, which can then affect other joints such as the ankle, knee, wrist and small joints of the hands. From a biological perspective, hyperuricemia (uric acid > 7 mg /dl) forms a part of diagnostic criteria in gout, although only one fifth of the patients with increased uric acid levels become symptomatic (1,4). The presence of inflammatory syndrome (high ESR, C-reactive

protein, and fibrinogen) and radiological changes (swelling of soft tissues in the early stages reaching erosions, "blown bone" appearance, tophi in advanced stages) suggest a diagnosis of gout. The laboratory tests for determining the urinary uric acid excretion are also discussed as they establish the type of excretion (hyperexcretion or normal excretion) important to know when choosing the hypouricemiant treatment (5). The correct diagnosis can be made only by demonstration of uric acid crystals in the synovial fluid of the joint. These crystals have an acicular appearance under optical microscope and negatively birefringent on polarizing examination (6, 7).

This study aimed to determine the prevalence of acute gout attack cases with normal serum uric acid levels, considering that data in the literature suggest that at least 10% of patients with acute gouty arthritis are normouricemic.

MATERIAL AND METHODS

We present a retrospective study conducted between October 2013 - June 2014 including 30 patients admitted to and diagnosed with acute gout attack in the Ist Rheumatology Clinic of Iasi/Rehabilitation Hospital. The diagnosis was made based on both clinical manifestations and favorable response to colchicine administration (therapeutic trial).

RESULTS

Of the 30 patients 25 (83.3%) were males and 5 (16.6%) females (fig. 1); mean age was 64.3 years (range 44-82 years). It is important to note that the age at the onset of gout attacks in men was younger in our study (44 years) than in the cases reported in the literature (50 years). Analyzing the home treatment in our patients, we found that only 4 (13.3%) were on different doses of allopurinol prior to admission.

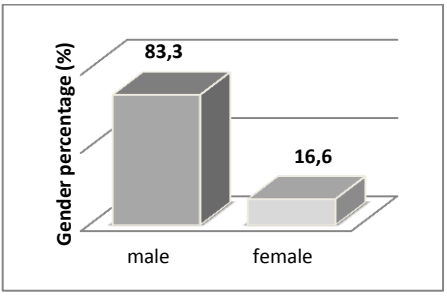

Fig. 1. Patient distribution by gender

The serum uric acid levels among the study patients was within normal range in 19 cases (63.3%) and elevated in 11 cases (36.6%) (fig.2). The inflammatory syndrome was present in most cases: elevated ESR and C-reactive protein were found in 76.6% and 30% of the cases, respectively (fig. 3). From this point of view, the limitation was the fact that C-reactive protein was not measured in all cases.

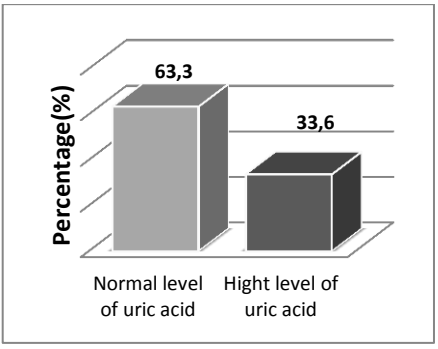

Fig. 2. Concentration of uric acid

In 11 of the 30 study patients the 24-hour urinary uric acid excretion was determined. It is known that this investigation gives us information on the hyper- or normo secretor status of the patient, which helps in selecting the therapeutic approach. The results showed that 55% of the 11

cases had increased levels of renal uric acid excretion.

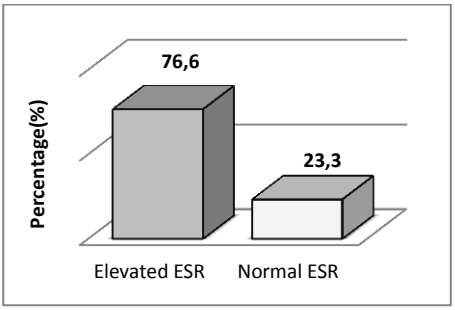

Fig. 3. Inflammatory syndrome

DISCUSSION

According to literature data, normal serum uric acid levels can be found in at least 10% of patients with acute gout attack; however, some studies show a proportion close to 50% of patients. A normal amount of uric acid during an acute gout attack can be explained by the increased urinary uric acid excretion resulting from ACTH release followed by adrenal stimulation caused by the stress of the painful process (1, 4, 5). Inflammation plays an important role in acute gout attack as it is believed that increased levels of inflammation markers (ESR, CRP), and IL6 and IL1β are directly correlated with lower serum uric acid levels (5).

An important aspect is the correct diagnosis, especially in elderly patients where symptoms can be intricate with a number of other co morbidities. A differential diagnosis of osteoarticular pains is required, as they can be of many causes, such as arthritis, multiple myeloma, autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus), bursitis, tendonitis and not the least gout. Elderly patients require a more detailed history not to miss a possible equivalence of angina pain (8). Also, the treatment of gout in elderly can be challenging in part because of increased colchicine and nonsteroidal antiinflammatory drug toxicity and low compliance to treatment and diet (9).

CONCLUSIONS

In our study we highlighted the fact that normal serum uric acid is not a criterion for exclusion of the diagnosis of acute gout attack, especially in the presence of a suggestive clinical picture or therapeutic response to colchicine. Low uric acid levels may be related in direct proportion to the elevated acute phase reactants as well as increased renal uric acid excretion.

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NEWS

ROSAI - DORFMAN DISEASE – A RARE CAUSE OF CERVICAL LYMPHADENOPATHY

Rosai-Dorfman Disease is a rare histiocytic disorder affecting various groups of lymphnodes in the human body. Rosai and Dorfman in 1969 described this disorder under the term Sinus Histiocytosis with Massive Lymphadenopathy. Majority of patients are children or young adults. Males are more commonly affected. Cervical Lymphnodes are commonly affected; however other lymphnodal groups like axillary, inguinal and mediastinal may also be involved. Extra-nodal involvement is seen in 25-40% cases. Various extranodal sites have been reported including the upper respiratory tract, gastrointestinal tract, paranasal sinuses, orbit and even meninges. In most of the cases patients are usually asyptomatic except for cervical lymphadenopathy however they may present with symptoms due to extranodal involvement and infiltration of vital organs. The disease usually has a self limiting, benign course and may not need any treatment. Episodes of remissions and exacerbations are characteristic however few patients may die from their disease. Etiology is not exactly known however like any other histiocytic disorder responds well to systemic steroids. Histologically it is characterised by pericapsular fibrosis with dilated sinuses, heavily infiltrated by large hitiocytes, lymphocytes and plasma cells. Emperipolesis is characteristic of lymphnodal involvement. Poor prognosis in the disease is due to wide spread dissemination and involvement of vital organs like kidney and liver or presence of immunological abnormalities. Otherwise the disease has a very stable and benign course. Diagnosis of Rosai-Dorfman disease is based on clinical suspicion and histopathological confirmation. Histologically there is infiltration of the tissue by lymphocytes, histiocytes and plasma cells. Demontration of emperipolesis i.e.engulfment of lymphocytes and erythrocytes by histiocytes, is usually diagnostic of Rosai-Dorfman Disease. Immunohistochemistry is usually necessary for confirmation of diagnosis. Characteristically S100 is always positive. Also some other markers like CD68, CD163, α1 antichymotrypsin and α1 antitrypsin may also be positive (2). Systemic symptoms in this disease may be related to enhanced production of such cytokines (5). In general the disease has a benign course and is self limiting. However massive lymphadenopathy and multisystemic involvement, especially vital organs like CNS, Liver, Kidney and lungs is usually associated with poorer prognosis. The treatment in Rosai-Dorfman Disease is non-specific and depends on the site of involvement. Isolated Lymphadenopathy may not be treated at all except for cosmetic reasons. However if any vital organs are involved or if the lesion is causing some obstructive symptoms or pressure symptoms, aggressive treatment may be indicated. In conclusion, Rosai-Dorfman Disease should be kept as a diffential diagnosis in young patients presenting with massive cervical lymphadenopathy. High degree of clinical suspicion with typical histopathological features and immunohistochemistry are diagnostic. These patients respond well to systemic steroids and aggressive surgical treatment is needed only in life threatening complications. However it is necessary that both clinicians and pathologist keep this entity in their list of differential diagnosis for massive lymphadenopathy (Shelkar R,. Ekhar VR, Rane S, et al. Rosai - Dorfman Disease – A Rare Cause of Cervical Lymphadenopathy. *MedPulse – International Medical Journal,* 2014; (2): 50-52).

Doina Butcovan

INFLAMMATORY MARKERS IN HYPERTENSIVE PATIENTS AND INFLUENCE OF SOME ASSOCIATED METABOLIC RISK FACTOR

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INFLAMMATORY MARKERS IN HYPERTENSIVE PATIENTS AND INFLUENCE OF SOME ASSOCIATED METABOLIC RISK FACTORS (Abstract): Some epidemiological studies have begun to show a possible correlation between systemic and vascular inflammation and the presence of essential hypertension, especially if it is associated with metabolic risk factors (obesity, dyslipidemia, diabetes mellitus). **Objectives:** The objective of this study was to evaluate the levels of C-reactive protein and fibrinogen as markers of inflammation in patients with essential hypertension, with or without associated metabolic risk factors. **Material and methods**: The retrospective study included 200 patients separated into five groups (control, hypertension, and respectively hypertension associated with obesity, or diabetes mellitus type II non-obese or obese type II diabetes). Anamnestic and anthropometric data, blood pressure and heart rate, blood glucose, lipid profile, fibrinogen, quantitative C-reactive protein and echocardiographic parameters have been reported and compared between groups. **Results:** Our study evaluated a total of 110 women and 90 men, each of the five groups comprising a total of 22 (55%) women and 18 (45%) males. Study of the inflammatory syndrome found the highest values of CRP in hypertensive and obese patients: 1.56 ± 3.08 ($p = 0.014$) in group 3 and 0.92 ± 1.11 ($p = 0.001$) in group 5 versus control group (0.30 ± 0.36). Fibrinogen values were significantly elevated in all groups of hypertensive, demonstrating the existence of an inflammatory syndrome, even in the absence of obesity or diabetes. **Conclusions:** All the patients showed a statistically significant relationship between elevated CRP and fibrinogen levels and the presence of hypertension, isolated, or in combination with obesity and diabetes mellitus. **Keywords**: C-REACTIVE PROTEIN, FIBRINOGEN, ESSENTIAL HYPERTENSION, METABOLIC RISK FACTORS.

Atherosclerosis is a pathological condition, caused by cardiovascular risk factors, which are associated to systemic and vascular inflammation. Observational studies have shown consistently that blood levels of systemic markers of inflammation is correlated with the risk of atherosclerosis. The association between C-reactive protein (CRP) and coronary heart disease has been the most intensively studied. Experimental studies indicated that atherosclerosis is not only a disorder of lipid accumulation, but

also a chronic inflammatory process (1, 2, 3). Inflammation plays an important role in atherothrombosis and measurement of inflammatory markers such as high sensitive CRP may be a new method for detecting individuals at high risk of plaque rupture. Several large prospective studies have shown that high-sensitive CRP is a strong independent predictor of future myocardial infarction and stroke in apparently healthy people, but with cardiovascular risk factors. Recent information about the CRP involvement in pathogenesis of atherosclerotic plaque, correlated with endothelial dysfunction, and having a direct role in molecular expression of cell adhesion, offer the possibility that CRP is also a potential therapeutic target. Thus, researchers have proposed that inflammatory markers such as CRP may be additional factors to assess global cardiovascular risk (1, 2, 3).

Hypertension is a major risk factor for developing atherosclerosis and its high prevalence (44% at the population level in Europe) will prone to many complications (4). In population studies, CRP was noted as a marker of inflammation that may enhance the risk of developing hypertension, not only the acceleration of atherosclerosis (1). In a prospective observational study, it was demonstrated the existence of higher values of CRP in individuals with high blood pressure. It is believed that the levels of CRP could increase blood pressure by reducing nitric oxide production in endothelial cells, resulting in vasoconstriction and increased production of endothelin-1. The CRP may also function as proatherosclerotic factor, by increasing the expression of the angiotensin receptor 1 (5). The inflammation has been shown to be correlated with the renin-angiotensin-aldosterone system (RAAS). Inflammation associated with oxidative stress and activation of the RAAS contributes to increased morbidity and mortality (6).

However, studies evaluating systemic inflammation and hypertension are still scarce and due to frequently associated risk factors (obesity, dyslipidemia, impaired glucose metabolism), the direct influence of elevated blood pressure was less evident in these studies. In this respect, our study aimed to assess the level of fibrinogen and CRP differentiating patients hypertensive only from those with associated risk factors.

MATERIAL AND METHODS

We conducted a retrospective study (between 01.07.2011 - 30.06.2013) which included 200 patients diagnosed with essential hypertension, hospitalized in the Cardiovascular Clinic, from Rehabilitation Clinical Hospital in Iaşi. At inclusion, patients were divided into five groups of 40 individuals, matched for age and gender, various by the association of metabolic risk factors as follows: group 1 (control group, after evaluation, hypertension and associated risk factors were excluded); group 2 (patients with hypertension, body mass index - BMI normal, without glucose metabolism disorders); group 3 (patients with hypertension and obesity, without glucose metabolism disorders); group 4 (patients with hypertension and non-obese type II diabetes); group 5 (patients with hypertension and obese type II diabetes). We excluded patients with recent infection or those with known inflammatory disease, diabetics with severe complications, patients with a history of cardiovascular disease (myocardial ischemia evidenced by electrocardiogram, heart attack or stroke), neoplasia, mental disorders, hepatic or renal failure. Also, in order to achieve ho-

mogeneity between groups, at the creation of the database, patients were evaluated with regard to the treatment scheme: for the treatment of hypertension and diabetes mellitus patients received drug treatment, but we excluded diabetic patients on insulin therapy and patients on the lipid-lowering treatment, regardless of class, knowing some antiinflammatory pleiotropic effects of hypolipemic drugs (7, 8).

Data reported and evaluated included: age, gender, anthropometric data (BMI, abdominal circumference - AC, obesity was defined by a BMI $\geq$ 30 kg/m^2), systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in supine using aneroid sphygmomanometers and heart rate. Biological evaluation included determination of inflammatory markers (CRP was determined quantitatively with latex immunoturbidimetric method by nephelometry, with normal range <1 mg/l and fibrinogen with koagulometers Clauss method, normal range 200-400 mg%) and metabolic parameters by the spectophotometric method (fasting plasma glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides). By transthoracic echocardiography were measured cardiac cavities, interventricular septum, posterior wall and left ventricle ejection fraction for assessing systolic function and left ventricular hypertrophy.

Statistical analysis of data was performed with SPSS version 17 and Statistics Microsoft Office Excel 2007. Chi square test and Student's t-test were used for means and frequency analysis, considering the level of significance for p value < 0,05.

RESULTS AND DISCUSSION

This study included 200 patients with a mean age of 60.37 ± 8.47 years and age limits between 40 and 79 years. The study evaluated a total of 110 women and 90 men, each of the five groups comprising a total of 22 (55%) women and 18 (45%) males. Study groups were homogeneous for age and gender. Among the variables studied, lipid profile was modified by the presence of other risk factors in hypertensive patients. Total cholesterol was 183 ± 17 values in the control group versus 207.6 ± 40, 5 (p = 0.001) in group 2, 197.8 ± 28.6 (p = 0.007) in group 3, 188.5 ± 40.4 (p = 0.437) in group 4 and 200.9 ± 33.94 (p = 0.004) in group 5. Except enrolled patients in group 4, the remaining hypertensive had significantly elevated cholesterol values. Evaluation of triglyceride levels showed the highest values in patients with hypertension and diabetes: 104 ± 36.8 in the control group, versus 121.8 ± 56.3 (p = 0.1) in group 2, 159.1 ± 96.7 (p = 0.001) in group 3, 168.5 ± 107.8 (p = 0.001) in group 4 and 155.7 ± 58.9 (p < 0,001) in group 5. Cholesterol levels were significantly increased in hypertensive patients, even in the absence of obesity or diabetes. These data are consistent with other studies in the literature showing the importance of dyslipidemia, in particular hypercholesterolemia, as a predictor of the installation of arterial hypertension and its complications (9). Some studies indicated also the presence of lipoprotein (a) in a greater proportion in hypertensive patients (10). Current medical guidelines highlights the significant increase in cardiovascular risk in hypertensive and dyslipidemic patients and recommend that LDL-cholesterol and even triglycerides should be maintained at levels below 100 mg/dl (11, 12). Atherogenic dyslipidemia determines maintenance of endothelial dysfunction (13), and the vascular prothrombotic status (14).

Study of the inflammatory syndrome found the highest values of CRP in hypertensive and obese patients: 1.56 ± 3.08 (p = 0.014) in group 3 and 0.92 ± 1.11 (p = 0.001) in group 5 versus control group (0.30 ± 0.36) (tab. I).

TABLE I
CRP in the study groups

CRP	Control group	Group 2	Group 3	Group 4	Group 5
Mean ± SD	0.30 ± 0,36	0.37 ± 0.42	1.56 ± 3.08	0.69 ± 1.16	0.92 ± 1.11
p	-	0.424	0.014	0.045	0.001

Fibrinogen values were significantly elevated in all groups of hypertensive, compared with the control group, demonstrating the existence of an inflammatory syndrome associated with hypertension, even in the absence of obesity or diabetes (tab. II).

TABLE II
Fibrinogen in the study groups

Fibrinogen	Control group	Group 2	Group 3	Group 4	Group 5
Mean ± SD	288.6 ± 53.09	319.5 ± 79.9	363.3 ± 144.4	326.0 ± 84.7	354.02 ± 85.2
p	-	0.046	0.004	0.021	0.001

Statistical analysis showed that, in the absence of obesity, diabetes should not influence as much CRP, compared with the presence of BMI change. Echocardiographic parameters of left ventricular hypertrophy were significantly higher in hypertensive patients with and without obesity.

In epidemiological studies, elevated CRP levels were consistently predictive of cardiovascular disease in different populations. Moreover, sensitive tests of CRP biological properties (such as half-life period of biological stability) make this protein to be a useful clinical marker of subclinical chronic inflammation (15, 16). Oxidative stress and inflammation have a central role in the pathogenesis of atherosclerosis. Reactive oxygen species, including superoxide and hydrogen peroxide, modify low-density lipoprotein to a vascular form of oxidized low-density lipoprotein. LDL stimulates endothelial cells to express leukocyte and vascular adhesion molecules, leading to the recruitment of monocytes and T lymphocytes, which invade the intima. Inflammation also triggers a cascade that leads to fibrosis and vascular remodeling (6). In our study, fibrinogen was a marker of inflammation in all types of hypertension, indicating that the inflammatory process accompanies early other cardiovascular risk factors, in order to achieve vascular complications. In a very recent study Hong L, et al. (17) demonstrated the relationship between fibrinogen levels and severity of coronary lesions, angiographycally proven, in patients with coronary heart disease and type II diabetes.

Recent observations on the immune response and hypertension have shown that, like many inflammatory stimuli, hypertension has a double effect: one is to promote T cell activation, and the second is to in-

crease the expression of chemokines and adhesion molecules in the target tissues to help access of the activated inflammatory cells to the tissue (18). Based on studies conducted by Harrison (18) or Pauletto (19), it was highlighted the hypothesis that hypertension causes accumulation of activated T cells in the perivascular "fat" and kidney. In these places, these cells release cytokines that affect adjacent vascular cells and renal tubular epithelium. According to this concept, many recent studies support the idea that the cytokines produced by T cells and other inflammatory cells contribute to hypertension installation. Some cross-sectional studies have shown elevated levels of CRP, TNF-α, amyloid-A in subjects with prehypertension and before the target organ lesions (20).

CONCLUSIONS

In the studied groups, we found a trend of increase in inflammatory markers in relation to the presence of hypertension. Both sexes showed a statistically significant relationship between elevated CRP and fibrinogen levels and the presence of hypertension, isolated, or in combination with obesity, which confirms that hypertensive patients present a proinflammatory and procoagulant status, which implies an increased risk of cardiovascular disease. Obese patients with hypertension and diabetes had significantly higher values of total cholesterol and triglycerides. There is a significant trend of dyslipidemia, with increased BMI and abdominal obesity, associated with high blood pressure and determining an increased risk of developing atherosclerosis.

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NEWS

CLINICAL RESULTS OF LINEZOLID IN ARTHROPLASTY AND TRAUMA MRSA RELATED INFECTIONS

Infections encountered in trauma surgery and implant related infection in arthroplasty, present a complex therapeutic challenge. Gram-positive organisms, particularly staphylococci and streptococci, are responsible for the majority of these infections encountered in orthopaedic practice in the United Kingdom and United States. Although the efficacy of linezolid has been well demonstrated in nosocomial pneumonia, bacteraemia, skin and soft tissue infections there is limited data supporting its use in complex orthopaedic infections. Using a retrospective analysis of twenty-two patients with orthopaedic related infections receiving a course of linezolid, the authors Joel J. and collaborators demonstrates that linezolid delivers excellent oral bioavailability, with good penetration into bone, joints and soft tissue. It exhibits action against gram-positive organisms, including methicillin resistant Staphylococcus aureus and vancomycin resistant enterococci, and it is ideally suited for the variety of infections encountered in orthopaedic practice and used in conjunction with surgical management, excellent results can be achieved in resolving infection. (Joel J, Graham SM, Peckham-Cooper A et al. Clinical results of linezolid in arthroplasty and trauma MRSA related infections. *World J Orthop*. 2014 Apr 18;5(2):151-7. PMID:248 29878)

Simona Tucaliuc

VITAMIN D STATUS IN PATIENTS ADMITTED IN A CLINIC OF ENDOCRINOLOGY

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VITAMIN D STATUS IN PATIENTS ADMITTED IN A CLINIC OF ENDOCRINOLOGY (Abstract)**:** Vitamin D deficiency has been known as a global health problem and there were reported moderate to strong inverse associations between 25(OH) D concentrations and cardiovascular diseases, serum lipid concentrations, inflammation, glucose metabolism disorders, weight gain, infectious diseases, multiple sclerosis, mood disorders, declining cognitive function, impaired physical functioning, and all-cause mortality. In Romania there are only a few published reports on vitamin D status among adult population**. Aim:** To evaluate vitamin D status in 440 patients those were admitted in our clinic for various endocrine pathology**. Material and method:** Serum 25-hydroxy vitamin D was measured using chemiluminescence assay. We categorized the vitamin D levels in 3 subgroups (deficiency, insufficiency and sufficiency)**. Results:** In our study there was a high prevalence of both vitamin D deficiency and insufficiency, while optimal level was observed only in a very small number of patients. **Conclusions:** We demonstrated a high frequency of vitamin D deficiency in general population, especially in elderly and children. There are still many controversies regarding the optimal vitamin D status and the supplementation dosage, so long term large scale studies are needed regarding efficacy and safety. **Keywords:** VITAMIN D, OSTEOPOROSIS, PHOSPHO-CALCIC METABOLISM.

Vitamin D was long known to be essential in bone metabolism, and its main function is the regulation of calcium and phosphate homeostasis, in conjunction with PTH, the gut, kidney and bone being the principal target tissues. Vitamin D deficiency has been recognized as a pandemic with various health consequences (1). Low vitamin D status has been associated with an increased risk of type 1 diabetes mellitus, cardiovascular disease, certain cancers, cognitive decline, depression, pregnancy complications, autoimmunity and allergy (2, 3)**.**

Vitamin D is a hormone precursor that is present in 2 forms: vitamin D2 (ergocalciferol) and vitamin D3 (chole-calciferol). Both are produced by photolysis from naturally occurring sterol precursors. Vitamin D can be manufactured in the skin by way

of ultraviolet B rays. There is very little vitamin D production from November to May throughout Europe. Also, we expose less than 5% of our skin to the sun and the vitamin D production in the skin decreases 4 times with age. Very few foods in nature contain vitamin D. The flesh of fatty fish (such as salmon, tuna, and mackerel) and fish liver oils are among the best sources. Small amounts of vitamin D are found in beef liver, cheese, and egg yolks. The major pathologic complication of vitamin D deficiency is rickets (in children) or osteomalacia (in adults), which results mainly from the deficiency of calcium and phosphate required for bone mineralization. A low vitamin D level is an established risk factor for osteoporosis and increasingly recognized as an important factor in fall status among elderly patients (tab. I).

TABLE I

Vitamin D levels and their impact on bone health

Definition (vitamin D)	Serum 25(OH)D level	Impact on bone health
Deficiency	< 25 nmol/L(< 10ng/mL)	Mineralization defect
Insufficiency	< 50 nmol/L(< 20 ng/mL)	Increased bone turnover and/or PTH
Sufficiency	50-75 nmol/L (20-30 ng/mL)	Neutral effect (bone turnover and PTH normalized), desirable benefits on fracture, falls and mortality
Sufficiency	> 75 nmol/L(> 30 ng/mL)	Desirable target in the fragile elderly due to optimal benefits on fracture, falls and mortality
Upper limit of adequacy	125 nmol/L	Possibility of adverse effects above this level

Vitamin D insufficiency is widespread (4), and is present in every region of the world, with a prevalence of 63.9% global and 57.7% in Europe (5). The rates of vitamin D deficiency are highest in the Middle East and South Asia (6). In elderly populations in Europe, vitamin D insufficiency is more common in the south than in the north, and more likely in women than in men (7).

In Romanian postmenopausal women, the mean concentrations of 25(OH)D were 20 ng/mL (50 nmol/L) and the prevalence of vitamin D deficiency was 32% (8), while in institutionalized seniors, we reported that mean concentrations of 25(OH)D were 28.5±10.8 nmol/L (9), far below the minimum 50 nmol/L that the Institutes of Medicine have set for everyone in the population. Our first study demonstrated 17,14% severe deficiency of 25(OH)D (10).

MATERIAL AND METHODS

Our study included 440 patients referred to us for different endocrine problems and was conducted in the Endocrinology Clinic of Saint Spiridon Hospital in Iasi from January 2013 till March 2014. The patients' ages ranged from 4 to 87 years. To determine vitamin D status we measured serum 25-hydroxyvitamin D3 (25-OH D3) by taking a single blood sample in the morning, in a fasting stage. Serum 25-OH D3 was measured by chemiluminescence assay with minimum detection limit of 3.0 ng/mL.

As seen in table I, we categorized vitamin D levels in 3 subgroups, based on the most recent studies – deficiency (< 10

ng/mL); - insufficiency (10-20 ng/mL) and sufficiency (> 20ng/mL)(11).

RESULTS AND DISCUSSION

The distribution by age was various, with the majority of patients (53%) in the 50-70 years group, followed by 13% (4-18 years and over 70), 9% (30-40 years), 7% (40-50 years) and 5% (18-30 years) (fig 1). The mean (SD) serum 25OHD was 17.21 ng/mL, with 242 patients in the insufficiency group (fig. 2). We found a high prevalence of both vitamin D deficiency (25OHD < 10 ng/mL) -16% and insufficiency (25OHD=10-20 ng/mL) -55 % (fig 2).

In our study, vitamin D deficiency (<10 ng/mL) was observed in:

- 15% postmenopausal women;
- 18% patients with osteoporosis;
- 7% children and adolescents.

Vitamin D insufficiency (10-20 ng/mL) was found in:

- 54% postmenopausal women;
- 63% osteoporotic patients;
- 54% children and adolescents.

39% of children and adolescents presented vitamin D levels of 20-30 ng/mL but none of them had vitamin D sufficient levels (>30ng/mL).

Vitamin D sufficiency (> 30 ng/mL) was observed only at 12% of all studied patients.

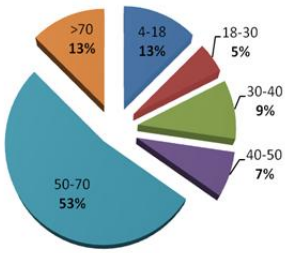

Fig. 1. Distribution of patients by age group

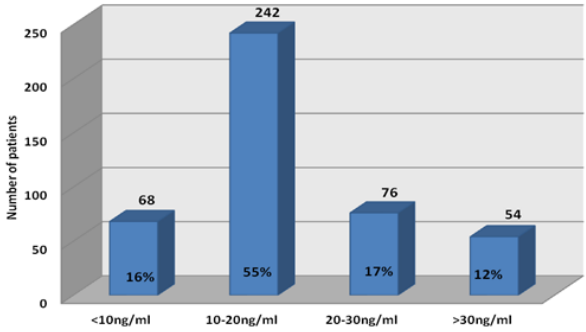

Fig. 2. Distribution of serum 25OHD3

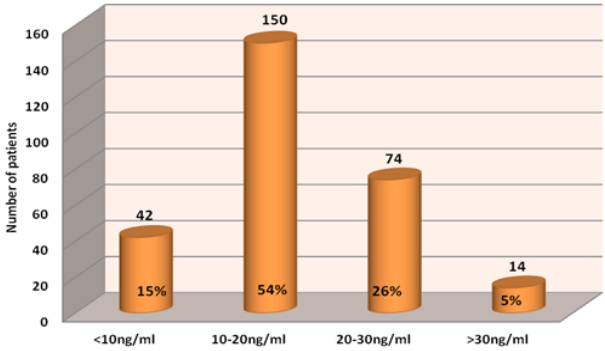

Fig. 3. Vitamin D deficiency in postmenopausal patients

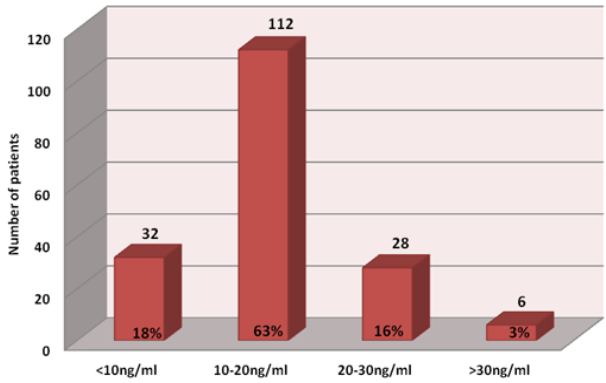

Fig. 4. Vitamin D deficiency in patients with osteoporosis

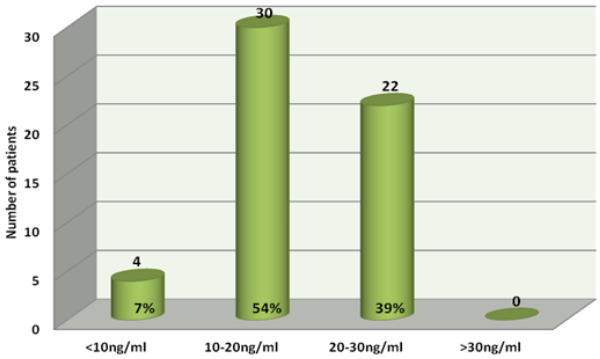

Fig. 5. Vitamin D in children and adolescents (4-18 years)

There are many controversies regarding the definition of different categories of low vitamin D and of the precise estimation of optimal vitamin D status (usually, in Europe a threshold of 20 ng/mL is agreed, although in the USA a 30 ng/mL is favored). In our country, dietary sources of vitamin D are scarce and there is no fortification of food with vitamin D. After giving bread fortified with 5000 IU (125 mcg) to nursing home residents, the medium 25(OH)D seric concentration were significantly higher after 3, 6 and 12 months (12). Another study measured the bone mineral density (BMD) before and after 12 months of serving bread fortified with 800 mg calcium carbonate and 5000 IU vitamin D to nursing home residents. After 1 year the hip BMD grew with 24 % of the initial value (13).

The greatest risk for bone and several major human diseases and preventable human health conditions are associated with 25OHD levels below 20 ng/mL (or 50 nmol/L).

The mean or median level of 25OHD around the world hardly exceeds 20 ng/mL (50 nmol/l) with slightly higher levels in US Caucasians (NHANES data) (14). An estimated number of at least 1 billion (and probably many more) people around the world have 25OHD levels < 20 ng/mL (50 nmol/l), so that their bone health could be improved by vitamin D supplements that

bring their 25OHD level above 20 ng/mL (50nmol/l). Several authoritative meta-analyses indicate that a daily dietary vitamin D supplementation (600-800 IU D3) plus calcium reduces the risk of fractures and falls. Such vitamin D dose is likely to increase mean 25OHD levels by 6-16 ng/mL (15-40 nmol/L) and bring serum 25OHD to ≥ 20 ng/mL (> 50 nmol/L) in most subjects. It is no unlikely that even higher vitamin D supplements, bringing 25OHD levels to over 30 ng/mL (75nmol/L) may convey further benefits without creating additional risk. This would however require vitamin D supplements ≥ 2,000 IU D3 per day for millions, if not billions of people, and until the completion of long term large scale RCTs the efficacy and safety of such interventions cannot be assured for the general population. Vitamin D supplementation (600-800 IU D3) plus calcium should be considered in elderly people (older than 75 years) with an increased fracture and/or fall risk, in particular people living in nursing homes. A Romanian study showed that after giving 200,000 IU Vitamin D 3 every 4 months for one year period, the 25(OH)D concentration grew 102%, but still didn't reach optimal range (> 75 nmol/L)(15).

CONCLUSIONS

Our study confirms the pre-existing data in literature and is showing once more that only a small number of patients are in the safe range regarding vitamin D, the majority of patients being in the deficiency or insufficiency area. 20 ng/mL should be the minimal serum 25-OHD concentration at the population level and in patients with osteoporosis to ensure optimal bone health, all individuals below this threshold requiring vitamin D supplementation. Since in our country we have only a few published reports on vitamin D status, we need long term large scale studies regarding the efficacy and safety of the vitamin D supplementation.

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NEWS

GASTROINTESTINAL STROMAL TUMOURS: FROM KIT TO SUCCINATE DEHYDROGENASE

Gastrointestinal stromal tumour (GIST) is the most common mesenchymal neoplasm of the gastrointestinal tract, with a worldwide annual incidence of 11–18 per million. The clinical behaviour of GIST is variable, ranging from entirely benign to highly aggressive. Several factors can aid in the stratification of the risk of malignant behaviour for GIST, including anatomical location, tumour size, mitotic activity, and genotype. Approximately 80% of GISTs harbor oncogenic mutations in KIT, and 8–10% harbor oncogenic mutations in PDGFRA, which encode tyrosine kinase receptors. The discovery of these mutations has served as an excellent model for the development of effective molecularly targeted therapies for solid tumours. Specific genotypes, in part, predict the response to treatment with the tyrosine kinase inhibitors imatinib and sunitinib. However, ~10% of GISTs lack KIT and PDGFRA mutations (often referred to as 'wild-type' GISTs); such tumours are generally resistant to imatinib therapy. Recent insights into the biology of 'wild-type' GISTs have resulted in clinically significant subclassification of this heterogeneous group of tumours, a large subset of which are now known to represent succinate dehydrogenase-deficient GISTs. Recognition of this distinctive class of tumours has critical implications for prognosis, therapy, clinical follow-up, and genetic counselling. This review provides an update on the diagnosis and pathogenesis of these less common classes of GISTs, summarizes the clinical and pathological features associated with particular genotypes, and discusses mechanisms of resistance to targeted therapies. (Leona A Doyle & Jason L Hornick. Gastrointestinal stromal tumours: from KIT to succinate dehydrogenase. *Histopathology* 2014; 64: 53–67)

Doina Butcovan

PREDICTIVE VALUE OF A POSITIVE EXERCISE STRESS TESTING AND CORRELATIONS WITH CARDIOVASCULAR RISK FACTORS

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PREDICTIVE VALUE OF A POSITIVE EXERCISE STRESS TESTING AND CORRELATIONS WITH CARDIOVASCULAR RISK FACTORS (Abstract): Electrocardiogram exercise stress test (EST) is a widespread technique in assessment of coronary artery disease, stratifying cardiovascular risk and prognosis. **Aim**: Evaluation of the impact of cardiovascular risk factors upon the results of EST. **Methods**: The 2-year retrospective study included 294 patients referred to an EST. All patients were assessed according to: presence of typical angina chest pain at admission, medical history of angina or myocardial infarction, resting electrocardiogram, global ejection fraction at echocardiographic exam and traditional cardiovascular risk factors. We compared the results between two groups: positive and negative EST. **Results**: The patients were age- and sex-matched. 160 patients (54.42%) had a positive EST. Positive EST was associated with typical angina chest pain at admission (88.12% vs. 76.11%, p=0.008), coronary artery disease history (61.87% vs. 41.04%, p=0.0003), resting ECG abnormalities (49.37% vs. 36.56%, p=0.026), arterial hypertension (85.62% vs. 74.62%, p=0.019). Ejection fraction was higher in the negative EST group (63.34±8.57% vs. 61.18±11.34%, p=0.035). Type 2 diabetes mellitus, smoking, obesity, dyslipidemia and inflammatory syndrome did not significantly influence the results of the EST. **Conclusions**: Typical angina, medical history of coronary artery disease, arterial hypertension, and reduced ejection fraction are strong predictors for a positive exercise stress test. **Keywords**: CARDIOVASCULAR RISK FACTORS, EXERCISE STRESS TESTING, PREDICTIVE VALUE.

Electrocardiogram exercise stress testing (EST) is an appropriate tool in diagnosis of coronary artery disease (CAD) (1). Even if the new European Society of Cardiology (ESC) guidelines recommend the use of stress imaging tests when available, EST still remains a useful option taking into account its widespread availability and easily interpretation (2). EST provides diagnostic and prognostic information like exercise capacity, chronotropic response to exercise, heart rate (HR) recovery, blood pressure (BP) response to exercise and for guiding cardiac rehabilitation (3). It offers quantitative and qualitative results, regarding chest pain (Kattus scale), perceived patient exertion (Borg scale), work-load (W and METs), maximum HR achieved and BP response, and electrocardiographic alterations. EST has an excellent safety profile, the risk being further reduced by a personalized careful medical history and clinical examination in order to define possible risks and contraindications (4). However, there are still de-

bates concerning categories of subjects that may benefit more from an EST and more studies emphasize on the limited clinical value of EST in asymptomatic adults (5).

CAD is related to the presence of risk factors. This led to developing total risk assessment charts, like the Framingham risk chart of total cardiovascular disease (CVD) and the SCORE risk chart of CVD death (6). Male sex and increasing age are unchangeable cardiovascular risk factors, very important determinants in clinical practice to stratify risk. Smoking affects 20% of the people worldwide and is responsible for one third of serious disease outcomes, mostly CVD, even in young adults and after an exposure of only a few years (7). Multiple studies showed that arterial hypertension causes endothelial injury which finally leads to cardiovascular events (8). Hypercholesterolemia is one of the most studied CV risk factors, predicting mortality, independently of age, both in subjects with and without preexistent CVD (9). Type 2 diabetes mellitus (DM) and metabolic syndrome are other conditions that favor the developing of coronary atherosclerosis (10). CAD is directly associated with obesity (body mass index > 30 kg/m²) but particularly with central obesity (waist circumference or waist-hip ratio) (11). Even if the cardio protective effects and health benefits of physical activity are irrefutable, increasing sedentary habits affect the quality of life and are correlated with a greater amount of chronic diseases, especially CVD (12). High resting heart rate has a worse prognostic in patients with or without CAD (13). Other cardiovascular risk factors, such as a positive family history, significant depression or reduced left ventricular ejection fraction influence the prognosis of CVD by accelerating the atherosclerotic processes, with worse outcomes in these patients (2).

However, very few studies appreciated comprehensively the influence of different traditional risk upon the EST. The aim of the study was to assess the impact of cardiovascular risk factors upon the positive EST results in patients referred for exercise testing.

MATERIAL AND METHODS

The current retrospective study included 294 patients, hospitalized over a two-year period (01.01.2011–31.12.2012) in the Cardiovascular Rehabilitation Clinic of the Rehabilitation Hospital at Iaşi, Romania, in whom an electrocardiogram EST was performed. We included patients with and without known CAD who were able to exercise and had no contraindications to performing the EST.

The patients were assessed according to: gender, age, rural or urban environment, obesity – body mass index (BMI) over 30 kg/m², smoking status, typical angina chest pain at admission, medical history of either angina or myocardial infarction, previous documented diagnostic or newly diagnosed arterial hypertension or type 2 DM. Dyslipidemia was defined, according to the ESC guidelines, as either total cholesterol over 190 mg/dl (4.9 mmol/L) or LDL-cholesterol over 115 mg/dl (3 mmol/L) or use of statin tratament for at least 6 months (6). According to the local laboratory, the inflammatory syndrome was present if the erythrocyte sedimentation rate was above 20 mm/1h or fibrinogen above 350 mg/dl or C-reactive protein (CRP) above 1 mg/dl. All patients performed a resting ecg and a transthoracic echocardiogram (Esaote MyLab50 ecocardiograph device) before the EST.

The EST was performed at the cycloergometer according to a standard protocol, starting with a power output of 25 W/min,

followed by an increment of 25 W every three minutes until endpoints were reached. We analyzed the maximal HR (according to the formula: 220 – patient' age) and BP, the Borg scale, work-load in Watts and METs, and the reasons for ending the EST. We considered a cut-off value for the submaximal exercise of 85% of age-predicted maximum HR. The EST was considered positive in the following situations: moderate to severe angina (≥ 3/4 on Kattus scale), severe exertional dyspnea, ST depression 80 ms after the J point more than 1 mm in three consecutive beats and in leads without diagnostic Q waves, pseudonormalization of T waves. The EST was terminated if systolic BP reached 240 mmHg or dropped more than 10 mmHg.

Statistical analysis was performed with SPSS 11.0 software. The mean values, standard deviations, frequencies were calculated. The significant differences were assessed by using the "t" Student test for two mean values and the "chi" square test for two frequencies, considering a "p" value < 0.05 statistically significant.

RESULTS

Mean age of patients was 55.75 ± 7.28 years (median 56 years); 51.7% were men. 294 ESTs were performed and 160 (54.42%) were positive, having at least one of the previous mentioned criteria for a positive EST.

In the positive EST (EST+) group, a submaximal HR was achieved in 59 patients (36.87%) while in the negative EST (EST-) group only 34 patients (25.37%) performed more than 85% of the maximal calculated HR (p=0.5). The two groups were matched for mean age, sex, and rural/urban origin (tab. I).

TABLE I

General data in the EST study groups

Parameter	EST+ Group	EST- Group
Age (years) (mean ± SD)	55.87 ± 6.50	55.61 ± 8.14
Male gender (no, %)	76 (47.5%)	76 (56.71%)
Urban origin (no, %)	76 (47.5%)	55 (41.04%)

As to the cardiovascular risk factors evaluated upon admission, we found statistically significant differences between the two groups. Typical angina chest pain was found in 88.12% of EST+ group compared with 76.11% in the EST- group (p=0.008). Personal medical history of angina or myocardial infarction was present in only 41.04% in the EST- group as to 61.87% in the EST+ group (p=0.0003). Arterial hypertension had a higher prevalence in the EST+ group (85.62% vs. 74.62%, p=0.01), as well as a lower ejection fraction in the EST+ group (61.18 ± 11.34 % vs. 63.34 ± 8.57 %, p=0.03). Resting ECG abnormalities (e.g. negative T waves, low ST segment depression, premature supraventricular or ventricular beats) were associated with a higher probability for a positive EST (49.37%) in the EST+ than in the EST- group (36.56%), p=0.02.

A positive EST was not significantly correlated with the presence of type 2 DM (p=0.12), with smoker status or with obesity (p=0.37). Patients with cardiovascular risk factors such as dyslipidemia or increased inflammatory markers were not prone for having a positive EST (tab. II).

TABLE II

Cardiovascular risk factors in the EST study groups

Parameter	EST+ Group	EST- Group	P
Typical angina chest pain at admission (%)	88.12	76.11	0.008
Medical history of angina or myocardial infarction (%)	61.87	41.04	0.0003
Rest ECG abnormalities (%)	49.37	36.56	0.026
Arterial hypertension (%)	85.62	74.62	0.019
Type 2 DM (%)	29.37	21.64	NS
BMI > 30 kg/m² (%)	50	44.77	NS
Current smokers (%)	47.5	47.01	NS
Dyslipidemia (%)	66.25	61.94	NS
Inflammatory syndrome (%)	16.87	17.16	NS
Ejection fraction (%)	61.18 ± 11.34	63.34 ± 8.57	0.035

The ECG stress test parameters were quite similar in the two groups. Maximum achieved HR was a bit lower in the EST- group (127.68 ± 19.51 / min) than in the EST+ group (129.85 ± 19.23 / min) but without statistical significance. Workload measured in METS showed no marked differences (5.65 ± 1.43 in EST+, 5.76 ± 1.60 in EST-) the same being true for the patient perceived exertion measured on Borg scale (14.27 ± 2.87 in EST+, 14.20 ± 2.67 in EST-).

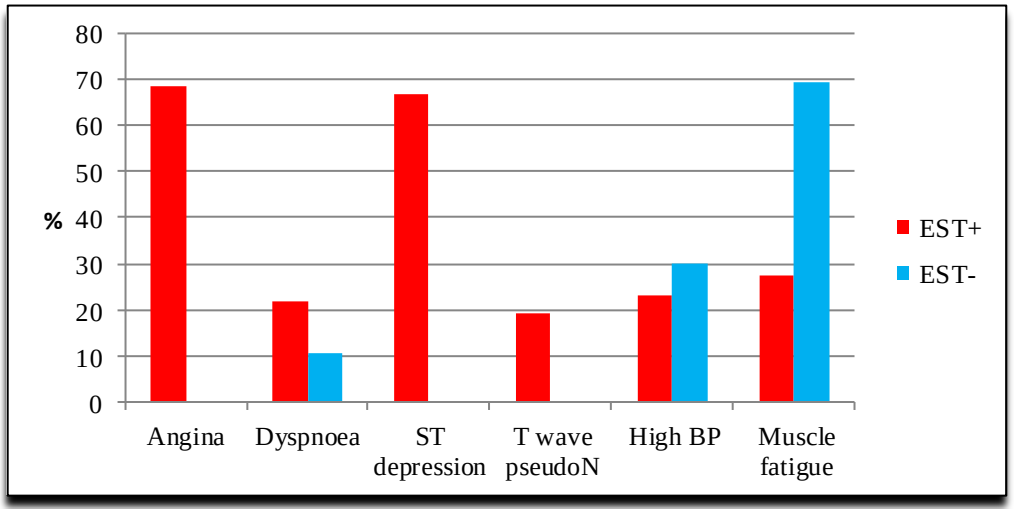

Fig. 1. Reasons for EST termination in the two groups

With regard to the reasons for terminating the EST, most patients in the EST- discontinued the test due to muscle fatigue (69.4%), severe arterial hypertensive re-

sponse (29.85%) or dyspnea (10.44%). In the EST+ group, most subjects presented moderate-severe angina (68.75%), ST segment depression more than 1 mm (66.87%), and less patients stopped because of muscle fatigue (27.5%) or high BP values (23.12%) (fig.1). Exercise-induced premature ventricular beats were present in about 30% of the patients in both groups.

DISCUSSION

According to the latest guidelines on the management of stable coronary artery disease, EST has a sensitivity of 45-50% and a specificity of 85-90% in detecting CAD, being especially indicated in patients with intermediate pre-test probability and left ventricular ejection fraction higher than 50% (2). However, if stress imaging testing is not available, ECG EST remains the first option in detecting CAD.

According to our study, 82.65% of the patients referred for an EST presented typical angina chest pain at admission, of which only 58% had a positive EST. This is in agreement with literature findings, EST being in about 50% non-conclusive with high false positive test results (14). The logical association between a positive EST and the presence of a medical history of angina or myocardial infarction is probably overrated in our study (64.28%), in comparison with 30-35% in other studies that made the same correlations (15). This is probably due to an overestimation of angina in patients presented with symptoms of chest pain.

Resting ECG abnormalities were seen in half of patients with positive EST, being a good marker for predicting a positive EST. In patients with known CAD, ST-segment abnormalities at rest are associated with a positive EST in more than 2/3 of cases (16).

Arterial hypertension is a strong predictor for CAD as well as for positive EST and is one of the major targets for treatment in both primary and secondary prevention (17).

As for type 2 DM and dyslipidemia, most patients having these risk factors associated a positive EST but without significant differences between groups. This may be due to the insufficient number of patients included in the study or to the high prevalence of these risk factors in patients with suspicious CAD (18).

Smoking and obesity also correlate with higher probabilities of CAD. In our study, they did not have a high predictive value for positive EST but it is well known that they are very important cardiovascular risk factors and could be integrated in risk scores in order to reach at least 80% negative predictive value for EST (19).

In our research, a high ejection fraction was associated with lower probability of positive EST. Our results are similar with other studies which state that patients with normal ejection fraction, without diabetes and good exercise capacity are unlikely to have a positive test result (20).

CONCLUSIONS

According to our research, typical angina chest pain, medical history of angina or myocardial infarction, resting ECG abnormalities, arterial hypertension and a lower ejection fraction are strong predictors for a positive EST.

Other important cardiovascular risk factors, such as type 2 DM, smoking, BMI over 30 kg/m², dyslipidemia or increased inflammatory markers, were not correlated with a positive EST and have a less predictive value.

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BACTERIAL MENINGITIS DURING SEPSIS IN DIABETIC PATIENT

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BACTERIAL MENINGITIS DURING SEPSIS IN DIABETIC PATIENT (Abstract): **The aim** of the study was to evaluate the etiology, clinical features and outcome in diabetic patients with bacterial meningitis, as a nervous system determination during invasive infections. **Material and methods:** In a retrospective study, conducted over a period of three years, we have analyzed clinical and etiological aspects of 445 patients over 18 years old, diagnosed with sepsis of known (positive cultures from normally sterile sites) or suspected etiology (positive cultures from pus), 95 of them being included in the diabetic group. **Results:** Bacterial meningitis was diagnosed in 16 of 95 diabetic patients (16.8%) and 43 of 350 (12.3%) non-diabetic patients (χ^2=0.98; GL=1; p=0.322). Among the multiple co morbidities associated in diabetic patients, as suggested by a higher Charlson score (5.44 *vs.* 3.25) (p=0.001), the most common underlying condition was chronic liver disease (31.3% *vs.* 25.6%) (p=0.916). The isolation of the microorganism concurrently from cerebro-spinal fluid and other sites (blood cultures and pus) was more frequently encountered in diabetics. The clinical picture was dominated by altered consciousness (68.8% *vs*. 23.3%) (p=0.003), while fever was less present (37.5% *vs*. 88.4%) (p=0.0003). The most frequently involved microorganism in the etiology of meningitis was *S. aureus* (31.3 *vs*. 23.3%) (p=0.771) and Gram negative bacilli: *E. coli* (12.5% *vs.* 4.7%) (p=0.629) and *Klebsiella* spp. (12.5% *vs.* 9.3%) (p=0.902). **Conclusions**: Altered consciousness was more frequent in diabetic patients group (68.8% *vs.* 23.3%) (p=0.003) where the absence of fever at admission was a more common finding than in non-diabetic septic patients with meningitis (37.5% *vs.* 88.4%) (p=0.0003).
Keywords: BACTERIAL MENINGITIS, SEPSIS, DIABETES

Bacterial infections of the central nervous system during invasive bacterial disease in diabetic population represents a challenge for the diagnosis, mainly because the clinical signs are often masked by acute and chronic complications of the diabetes, which may delay early recognition and adequate treatment. The epidemiology of bacterial meningitis varies in different populations. Several factors such as age, geographic distribution, underlying medical and surgical conditions, way of acquisition and the vaccination status may influence the prevalence of the involved pathogens (1). Association with diabetes mellitus as a co morbidity was found to be around 4-5% in patients with adult community-acquired bacterial meningitis in western reports, and as high as 25% in patients with bacterial meningitis from Taiwan (2). Therefore, the aim of the study was to evaluate the etiology, clinical features and outcome in diabet-

ic patients with bacterial meningitis, as a nervous system determination during invasive infections.

MATERIAL AND METHODS

In a retrospective study, conducted over a period of three years (2008-2010), we have analyzed the clinical and etiological aspects of 445 patients over 18 years old with sepsis admitted to the Clinical Hospital of Infectious Diseases Iaşi. Only cases of sepsis with known etiology were included in the study, documented by positive cultures from different normally sterile sites or suspected etiology (positive cultures from pus). According to American Diabetes Associations criteria, 95 of patients were included in the diabetic group. Patients with bacterial meningitis were identified based on the microbiological records of the cerebrospinal fluid (CSF): direct examination, positive cultures and latex agglutination assay.

The associated Chronic co morbid medical conditions were assessed by Charlson co morbidity score (3). Other variables such as onset-admission period longer than 48 hours, length of stay, discharged status, adequate treatment, mortality were collected. All the information represented data available from medical charts during hospitalization and long term outcome information was not available. For statistical analysis, all information was centralized in an *Excel* data base and differences between patient groups (diabetics and non-diabetics) were assessed using *SPSS* application, version 17.0. A p-value of < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

During the period of study of three years, among 445 patients diagnosed with sepsis of known or suspected etiology, bacterial meningitis was documented in 95 cases, 16 (27.1%) of them being patients with diabetes mellitus. Regarding the frequency of meningitis diagnosed during invasive disease, no statistically significant difference was noted between the studied groups: 16 of 95 diabetic patients (16.8%) and 43 of 350 (12.3%) non-diabetic patients (χ^2=*0.*98; GL=1; p=*0.*322) (fig.1).

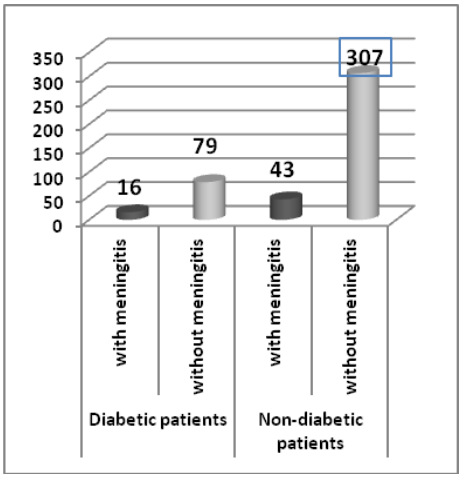

Fig. 1. The structure of the studied groups with bacterial meningitis

The majority of cases in both groups were males (68.8% respectively 74.4%). The median age of the patients with diabetes was significantly higher (63.88 *vs.* 52.07 years) (p=*0.*050).

Among the multiple co morbidities associated in diabetic patients, as suggested by a higher Charlson score (5.44 *vs.* 3.25) (p=0.001), chronic liver disease was the most commonly noted (31.3% *vs.* 25.6%) (p=0.916). Other underlying conditions and predisposing factors are shown in table I. Clinical evaluation in the first 24 hours of hospitalization, appreciated with SAPS II (Simplified Acute Physiology Score), revealed more severe forms of sepsis in diabetic patients (54 *vs.* 26.62) (p<0.001) (tab. I).

TABLE I

Characteristics of diabetic and non-diabetic patients with bacterial meningitis

Characteristic	Diabetic patients (n=95) (%)	Non-diabetic patients (n=350) (%)	p
Patients with bacterial meningitis (no/% of the whole group)	**16 (16.8%)**	**43 (12.3%)**	*0.*322
Median age (years)	**63.88±6.91**	**52.07±15.46**	***0.*050**
Gender			
Male	11 (68.8%)	32 (74.4%)	*0.*916
Female	5 (31.2%)	11 (25.6%)	
Predisposing conditions			
Chronic liver disease	5 (31.2%)	11 (25.6%)	0.916
Alcoholism	2 (12.5%)	6 (13.9%)	0.777
Chronic kidney disease	1 (6.3%)	1 (2.3%)	0.945
Tumors	1 (6.3%)	2 (4.7%)	0.676
AIDS	-	2 (4.7%)	0.945
Charlson Score	5.44±1.75	3.25±1.83	**0.001**
SAPS II	54.0±7.27	26.62±10.13	**0.001**
Clinical features			
Fever	6 (37.5%)	38 (88.4%)	**0.0003**
Altered consciousness	11 (68.8%)	10 (23.3%)	**0.003**
Seizures	2 (12.5%)	7 (16.3%)	0.961
Shock	3 (18.8%)	7 (16.3%)	0.869
MODS[a]	4 (25.0%)	5 (11.6%)	0.388
Brain abscess	-	1 (2.3%)	0.604
Epiduritis	1 (6.3%)	1 (2.3%)	0.945
Positive cultures from CSF and other pathological products	13 (81.3%)	14 (32.6%)	**0.0003**
Duration onset-admittance >48 ore	6 (37.5%)	10 (23.3%)	0.444
Hospital stay	15.75±10.22	12.23±8.75	0.888
Adequate initial therapy	11 (68.8%)	30 (69.8%)	0.808
Mortality rate	2 (12.5%)	11 (25.6%)	0.469
Transfer	4 (25%)	4 (9.3%)	0.255

[a]MODS – multiple organ dysfunction syndrome, [#]CSF – cerebrospinal fluid

A period of more than 48 hours between the onset of clinical signs and admission in hospital was noticed in diabetic patients (37.5% *vs.* 23.3%) (p=0.444). This is probably due to the fact that classical clinical signs of meningitis were less obvious, metabolic imbalance being also a cause of altered consciousness, which may be misleading, delaying thus the diagnosis of meningitis.

Before admission, the clinical picture was dominated by altered consciousness (68.8% *vs.* 23.3%) (p=0.003), while fever was less present (37.5% *vs.* 88.4%) (p=0.0003). Seizures were more often encountered in non-diabetic patients 16.3% *vs.* 12.5%, but without statistical significance (p=0.961).

Patients with diabetes were more likely to have other foci of infection than patients without diabetes, multiple organ dysfunction syndrome being documented in 25% of this immunocompromised group *vs.* 11.6% in the non-diabetic group (p=0.388). It is noteworthy that the isolation of the same microorganism concurrently from cerebrospinal fluid and other different pathologic collections (blood cultures and pus) was also more frequently encountered in diabetic group (81.3% *vs.* 32.6%) (p=0.0003). Complications of bacterial meningitis were epiduritis (one case in each group) and brain abscess (one case in the non-diabetic group).

TABLE II

The etiology of bacterial meningitis in diabetic and non-diabetic patients

Etiology	Diabetic patients (n=16)	Non-diabetic patients (n=43)	P
S. aureus	5 (31.3%)	10 (23.3%)	0.771
Coagulase-negative staphylococci	2 (12.5%)	3 (7.0%)	0.880
Streptococcus grup B	1 (6.3%)	-	0.604
Streptococcus pneumoniae	2 (12.5%)	12 (27.9%)	0.372
E. coli	2 (12.5%)	2 (4.7%)	0.629
Klebsiella spp.	2 (12.5%)	4 (9.3%)	0.902
Neisseria spp	1 (6.3%)	2 (4.7%)	0.676
Aerococcus viridans	1 (6.3%)	1 (2.3%)	0.945
Enterococcus spp	-	3 (7.0%)	0.676
Acinetobacter spp	-	3 (7.0%)	0.676
Pseudomonas aeruginosa	-	2 (4.7%)	0.945
Citrobacter freundii	-	1 (2.3%)	0.604

The mean hospital period of stay varied between 12 and 16 days in both groups (p>0.05).

Unfavorable outcome was not signifi-

cantly different in diabetic and non-diabetic patients.

Transfer to other medical or surgical clinics was necessary in 25% of diabetic cases (neurosurgery - 1 case of epiduritis, and in intensive care service - 3 cases, for acute respiratory distress syndrome).

S. aureus was the most common pathogen responsible for meningitis in diabetic patients with sepsis (31.3 *vs.* 23.3%) (p=0.771) followed by Gram negative bacilli: *E. coli* (12.5% *vs.* 4.7%) (p=0.629) and *Klebsiella* spp. (12.5% *vs.* 9.3%) (p=0.902). We did not find significant differences between the two groups of patients regarding the etiology (tab. II).

DISCUSSION

A report from Koreea (4) analyzing cases of community-acquired meningitis from 1998 to 2008 showed that 31.3% of patients were diabetic compared with 27.1% in our cohort of patients with meningitis during sepsis. It is worth noting that we included in our study only cases with known etiology (positive cultures of CSF or blood), therefore, true prevalence of bacterial meningitis may be underestimated. In a previous Romanian study, conducted on 844 cases of meningitis, diabetes was associated in only 1.5% of cases. These important differences may be due to the fact that in the study patients younger than 18 years were also included, children under 15 years representing 43.5% and only 7.7% being patients over 65 years (5).

Similar studies from Taiwan showed a high incidence of diabetes (34-36%), among the adult patients with community-acquired spontaneous bacterial meningitis (1, 6). In a prospective study from the Netherlands on adult community-acquired bacterial meningitis, diabetes was reported in 7% of cases (7).

A lot of reports support the concept that changes in mental status are the strongest clinical indicators of bacterial meningitis in elderly, the same findings being noted in our study in diabetic patients, whose median of age was higher than in non-diabetics (8. 9). In this category of patients, altered consciousness was noted in almost 70% of cases.

Beside diabetes, alcohol consumption and/or liver cirrhosis were frequent additional underlying conditions in our study, being present in more than 80%.

The etiology was related to the most prevalent pathogen causing nosocomial infections (*Staphylococcus aureus*/ coagulase negative staphyloccocci) in diabetics group, whereas in non-diabetic patients, *S. pneumonia* was more frequently isolated. In community-acquired meningitis in the same region, staphylococcus accounted for only 5% of cases with known etiology (5).

This figure is in contrast with the findings of the Taiwanese report that mentioned *Klebsiella* species as the most frequent etiological agent of meningitis in diabetic patients (2).

An investigation concerning community-acquired meningitis in the Hospital of Infectious Diseases Iaşi during 1998-2007 reported that the overall mortality rate was 13.1%, lower than that found in the Korean study (19%) (4, 5).

Kragsbjerg et al. in Sweden, revealed a mortality rate of 16% in patients over 14 years of age with pneumococcal meningitis, while, the mortality rate was 33% for those over 70 years of age (10). This value contrasts with the mortality rate of 30% reported for patients with pneumococcal meningitis in USA (11).

The rate of death in our study was not

statistically different among patients with and without diabetes (12% *vs.* 25%).

There are some controversies among the studies concerning the analysis of variables associated with prognosis. In van de Beek study, the strongest risk factors for an unfavorable outcome are those that are indicative of systemic compromise, a low level of consciousness and *S. pneumoniae* etiology (11). Factors associated with poor prognosis in a previous local analysis were: pneumococcal etiology, age over 60 and the presence of seizures or coma at admission (5).

Other risk factors associated with fatality rate were the absence of headache (finding probably associated with the delay in diagnosis), immunocompromised status, presence of coma and pneumonia – in the study of Papaiordanou (12).

A high index of suspicion for meningitis must be maintained during the healthcare of diabetic patients with changes in mental status and/or fever, because a delay in diagnosis and therapy will result in a devastating illness.

Accurate knowledge of epidemiology of sepsis and meningitis in diabetic patients and local pattern of antibiotic susceptibility may help us in filling the gaps related to the outcome of this particular category of patients.

CONCLUSIONS

This study shows a relatively high frequency of meningitis during sepsis in diabetic patients.

Bacterial meningitis was more frequently microbiologically documented (*S. aureus*, *Coagulase-negative staphylococci*, *Streptococcus pneumoniae*, *E. coli*, *Klebsiella spp*.) from the CSF in diabetic population.

The risk of late recognition was higher, mainly due to chronic complications of diabetes. Altered consciousness was more frequently in diabetic patients (68.8%) in whom the absence of fever at admission was a more common finding (88.4%) than in non-diabetic septic patients with meningitis.

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NEWS

METASTATIC CROHN DISEASE - A RARE CUTANEOUS ENTITY

Metastatic Crohn disease is a rare cutaneous disorder characterized by noncaseating, granulomatous skin lesions present at sites anatomically separate from the gastrointestinal tract.First described by Crohn et al, Crohn disease is a chronic granulomatous inflammatory bowel disorder that may involve any segment of the gastrointestinal tract. Along with the characteristic gastrointestinal findings of this disease, patients with Crohn disease may also present with extraintestinal manifestations including ocular findings, musculoskeletal pathology, and mucocutaneous manifestations. Mucocutaneous findings are the most frequent extraintestinal manifestation of Crohn disease; 22% to 44% of patients present with mucocutaneous changes, which may be categorized as granulomatous skin disease. Included within the granulomatous cutaneous category are perianal, peristomal, and perifistular inflammatory lesions, which are contiguous with the gastrointestinal tract and are the most common cutaneous manifestations of Crohn disease. Parks et al were the first to describe the presence of sterile, noncaseating, granulomatous lesions of the skin at sites noncontiguous with the gastrointestinal tract in patients with Crohn disease. This entity is known as metastatic Crohn disease (MCD), the name first coined in 1970 by Mountain. It is characterized by cutaneous, noncaseating granulomas at sites anatomically separate from the gastrointestinal tract. It is the least common dermatologic manifestation of Crohn disease, and the differential diagnosis includes numerous similarly appearing, granulomatous skin entities. Males and females appear to be equally affected, and children tend to present with clinical lesions different from those of adults. An integration of clinical information, microscopic findings, and ancillary studies is necessary to accurately diagnose this rare cutaneous disease. Our objective is to review the clinical features, histopathologic characteristics, suggested pathogenesis, differential diagnosis, and current therapeutic options of metastatic Crohn disease. (Alan Siroy, Jay Wasman. Metastatic Crohn Disease - A Rare Cutaneous Entity. Arch Pathol Lab Med. 2012;136:329–332)

Doina Butcovan

PLATELET INDICES IN PATIENTS WITH DE NOVO PORTAL VEIN THROMBOSIS AND LIVER CIRRHOSIS

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PLATELET INDICES IN PATIENTS WITH DE NOVO PORTAL VEIN THROMBOSIS AND LIVER CIRRHOSIS (Abstract) Platelet indices are markers of platelet reactivity used for thrombotic risk assessment in patients with cardiovascular diseases, and recently in venous thrombosis. **Aim:** To assess the diagnostic value of platelet indices in patients with non-malignant de novo portal vein thrombosis and liver cirrhosis. **Material and methods**: We conducted a prospective, case-control study on patients admitted to a tertiary center in the interval January, 2010 – December, 2012. Included in the study were 54 patients with portal vein thrombosis (PVT) and 54 controls. Patients with known malignancy, sepsis, thrombophilia, on anticoagulant or antiaggregant therapy, acute or chronic inflammatory diseases, severe anemia, renal failure, acute coronary syndrome, and chronic pulmonary disease were excluded from the study. **Results**: Both groups were comparable for baseline characteristics. Mean platelet volume, platelet distribution width (PDW) and plateletcrit were higher in the PVT group. In a multivariate logistic regression analysis, significant predictors of the presence of PVT were mean platelet volume (MPV), PDW, and procalcitonin (PCT). **Conclusion**: Our data suggest that increased platelet indices contribute to the prethrombotic state in liver cirrhosis and that larger platelets may play a specific role in thrombosis despite thrombocytopenia. **Keywords:** PORTAL VEIN THROMBOSIS, PLATELET INDICES, LIVER CIRRHOSIS, THROMBOCYTES.

Portal vein thrombosis (PVT) is an important cause of morbidity and mortality in patients with liver cirrhosis (LC) (1). The incidence ranges between 5% and 26%, and is higher in patients on transplant waiting list (2,3). Predisposing clinical conditions for PVT are advanced LC, low portal vein velocity, malignancy, and a wide variety of acquired and inherited hematological diseases (4). Despite thrombocytopenia, platelets are important in LC, playing a major role in hemostasis and other important functions such as inflammation, host defense and anfiogenesis and fibrosis (5,6). The routine platelet function tests including bleeding time and aggregometry have a low positive predicting value in differentiating between bleeding and thrombotic risk in LC, and new methods for assessing platelet function are needed (6).

Platelets are heterogeneous in size, density, and reactivity (6). Mean platelet volume (MPV) shows platelet size and may reflect platelet function and activity (7).

Since larger platelets are more reactive than the normal size ones, elevated MPV values were demonstrated to be a risk factor for arterial thrombosis and cardiovascular diseases (8,9). However, new data sustain that MPV and other parameters including platelet distribution width (PDW) and plateletcrit (PCT) may be used as indicators of platelet function and activation in patients with vein thrombosis (10). PDW directly measures the variability in platelet size and is a marker of platelet activation (11). A high PDW value could suggest a greater production of larger reticulated platelets. Of all platelet indices, PCT provides more comprehensive data about total platelet mass because it is equivalent to MPV and platelet count (PLT), where $PCT=PLTxMPV/10^7$ (11).

Recently it has been demonstrated that MPV is elevated in patients with deep vein thrombosis and significantly higher in patients with non-cirrhotic PVT. Therefore, in the present study we aimed at investigating whether platelet indices (MPV, PDW, and PCT) could have a diagnostic value for PVT in cirrhotic patients, despite thrombocytopenia.

MATERIAL AND METHODS

Study population

We conducted a prospective, case-control study on patients with liver cirrhosis admitted to a tertiary hospital in the interval January, 2010- December, 2012 in which a diagnosis of PVT was made during the study interval. Excluded from the study were the patients previously diagnosed with hereditary thrombophilia, hepatocellular carcinoma or other known malignancies, patients on anticoagulant or antiaggregant therapy, and patients with acute or chronic inflammatory diseases, severe anemia, renal failure, acute coronary syndrome, and chronic pulmonary disease. Between January, 2012- December 2012, 3124 cirrhotic patients were admitted to our department, of which a control group was randomly selected. After a detailed history and physical examination, all patients were subjected to hematological and biochemical blood tests and abdominal ultrasound. The study was performed in accordance with the Declaration of Helsinki and our local ethical committee approved the study. A written consent was obtained from all patients.

Portal vein thrombosis diagnosis

Portal vein thrombosis was diagnosed by abdominal ultrasound and Doppler ultrasound after 8 hours fasting. A Philips HD11XE ultrasound machine equipped with a 3.5 MHz convex transducer was used. PVT was confirmed by computed tomography with IV contrast. PVT was classified into 2 categories: complete and partial.

Laboratory data

Fasting peripheral blood sample was drawn from the antecubital vein using a 21-guage needle with a vacutainer and minimal stasis. The blood sample was collected into tubes containing 2.7 mL EDTA for platelet count, MPV, PDW, and PCT analyses. Platelet indices were measured using an automated system (Sysmex XS-1000i), by flow cytometry (11). The measurements were performed within 30 minutes after vein puncture to rule out the possible swelling of platelets. The reference ranges in our hematology laboratory are: MPV: 8.5 to 12.0 fL, PDW: 10.0 to 18.0 %, and PCT: 0.12 to 0.40 %.

Statistical analysis

Data were analyzed using SPSS Software Version 17.0 for Windows (SPSS Inc.,

Chicago, Illinois). Continuous variables were presented as mean ± standard error and categorical variables as frequency and percentage. Student's t test was used to compare normally distributed continuous variables, and the Mann-Whitney U test for variables without normal distribution. The χ^2 test or the Fisher exact test was used to compare categorical variables. Pearson analysis was used to analyze the correlations between platelet indices. Separate logistic regression analyses were used to identify univariate predictors of PVT, and a subsequent stepwise (forward conditional) regression analysis was performed (parameters with a *p* value of <0.1 in univariate analysis were included in the model). Receiver–operating characteristic (ROC) analyses were used to determine the cutoff values and the sensitivity/ specificity of platelet indices. The odds ratios (OR) and the 95% confidence intervals (CI) were calculated. A 2-tailed *p* value of <0.05 was considered statistically significant.

RESULTS

There were no significant differences between the two groups in age, sex, clinical risk factors (hypertension, diabetes, smoking), and laboratory parameters (cholesterol, fasting plasma glucose, white blood cell count, hemoglobin, platelet count, etiology of LC, MELD score and Child-Pugh class) (tab. I).

TABLE I

Baseline demographic, clinical and laboratory characteristics of the study population

Characteristics	PVT group (n=54)	Control Group (n=54)	p value
Age, y	61.5±11.0	62.3±12.3	0.471
Male,%	34(62.9%)	33(61.1%)	0.755
Diabetes,%	7(12.9%)	8(18.4%)	0.438
Smoking,%	22(40.7%)	18(33.3%)	0.122
Etiology (alcoholic/virus B/C/other)	23/4/25/2	23/6/22/3	0.246
MELD score	12.6±5.5	11.9±7.5	0.685
Score Child	7.6±2.3	7.8±2.1	0.724
Child-Pugh class (A/B/C)	20/10/24	18/14/22	0.111
Blood glucose (mg/dl)	128.9±60.1	137±83.7	0.366
WBC count, per ml	9860±3240	8844±3498	0.229
Hemoglobin, g/dl	12.2±2.07	11.4±2.5	0.584
AP	75.2±19.6	73.7±20.6	0.256
Platelet count,x10^9 per L	176±11.2	141±14.3	0.239

Abbreviations: PVT, portal vein thrombosis, WBC, with blood cell, AP prothrombin activity.

According to the time scale of PVT, 25 (46.2%) patients had acute and 29 (53.8%) chronic PVT. In all, PTV was complete in 15 (27.7%) cases and partial in 39 (72.3%).

MPV was significantly higher in the PVT group compared with the controls (811.2 + 1.0 fL *vs* 9.7+1.2 fL [95% CI

0.07-0.99], p=0.05). PDW was also significantly higher in the PVT group compared with the control group (14.3 + 3.13 % *vs* 12.2 + 2.38 % [95% CI 0.10-2.48], p=0.048, respectively). PCT was, however, significantly lower in PVT group compared with the controls (0.19+0.12% *vs* 0.14+0.06% [95% CI 0.08-0.09], p=0.015, respectively). In addition, there was a significant inverse relationship (fig. 1).

In a multivariate logistic regression analysis, significant predictors of the presence of PVT were MPV, PDW, and PCT (tab. II). For MPV, the multiple-adjusted OR of the presence of PVT was 2.3 (95% CI, 1.77-2.37); for PDW, the multiple-adjusted OR of the presence of PVT was 2.4 (95% CI, 1.435-3.85); and for PCT, the multiple-adjusted OR of the presence of PVT was 1.79 (95% CI, 0.95-2.86).

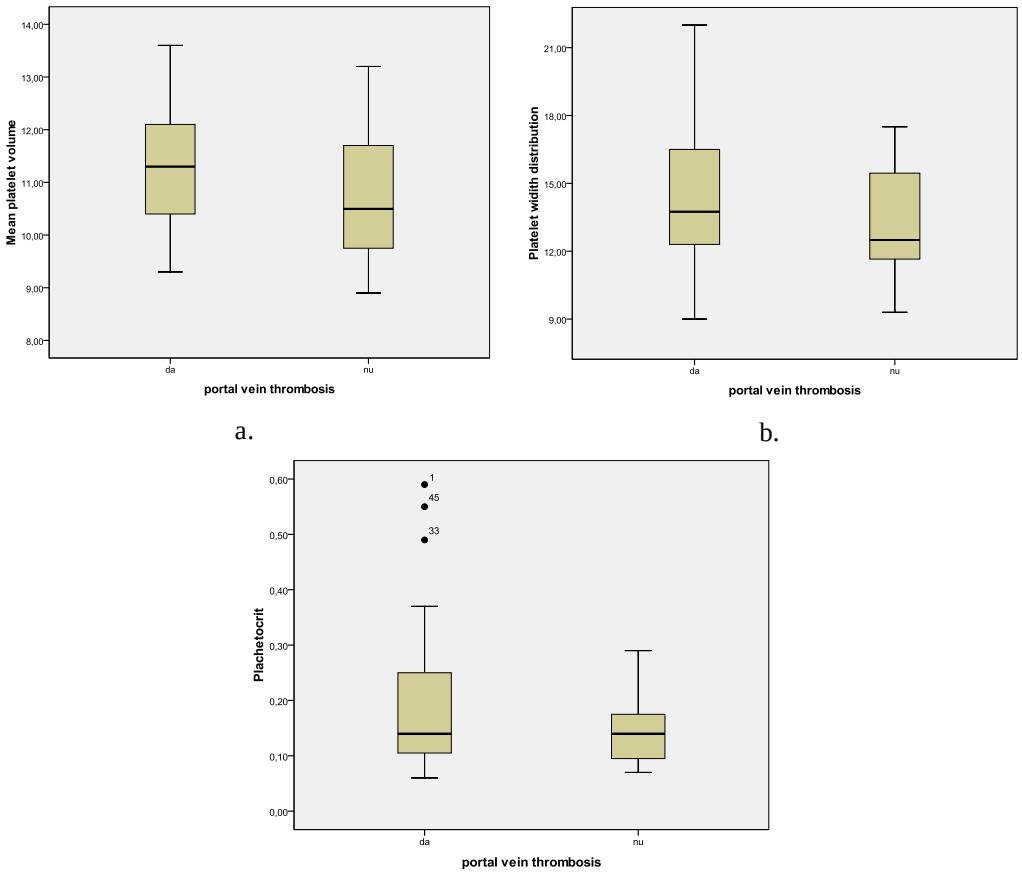

Fig. 1. Platelet indices in patients with liver cirrhosis with and without PVT: a-MPV, b- PDW, c- plachetocrit**.**

The cutoff values of 11.0 fL, 11.7%, and 0.11% for MPV, PDW, and PCT, respectively, were found to be moderately sensitive and specific for predicting PVT.

TABLE II
Multivariate regression analysis of the presence of PVT

Variable	β	±SE	p Value
MPV	-0.053	0.740	0.046
PDW	0.011	0.258	0.012
PCT	-7.584	5.972	0.026

Finally, to determine the best cutoff values of platelet indices for predicting PVT, ROC analyses were performed. The areas under the ROC curves for these indices used to detect PVT were calculated (tab. III) and the sensitivity and specificity of the best cutoff values were determined (tab. IV).

TABLE III
Receiver operating characteristic analysis for platelet indices to predict PVT

Variable	Area under the ROC curve	95% CI	p Value
MPV	0.610	0.46-0.75	0.022
PDW	0.579	0.43-0.71	0.035
PCT	0.576	0.44-0.71	0.038

The cutoff values of 11.0 fL, 11.7%, and 0.11% for MPV, PDW, and PCT, respectively, were found to be moderately sensitive and specific for predicting PVT.

TABLE IV
Sensitivity and specificity of the cutoff values of platelet indices

Variables	Cutoff value	Sensitivity (%)	Specificity (%)
MPV	11.0	53%	37%
PDW	11.2%	72%	63%
PCT	0.1%	69%	55%

DISCUSSION

In the present study, we have found that the platelet activity indices were significantly and independently associated with the presence of PVT. Platelet activity can be influenced by a multitude of variables (12), therefore it is difficult to predict the extent of platelet reactivity in an individual. A practical, reliable, and available index of platelet activation has not been discovered although considerable amount of research has been performed (11).

Various laboratory methods including platelet count and size, aggregates, and released substances from activated platelets have been introduced for the detection of platelet activity (11). However, they have different advantages and disadvantages. MPV is an inexpensive and easily available biomarker. It is routinely available in inpatient and outpatient setting at a relatively low-cost. Increased MPV values are related to various cardiovascular risk factors, disorders and inflammatory processes resulted in arterial and venous thromboses (9,10). Circulating platelets are heterogeneous in size, metabolism, functional activity, and density. Larger platelets are metabolically and enzymatically more active than smaller platelets, containing more prothrombotic material, with increased thromboxane A2 and B2 per unit volume and glycoprotein IIb-IIIa receptor expression (13). In healthy participants, there is a nonlinear inverse

correlation between MPV and platelet concentration: MPV tends to decrease in participants with higher platelet counts, as found in our study (14,15).

In previous studies, increased MPV was found to be associated with non-alcoholic fatty liver disease, hepatits C and B and advanced fibrosis (16). Recently, Braekkan et al. reported that MPV is a risk factor in venous thromboembolism (10). Our findings provide further evidence that platelet activation, measured by elevated MVP, PDW and PCT may contribute to the pathogenesis of venous thrombosis.

To our knowledge, this study has examined for the first time the relationship between all platelet indices, PVT and liver cirrhosis. Pizzuli et al. suggested that because platelets stay in the circulation 7-11 days, they might be detected days before symptoms appear (17). We believe that the same correlation might exist between platelet indices and PVT. At routine checkup a cirrhotic patient with elevated MPV, PCT and PDW might be a suspect for PVT, despite thrombocytopenia. However, to demonstrate the possible predictive value of platelet indices for PVT more prospective studies on larger patient groups should be performed.

Our data suggested that increased platelet indices contribute to the prethrombotic state in liver cirrhosis and that larger platelets may play a specific role in thrombosis despite thrombocytopenia.

Platelet indices could provide useful clinical information and be built into a risk assessment algorithm for PVT. This study supports the fact that platelet reactivity is important in the pathogenesis of PVT.

An important limitation concerning the predictive value of MPV, MPM, and MPC is the absence of well-defined limits to differentiate between activated and nonactivated platelets. Other platelet activity indices, such as radio-labeling methods, aggregometry procedures, platelet-specific eicosanoids, other release reactions such as serotonin and histamine, adhesion molecules, flow cytometric investigations, platelet function analyzers, thromboelastography, and plasma factors influencing platelet activation such as fibrinogen were not used in our study.

CONCLUSIONS

Patients with PVT and liver cirrhosis had abnormalities of platelet activation compared with the group without PVT, and these platelet abnormalities may partly contribute to the pathophysiology of PVT. Platelet activity indices including MPV, PDW, and PCT may be used in predicting PVT in patients with liver cirrhosis. However, these results have to be confirmed in larger, prospective, and more homogenous study groups.

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NEWS

LASER TREATMENT OF ONYCHOMYCOSIS

The efficacy of in vitro laser irradiation against fungi, for treatment of onychomycosis, was investigated by Paasch *et al*. For this puspose, 808, 980 and 1064-nm lasers were used to heat cell culture media and nail clippings and several *Trichophyton, Microsporum* and *Candida* species were subjected to laser irradiation, followed by incubation for 6 days. The results showed a thermal effect for 980-nm and 1064-nm laser systems on cell culture media and nail clippings. Growth inhibition was detected for *Candida guilliermondii* and *Trichophyton interdigitale* and complete growth impairment was achieved at temperatures above 50°C. For other pathogens, only reduced growth was observed. (Paasch U, Mock A, Grunewald S, Bodendorf MO, Kendler M, Seitz AT, et al. Antifungal efficacy of lasers against dermatophytes and yeasts in vitro. (*Int J Hyperthermia.* 2013 Sep;29(6):544-50. doi: 10.3109/02656736.2013.823672)

Teodora Vremera

BODY COMPOSITION ALTERATIONS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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BODY COMPOSITION ALTERATIONS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (Abstract): Metabolic disorders are involved in the pathogenesis of some comorbid conditions such as atherosclerosis and cardiovascular diseases, which frequently occur in COPD patient. These disorders may also associate alterations in body composition. **Aim:** To determine the particularities of body composition, assessing its relationship with respiratory function and clinical status in COPD patients. **Material and methods:** A prospective case-control study including 31 patients with COPD admitted to the Clinic of Pulmonary Diseases Iasi from April 2011 to September 2012, and 29 subjects without lung diseases which served as controls. Body composition (body fat, lean body mass, body water) and basal metabolic rate were measured using bioelectrical impedance analysis. Other assessments included anthropometric parameters, spirometry, 6-minute walk test. In COPD patients, dyspnea scale and health impairment assessment by St George's Respiratory Questionnaire (SGRQ) were used. **Results:** In COPD group, 67% of the patients were normal weight, 10% underweight, 10% overweight and 13% obese. The results showed that lean body mass ($p<0.001$), dry lean mass ($p<0.001$), total body water ($p<0.001$) and basal metabolic rate ($p<0.001$) were lower in the COPD patients as compared to the controls. These findings significantly correlate with age, dyspnea, severity of airway obstruction and exercise capacity in patients with COPD. No significant differences between study groups were observed in body fat mass. **Conclusions:** Lean body mass, dry lean mass, and total body water are altered in COPD patients and significantly correlate with age, clinical and functional severity of COPD. **Keywords:** COPD, BODY COMPOSITION, FAT MASS, LEAN MASS.

Chronic obstructive pulmonary disease (COPD) is associated with an increasing prevalence and a high mortality in the coming decades, being a challenge for the clinicians and a leading public health problem worldwide (1). One of its specific features associated with increased morbidity and mortality is the presence of chronic comorbidities. The complex pathogenic link between COPD and comorbidities seems to involve systemic inflammation which represents another important feature of COPD (2).

The metabolic syndrome is frequently diagnosed in overweight COPD patients, its association with increased systemic inflammation independent of lung function impair-

ment being thus proved (3). However, data on metabolic alteration in COPD are still poor and inconsistent. The "obesity paradox", defined as an inverse association of good health, survival and obesity in COPD patients was described. A higher mortality was found among underweight COPD patients, whereas overweight patients had a lower overall mortality (4). Other researches have found a low fat-free mass frequently associated in COPD patients, even when body mass index (BMI) was normal. Furthermore, low fat-free mass is considered an independent predictor of mortality in COPD (5).

Plasma adiponectin, an adipose tissue-derived specific protein with anti-inflammatory and anti-atherosclerotic effects, correlates with body weight loss and systemic inflammation in COPD, some researches concluding that adiponectin may reduce cardiovascular events in underweight patients (6).

However, cardiovascular diseases, many of these involving atherosclerosis and metabolic disorders, represent an important comorbidity accounting for 26% of the cause-specific mortality in COPD patients (7).

COPD patients have excessive visceral fat even in advanced stages of the disease despite the absence of obesity (8). Physical inactivity, a commonly observed feature in COPD, might be a determinant of visceral fat accumulation (9).

Given the pathogenic and prognostic implications of fat mass alteration, our study aimed to determine the particularities of body composition, including fat body mass, lean body mass, body water and basal metabolism, in view of assessing their relationship with respiratory function and clinical status in COPD patients.

MATERIAL AND METHODS

We conducted a prospective case-control study in which we determined and compared body composition in patients with COPD and subjects without lung disease. The study included patients admitted to the Clinic of Pulmonary Diseases Iasi in the interval April 2011 - September 2012, diagnosed with COPD according to the criteria of Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines: chronic dyspnea, cough, or sputum production, and persistent airflow limitation, confirmed by spirometry (a ratio of forced expiratory volume in 1 second (FEV1) to forced vital capacity (FVC) of <0.7 after bronchodilator administration) (1).

The control group included subjects with no history of lung diseases, clinical symptoms suggestive of COPD or respiratory dysfunction, based on the results of pulmonary function tests. COPD patients and controls were matched for sex and age. Male gender and age between 45 and 85 years were inclusion criteria for both COPD patients and controls. Cachexia was the exclusion criterion for the control group.

The study was approved by the Ethics Committee of the Clinic of Pulmonary Diseases Iasi and the patients were enrolled only after they provided appropriate informed consent.

The study protocol included the clinical and anthropometric assessment of the subjects: body height and weight were measured and BMI was calculated as weight/height^2 (kg/m^2). We used the World Health Organization classification of BMI which categorizes BMI into the following categories: underweight (BMI≤18.5 kg/m^2), normal weight (BMI: 18.5-24.99 kg/m^2), overweight (BMI: 25-29.9 kg/m^2), and obesity (BMI>30 kg/m^2) (10). Smoking history was expressed as the number of pack-years (py). For the COPD group the

degree of dyspnea was estimated using the Modified Medical Respiratory Council (MMRC) dyspnea scale (11).

Pulmonary function was assessed by spirometry, including postbronchodilator FEV1 measuring, to confirm the diagnosis of COPD in the corresponding group. FEV1 was expressed as percentage of predicted value. Exercise capacity was assessed in all subjects by the 6-minute walk test (6MWT). This was performed according to the standards of the American Thoracic Society (12). Health impairment in COPD patients was measured by St George's Respiratory Questionnaire (SGRQ). This provides three component scores: symptoms, activity, and impacts, in addition to SGRQ total score (13). Body composition was measured using bioelectrical impedance analysis with BodyStat 1500 analyzer (Bodystat Ltd, Douglas, Isle of Man). The device measures/estimates the following parameters: body fat percentage and fat weight (kilograms), lean mass percentage and lean mass (kilograms), body water percentage and total body water (liters), basal metabolic rate (kilocalories), and body impedance.

For analysis data were expressed as mean ± standard deviation (SD). Quantitative variables were compared using the unpaired Student's t-test. When the assumption of normality was not met, a non-parametric test was applied (Mann–Whitney U-test). Pearson correlation coefficient or Spearman rank correlation coefficient were calculated to assess the relationships between various clinical and functional parameters in COPD patients. All statistical tests were 2-tailed, and p value < 0.05 was considered significant for all analyses.

RESULTS

We enrolled a total of 60 male subjects, of which 31 were COPD patients and 29 controls. The most important clinical and anthropometric characteristics, respiratory function and body composition data are shown in table I.

TABLE I

Clinical characteristics, respiratory function and body composition

	Case	Control	p value
Age (years)	65.77 ± 8.86	61.41 ± 8.69	0.056
Smoking (py)	37.95 ± 18.83	19.34 ± 20.23	<0.001
Weight (kg)	66.80 ±16.27	81.51 ± 16.45	<0.001
Height (cm)	168 ± 6.11	171.34 ± 6.00	0.061
BMI (kg/m^2)	23.57 ± 5.09	27.74 ± 5.26	0.001
FEV1 (%)	37.59 ± 13.47	102.28 ± 15.02	<0.001
FEV1/FVC (%)	51.87 ± 10.90	80.95 ± 7.43	<0.001
6MWT distance (m)	276.09 ± 106.68	424.2 ± 77.9	<0.001
Fat (%)	17.89 ± 8.70	18.10 ± 7.24	0.842
Fat (kg)	12.83 ± 8.11	15.45 ± 9.24	0.185
Lean (%)	81.92 ± 8.56	81.89 ± 7.24	0.750
Lean (kg)	54.44 ± 11.21	66.22 ± 10.56	<0.001
Dry lean (kg)	8.35 ± 5.50	12.86 ± 4.33	0.001
Water (%)	70.60 ± 11.19	66.48 ± 8.2	0.111
Water (L)	46.09 ± 7.08	53.15 ± 6.86	<0.001
Basal metabolism (kcal)	1626.8 ± 290.9	1915.8 ± 262.9	<0.001
Impedance	410.8 ± 69.1	357.1 ± 57.2	0.002

A higher smoking index and lower weight were found in the COPD as compared to the control group. The 6-minute walking distance was significantly higher in the control group. BMI calculation in the patients in the COPD group showed: 67% were normal weight, 10% underweight, 10% overweight and 13% obese (fig. 1).

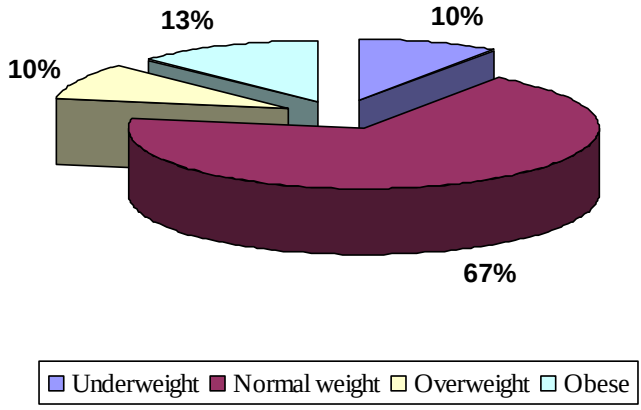

Fig. 1. Distribution of COPD patients according to BMI

The results of body composition assessment revealed certain particularities in COPD patients. The dry lean mass was significantly lower than in the healthy subjects. The absolute lean mass value, expressed in kilograms, was also lower in COPD patients, but no significant differences in percentage values between the two study groups were found.

Body water, in liters, was higher in the control compared with COPD group. However, body water percentage was higher in COPD patients than in controls, without reaching statistical significance. No difference in body fat mass, expressed either as percentage or absolute value, between COPD patients and healthy subjects was found. The different patterns of body composition determined a significant difference in body impedance between the study groups, which was significantly higher in COPD patients. Bioelectrical impedance analysis allowed the estimation of the basal metabolic rate. This was significantly higher in the control group than in COPD patients.

We also assessed the relationships between the results of body composition analysis and other clinical or functional parameters in COPD patients (tab. II).

In COPD patients, lean mass and dry lean mass, expressed in kilograms, were inversely correlated with age and dyspnea score (MMRC), and positively correlated with FEV1 and 6MWT distance (6MWTd). Body water percentage correlated with age and water volume (liters), inversely correlated with dyspnea score and positively correlated with FEV1 and 6MWTd. Basal metabolic rate, determined by bioelectrical impedance analysis, inversely correlated with age and MMRC and positively correlated with FEV1 and 6MWTd. No significant correlations between body composition components and health impairment in COPD patients, estimated by the three SGRQ component scores including symptoms, activity and impacts were found.

TABLE II

Correlations between body composition, clinical and functional parameters in COPD patients

		Age	MMRC	FEV1%	FEV1/FVC	6MWTd
Lean (kg)	Correlation Coefficient	-0.395	-0.473	0.47	0.475	0.505
	p value	0.002	<0.001	<0.001	<0.001	<0.001
Dry lean (kg)	Correlation Coefficient	-0.523	-0.398	0.361	0.402	0.474
	p value	<0.001	0.002	0.005	0.001	<0.001
Water (%)	Correlation Coefficient	0.329				
	p value	0.01				
Water (L)	Correlation Coefficient		-0.482	0.491	0.465	0.415
	p value		<0.001	<0.001	<0.001	0.001
Basal Metabolic Rate (kcal)	Correlation Coefficient	-0.37	-0.459	0.464	0.465	0.493
	p value	0.004	<0.001	<0.001	<0.001	<0.001
Impedance	Correlation Coefficient		0.392	-0.42	-0.34	-0.318
	p value		0.002	0.001		0.013

DISCUSSION

Even though the majority (67%) of COPD patients had a BMI within normal limits, the subjects in the control group presented a significantly higher BMI, which is in line with the results reported by Engelen et al. in patients with pulmonary emphysema (14).

Unlike Ischaki et al. we have proved a significant correlation of BMI, not only with 6MWTd but also with FEV1 which is the main determinant of COPD stage (15). A major difference is that, unlike previous studies, we found no differences in body fat mass between COPD patients and healthy controls and no correlation between body fat mass and clinical or functional parameters (8).

Ischaki et al. have demonstrated correlations between free-fat mass index and MRC dyspnea scale, 6MWT distance and percentage predicted FEV1 (15). Our results support these findings, proving an inverse correlation between lean mass/dry lean mass, expressed in kilograms, and dyspnea score (MMRC) and a positive correlation with FEV1 and 6MWTd. We also found a significantly lower lean mass in the COPD than in the control group.

Body water was also significantly lower in COPD patients than in healthy subjects. As lean body mass, body water inversely correlated with dyspnea score and positively correlated with FEV1 and 6MWTd. Previous researches demonstrated that COPD patients associating fat-free mass depletion are characterized by increased extracellular/intracellular water ratio (16).

Body impedance analysis also provided an estimation of the basal metabolic rate.

This was lower in COPD group, inversely correlated with age and dyspnea severity and positively correlated with FEV1 and exercise capacity. However, other researchers have used more accurate methods for assessing metabolic rate in COPD. Using indirect calorimetry adjusted for the influence of fat-free mass, Creutzberg et al. have proved that hypermetabolism commonly occurs in COPD but cannot be linked to aging and fat-free mass depletion (17).

CONCLUSIONS

Although more than a half of our COPD patients are normal weight, they may present important disturbances in body composition. Lean body mass, dry lean mass and body water are significantly lower in COPD patients than in healthy subjects. These significantly correlate with age, dyspnea, severity of airway obstruction, and exercise capacity in patients with chronic obstructive lung disease.

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NEWS

CONE-BEAM COMPUTED TOMOGRAPHY ANALYSIS OF THE VESTIBULAR SURGICAL PATHWAY TO THE PALATINE ROOT OF THE MAXILLARY FIRST MOLAR

Surgical endodontic treatment has been considered difficult to perform for the maxillary molars because the operative site is frequently close to important anatomical structures, such as the major palatine vascular-nervous bundle and the maxillary sinus. Therefore clear visualization of the operative area as well exact localization of the anatomic structures surrounding the area before surgery is crucial to prevent surgical complications. Cone-beam computed tomography (CBCT) has been specifically designed to produce undistorted 3D information of the maxillofacial skeleton, including the teeth and their surrounding tissues, with a significantly lower effective radiation dose as compared with conventional CT. The aim of this study was to evaluate the anatomical distance between the apices of the palatine root of the maxillary first molars to the buccal bone plate in order to identify the vestibular surgical pathway using cone-beam computed tomography (CBCT) images. CBCT scans of 107 (48 male and 59 female) patients were retrospectively analyzed. A Pearson's chi square test and Mann-Whitney U-test were performed to evaluate differences among age, localization, and measurements. Differences were considered statistically significant at $P<.05$. The mean distance between the buccal cortex and the buccal side of the palatine root of the superior first molar in both male and female patients was 10.13 and 9.70 mm, respectively. The incidence of interposition of the lateral recess of the maxillary sinus between the roots was 42.2% and 49% in men and women, respectively. Statistically significant sex-related differences were found in the vestibular surgical pathway ($P<.05$). These results suggested that CBCT could be a powerful tool for the evaluation of surgical cases and identification of the most appropriate surgical pathway. However the authors point out that CBCT images should not necessarily replace conventional radiographic images when there is no need for a surgical procedure to eliminate infected tissues by resecting the root apices (Kalender A, Aksoy U, Basmaci F, Orhan K, Orhan AI**:** Cone-beam computed tomography analysis of the vestibular surgical pathway to the palatine root of the maxillary first molar. *Eur J Dent* 2013; 7(1): 35-40).

Gianina Iovan

SIGNIFICANCE OF ARTERIAL STIFFNESS AND RELATIONSHIP WITH OTHER NONINVASIVE METHODS FOR THE ASSESSMENT OF SUBCLINICAL ATHEROSCLEROSIS INPATIENTS WITH METABOLIC SYNDROME

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SIGNIFICANCE OF ARTERIAL STIFFNESS AND RELATIONSHIP WITH OTHER NONINVASIVE METHODS FOR THE ASSESSMENT OF SUBCLINICAL ATHEROSCLEROSIS IN PATIENTS WITH METABOLIC SYNDROME (Abstract): Measurement of arterial stiffness is an accurate method of assessment of endothelial dysfunction, together with other noninvasive methods, in the diagnosis of atherosclerotic burden in patients with MetS. **Material and methods**: The study included 63 patients: MetS group (18 men, 20 women, mean age 58.86±8.86 years) and the control group (14 men, 11 women, mean age 59.68±10.0 years). They underwent the following examinations: assessment of arterial stiffness - pulse wave velocity (PWVao), augmentation index of brachial artery (Aixbr) and aorta (Aixao), central systolic blood pressure (SBPao); carotid ultrasound for detection of plaques and measurement of intima-media thickness (IMT); echocardiography - left ventricular hypertrophy (LVH); ankle-brachial index (ABI); biochemical parameters: C-reactive protein (CRP), fibrinogen (Fb), cholesterol (Col), HDLcol, LDLcol and triglycerides. **Results**: MetS patients had higher PWVao (10.06±2.12 m/s vs 8.29±1.33 m/s, p=0.0001) and SBPao (135.06±19.80 mmHg vs. 121.76±18.62 mmHg, p=0.009). Carotid IMT was higher in MetS group (0.92±0.11 vs. 0.83±0.10 mm, p=0.003). Almost all MetS patients were hypertensive (94.7% vs. 52%, p=0.01); LVH was present in 57.9% of MetS patients and 20% of the controls (p=0.05). The MetS group presented higher Col (208.76±38.41 vs. 176.20±30.08 mg/dl, p=0.0003) and CRP levels (0.872±0.852 mg/dl vs. 0.476±0.392 mg/dl, p=0.01). **Conclusions**: In MetS patients the most reliable marker of arterial stiffness was PWVao, followed by SBPao. Higher values of carotid IMT are also parameters of high atherosclerotic risk. CRP and Col can be considered biomarkers of high risk in MetS. **Keywords**: METABOLIC SYNDROME, ARTERIAL STIFFNESS, SUBCLINICAL ATHEROSCLEROSIS.

Metabolic syndrome (MetS) consists in a constellation of cardiovascular risk factors with a direct impact on the initiation and progression of atherosclerotic cardiovascular disease. Several large studies showed that subjects with MetS are prone to adverse cardiovascular events. Such a patient is vulnerable, prone to acute coro-

nary syndrome, sudden death, or stroke, due to vulnerable atherosclerotic plaque (prone to disruption or thrombosis), vulnerable blood (prone to thrombosis) or vulnerable myocardium (prone to arrhythmia) (1, 2, 3).

The present cardiovascular risk charts, as Framingham in the U.S.A. or SCORE in Europe, can identify subjects at very high risk or low risk of cardiovascular events in the next ten years (4, 5). Yet, there are a large number of subjects with an intermediate risk score, often underestimated by the risk charts. In this group of individuals, noninvasive detection of asymptomatic atherosclerosis is indicated, before the occurrence of major adverse events (6, 7). The investigations suggest that carotid ultrasound (CU), ankle-brachial index (ABI), cardiac CT for calcium scoring, or magnetic resonance imaging is useful in detecting subclinical atherosclerosis. While ABI, intima-media thickness (IMT) and carotid plaque ultrasound assessment are widely used, the other two are less available. The measurement of arterial stiffness provides additional useful information in the evaluation of atherosclerotic risk together with the above mentioned noninvasive methods (8).

The aims of the study are: - assessment of arterial stiffness in patients with MetS by measuring aortic pulse wave velocity (PWV) as the gold standard for assessing arterial stiffness; - importance of arterial stiffness analysis as a parameter of cardiovascular risk in MetS; - significance of other noninvasive methods for the detection of subclinical atherosclerosis and some biochemical markers in MetS.

MATERIAL AND METHODS

The study included 38 patients with MetS (18 men, 20 women), group S, and 25 control patients (14 men, 11 women) – group C. Patients in group S met the MetS criteria according to ATP III: fasting glucose $\geq$ 110 mg/dl or known type 2 diabetes mellitus (type 2 DM); waist circumference (WC) $\geq$ 102 cm in men and $\geq$ 88 cm in women; serum triglycerides $\geq$ 150 mg/dl; HDL $<$ 40 mg/dl in men and $<$ 50 mg/dl in women; arterial pressure $\geq$ 130/85 mmHg or treatment for arterial hypertension (AHT). Patients were included in group C when at least three MetS criteria were not present.

The exclusion criteria were: secondary AHT, significant chronic renal disease, major cardiovascular complications (myocardial infarction, stroke, heart failure, cardiac or peripheral revascularization).

The following investigations were made: medical history and clinical examination; electrocardiography; ABI. Echocardiography studied markers of left ventricular hypertrophy (LVH), systolic and diastolic function, aortic and valvular atherosclerosis. CU measured IMT of the distal common carotid artery and bifurcation and revealed atherosclerotic plaques. The echographic examinations were performed with an Esaote MyLab50. The biochemical tests included determinations of fasting blood glucose, cholesterol (Col), HDL cholesterol, triglycerides (TG), calculation of LDL cholesterol with the formula Col – HDL – TG/5, C reactive protein (CRP), fibrinogen (Fb), plasma urea and creatinine, hepatic enzymes - alaninaminotransferase (ALAT), aspartataminotransferase (ASAT).

Arterial stiffness was assessed by a TensioMedArteriograph TL2. The following parameters were measured: brachial augmentation index (AIXbr), aortic augmentation index (AIXao), aortic pulse wave velocity (PWVao), central pulse pres-

sure (PPao), and systolic blood pressure (SBPao).

Statistical analysis was performed in *SPSS 11.0* software. The mean values, frequencies, standard deviations were calculated. *"t"* Student test was used to assess the significance of difference between two mean values; chi square test was used to analyze the difference between two frequencies.

RESULTS

Mean age of group S was 58.86 ± 8.86 years, and of group C 59.68 ± 10.0 years, age-matched controls.

Anthropometric data. In all group S patients WC was above the maximum admitted values, with a mean of 109.05 ± 9.53 cm in men and 110.10 ± 15.48 cm in women. In group C, mean WC was significantly lower (p=0.006). In group C most subjects were normal weight (36%) or overweight (32%), whereas in group S most were overweight (28.95%) or obese (68.42%) (tab. I).

TABLE I

General and anthropometric data in the two study groups

Parameter	Group S	Group C	P
Age (years) (mean ± SD)	58.86 ± 8.86	59.68 ± 10.0	NS
Male gender (nr, %)	18 (47.4%)	14 (56%)	NS
Weight (kg)	90.89 ± 17.03	73.76 ± 20.51	0.001
BMI (W/H^2)	33.36 ± 6.61	26.36 ± 6.33	NS
Waist circumference in men (cm)	109.05 ± 9.53	92 ± 18.88	0.006
Waist circumference in women (cm)	110.10 ± 15.48	94.54 ± 10.15	0.002

Analysis of *arterial stiffness parameters* showed that PWVao was 10.06 m/s in group S, and significantly lower (p=0.0001), 8.29 m/s, in group C. According to the reference values, in 64% of the controls it was optimal, in the remaining 36% being normal or elevated. In group S, in 50% it was normal, in 34.2% elevated and in 13.1% pathologic, above 12 m/s (with a maximum of 17.1 m/s) (fig. 1).

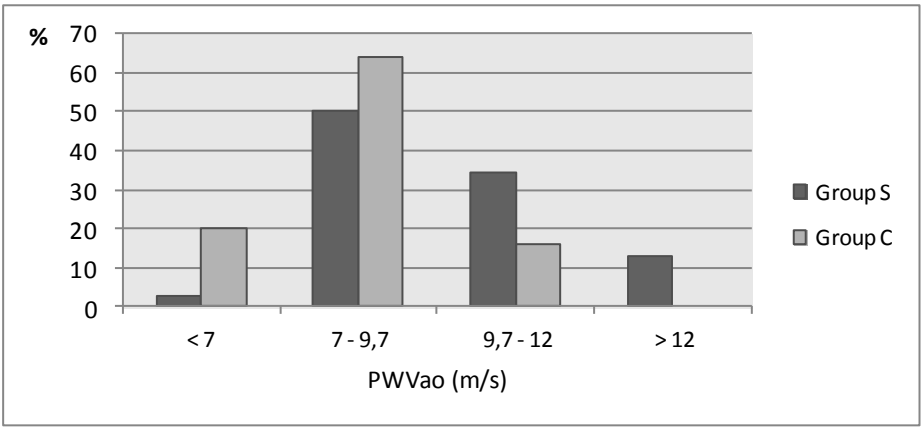

Fig. 1. The prevalence of PWVao according to value categories

SBPao in group S was 135.06 mmHg, higher than in group C (121.76 mmHg, p=0.009). There were no significant differences in AIXbr and AIXao between the two groups (tab. II).

Carotid intima-media thickness (IMT) in group S was 0.92 ± 0.11 mm, significantly higher than in the C group (0.83 ± 0.10 mm, p=0.003) (tab. II). *Carotid plaques* were present in both groups, more frequent in group S (86.8%) compared with C group (72%), but without statistical significance.

Mean values of *ABI* were not significantly different, but the analysis showed that in group S 9 patients (23.68%) had values outside the normal range (0.9 – 1.3) compared with only two subjects in group C (8%) (tab. II).

AHT was present in almost all group S (94.7%) and less in group C patients (52%, p=0.01). LVH was defined in the presence of a left ventricular mass index (LVMI) above 122 g/m^2 in women and 149 g/m^2 in men. LVH was found in 57.9% of the patients with MetS*versus* 20% in the controls (p=0.05) (fig. 2).

Echocardiography revealed *aortic atherosclerotic alterations* in almost all patients with MetS (97.4%), and in most controls (80%). Diastolic dysfunction, assessed by E/A ratio < 0.9, was present in 68.4% group S and 52% group C patients (fig. 2).

Type 2 DM, another component of MetS, was found in 42% of group S patients. No subject in group C had type 2

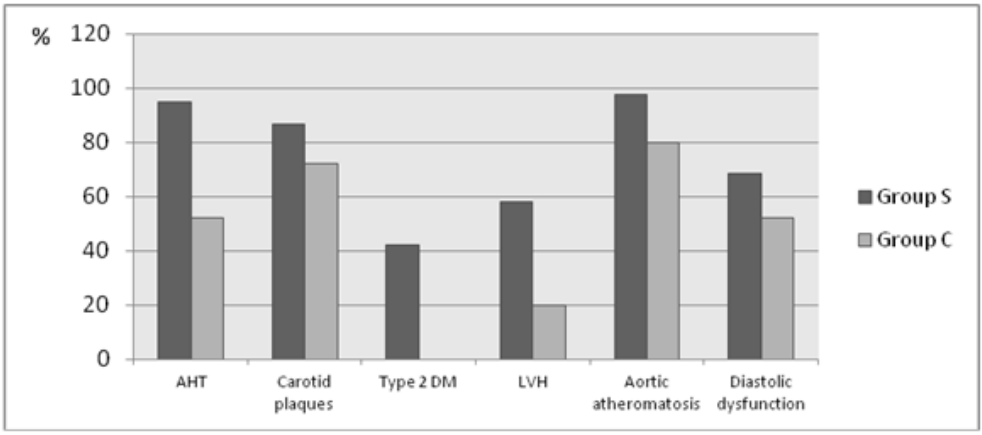

Fig. 2. Prevalence of different parameters in the two study groups

Analysis of *serum lipids* showed the following. In group S, 63% presented Col levels above 190 mg/dl, compared with 32% in group C, with mean values higher in group S. HDL was slightly higher in group S. Triglycerides were also higher in group S but without statistical significance (tab. II).

The studied *inflammation markers* were C reactive protein (CRP) and serum fibrinogen (Fb). CRP levels higher than 1 mg/l were found in 44% in group S and in only 16% in group C; no differences in Fb levels were found between the two groups (tab. II). When comparing the hepatic enzymes levels, higher ALAT values were found in group S, with ASAT values were similar in the two groups (tab. II).

TABLE II
Imaging and biochemical data in the two study groups

Parameter	Group S	Group C	p
PWVao (m/s)	10.06 ± 2.12	8.29 ± 1.33	0.0001
SBPao (mmHg)	135.06 ± 19.80	121.76 ± 18.62	0.009
AIXbr (%)	10.99 ± 24.28	10.54 ± 37.44	NS
AIXao (%)	42.03 ± 12.76	42.92 ± 18.96	NS
Carotid IMT (mm)	0.92 ± 0.11	0.83 ± 0.10	0.0063
Cholesterol (mg/dl)	208.76 ± 38.41	176.20 ± 30.08	0.0003
HDL col (mg/dl)	54.56 ± 13.52	50.08 ± 7.98	NS
LDL col (mg/dl)	114.08 ± 34.03	103.08 ± 27.88	NS
Triglycerides (mg/dl)	199.16 ± 100.01	115.96 ± 40.42	NS
CRP (mg/l)	0.87 ± 0.85	0.47 ± 0.59	NS
Fibrinogen (mg/dl)	367.78 ± 85.71	336.08 ± 46.94	NS
ABI	1.11 ± 0.18	1.08 ± 0.11	NS
ALAT (UI)	39.41 ± 19.36	28.44 ± 9.89	0.004
ASAT (UI)	29.14 ± 19.81	25.72 ± 12.34	NS

DISCUSSION

The analysis of the clinical and biochemical features related to the criteria used to define MetS showed, as expected, higher WC and BMI in the MetS group. WC is a better predictor of cardiometabolic risk than BMI. Type 2 DM was present in about half of MetS group and this could be explained by insulin resistance. The risk of developing DM is three times higher in MetS compared to general population. AHT was present in almost all MetS patients and it doubled the cardiovascular risk (9).

The assessment of asymptomatic atherosclerosis by the noninvasive methods described in the study showed some peculiarities. LVH is more frequent in MetS and is an independent cardiovascular risk factor (10). IMT is another marker of atherosclerosis, together with carotid plaques, and their presence has to be interpreted as atherosclerotic disease, even if asymptomatic and adequate measures must be taken (11).

Mean serum cholesterol is higher in MetS patients and increases cardio metabolic risk. In this study there were not significant differences in HDL and TG between the two groups; this can be partially explained by the relative small groups, but also by the fact that most patients were under hypolipemiant treatment. The finding of higher CRP levels is in agreement with other studies that showed a correlation between the inflammation markers and MetS (12).

Focusing on the results of arterial stiffness in MetS patients, it could be asserted that PWVao is the parameter that correlates best with the presence of MetS, followed by SBPao. The differences between groups could be explained in part by the higher prevalence of AHT in the MetS group. Arterial stiffness is known to be a marker of early endothelial dysfunction, which is influenced by several factors, not only by elevated arterial pressure levels (13). AIXbr and AIXao are mentioned in studies as indicators of arterial stiffness and cardi-

ovascular risk; in the present study they did not prove to have diagnostic significance.

CONCLUSIONS

Increased arterial stiffness is an early finding in patients with MetS compared with controls. The most reliable parameter is PWVao, followed by SBPao; AIXbr and AIXao have a less predictive value.

Other markers of early atherosclerosis in MetS are carotid IMT and LVH. Of the biochemical investigations, CRP and cholesterol have better predictive value while LDL and TG have less predictive value.

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THE RISK OF THROMBOTIC EVENTS IN PATIENTS WITH LIVER CIRRHOSIS

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THE RISK OF THROMBOTIC EVENTS IN PATIENTS WITH LIVER CIRRHOSIS (Abstract). **Aim**: To evaluate the incidence and risk factors for thrombotic events (deep vein thrombosis and portal vein thrombosis) in patients with liver cirrhosis. **Material and methods**: we studied patients diagnosed with liver cirrhosis admitted in our department between January 2010-December 2011, which were divided in two groups: liver cirrhosis with thrombotic events and without thrombotic events. **Results**: we included 3108 patients, the incidence of deep vein thrombosis was 0.99% and portal vein thrombosis was 1.51%, the incidence of all thrombotic events was 2.5%. In the univariate analysis serum albumin was significantly lower in cases than controls, and MELD score, mean platelet volume were higher in cases than controls. The presence of sepsis and diabetes mellitus were demonstrated like risk factors by the univariate analysis. In multivariate analysis, albumin level< 3mg/dl (HR=1.65, CI 1.10-2.51, p=0.018) and MELD score >13 (HR=2.94, CI 1.61-5.47, p=0.001) remained independently predictive of thrombotic events. **Conclusions**: The incidence of thrombotic events in patients with liver cirrhosis was 2.5%. Low serum albumin and high MELD score could predict the development of thrombotic events in patients with liver cirrhosis. **Keywords**: DEEP VEIN THROMBOSIS, PORTAL VEIN THROMBOSIS, LIVER CIRRHOSIS, RISK.

In the last years, the axiom that says that the patient with liver cirrhosis is predisposed to bleeding has proved to be wrong (1). The influence of liver disease on hemostasis is complex, the changes being determined by lower serum levels of both procoagulant and anticoagulant factors, especially in the advanced stages of liver cirrhosis (2).

In cirrhotic patients also, the pathogenesis of venous thrombosis is based on Virchow's triad: venous stasis, hypercoagulability, and venous injury. In portal vein thrombosis (PVT) venous stasis is caused by the decreased portal flow as a consequences of portal hypertension (3), and in cirrhotic patients with deep vein thrombosis (DVT) limited physical activity along with advanced liver cirrhosis are leading to venous stasis and thrombosis. Procoagulant status in cirrhotic patients is characterized by low anticoagulant factors (protein C,

protein S, antithrombin III), and high values of factor VIII and von Willenbrand factor (4). Endothelial dysfunction associated with cirrhosis is based on increased nitric oxide and proinflammatory cytokines and contributes to vascular wall damage and increases the risk of thrombosis (4).

The accumulation of thrombotic risk factors results in an incidence of DVT in cirrhotic patients ranging from 0.9 to 1.8%, increasing to 8.1% in hospitalized patients (5-7), while the incidence of PVT is 1% in patients with compensated liver cirrhosis, reaching 25% in those on the liver transplant list (3).

Although the cirrhotic patient is no longer considered "anticoagulated" (8), his thrombotic risk being similar to that in general population (9), the American College of Chest Physicians (ACCP) does not specify prophylactic measures and a curative treatment of thrombosis in this population (9), due to the lack of concrete data on the risk factors associated with thrombosis and the impossibility to assess the risk of bleeding associated with anticoagulant therapy.

The aim of this study was to determine the incidence and risk factors associated with venous thrombosis in hospitalized patients diagnosed with liver cirrhosis.

MATERIAL AND METHODS

In the study we enrolled patients admitted to the Center of Gastroenterology and Hepatology Iasi between January 2010 and December 2011, who were diagnosed with liver cirrhosis.

Exclusion criteria were previous diagnosis of hereditary thrombophilia, hepatocellular carcinoma or other known malignancies and patients on anti-coagulation or antiplatelet therapy.

The diagnosis of liver cirrhosis was made on clinical manifestations, biological, endoscopic and ultrasound changes suggestive for advanced liver disease and portal hypertension, and confirmed, in some cases, by elastography (Fibroscan), a non-invasive method for the assessment of liver fibrosis.

Thrombotic events, defined as deep vein thrombosis and portal vein thrombosis, were diagnosed clinically and confirmed by venous Doppler ultrasound for DVT and abdominal ultrasonography and contrast computer tomography for PVT.

Liver cirrhosis severity was evaluated using the MELD score (Model of End Life Disease) and Child-Pugh score which is based upon the presence of ascites, hepatic encephalopathy, serum bilirubin, INR and serum albumin levels.

To determine the thrombotic risk factors, the group of patients diagnosed with venous thrombosis was compared with a control group of cirrhotic patients without thrombotic events, randomly selected from all cirrhotic patients admitted during the above mentioned interval.

Statistical analysis was performed using SPSS 17.0 (SPSS, Inc., Chicago, IL). For comparing the two groups Chi-square test for categorical variables and *t* Student test for numeric values were used. The thrombotic risk factors were determined using logistic regression for univariate analysis, and Cox regression analysis method for multivariate analysis.

RESULTS

Out of the 3108 patients included in the study, 31 patients were diagnosed with DVT and 47 patients with PVT, which corresponds to a 2.5% incidence of thrombotic events in patients diagnosed with

liver cirrhosis, 0.99% for DVT and 1.51% for PVT.

DVT was located in the calf in 28 patients, 3 patients had ileo-femoral DVT; the right leg was the most frequently affected, 58% of cases (18 patients).

In 56% of cases PVT was complete and 5 cases were diagnosed with portal cavernoma. Excluding the 5 cases diagnosed with portal cavernoma, imaging evaluation by Doppler ultrasound and CT could not establish the age of portal vein thrombosis.

None of the patients diagnosed with DVT or PVT had a history of recent surgery (less than one month) or prolonged immobilization.

Out of the 3,030 patients with liver cirrhosis without thrombotic events we have randomly selected a control group of 160 patients in order to establish the risk factors involved in the occurrence of DVT and PVT.

The two groups were comparable in terms of demographic characteristics (age, sex), but the group with thrombotic events was characterized by a higher MELD score ($p < 0.0001$), a higher prevalence of Child-Pugh classes B and C, presence of ascites ($p = 0.083$), hypoalbuminemia ($p = 0.005$), sepsis ($p < 0.0001$), mean platelet volume (MPV) increased ($p < 0.0001$), and diabetes mellitus ($p = 0.008$) (tab. I).

TABLE I

Clinical and biological characteristics of the study groups

	with DVP/TP n=78	**without TVP/TP n=160**	**p**
Age (years)	59.6±11,2	55.2±11.7	0.164
Male gender	49 (62.82%)	101 (63.12%)	0.780
Etiology - toxic	28 (35.9%)	60 (37.5%)	0,083
- viral	33 (42.3%)	66 (41.2%)	0.120
- toxic+viral	15 (19.2%)	29 (18.1%)	0.258
- other	2 (2.6)	5 (3.2%)	0.138
Ascites	45 (57.69%)	78 (48.7%)	0.075
Hepatic encephalopathy	33 (42.3%)	66 (41.2%)	0.577
Sepsis	17 (21.7%)	11 (6.8%)	**<0.0001**
Diabetes mellitus	7 (8.9%)	8 (5%)	**0.008**
Albumin (mg/dl)	2.5±0.5	3.2±0.7	**0.005**
Platelets (/mm3)	101,110±51,937	109,100±46,833	0.122
MPV (fl)	10.7±1.27	8.9±0.14	**<0.0001**
INR	1.6±0.2	1.5±0.4	0.385
MELD score	13.17±5.39	10.33±4.24	**<0.0001**
Child-Pugh score	7.7±1.74	7.9±0.70	0.680

Univariate analysis identified as risk factors for the development of thrombotic events: MELD score>13, albumin <3mg/dl, VPM> 10.5 fl, presence of sepsis and diabetes mellitus (tab. II); the remaining clinical and biological parameters did not reach statistical significance.

Of the above mentioned factors, multi-

variate analysis has confirmed as independent risk factors of thrombosis in cirrhotic patients: albumin <3 mg/dl, and the presence of sepsis, as a reflection of their advanced liver disease (tab. III).

None of the patients with DVT was diagnosed with pulmonary embolism based on clinical symptoms, but asymptomatic pulmonary embolism has not been evaluated.

TABLE II

Univariate analysis of thrombotic risk factors

	OR	95% CI	p
MELD>13	3.955	1.131-15.753	0.009
Albumin<3mg/dl	3.900	0.981-12.182	0.012
MPV>10,5fl	2.214	0.269-13.850	0.044
Sepsis	2.129	0.755-10.468	0.032
DM	3.875	1.278-11.336	0.021

TABLE III

Multivariate analysis of risk factors for venous thrombosis in cirrhotic patients (Cox regression model)

	Coefficient (β)	Wald	HR	95% CI	p
MELD>13	1.087	12.089	2.94	1.61-5.47	**0.001**
Albumin<3mg/dl	0.504	5.565	1.65	1.10-2.51	**0.008**
MPV>10,5fl	0.301	3.634	1.35	1.00-1.84	0.057
Sepsis-yes	0.262	2.525	1.31	0.94-1.80	0.116
DM	0.189	1.498	1.20	0.89-1.64	0.221

DISCUSSION

So far there are several studies that have attempted to assess the thrombotic risk in cirrhotic patients, and some of the results are contradictory.

A population-based study conducted in Denmark on 99,000 patients showed that cirrhotic patients have a 1.74 times higher risk of developing DVT than the general population (10), and the risk was 2.10 times higher in patients aged <55 years. These data, however, were not confirmed by the results of a smaller case-controlled study, carried out by Heit et al., which demonstrated that the association of liver cirrhosis in general population reduced the risk of thrombotic events (RR 0.10) (11). The two studies had some limits because they were not aimed primarily to assess thrombotic events in cirrhotic patients, and the data were extracted from a study subanalysis, so that their relevance is low.

DVT incidence in cirrhotic patients was assessed in eight studies, including two case-control and six retrospective studies, the reported incidence of DVT in cirrhotic patients ranging between 0.5 and 8.1% (5, 7, 12, 13). In our study the incidence of DVT was 0.9%, similar to data reported in the literature.

Non-malignant portal vein thrombosis in cirrhotic patients has an incidence of 5-

26% (3). The incidence of 1.51% found in our study was lower than that reported in the literature, but it must be mentioned that previous studies have been conducted in patients on liver transplantation list, diagnosed with advanced liver cirrhosis .

Although surgical interventions and prolonged immobilization are major risk factors for the development DVT in the general population (9), in our study no patient diagnosed with DVT or PVT had a history of recent surgical intervention or prolonged immobilization, supporting the hypothesis that the risk factors involved in the occurrence of thrombosis in cirrhotic patients are different from the general population.

Our study showed that sepsis is a risk factor for the development of thrombotic events in patients with cirrhosis, confirming the results of the study conducted by Garcia-Fuster et al, in which 6 of the 17 patients with DVT were diagnosed with sepsis (6). Sepsis acts by increasing the direct effect of proinflammatory cytokines, vascular endothelial damage, and also by stimulating platelet aggregation (14). It has been shown that coagulation disorder, defined by elongation of prothrombin time and INR, does not predict the risk of bleeding in cirrhotic patients and does not reflect the true coagulation status in this population (1); moreover they do not protect patients from the occurrence of thrombotic events (5,15). In our study, INR values, prothrombin time, as well as platelet count were similar in the two groups. The difference between the two groups occurred when we used mean platelet volume as a method for assessing platelet trombogenity. MPV was higher in patients with thrombotic events, and univariate analysis recognized MPV as a thrombotic risk factor. Mean platelet volume is used to assess the risk for thrombosis in patients with ischemic heart disease (16), without being used, until now, in cirrhotic patients. The role of MPV in these patients remains to be established.

Of the biological parameters used to evaluate cirrhotic patients only albumin level <3mg/dl was an independent risk factor for the development of thrombotic events, and reflected poor protein synthesis, including anticoagulant factors (protein C, protein S, antithrombin III).

This is the first national study on the incidence of thrombotic events in patients with cirrhosis and the first in which mean platelet volume as a risk factor for thrombosis in patients with liver cirrhosis was used.

The development of a thrombotic risk scale and a method for the assessment of bleeding risk are essential in DVT and PVT prophylaxis and curative treatment in patients with liver cirrhosis.

CONCLUSIONS

The incidence of thrombotic events in patients with cirrhosis was 2.5%, confirming that this population is not "anticoagulated" by definition.

Cirrhotic patients with serum albumin level <3mg/dl and MELD score> 13 have an increased risk for developing thrombotic events, correlated with the severity of liver disease. Thrombosis prophylaxis should be considered in the management of cirrhotic patients and the current guidelines for thromboprophylaxis should also include this special patient category.

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ANTIBIOTIC THERAPY IN SEVERE SEPSIS IN HIV-POSITIVE PATIENTS

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ANTIBIOTIC THERAPY IN SEVERE SEPSIS IN HIV-POSITIVE PATIENTS (Abstract): Severe sepsis has become one of the most frequent causes of hospitalization in intensive care units for patients diagnosed with HIV infection. The difficulty in setting a sepsis diagnosis in HIV-positive patients led to the systematic exclusion of these patients from studies on sepsis, which limited the understanding of its impact on the evolution of the disease. Our study aims to evaluate the etiology of sepsis in immunocompromised HIV-positive patients and the evolution after antibiotic therapy. 30 patients diagnosed with HIV infection and sepsis, admitted to our clinic between January 2008 and April 2012, were followed. Severity of illness, time since diagnosis, CD4 count, antiretroviral treatment, incidence of severe sepsis, and organ dysfunctions were registered. Patients were between 1 and 61 years of age, most of them were classified into stages B2, B3 and C3, requiring hospitalization for a period ranging from 14 to 28 days, with an average of 16.7 days and a median of 18 days, while 8 required monitoring in the intensive care unit. In about 40% of cases, the starting point was an infection of the lower respiratory tract, but also of the upper urinary tract and skin infections. Evolution and mortality in sepsis associated with HIV/AIDS infection depend on the presence of organ failure and are less influenced by the level of immunodepression, complex antibiotic therapy being the cornerstone in controlling patients. **Key words**: HIV INFECTION, SEPSIS, ORGAN FALURE, ANTIBIOTIC THERAPY

In the past 20 years, long term survival in HIV-positive patients has been marked by the introduction of the HAART therapy and it is expected that the number of infected persons should increase, especially in developing countries, but also in the so called "third world" countries (1).

Recent studies carried on HIV-positive patients have analyzed the characteristics of critical stages, comparing pre- and post-HAART moments and focusing on the hospitalization reasons and mortality risk factors (2). Analysis of HIV infection in critical patients showed that sepsis is among the causes of hospitalization in intensive care units with a rising incidence, contrary to the decreasing tendency observed in patients with acute respiratory failure (3). Other studies proved that bacterial infections became more frequent in HIV patients irrespective of HAART use (4).

Despite a significant growth of the sur-

vival rate and the quality of life, patients with HIV/AIDS have been systematically excluded from studies focused on sepsis, thus limiting the understanding concerning the impact that the association of the two entities has in the population, and, up to now, few studies have prospectively evaluated the determinant factors in the survival of such patients (4).

Different anomalies detected in the HIV/AIDS infection defense mechanisms could account for the presence of bacterial infections with germs developing intracellularly or with encapsulated germs. These infections are also very common because of the frequent use of antibiotics and invasive procedures during repeated hospitalization.

The latest evaluations made on cohorts demonstrated that severe sepsis is a risk factor for intrahospital mortality affecting survival in the short and long term in HIV-positive patients. Mortality has rather depended on the critical status associated to the severity of organ failure than on the modifications caused by HIV/AIDS infection, irrespective of the immunodeficiency level, HAART use or the time when the HIV infection was diagnosed. Thus, death due to severe sepsis associated to HIV/AIDS infection was found by studies to range from 28 to 56 % (5).

MATERIAL AND METHODS

We have carried out a retrospective study on HIV-positive patients diagnosed with sepsis, hospitalized in the Clinical Hospital of Infectious Diseases of Iaşi, between January 2008 and April 2012.

Criteria for a sepsis diagnosis were given by the association of at least two of the following modifications: temperature>38ºC or temperature < 36ºC, cardiac frequency > 120/minute, systolic arterial pressure < 110 mmHg, respiratory frequency > 30/min, leukocytosis > 12.000/mm³ or < 4000/mm³ with the risk of aggravation should a portal of entry be identified, pulmonary or intra-abdominal, with the association of thrombocytopenia < 150.000/mm³, hypernatremia > 145mmol/l or bilirubinemia > 50mg‰.

We analyzed the clinical characteristics, context of immunodepression, and criteria of circumscription to sepsis, period and evolution under antibiotics therapy by means of APACHE II scoring evaluation.

Upon hospitalization, patients gave their written consent for their personal data to be used for scientific purposes.

RESULTS AND DISCUSSIONS

Out of 2028 HIV-positive patients hospitalized in the Clinical Hospital of Infectious Diseases of Iaşi in the above mentioned period, 30 met the sepsis diagnosis criteria. These 30 patients were aged from 1 to 61 years, with an average age of 35.6, a median of 34 years and the predominant age (module) of 32 years.

The analysis of distribution by sex showed a prevalence of the female sex (17 cases), whereas the place of residence was mainly rural (22 cases).

Patient classification according to their immunodepression stage upon hospitalization are registered in table I.

TABLE I

Patient stage of immunodepression

HIV stage	Number of patients
A3	3
B2 and B3	10
C3	17

According to whether the etiologic agent involved in the outbreak of sepsis was identified or not, three large groups of

patients were distinguished:

I. Patients with sepsis of unspecified etiology;

II. Sepsis patients with *Staphylococcus aureus Meti-R*, or with other *Gram positive* bacteria, of whom 3 cases with cutaneous staphylococci as starting point

2 cases with face staphylococci;

1 case with left chronic suppurative hemimandible as starting point;

1 case with incised palatine abscess;

III. Group of patients with sepsis originating in the urinary tract associated with urinary infection caused by *Escherichia coli*, and renal microlithiasis in 3 patients, in whom *Escherichia coli* sepsis was also present and originated in the lungs (2 cases).

Complex large spectrum antibiotic therapy, based on the principle of de-escalation, was preferred in all the three groups identified. First intention antibiotic therapy was mostly empirical and recommended from the very first signs of evolution to sepsis in HIV patients. Whenever the etiologic agent was identified, the antibiotic scheme was adjusted according to the bacterial sensitivity test to antibiotics (tab. II).

TABLE II

Antibiotic scheme

1st Group of patients	Cephalosporins GIII + Aminoglycosides Carbapenems + Quinolons
2nd Group of patients	Oxazolidinons (Linesolid) + Quinolons Lincosamides (Clindamycin) + Quinolons
3rd Group of patients	Cephalosporins GIII + Quinolons Carbapenems + Quinolons

Here are the associated diseases in the studied patients:

- infection of the lower respiratory tract (12 cases)
- thrombocytopenia (5)
- HIV encephalopathy (4)
- decompensated hepatic cirrhosis of different etiologies(4)
- oropharyngeal candidiasis (4)
- microcytic hypochromic anemia (4)
- hepato-renal failure (3)
- chemical polyradiculoneuritis (3)
- chronic prurigo (3)
- diabetes mellitus (2)
- mononucleosis syndrome (2)
- toxoplasmosis (2)
- chronic heart failure (2)

Overall, 8 patients needed monitoring in the intensive care unit, in 4 of whom the etiologic agent was identified in blood cultures (2 *Staphylococcus aureus Meti-R* cases and 2 *Escherichia coli* cases) (table III).

TABLE III

APACHE II Scoring according to the etiological agent

Germ type	<20	20-29	>30
	NUMBER OF PATIENTS		
known	2	2	0
unknown	20	4	2

Patients in the intensive care unit had an average hospitalization period of 17.4 days with defervescence occurring after 4.7 days of the average.

In patients in whom the etiologic agent was identified defervescence occurred after

an average period of 3.2 days, as compared to 3.7 days in those with unknown etiologic agent, whereas 9 became afebrile. The hospitalization period ranged between 16 and 28 days, with an average of 16.5 days and a median of 17 days.

The paraclinical evaluations revealed:

- leukocytosis >12.000/mm³ in 16 cases, leukopenia<4000/mm³ in 4 patients; agranulocytosis associated with the most typical proteiform clinical presentation and the longest periods of hospitalization (between 23 and 28 days);
- the hepatic function underwent deterioration in 6 cases, the maximum transaminases levels reaching 2580 UI/L for TGP and 1750 UI/L for TGO;
- a hepato-renal syndrome was present in 3 cases, in 2 of whom the disease evolved towards death;
- the lymphocytes TCD4 level evaluated at the beginning of the antibiotic therapy varied; thus, in 12 patients, the lymphocytes TCD4 level decreased by 20-75 units during sepsis evolution.

CONCLUSIONS

Evolution and mortality rates in sepsis associated with HIV/AIDS infection depend on the presence of organ failure and are less influenced by the level of immunodepression, complex antibiotic therapy being the cornerstone in controlling patients.

Even though the identified number of cases in which sepsis was associated with HIV was relatively low in our study, additional effort is required in approaching these patients, considering their chronicization profile in the evolution of the HIV infection and, consequently, their exposure to recurring infections.

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PREVALENȚA BOLILOR CARDIOVASCULARE LA PACIENȚII CU HIPOTIROIDISM ASISTAȚI ÎN CLINICA A VI-A MEDICALĂ

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PREVALENCE OF CARDIOVASCULAR DISEASES TO THE PATIENTS WITH HYPOTHYROIDISM (Abstract): The cardiovascular risk in patients with hypothyroidism is related to an increased risk of functional cardiovascular abnormalities and atherosclerosis**. Aims** – The purpose of the present study was to examine a possible association between subclinical and clinical hypothyroidism and cardiovascular disease (CVD). The retrospective study, which covered a 12 months period, had the purpose to establish the prevalence of various risk factors for CVD, within subclinical and clinical hypothyroidism. **Material and methods**: We have studied 64 subjects with subclinical and clinical hypothyroidism admitted to the Rehabilitation Hospital, in the Cardiovascular Clinic. They were divided by sex, age, living environment (urban, rural), value of cholesterol, heart rate, BMI, blood pressure. **Conclusions** - The study revealed an increased risk of cardiovascular abnormalities and an increased risk of atherosclerosis: 65,61% subjects were obese, 90,6% with high blood pressure, 62,5% had dyslipidaemia. Subclinical thyroid dysfunction is common in older people. The identification of subclinical and clinical hypothyroidism earlier, pursuit of markers for subclinical atherosclerosis and deployment of lifestyle changes, are elements that can also be the key in improving clinical and paraclinical parameters. **Key words**: SUBCLINICAL HYPOTHYROIDISM, CLINICAL HYPOTHYROIDISM, CARDIOVASCULAR ABNORMALITIES, ATHEROSCLEROSIS

Hipotiroidismul se caracterizează printr-o producție scăzută de hormoni tiroidieni la nivelul glandei tiroide, ce se traduce printr-o concentrație scăzută a hormonilor la nivelul celulelor şi țesuturilor, şi poate varia ca severitate de la forma uşoară (mild subclinical hypotiroidism-SCH) până la cea mai severă, respectiv mixedemul (1). Sistemul cardiovascular este una din principalele ținte de acțiune ale hormonilor tiroidieni, şi în acelaşi timp un marker sensibil al acțiunii periferice a acestora (2). Riscul cardiovascular la pacienții cu hipotiroidism este determinat atât de modificări la nivelul sistemului cardiovascular, cât şi de modificările aterosclerotice. La nivelul sistemului cardiovascular sunt menționate favorizarea şi apariția disfuncției endoteliale, afectarea atât a funcției sistolice, cât şi a celei diastolice globale, în special la nivelul ventriculului stâng, creşterea rezistenței arteriale periferice cu scădere toleranței la efort. Creşterea riscului

aterosclerotic rezultă din predispoziția spre HTA (şi în special HT diastolică) şi modificarea profilului lipdic (3). T3 inflențează frecvența cardiacă, funcția sistolică, diastolică şi rezistența vasculară sistemică, ducând la scăderea performanței cardiace şi tulburări la nivelu contractilității miocardului. De asemenea la pacienții cu hipotiroidie se constată o scădere a presarcinii, cu scăderea volumului total circulant şi a funcției diastolice chiar la niveluri incipiente ale hipotiroidiei (4). Rezistența vasculară periferică şi timpul de circulație crescute se asociază cu redistribuția fluxului sanguin, scăderea fluxului plasmatic renal, concentrația plasmatică a reninei şi aldosteronului scăzute. În acelaşi timp, apariția disfuncției endoteliale favorizează atât procesul aterosclerotic cât şi rigiditatea arterială, ceea ce va contribui la accentuarea HTA în contextul hipotiroidismului (5).

MATERIAL ŞI METODE

Studiul şi-a propus cuantificarea bolilor cardiovasculare, cât şi a factorilor de risc cardiovasculari cu valoare predictivă asupra morbidității şi mortalității prin boli cardiovasculare, la pacienții cu hipotiroidie internați în Clinica de Recuperare Cardiovasculară. Din numărul total de pacienți internați în Clinica de Recuperare Cardiovasculară a Spitalului de Recuperare Iaşi în perioada 1.01.2011-1.01.2012 au fost incluşi în studiu 64 pacienți cu diagnostic confirmat de hipotiroidism. S-a urmărit distribuția acestor pacienți în funcție de mediul de proveniență, indicele de masă corporală (IMC), circumferința abdominală, colesterolul, TA, frecvența cardiacă, modificările ecocardiografice, diagnosticele la externare.

REZULTATE

În lotul de studiu, din cei 64 pacienți, 90% (58) pacienți au avut vârsta peste 50 ani, 63 (98,4%) au fost femei şi 1 (1,5%) bărbat (diferență semnificativă statistic între cele 2 loturi, $p<0.01$), distribuția pe mediul de proveniență fiind: 46 (72%) din mediul urban şi 18 (28%) din mediul rural (diferența semnificativă statistic se păstrează şi între medii, $p<0.01$) (fig. 1).

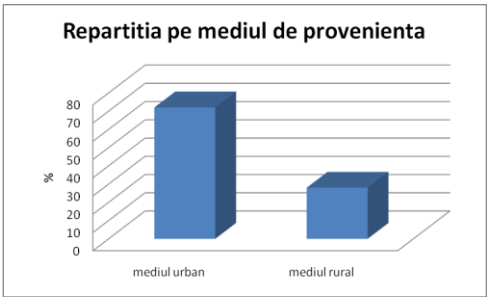

Fig. 1. Repartiția pacienților pe mediul de proveniență

În funcție de IMC (indice de masă corporală), repartiția pacienților a fost următoarea: 5 (7,85%) normoponderali, 17 (26,5%) supraponderali, 28 (43,79%) obezitate gradul I, 11 (17,18%) obezitate gradul II, 3 (4,68%) obezitate gradul III, procentul total de pacienți obezi fiind de 65,6% (fig. 2).

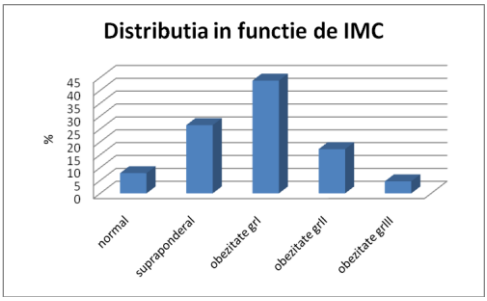

Fig. 2. Distribuția procentuală a pacienților în funcție de IMC

Din cei 64 pacienți introduşi în studiu 58 (90,60%) au fost diagnosticați cu HTA, dintre care 16 (27,5%) cu HTA gradul I, 21

(36,2%) cu HTA gradul II şi 21 (36,2%) cu HTA gradul III. Nu s-a constatat o diferență semnificativă statistic între loturi. Important de menționat este procentul de 45,3% (29) pacienți cu valori ale tensiunii arteriale diastolice (TAD) peste 90 mmHg (fig. 3,4).

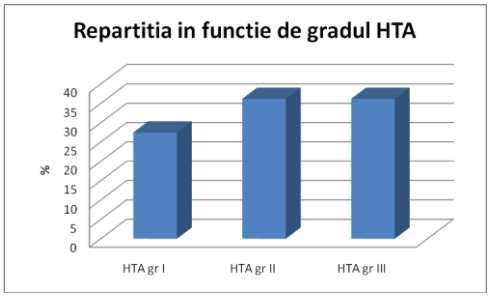

Fig. 3. Repartiția pacienților în funcție de gradul HTA

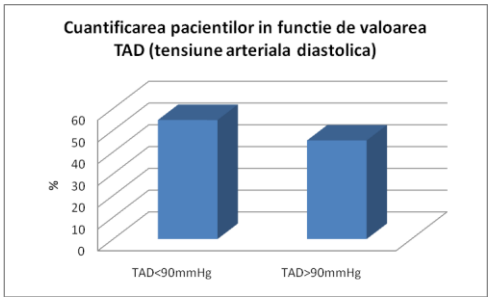

Fig. 4. Cuantificarea pacienților cu valori ale TAD>90mmHg

Pe lotul studiat ne-am propus şi cuantificarea pacienților cu frecvență cardiacă peste 70 bătăi pe minut în vederea evaluării acestui parametru ca factor de risc independent pentru bolile cardiovasculare. Nu s-a constatat o diferență statistic semnificativă între cele două loturi (fig. 5).

Au fost diagnosticați pe parcursul internării un număr de 21 (32,8%) pacienți cu diferite forme de angină pectorală. De menționat că restul de 67,2% pacienți au prezentat la externare diferite alte componente ale afectării cardiovasculare, precum HTA sau insuficiență cardiacă (fig. 6).

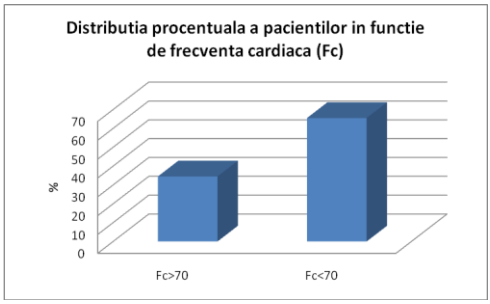

Fig. 5. Distribuția procentuală a pacienților cu FC>70 bătăi/minut

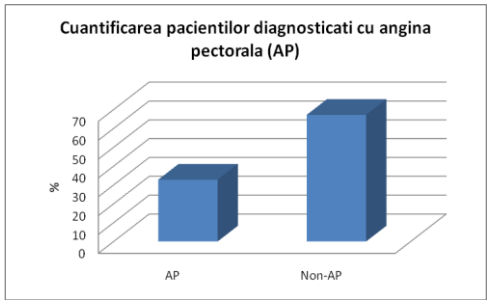

Fig. 6. Cuantificarea pacienților diagnosticați cu angină pectorală la externare

Din lotul studiat a reieşit un număr de 18 (28,12%) pacienți care prezentau scăderea toleranței la glucoză (STG) sau diabet zaharat tip 2 (DZ) (sub antidiabetice orale sau insulino-necesitant) (fig. 7).

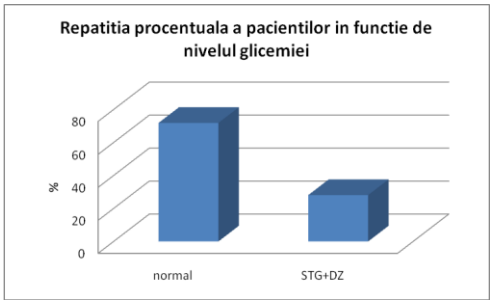

Fig. 7. Incidența modificărilor metabolice la pacienții hipotiroidieni

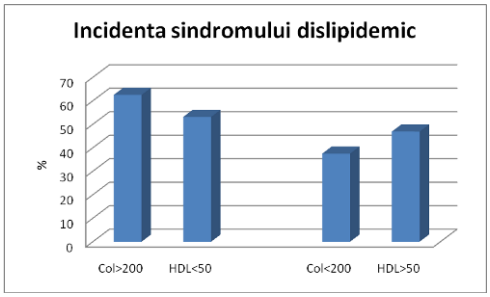

Fig. 8. Incidența sindromului dislipidemic

La lotul studiat s-a dorit şi evaluarea profilului lipidic: din cei 64 pacienți 40 (62,5%) au avut colesterolul peste 200 mg/dl, 24 (37,5%) au avut valori ale colesterolului sub 200mg/dl, 34 (53,12%) pacienți au avut valori ale HDL sub 50 mg/dl, şi respectiv 30 (46,87%) peste 50 mg/dl (fig. 8). Nu s-a constatat o diferență semnificativă între loturi.

Evaluând fracția de ejecție determinată prin ecocardiografie s-au identificat un număr de 26 (40,62%) pacienți cu fracție de ejecție sub 60% şi respectiv 38 (59,38%) pacienți cu fracție de ejecție peste 60% (fig. 9).

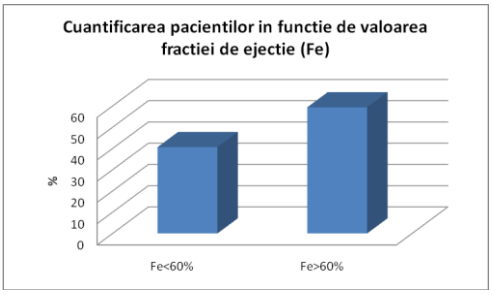

Fig. 9. Cuantificarea procentuala a pacienților în funcție de valoarea FE

DISCUȚII

Bolile cardiovasculare rămân pe primul loc între cauzele de morbiditate şi mortalitate la nivel mondial. În 2003 au fost înregistrate nu mai puțin de 16,7 milioane decese prin boli cardiovasculare (peste 29% din toate cauzele de deces) (6). Plecând de la studiile din literatură, în care se citează implicarea hipotiroidismului în determinismul bolilor cardiovasculare cu impact în special asupra funcțiilor miocardului, favorizarea patologiei coronariene şi ateroscleratice, studiul şi-a propus o cuantificare a acestor patologii raportate la cercetările deja existente, la pacienții din Clinica de Recuperare Cardiovasculară (7). În mod similar studiilor internaționale, distribuția pe grupe de vârstă (90% peste 50 ani) şi sex este concordantă (8). Chiar dacă nu există foarte multe referințe cu privire la mediul de proveniență, care la noi poate implica atât regiunea geografică (cu riscul deficitului de iod), cât şi diferențele semnificative între mediul urban şi rural (nivel de trai şi stil de viață), rezultatele sunt concordante cu datele existente (6): 72% provin din mediul urban, respectiv 28% din mediul rural. Se ştie că mediul urban, asociat hipotiroidismului, este un factor de risc cardiovascular în sine, fiind cunoscută şi dovedită importanța stilului de viață (fumat, sedentarism, stres). IMC şi circumferința abdominală aşa cum se ştie sunt markeri cu valoare predictivă pentru bolile cardiovasculare, iar procentele de 65,61% (42 pacienți) cu obezitate de diferite grade şi respectiv 92% (59 pacienți) cu circumferință abdominală peste 88 cm, indică la aceşti pacienți o asociere semnificativă şi un teren favorizant atât pentru disfuncția endotelială, cât şi pentru apariția insulinorezistenței, respectiv scăderea toleranței la glucoză şi diabet zaharat (incidență de 28,12%) (9, 10). Datele din literatură asociază în mod constant hipotiroidia disfuncției endoteliale şi scăderii disponibilului de NO (11). La acestea se adaugă rigiditatea vasculară sistemică şi rigiditatea arterială la

nivelul aortei, toate conlucrând la crearea unui teren favorizant dezvoltării HTA (12). Rezultatele pe grupul de studiu sunt concordante, cu un procent semnificativ de 90,6% (58 pacienți), cu grade variate de HTA. În acelaşi timp, de subliniat este procentul pacienților cu valori ale TAD peste 90 mmHg – 45,3% (29), care corespunde studiilor existente cu privire la acest parametru (13). Având în vedere că FC>80/min şi masa VS>270 g, se asociază cu risc crescut de ruptura de placă, la pacienții cu boală coronariană documentată, şi că o frecvență cardiacă de peste 70/min creşte cu 46% riscul de infarct miocardic la pacienții cu cardiopatie ischemică, procentul de 62,5% pacienți cu frecvență cardiacă peste 70/min este un marker important al riscului cardiovascular (14). Există dovezi că hormonii tiroidieni afectează în mod direct contractilitatea cordului, atât funcția sistolică cât şi cea diastolică, acționând prin canalele Ca-ATP-azice de la nivelul reticulului sarcoplasmatic şi de aceea a fost importantă cuantificarea pacienților cu diferite forme de angină pectorală, în studiul nostru aceştia reprezentând 32,8 % din totalul pacienților (15). Cele mai multe referințe bibliografice asociază hipotiroidiei sindromul dislipidemic şi favorizarea procesului de ateroscleroză (16, 17), chiar dacă există şi trialuri ce contrazic aceste date (18, 19). Cauza acestor modificări poate fi creşterea sintezei de colesterol în paralel cu scăderea activității LPL (lipoproteinlipazei) hepatice în contextul hipotiroidiei şi scăderea receptorilor hepatici-LDL (20). Rezultatele pe grupul nostru de studiu sunt în concordanță cu aceste date, un procent de 62,5% pacienți fiind cu valori ale colesterolului peste 200 mg/dl şi 53,12% pacienți cu valori ale HDL sub 50 mg/dl. Există o serie de studii ce asociază modificările la nivelul cordului, cu afectarea funcției sistolice şi diastolice la pacienții hipotiroidieni, şi cu uşoara scădere a FE (fracției de ejecție), ceea ce în studiu de față se regăseşte la 40,62% dintre pacienți (21).

CONCLUZII

Studiul asupra prevalenței bolilor cardiovasculare la pacienții cu hipotiroidism, internați în Clinica de Recuperare Cardiovasculară a Spitalului de Recuperare Iaşi, relevă un risc crescut de a dezvolta boli cardiovasculare şi complicații ale acestora plecând de la procentele semnificative ale markerilor relevanți atât cardiovasculari cât şi metabolici: un procent de 65,61% pacienți obezi, 28,12 % pacienți cu modificări ale profilului glucidic, 40,62% pacienți cu valori peste medie ale frecvenței cardiace.

Doi parametrii esențiali în evaluare şi cu implicații în prognosticul individual al pacienților sunt HTA şi sindromul dislipidemic. Faptul că la diagnosticare un procent de 90,62% aveau HTA de diferite grade implică necesitatea unui diagnostic mai precoce, cu prevenție primară şi secundară, şi în acelaşi timp acest parametru duce la modificarea riscului individual pe grila SCORE, de unde implicații asupra comorbidităților şi bineînțeles asupra calității vieții pacientului.

Modificarea profilului lipidic atrage după sine favorizarea procesului aterosclerotic şi riscul de evenimente cardiovasculare majore. Este deci necesară o cât mai bună evaluare a pacienților cu diagnosticul de hipotiroidie (chiar dacă şi numai subclinică), cu o cuantificare riguroasă a parametrilor clinici, inflamatori (aterosclerotici) şi ecocardiografici, permițând astfel o privire de ansamblu asupra riscului de boală cardiovasculară şi metabolică a pacientului.

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SCORUL PROGNOSTIC ÎN ENDOCARDITELE INFECȚIOASE – NOU FACTOR DE PREDICTIBILITATE ÎN EVOLUȚIA BOLII

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PROGNOSTIC SCORE IN INFECTIVE ENDOCARDITIS – INNOVATIVE AND USEFUL PREDICTIBILITY FACTOR FOR DISEASE´S EVOLUTION (Abstract): **Aim:** Evaluate the potential of evolution in infective endocarditis (IE) according to patient´s personal parameters (biological, clinical and paraclinical), and Carmeli score, as purpose in establishing an appropiate antibiotherapy. **Material and methods:** Clinico-therapeutic observations concerning a cohort of 868 patients admitted between 1995 and 2008 in three hospital services of Iaşi. The following guide marks were included in the study: age, sex, previous state of heart, left/right sided IE, number of valves implicated, presence of major complications, health care associated IE/nosocomial IE, major associated diseases, type of microorganism implicated/negative blood cultures. According to their importance, the score varied for each between 0 and 2, resulting a global minimum of 1 and a maximum of 10. All scores obtained were correlated with mortality and Carmeli score. **Results:** Patients with score 1-4 had a mild evolution, > 4 - < 7 usual trends of evolution with mortality close to medium rate, and 7-10 severe , mortality growing at every point with 15-18%. Carmeli score 1 was associated with 1-4 new score, 2 with > 4 - < 7, and 3 with 7-10 score. Antibiotic regimens changed usually during therapy, higher succes rates being obtained in IE with isolated pathogen. **Conclusions:** As new score may vary during patient's evolution, there is an important connection (especially at the time of admission) – but not similarity – between this new factor and Carmeli score, therapy being highly related to guide proposed regimens. However, antibiotherapy in IE should be applied using a mix of these scores, as there are important interferences due to major prognostic factors proposed in the new score, often needing next level's Carmeli score related antibiotherapy. **Key words:** INFECTIVE ENDOCARDITIS, NEW SCORE, CARMELI, ANTIBIOTHERAPY, PROGNOSTIC

Definită ca infecție a suprafeței endocardice a cordului, endocardita infecțioasă (EI), rămâne, în ciuda avansului constant în ceea ce priveşte diagnosticul şi tratamentul medical şi/sau chirurgical, una dintre cele mai severe infecții cu potențial de evoluție imprevizibil, fiind grevată de o mortalitate crescută. Acest fapt se datorează, în cea mai mare măsură, complicațiilor majore cardiace/extracardiace, a imposibilității frecvente de stabilire a diagnosticului etiologic (aprox. jumătate din cazuri având hemoculturi negative), precum şi a implicării din ce în ce mai frecvente a microorganismelor cu rezistență crescută la antibiotice, adesea cu origine nosocomială (1, 2).

MATERIAL ŞI METODE

Studiul retrospectiv a inclus 868 pacienți cu EI internați între 1995 şi 2008 în trei unități spitaliceşti din municipiul Iaşi. Au fost incluşi în lot doar pacienți cu vârsta peste 15 ani, EI survenind fie izolat, fie în contextul unei infecții sistemice (două sau mai multe determinări septice). În fiecare din cazuri am stabilit – din analiza foilor de observație – un scor prognostic individual, corelat cu 9 factori (tab. I).

TABELUL I

Valoarea factorilor predictivi în noul scor din endocardita infecțioasă

Criteriu	0 puncte	0.5 puncte	1 punct	2 puncte
Vârsta	< 45 ani	> 45 ani	-	-
Sexul	Feminin	Masculin	-	-
Îngrijiri medicale anterioare	Nu	Extraspitaliceşti (tratament la domiciliu, sonde urinare permanente, tratament stomatologic, etc)	Nosocomiale (cateterism venos/urinar, IOT, bronhoscopii, intervenții chirurgicale laborioase)	-
Comorbidități importante	Nu	Utilizatori droguri intravenoase, corticoterapie prelungită	Neoplazii, diabet zaharat, dializați cronic, ciroză hepatică, colagenoze, SIDA, TBC	-
Complicații majore	Nu	-	-	Tromboembolism, ruptură, anevrisme micotice cu metastazarea principalelor organe, abcese perivalvulare, perforări/distrugeri valvulare, insuficiență cardiacă, etc
Starea anterioară a cordului	Indemn	Extraendocardice uşoare/moderate	-	Endocardice şi extraendocardice severe -insuficiență cardiacă cronică, dispozitive antiaritmice, MCC
Preponderență	-	Cord drept	Cord stâng	-
Număr valve afectate	-	1	2 sau mai multe	-
Microorganism implicat	Streptococi cu excepțiaVREF, MSSA, staf. coagulazo-negativi, HACEK, BGN, Mycobacterium, Chlamydii, Mycoplasma	MRSA, VREF, BGN producători de betalactamaze cu spectru extins, fungi, Coxiella	Hemoculturi negative	-

Fiecare caz a fost, de asemenea, încadrat şi în scorul Carmeli (cit. 3) (tab. II).

S-au corelat cele două scoruri între ele şi cu antibioterapia propusă prin scorul Carmeli încercând a se stabili legătura între acestea şi evoluția ulterioară a pacientului, având ca principal reper rata mortalității (tab. III).

TABELUL II

Scorul Carmeli (valoarea cea mai mare indiferent de parametru)

Contact cu sistemul de îngrijiri de sănătate	Fără	1
	Fără proceduri invazive	2
	Contact prelungit cu proceduri invazive	3
Terapie antibiotică anterioară	Nu	1
	Da	2
Caracteristicile pacientului	Tânăr fără comorbidități	1
	Vârstnic ± comorbidități	2
	Imunosupresie (SIDA, BPOC, neoplazie, etc)	3

TABELUL III

Scorul Carmeli şi antibioterapia (cit. 3)

Carmeli 1	Carmeli 2	Carmeli 3
⇩	⇩	⇩
Infecții comunitare	Infecții asociate îngrijirilor medicale	Infecții nosocomiale
⇩	⇩	⇩
Amoxicilină/clav, Fluoroquinolone, Cefalosporine generația II/III, Macrolide	Ertapenem, Aminoglicozide±Fluoroquinolone, Cefalosporine de generația III/IV, Betalactamine + Inhibitori de betalactamază, Ureidopeniciline, Antistafilicocice majore, Macrolide, Fluconazol/Itraconazol	Imipenem/Meropenem, Aminoglicozide, Moxifloxacin iv, Cefalosporine generația III antipseudomonas, Cefalosporine gen. IV (Cefepim, Cefpirom), Linezolid/Vancomicină, Tigeciclină,Colistin, Antifungice majore (Voriconazol, Caspofungin)

Astfel se poate aborda o terapie antibiotică de primointenție corelând cele două scoruri, folosind uneori de-escaladarea (mai ales când diagnosticul etiologic este cert, având şi o antibiogramă utilă) sau menținerea în cazul EI cu hemoculturi negative (EIHN), atât timp cât evoluția este favorabilă (4,5,6).

Modelul local (incidență de aprox. 3 cazuri la 100000 locuitori) se suprapune în

mare măsură unui model național de tranziție, fiind de tip intermediar între cele două modele clasice, cel occidental şi cel auster, diagnosticul fiind pus prin criteriile Duke modificate (7).

Din cei 868 pacienți, 597 (68.7%) au fost bărbați şi 271 femei (31.3%), cu un raport mediu de 2.2:1 în favoarea sexului masculin. Ca şi vârstă, 2.5% aveau 15-20 ani, 14.5% 20-35 ani, 23% 35-49 ani, 35% 50-65 ani şi 25% > 65 ani, media în lotul studiat fiind de aproximativ 51 ani (peste media națională).

Factori locali predispozanți: valvulopatii reumatismale (26.8%), proteze valvulare (15.3%), dispozitive antiaritmice (2.8%), MCC (1.7%).

Factori generali: cateterism venos (15.1%), cistoscopii, sondaj vezical, chirurgie prostatică (14.3%), endoscopii digestive (12.4%), bronhoscopii, IOT (8,4%), extracții dentare, chirurgie oromaxilofacială (9.6%) şi altele sub 10% fiecare, intervenții chirurgicale digestive/obstetricale, neoplazii, diabet zaharat, hemodializă cronică, ciroză hepatică, SIDA, corticoterapie prelungită, etc.

REZULTATE

În ceea ce priveşte afectarea valvulară şi profilul etiologic, s-au obținut următoarele rezultate (tab. IV, tab. V):

TABEL IV

Afectarea endocardică

Cord stâng 67.2%	Mitrală	37.1%
	Aortică	30.1%
Cord drept 15.9%	Tricuspidă	14.7%
	Pulmonară	1.2%
Mixtă	Mitroaortică	12.1%
Extravalvulară	-	3.7%

TABELUL V

Profilul etiologic al endocarditelor infecțioase

Staphylococcus aureus	53.6% din EIHP
Stafilococ coagulazo-negativ	5.3% din EIHP
Streptococi (exceptând enterococi)	9.8% din EIHP
Enterococcus spp	18.9% din EIHP
BGN	4.8% din EIHP
HACEK	2.1% din EIHP
Fungi	3.04% din EIHP
Altele	2.46% din EIHP
Etiologii mixte	2.75% din EIHP
Hemoculturi negative	45.9% din total

Etiologie: EI cu hemoculturi pozitive (EIHP) s-a regăsit în 470 de cazuri (54.1%), cel mai frecvent implicat fiind stafilococul (277 tulpini, 123 fiind meticilino-rezistente: 44.4%), hemoculturi negative (EIHN) fiind regăsite în 45.9% din cazuri.

S-au înregistrat 224 de decese (25.8%), peste valoarea medie din modelul occidental (~ 14-15%), în special la cei cu vârste > 50 ani (tab. VI, VII).

TABELUL VI

Corelația scor Carmeli – mortalitate

	Număr cazuri	Mortalitate
Carmeli 1	437	11.2% (49 cazuri)
Carmeli 2	293	29.3% (86 cazuri)
Carmeli 3	138	65.9% (91 cazuri)

TABELUL VII

Corelația noului scor propus cu mortalitatea

Scor inițial	Număr cazuri	Mortalitate
1-1.5	48	6.25% (3 cazuri)
2-2.5	96	10.4% (10 cazuri)
3-3.5	112	13.4% (15 cazuri)
4-4.5	140	17.1% (24 cazuri)
5-5.5	168	25% (42 cazuri)
6-6.5	161	32.3% (52 cazuri)
7-7.5	96	44.8% (43 cazuri
8-8.5	31	67.7% (21 cazuri)
9-9.5	11	81.8% (9 cazuri)
10	5	100%
Total	868	224

DISCUȚII

Schemele antibiotice folosite, mai ales în ultimii ani ai studiului, au corespuns în destul de mare măsură recomandărilor din scorul Carmeli însă numărul lor a fost extrem de variat, mai adesea în asociere (două antibiotice în 596 cazuri, trei antibiotice în 219 cazuri, antibiotic+antifungic 198 cazuri).

Monoterapia a fost o excepție (53 cazuri), mai ales în primii ani de studiu. S-a semnalat tendința schimbării cel puțin a unei scheme antibiotice pe parcurs (datorită fie izolării unui agent patogen, fie evoluției nefavorabile în cazul EIHN, adeseori şi datorită „plierii" clinicienilor pe mijloacele terapeutice avute la un moment dat la dispoziție), rezultând un adevărat "cocktail" de asocieri şi replieri (8,9).

Exista o corelație bună între scorul Carmeli şi noul scor (tab. VIII).

TABELUL VIII

Corelația scor nou-Carmeli-mortalitate

Scor Carmeli			Scor nou		
	Număr cazuri	Mortalitate		Număr cazuri	Mortalitate
1	437	11.2%	1-4	396	14.3%
2	293	29.3%	>4-<7	329	28.1%
3	138	65.9%	7-10	143	58.3%

Scorul nou este adaptabil pe toată perioada internării, unii factori predictivi, putând fi introduşi / modelați pe parcurs. În absența cunoaşterii agentului etiologic, toate cazurile au fost interpretate drept EIHN (1 punct).

Corelarea celor două scoruri cu antibioterapia s-a făcut într-un mod mai elastic, existând perioade foarte diferite în studiu, în ceea ce priveşte posibilitățile terapeutice şi modul de abordare a antibioterapiei (10,11). În cazul EIHN, dificilă de tratat, în ciuda multiplelor asocieri antibiotice/antifungice, abordarea se poate simplifica (tab. IX).

TABELUL IX

Tratamentul endocarditelor infecțioase cu hemoculturi negative

Proteză valvulară	**Durata (săptămâni)**
Vancomicină	Minim 4
+Aminoglicozid	2-4
+Rifampicină ± fluoroquinolonă (debut tardiv > 1 an	Minim 4
SAU	
Penicilină M/Cefalosporine gen III/IV	4-6
+ Aminoglicozid	2-4
+Rifampicină (suspiciune MSSA)	4-6
Valvă nativă	
Aminopenicilină+sulbactam	Minim 4
+aminoglicozid	2-4
SAU	
Vancomicină	4-6
+aminoglicozid	2-4
+fluoroquinolonă	4-6
EI nosocomială	
Cefalosporină gen III/IV+vancomicină +aminoglicozid	4
Purtător pacemaker	
Vancomicină+fluoroquinolonă	4-6

Antibioterapia trebuie stabilită cu prioritate funcție de scorul nou, fără a ne cantona întotdeauna în recomandările scorului Carmeli, existând posibilitatea abordării antibiotice de nivel superior Carmeli-asociată, funcție de o serie de particularități ale unor pacienți.

Mortalitatea este mult crescută în cazul stafilococilor meticilino-rezistenți, cu afectare a cordului stâng, protezaților valvular sau cei cu afecțiuni cardiace majore în antecedente. În ordine descrescătoare, în cazul EIHP, mortalitatea se întâlneşte în cazul MRSA, fungilor (12) şi a enterococilor rezistenți. Regimurile terapeutice antimicrobiene au avut o bună bucată de timp o orientare oarecum „caracteristică" fiecărei clinici în parte, existând o mare diversitate de opțiuni. În ultimii ani se poate remarca o remodelare a abordării terapeutice pe principii mult mai obiective, cu o colaborare interdisciplinară mai strânsă, rezultatele neîntârziind astfel să apară.

CONCLUZII

Cercetarea lotului de 868 de cazuri de EI, reliefează faptul că scorul Carmeli nu era perfect adaptat acestei infecții severe, fapt ce m-a determinat să extind plaja factorilor prognostici şi să îi corelez cu terapia antibiotică şi cu mortalitatea. Ca atare, o evaluare rapidă a pacienților încă de la

internare – dar şi pe parcurs – conform noului scor, a ajutat, împreună cu recomandările antibiotice din scorul Carmeli, aprecierea posibilităţilor evolutive cât şi abordarea terapeutică. Stabilirea noului scor, care nu a necesitat mijloace deosebite, ci doar o anamneză şi un diagnostic riguros, a reprezentat un reper important în monitorizarea acestei infecţii cu potenţial foarte sever.

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RESTRICTIVE FILLING PATTERN - A MAJOR NEGATIVE PROGNOSIS FACTOR IN DILATED CARDIOMYOPATHY

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RESTRICTIVE FILLING PATTERN-A MAJOR NEGATIVE PROGNOSIS FACTOR IN DILATED CARDIOMYOPATHY (Abstract): Dilated cardiomyopathy (DCM) represents an important cause of chronic heart failure and, thereby, of morbi-mortality worldwide, not only because of its high-risk for sudden death, but also by its evolutive complications (arrhythmias, thromboembolic accidents). The objectives of the study: selection of patients with DCM in sinus rhythm and with a restrictive pattern at echo-Doppler examination, analysis of relevant epidemiological, clinical and echocardiographic parameters and their correlation with a negative prognosis. **Material and methods**: Retrospective evaluation of a total of 300 patients admitted to the Cardiology Clinic of "Sf. Spiridon" Hospital between January 2017 and December 2017 with the confirmed diagnosis of DCM. Of these, applying the exclusion criteria, we selected 120 patients for further evaluation. **Results**: Of the analyzed patients, 85 were men and 35 were women, the proportion of those > 60 years old being 71.66%, while those <60 years representing 28.34% of the total, the mean age being 64.4 years. Male sex was significantly correlated with ischemic ($p = 0.000001$) and ethanolic ($p = 0.05$) etiology of DCM, as well as with a more severe symptomatology (NYHA III-IV, $p = 0.007$) compared to women. The restrictive pattern was echocardiographically detected in 79 patients (65.83% of cases) and showed a statistically significant association with the male sex ($p = 0.03$), the older age ($p = 0.00006$), but also with an ejection fraction <25% ($p = 0.007$) or with the presence of the left bundle branch block ($p = 0.05$). All patients with unfavorable evolution (7.5%) or who died later (4.15%) showed a restrictive pattern, showing a clear link between its presence and a poor prognosis. **Conclusions**: The present study reveals the major negative influence of the restrictive pattern on the prognosis and subsequent evolution of DCM patients, as well as its interdependence with other clinical and epidemiological parameters. **Keywords:** DILATATIVE CARDIOMYOPATHY, RESTRICTIVE PATTERN, POOR PROGNOSIS.

Dilated cardiomyopathy (DCM) represents an important cause of chronic heart failure and, thereby, of morbi-mortality worldwide, not only because of its high-risk for sudden death, but also by its evolutive complications, which may range from severe rhythm disorders (atrial fibrillation, ventricular tachycardia) to thromboembolic accidents.

Several factors that could influence the

progression and prognosis of patients with DCM have been studied, recalling systolic dysfunction (FEVS <45%), old age, NYHA III-IV grades of heart failure, arrhythmias and diastolic left ventricular dysfunction (1, 2).

Regarding the importance of diastolic dysfunction as a prognosis factor, these stems from the fact that most patients with DCM present at the echocardiographic examination a certain degree of alteration of the diastolic function, the most important severity being associated with the restrictive type, which also has the most reserved long-term prognosis (3).

The objective of our study was to identify patients with DCM in sinus rhythm, to assess certain relevant echocardiographically, clinical and epidemiological parameters in pathological context, to select those patients who had a restrictive pattern in the eco-Doppler examination and to correlate the presence of this pattern with subsequent progression and prognosis of the patients.

MATERIAL AND METHODS

Our study retrospectively evaluated a total of 300 patients admitted to the Cardiology Clinic of "Sf. Spiridon" County Clinical Emergency Hospital from Iasi between January 2017 and December 2017 with the confirmed diagnosis of DCM. Of these, we selected for subsequent evaluation a total of 120 patients with sinus rhythm, the absence of which was an exclusion criterion. Patient noncompliance, their refusal to be echocardiographically examined or the low-quality echographic window were other exclusion criteria. The DCM diagnosis was primarily based on echocardiographic appearance and data (dimensions, volumes- the evidence of cavity enlargement, other suggestive parameters for systolic and / or diastolic dysfunction) supported by other clinical data (signs and symptoms of left or global heart failure, mitral valve insufficiency, third heart sound-gallop etc.) and electrocardiographic changes (indirectly by signs of left ventricular hypertrophy). In addition, biological determinations (hem leucogram → macrocytosis, electrolytes, renal function) were performed. Anamnesis was useful in some situations to objectify the etiologies (ethanolic, viral, ischemic, cardiotoxic medication). For the determinations, we used the Samsung Ugeo H60 echocardiograph, with the Doppler pulsed-wave function cursor placed on the tip of the mitral valve. A restrictive pattern was considered if E / A> 2, with DTE <140 ms and IVRT <60 ms. Patients signed and agreed on admission to participate in the research process, their data being processed anonymously. All data was statistically analyzed using the *Microsoft Excel* 2010 and *Epi Info 7* software and for p-value we used the two-tailed test.

RESULTS

From the point of view of epidemiological aspects, out of a total of 120 patients analyzed, 85 (70.83%) were men, while women were 35 (29.17%).

For the comparative analysis by age groups, we divided the patients into two groups: those aged more than or equal to 60 years (86 cases, 71.66%), respectively patients under 60 years (34 cases, 28.34%), mean age being 64.4 years.

Following the echocardiographic evaluation, the restrictive pattern was detected in 79 patients (65.83% of cases), while an ejection fraction (EF) of less than 25% was observed in only 20 patients (16.66%).

In terms of evolution, 111 (92.5%) showed a favorable evolution at 3 months, compared to the remaining 9 patients (7.5%) who had a severe evolutionary path, of these remaining cases, 5 being followed by death (4.16%) (Fig.1).

We noticed that the clinical manifestations of heart failure correlate with age, the share of patients in NYHA III-IV classes being significantly more important in the age group> 60 years (64.1% versus 20.9%, p = 0.027). Also, regarding DCM etiologies, although we have noticed certain percentage differences when assessing the age groups, they have failed to reach the statistical significance threshold (tab. I).

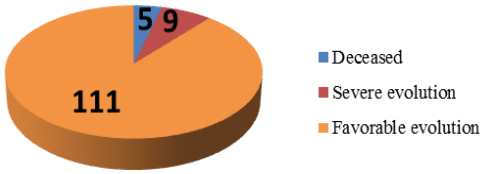

Fig. 1. Evolution of the patients

TABLE I

Severity of symptoms and etiology of DCM related to age

	>60 years No=86 (%)	<60 years No=34 (%)	Total No=120 (%)	p
NYHA III-IV	77 (64.1%)	25 (20.9%)	102 (85%)	0.027
Ischemic DCM	15 (12.5%)	11 (9.2%)	26 (21.7%)	0.075
Mixed DCM	18 (15%)	6 (5%)	24 (20%)	0.68
Ethanolic DCM	37 (30.8%)	15 (12.5%)	52 (43.3%)	0.91
Valvular DCM	13 (10.8%)	5 (4.2%)	18 (15%)	0.95

Analyzing the same parameters but focusing on the influence of the patients' sex, we revealed the direct correlation between male sex and the presence of ischemic etiology (p = 0.05), respectively the ethanolic DCM (p = 0.000001), these statistically significant values confirming the higher incidence of ischemic events in men and, also, certain social behaviors (chronic ethylism). The same trend can be observed for the symptoms of heart failure, the proportion of male patients in NYHA III-IV classes being significantly higher than that of women (90.6% of men vs. 71.4% of women, p = 0.007). Unlike the previous aspects, no statistically significant differences regarding sex of the patients were observed for valvular and mixed etiology of DCM (tab. II).

TABLE II

Severity of symptoms and etiology of DCM related to sex

	Men No=85 (%)	Women No=35 (%)	Total No=120 (%)	p
NYHA III-IV	77 (64.1%)	25 (20.9%)	102 (85%)	0.007
Ischemic DCM	23 (19.1%)	4 (3.3%)	27 (22.4%)	0.05
Mixed DCM	17 (14.15%)	7 (5.85%)	24 (20%)	0.95
Ethanolic DCM	47 (39.1%)	4 (3.3%)	51 (42.4%)	0.000001
Valvular DCM	12 (10%)	6 (5%)	18 (15%)	0.67

Of the 79 patients with restrictive pattern, premature ventricular contractions (PVC) on ECG were identified in 31 cases (25.83% of total patients, p = 0.03), while

the left bundle branch block (LBBB) was present in 19 cases (15.83%, p = 0.05), this data revealing the statistically significant correlation between the presence of arrhythmias and severe diastolic dysfunction in patients with DCM (tab. III).

TABLE III

Correlation between ECG anomalies and restrictive pattern

	With restrictive pattern No=79 (%)	Without restrictive pattern No=41 (%)	Total No=120 (%)	p
With PVC	31 (25.83%)	8 (6.66%)	39 (32.5%)	0.03
With LBBB	19 (15.83%)	4 (3.33%)	23 (19.16%)	0.05

The proportion of male patients with restrictive pattern was significantly higher than that of women (61 *vs.* 18, p = 0.03), objectifying a cumulative effect of negative prognosis factors in these patients. Also, the elderly patients were correlated with an increased frequency of restrictive type diastolic dysfunction, out of a total of 86 patients > 60 years of age, 66 presenting this pattern (p = 0.00006) (tab. IV).

TABLE IV

Association between restrictive pattern and some epidemiological data

	With restrictive pattern No=79 (%)	Without restrictive pattern No=41 (%)	Total No=120 (%)	p
Men	61 (50.83%)	24 (20%)	85 (70.83%)	0.03
Women	18 (15%)	17 (14.15%)	35 (29.15%)	
Age>60 years	66 (55%)	20 (16.66%)	86 (71.66%)	0.00006

In patients with a restrictive pattern in the echocardiographic analysis, we noticed a statistically significant correlation between the presence of this diastolic dysfunction and an EF lower than 25% (p = 0.007), thus evidencing the presence of a severe double dysfunction, systole-diastolic, in patients with DCM. Also, the restrictive pattern correlated with an unfavorable prognosis, basically all patients with severe evolution (7.5%) or deceased (4.15%), presenting the echocardiographic characteristics of this type of diastolic dysfunction (tab. V). The presence of spontaneous echo-contrast in LV, a marker of severity, should be noted, its presence being found predominantly in cases with restrictive pattern (9 *vs.* 1, p = 0.09).

TABLE V

The influence of restrictive pattern on some echocardiographic findings and the evolution of the patients

	With restrictive pattern No=79 (%)	Without restrictive pattern No=41 (%)	Total No=120 (%)	p
EF<25%	17 (14.15%)	3 (2.5%)	20 (16.65%)	0.047
Spontaneous LV echo-contrast	9 (7.5%)	1 (0.85%)	10 (8.35%)	0.09
Severe evolution	9 (7.5%)	0	9 (7.5%)	0.02
Death	5 (4.15%)	0	5 (4.15%)	0.09

DISCUSSION

Although literature data is limited in terms of the actual prevalence of restrictive diastolic dysfunction in DCM patients, some studies have identified some epidemiological trends. Thus, both in our study and in the research carried out by Pinamonti and Mehani, there is a net predominance of male DCM patients with restrictive pattern (3, 4), an explanatory aspect being the overall prevalence of DCM among men.

Several previous studies have highlighted the role of systolic dysfunction as a negative prognosis factor in patients with DCM (9, 10). In our study, we identified 20 patients with a severely diminished ejection fraction, significantly correlated with the presence of the restrictive type pattern and thus with a reserved prognosis. A Rihal study on a group of >100 DCM patients revealed the negative prognosis role of an EF <25% in patients with DCM and diastolic dysfunction (5), due to the interdependence between LV stiffness, high filling pressures and volume-pressure curve modification. Janicki also demonstrated that when all four cavities are dilated, parietal rigidity is also influenced by the pericardial restriction, as a result globally affecting the systolic-diastolic function (6).

As for CMD etiology, most of the data in the literature plead for the association between ischemic origin and male gender, this fact being confirmed by the data from our study ($p = 0.05$). However, considering that the analyzed population originates from the NE region of Romania, two researches made by Costache *et al.* must be mentioned regarding the prevalence of cardiovascular risk factors in the two sexes, highlighting a predominance of coronary ischemia in women, which represents an important risk factor for the further development of DCM (7, 8).

Although the association between DCM and arrhythmias was confirmed by multiple evidence from literature, relatively few studies highlighted their influence on patient prognosis. In the present paper we underlined the statistically significant association between the restrictive filling pattern and the arrhythmias (premature ventricular contraction-> $p = 0.03$ or left bundle branch block (LBBB)-> $p = 0.05$), but we could not quantify their influence on the subsequent evolution. However, one study from 2014 indicates that LBBB *"per se"* negatively influences the long-term prognosis, especially as it is established after initiation of therapy with inhibitors of the conversion enzyme (14). Moreover, Costache *et al.* also demonstrated a clear association between LBBB and an infaust prognosis in patients with DCM ($p < 0.0001$) (13). In addition, considering that all DCM patients had enlarged LV, with diameters >55 mm, it is important to mention that Aleong *et al.* demonstrated the superior predictive value of LV diameter compared to diminished EF in prediction of ventricular arrhythmias, thereby being a marker for a reserved prognosis in DCM patients (16).

The restrictive pattern was associated with a negative prognosis not only in the present study but also in the studies of Shen and Pinamonti on cohorts of patients with DCM, mortality being recorded only in the groups presenting this type of filling ($p < 0.0001$). One-year survival rates were similar between our study and those of Pinamonti (95.83% *vs.* 89%), a significantly higher mortality being seen in Shen's research (survival rate of 60.3%), with the indication that the follow-up period and the

final balance respectively in the latter case was 31±12 months (3, 11).

Similar data regarding the future evolution of patients with restrictive pattern were obtained by Klein and Costache in patients with amyloidosis, confirming that restrictive-type diastolic dysfunction is a global negative prognostic factor in multiple cardiac pathologies (12, 15).

CONCLUSIONS

In conclusion, the present study reveals the major influence of the restrictive pattern on the prognosis and subsequent evolution of DCM patients, as well as its interdependence with other clinical and epidemiological parameters. Thus, the restrictive type was significantly correlated with the presence of other negative prognosis factors (systolic dysfunction, male gender, advanced age), defining a complex set of risk factors that further influence the morbidity of DCM patients. Subsequent multiannual researches with a significantly increased number of patients are needed to highlight the dynamical evolution of diastolic dysfunction as a poor prognosis factor in DCM patients and for a better risk stratification in patients at risk of sudden death or candidates for a cardiac transplantation.

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NEWS

ENVIRONMENTAL POLLUTANTS AND PREMATURE OVARIAN INSUFFICIENCY

Until now several etiological factors were described for premature ovarian failure, but not the impact of environment on this pathology which is the aim of this review. Environmental pollutants (pesticide, phenol or bisphenol, flame retardant, dioxin, cosmetic, xenobiotic, cigarettes smoke) can be deleterious to women health especially altering the reproductive function and steroidogenesis with the impact on ovarian function by three ways: endocrine disrupting chemicals; induction of oxidative stress an epigenetic modification. These ways can coexist and influence the ovarian reserve by increasing follicular atresia; increasing activation of primordial follicles; by blocking of folliculogenesis before the antral stages or by decreasing of the pool of resting primordial follicles. Moreover, some pollutants such as phthalates have a transgenerational effect by the effect on DNA methylation of imprinted genes. This review reinforces the notion that the environmental pollutants act as endocrine disruptors with a key role in the induction of POI. The severity of the syndrome depends on dose and window of exposure. Exposure to environmental pollutants is a critical public health problem that need an information of patients about the risk of POI with the possibility to prevent the consequences of this syndrome by preserving fertility (Pauline Vabre, Nicolas Gatimel, Jessika Moreau, Veronique Gayrard, Nicole Picard-Hagen, Jean Parinaud and Roger D. Leandri. Environmental pollutants, as a possible etiology for premature ovarian insufficiency: a narrative review of animal and human data. *Environmental Health* (2017): 16-37).

Natalia Darii

ERIZIPELUL - CARACTERISTICI DEMOGRAFICE, CLINICE ŞI PARACLINICE ÎN PERIOADA 2001-2010

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DEMOGRAPHIC, CLINICAL AND LABORATORY CHARACTERISTICS OF ERISYPELAS IN THE PERIOD 2001-2010 (Abstract): **Aim**: to describe demographic and clinical characteristics of erysipelas and to compare the sensitivity of two laboratory tests. **Material and methods:** we studied patients with erysipelas hospitalized in the Dermatological Clinic between 2001 and 2010. **Results:** the investigated 140 cases represented 8% of all skin infections hospitalized. Distribution of annual admissions has shown a gradual reduction in the number of admissions in the second half of the decade. A higher frequency was found in the second half of the year. Most patients were from rural areas (55%). The female gender predominated (69.3%).With few exceptions, the patients were adults averaging 59 years. The most common location was the lower leg (91.4%). Most clinical forms were mild and medium. Septic complications were absent. Recurrent erysipelas occurred in 5% of cases. Comorbidity included: chronic venous insufficiency (14.2%), hypertension (10%), obesity (8%), diabetes mellitus (5.7%) or chronic hepatitis (3.5%). **Conclusions:** the "benign" appearance of our cases is contrary to the trend rate of severe cases of cutaneous streptococcal infections in some western areas. From comparing laboratory tests results we observed their frequent mismatch. The ESR and the increase in percentage of polymorphonuclear neutrophils were more significant than the total number of leukocytes. Etiological therapy with penicillin G at a dose of 4 million UI/day was effective. The analysis allows the establishment of demographic and clinical features of erysipelas during the period and given territory.
Key words: SKIN, INFECTION, STREPTOCOCCUS

Infecţiile cutanate bacteriene sunt răspândite pe tot globul. Afectează toate vârstele. Pot fi primare sau secundare, superficiale sau profunde. Agenţii etiologici cel mai frecvent implicaţi sunt *Staphylococcus aureus* şi *Streptococcus pyogenes*. Mai rar sunt cauzate de alte bacterii Gram pozitive sau Gram negative, aerobe sau anaerobe.

Obiectivul lucrării a constat în cunoaşterea caracteristicilor demografice, clinice şi a unor aspecte paraclinice ale erizipelului într-un teritoriu din Estul României în perioada 2001-2010, dată fiind posibilitatea variaţiei acestor parametrii în raport cu spaţiul şi timpul de evoluţie. Totodată neam propus compararea sensibilităţii testelor paraclinice (hemoleucograma şi viteza de sedimentare a hematiilor) în aprecierea intensităţii procesului inflamator.

MATERIAL ŞI METODE

Am realizat un studiu retrospectiv (pentru perioada 2001-2003) şi prospectiv (2004-2010) ce a urmărit pacienţii diagnosticaţi cu erizipel în Clinica Dermatologică Iaşi.

S-au evaluat caracteristicile demografice, clinice (aspect, localizare, evoluţie) şi semnificaţia unor date paraclinice (hemoleucograma şi VSH) pentru aprecierea intensităţii procesului inflamator şi utilitatea lor în diagnosticul afecţiunii.

Analiza statistică a folosit programe computerizate specializate (Microsoft Excel, Analyse-It) pentru calcularea de valori medii, mediane, intervale de încredere 95%, testul *χ2, t-Student*. Un $p<0,05$ a fost considerat semnificativ.

REZULTATE

În perioada studiată, infecţiile cutanate bacteriene diagnosticate la 1120 de pacienţi au constituit 6,19% din totalul de 18083 internaţi. În 30% din infecţiile cutanate au fost incriminate streptococul, erizipelul fiind cea mai frecventă afecţiune, totalizând 41,6% din cazuri (140 din 336)(tab. I).

TABELUL I

Distribuţia anuală a cazurilor de piodermită streptococică

Forma clinică	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Total
Erizipel	25	19	20	9	15	7	12	11	13	9	**140**
Impetigo	14	15	18	20	13	15	9	7	6	7	**124**
Intertrigo	2	4	1	-	1	6	2	4	5	3	**28**
Ectimă	-	5	6	5	2	2	3	1	-	2	**26**
Streptococia scalpului	2	-	1	-	3	-	-	2	1	-	**9**
Pitiriazis alb al feţei	2	2	3	-	1	-	1	-	-	-	**9**
Total	**45**	**45**	**49**	**34**	**35**	**30**	**27**	**25**	**25**	**21**	**336**

Variaţia numerică anuală a internărilor pentru erizipel se situează între 11 şi 25 de cazuri cu excepţia anilor 2004 şi 2006 când s-au internat doar 9 respectiv 7 bolnavi cu această afecţiune. Numărul mediu anual de cazuri a fost de 14 (95%CI 9.9 - 18.1).

În ceea ce priveşte repartiţia lunară a internărilor rezultă preponderenţa acestora în lunile VI-XII: 62,1% din cazuri.

Cu câteva excepţii, toţi pacienţii au fost adulţi (tab. II). Vârsta medie a pacienţilor a fost de 59 ani (54 ani pentru bărbaţi, 60 pentru femei). Majoritatea pacienţilor (79,2%) au depăşit vârsta de 50 de ani.

Mediul de proveniență al bolnavilor a fost în 55 % din cazuri rural şi 69,3% au fost de sex feminin (tab. III).

Localizarea cea mai frecventă a placardului eritemato-edematos a fost la nivelul gambelor (91,4%) şi la 8.6% acesta s-a situat la coapsă, picior, faţă, sân.

Formele clinice apreciate în raport cu simptomele generale şi locale, au fost uşoare şi medii.

TABELUL II

Distribuția pe grupuri de vârstă a cazurilor de erizipel

	<15	**15-19**	**20-29**	**30-39**	**40-49**	**50-59**	**60-69**	**70-79**	**>80**	**Total**
2001	-	1	1	4	5	2	6	4	2	25
2002	-	-	1	-	-	6	9	2	1	19
2003	-	-	-	1	2	4	11	2	-	20
2004	-	-	-	1	-	1	2	1	-	5
2005	-	-	-	1	3	3	2	6	-	15
2006	-	-	-	-	-	3	2	2	-	7
2007	-	-	-	2	-	6	2	5	1	16
2008	-	1	-	-	1	4	1	4	-	11
2009	-	-	-	1	2	2	3	4	1	13
2010	-	-	-	1	1	2	2	3	-	9
Total	-	2	2	11	14	33	40	33	5	140

TABELUL III

Unele caracteristici evaluate pe un lot de 140 pacienți cu erizipel

Caracteristici demografice		**Comorbidități**	**Nr. %**
Vârsta în ani (mediana) 59 (min 20 , max 85)		IVC	20 (14,2)
Masculin	54	HTA	14 (10)
Feminin	60	Obezitate	11 (8)
Sexul		Diabet	8 (5,7)
Masculin	43 (30,7%)	Hepatită cronică	5 (3,5)
Feminin	97 (69,3%)	Etilism	2 (1,4)
Mediul de proveniență		Neoplazii	1 (0,7)
Urban	77 (45%)	TBC	1 (0,7)
Rural	63 (55%)	Ulcer gastro-duodenal	1 (0,7)
Localizarea infecției	**%**	**Forme clinice**	**%**
Gambe	91.4	Uşoare	30
Alte localizări (coapsă, picior, față, sân)	8.6	Medii	70
Erizipel recidivant	5	Letalitatea	0

Erizipelul recidivant s-a înregistrat la 5% din cazuri. Într-un singur caz s-au constatat 2 recidive, primul erizipel în 2002, al doilea după un an şi al treilea după alte 4 luni.

Comorbiditățile constatate au fost: insuficiența venoasă cronică (14,2%), hipertensiunea arterială (10%), obezitatea (8%), diabetul zaharat tip II (5,7%) şi hepatita cronică (3,5%)(tab. III).

Valorile scăzute ale VSH la 2 ore (între 15-29 mm) s-au înregistrat la 10,5% din cazuri, 39% au avut între 30-50 mm, 27,5%, 60-90 mm iar 23%, 100-150 mm. În 50,5% din cazuri valorile VSH-ului au fost de 60-150 mm (tab.IV). Valorile crescute ale VSH-ului au fost, în 29% din cazuri, concordante cu hiperleucocitoza şi procentul crescut al polimorfonuclearelor neutrofile.

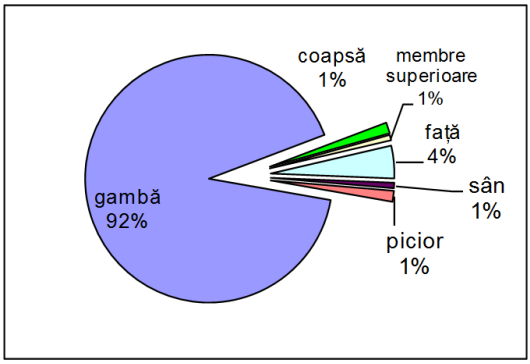

Fig. 1. Repartiția pacienților în relație cu localizarea infecției

TABELUL IV

Relația VSH-leucogramă la 57 bolnavi cu erizipel

	VSH/2h (mm)				Total
	<30	30-50	60-90	100-150	
LN PN	5	12	10	2	33
LN PC	1	3	1	1	10
LC PN	2	1	2	3	8
LC PC	2	-	2	2	6
Total	10(17.5%)	16(28%)	15(26.3%)	16 (28%)	57

LN- nr. normal leucocite, LC-nr. crescut leucocite
PN - nr. normal de polimorfonuclearеneutrofile PC- nr. crescut de polimorfonuclearеneutrofile

Terapia etiologică a erizipelului variază atât în ceea ce priveşte antibioticul folosit, dozaj, calea de administrare şi durată.

Cel mai frecvent folosite sunt betalactaminele, pe primul loc situându-se penicilina G şi V (tab. V).

TABELUL V

Antibiotice folosite în terapia erizipelului

Antibiotic	Nr. Pacienți	%
Penicilina G		
7 zile	54	38.5
10 zile	15	10.7
7 zile + Moldamin	40	28.6
Oxacilină	4	2.8
Amoxicilină	10	7.1
Ciprofloxacină	11	7.6
Eritromicină	2	1.4
Unasyn	2	1.4
Ceftriaxonă	2	1.4

În cazuistica noastră 77% din bolnavi au primit penicilina G în doză zilnică de 4 milioane UI administrată intra muscular în 4 prize; 67 % timp de 7 zile, iar pentru 10,7 % a fost de 10 zile; majoritatea dintre aceştia primind benzantin penicilina (Moldamin 1200000 U) după 7 zile de penicilina G.

Aminoglicozidele (Amoxil şi Unasyn) au fost folosite în 8,56% din cazuri, fiind utilizate în multe țări datorită căii orale de administrare şi a bio-disponibilității ridicate.

DISCUȚII

Erizipelul, în perioada studiată (2001-2010), a fost cea mai frecventă infecție bacteriană cutanată spitalizată în Clinica de Dermatologie din Iaşi - 140 (41,6% din total), dar a reprezentat numai 0,8% din patologia cutanată tratată.

S-a remarcat o reducerea numărului de internați în a doua parte a deceniului; în prima jumătate numărul mediu anual fiind de 17,6, iar în ultimii 5 ani de 10.4, diferența fiind semnificativă statistic (t=2,5, p=0,03). Această scădere a numărului de internări pentru erizipel s-ar putea datora unui acces mai facil al pacienților la o terapie orală cu antibiotice în ambulatoriu, îmbunătățirii măsurilor de prevenție sau de educație pentru sănătate a populației dar şi urmarea adresabilității spre alte specialități. Date ale unor cercetători belgieni (1), dintr-un studiu similar, au constatat o creştere a incidenței erizipelului în anii 2000-2004.

Au fost afectați în special persoane vârstnice – media 59 ani, o proporție semnificativă (45%) având şi alți factori favorizanți pentru grefarea unei infecții streptococice cutanate: insuficiență venoasă cronică – 14.2%; obezitate – 8%; diabet zaharat – 5.7%, ceea ce concordă cu datele din literatură (2).

Marea majoritate au avut ca localizare a erizipelului gamba şi doar 4,3% în aria facială. Această observație este în acord cu a lui Jorup-Ronstrom.(3) care a constatat reducerea frecvenței erizipelului feței.

Aspectul "benign" al cazurilor studiate nu corespunde cu datele publicate în ultimele decenii în Europa, Asia şi America de Nord (2).

Recidivele erizipelului au fost rar semnalate de noi (5%), comparativ cu cele ale altor specialişti care sunt în medie sunt de 18%. (3)

Cel de-al doilea obiectiv al studiului a fost stabilirea sensibilității testului VSH comparativ cu datele hemoleucogramei. Datele noastre sunt similare cu ale lui Concheiro (4,5) în ceea ce priveşte sensibilitatea mai mare a VSH-ului comparativ cu hemoleucograma dar diferă de ale acestuia în privința corelării valorilor testului cu intensitatea inflamației apreciată clinic, întrucât în cadrul cazurilor uşoare şi medii aceste valori au fost în mod egal reprezentate.

Doza de penicilină G folosită de noi se încadrează în recomandarea infecționiştilor şi dermatologilor români (1,6-3 milioane, Voiculescu; 2,4-4 milioane, Forsea; 1,6-4 milioane, Diaconu)(6, 7, 8). Efectul terapeutic benefic asupra evoluției fenomenului inflamator local, cât şi evitarea complicațiilor septice locale a fost constant.

Unii specialişti sugerează reducerea timpului de administrare la 5 zile iar alții consideră că prelungirea terapiei cu benzantin penicilină pentru prevenția recăderilor este neconvingătoare (3).

Folosirea eritromicinei la persoanele alergice la penicilină este limitată urmare a răspândirii tulpinilor de streptococ de grup A eritro-rezistente, situație remediată prin administrarea de cefalosporine din grupul I sau II (9).

Schemele terapeutice utilizate s-au dovedit eficiente realizând reducerea inflamației locale şi prevenind complicațiile septice.

CONCLUZII

Analiza caracterelor demografice, clinice şi paraclinice din Clinica ieşeană au permis evidențierea unor particularități ale erizipelului în primul deceniu al secolului XXI:

- în a doua perioadă a deceniului se constată o reducere anuală a internărilor, în special la vârsta copilului şi adolescentului;
- creşte în mod important localizarea infecției la membrele inferioare şi scade pentru extremitatea cefalică;
- se constată predominanța formelor medii şi uşoare;
- se confirmă utilitatea redusă a modificărilor hemoleucogramei în diagnosticul acestei afecțiuni;
- doza de penicilină G de 4 milioane de UI/zi i.m. a fost eficientă.

Studiul efectuat permite conturarea aspectului general al erizipelului într-o zonă estică a țării, în primul deceniu al secolului XXI.

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CANCERUL COLORECTAL – CONSIDERAŢII ASUPRA TENDINŢELOR EVOLUTIVE ALE PROFILULUI EPIDEMIOLOGIC ŞI ANATOMOCLINIC

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COLORECTAL CANCER – THE EVOLUTION TENDENCIES OF THE EPIDEMIOLOGIC AND ANATOMOCLINICAL PROFILE (Abstract): In the past 3 years colorectal cancer became the second cause death (after the broncho-pulmonary cancer) exceeding gastric cancer by 4150 deaths in 2002 (19,05%ooo inhabitants) and 4860 deaths in 2006. **Material and methods:** 644 of the 18,978 patients coloscopically investigated at the Iasi Gastroenterology and Hepatology Institute in the interval 2000-2007 in which pathology results revealed colorectal cancer, and 279 patients operated at the First Clinic Surgery of the Iasi "Sf. Spiridon" Hospital during 2008-2010 were selected for this study. **Results:** Colorectal carcinoma has a high incidence in the patients over 60 years. Sex distribution of the tumors showed that this disease most commonly affects males. The male/female ratio was 1.3. In less than 3% of the cases the disease occurred at ages under 40 years. The most frequent clinical manifestations were intestinal transit disorder (97%), pain syndrome on the left side of the abdomen (82.82%), and progressive severe constipation (67.89%). 24.07% of the colorectal cancer cases were located at the level of the proximal colon (cecum, ascending colon, hepatic flexure, transverse colon and splenic flexure), while 75.9% were located at the distal level. The most frequent macroscopic form was the vegetative one (89.91%). **Key words**: CANCER, CANCER COLORECTAL, EPIDEMIOLOGY.

Patologia tumorală reprezintă o problemă majoră de sănătate publică pentru care se alocă enorme resurse materiale şi umane în vederea descifrării mecanismelor de apariţie, dezvoltare şi progresie.

Cancerul a provocat decesul a 7,6 milioane de persoane la nivel mondial în 2008, mai ales în ţările în curs de dezvoltare, potrivit unui studiu al Centrului internaţional de cercetări asupra cancerului (CIRC, organism al OMS), dat publicităţii în 2008. 56% dintre cele 12,7 milioane de noi cazuri de cancer şi 63% din totalul de 7,6 milioane de decese asociate unui cancer au survenit în ţări în curs de dezvoltare. Potrivit CIRC, mortalitatea asociată cancerului a crescut în ultimii ani în lume, cele mai frecvente forme de cancer fiind cel pulmonar, de sân, colorectal, de stomac şi de prostată.

Cancerul colorectal este ce mai frecventă tumoră malignă şi cea mai frecventă cauză de deces prin cancer în ţările industrializate din Europa (58.300 decese/an) şi Statele Unite ale Americii(156.000 cazuri noi/an) (2). În România, frecvenţa CCR este în creştere rapidă (dublarea incidenţei şi mortalităţii în ultimii 20 de ani) atingând

în anul 2000 o incidenţă de 17,74%ooo pe an, ceea ce plasează ţara noastră în rândul ţărilor cu incidenţă medie a bolii. În ultimii 3 ani, CCR a devenit a doua cauză de deces (după cancerul bronho-pulmonar, devansând cancerul gastric cu un număr de 4150 de decese în 2002 (19,05%ooo locuitori). şi 4860 decese în 2006. Circa 60% din pacienţii cu CCR se prezintă cu boală avansată, metastazele hepatice fiind cele mai frecvente (1).

Incidenţa medie a cancerului colorectal în judeţul Iaşi în perioada (2004 - 2007) a fost de 15,23 la 100.000 de locuitori. Această valoare aşează judeţul Iaşi într-o zonă de incidenţă medie în raport celelalte zone ale ţării.

Mortalitatea medie în aceasta perioadă a fost de 12,5 ‰ în judeţul Iaşi faţă de 18,25 ‰ în ţară (3).

MATERIAL ŞI METODE

Pentru studiul tendinţelor evolutive ale profilului epidemiologic şi anatomoclinic al cancerului colorectal în studiu s-a avut în vedere perioada 2000 – 2007 interval în care au fost investigaţi colonoscopic în cadrul Institutului de Gastroenterologie şi Hepatologie Iaşi 18.978 pacienţi dintre care pentru studiul de faţă au fost selectaţi 644 pacienţi la care rezultatul anatomopatologic a relevat cancer colorectal şi un alt lot de 279 pacienţi operaţi în Clinica nr.1 Chirurgie a Spitalului „Sf. Spiridon” Iaşi în perioada 2008-2010.

Cele 644 de cazuri au beneficiat toate de examen anatomo-patologic efectuat pe specimene rezultate în urma examenului colonoscopic iar cele 279 de cazuri au beneficiat pe lângă examenul histopatologic al tumorii primare şi de examenul histopatologic al metastazei la distanţă.

Realizarea studiului a necesitat cunoaşterea principalelor date clinice (vîrstă , sex, domiciliu, data diagnosticului, sediul anatomic al tumorii şi a aspectelor anatomopatologice tumorale – macroscpia, microscopia şi stadiul patologic. Pentru aceasta au fost studiate foile de observaţie a bolnavilor, registrul de examene endoscopice, registrul de protocoale operatorii şi registrul examenelor anatomo-patologice.

Prelucrarea datelor s-a efectuat cu ajutorul programului de statistic *S.P.S.S.* şi programului MS EXCEL 2007.

REZULTATE

Se remarcă faptul că frecvenţa sexului masculin în lotul de studiu este uşor mai crescută (59,63%). Repartiţia cazurilor în funcţie de sexul pacienţilor pe anii de studiu a evidenţiat o menţinere aproximativ constantă a frecvenţei cazurilor în perioada studiată cu excepţia anului 2000. În acest interval pacienţii de sex masculin au prezentat o frecvenţă uşor mai ridicată (50%-63,3%) comparativ cu frecvenţa pacienţilor de sex feminin (40%-50%), excepţie făcând anul 2000 când frecvenţa pacienţilor de sex masculin ce prezentau cancer colorectal a fost de 80,65%, valoare semnificativ mai mare comparativ cu valoarea corespunzătoare pacienţilor de sex feminin (19,35%).

Analiza distribuţiei cazurilor diagnosticate cu cancer colorectal în funcţie de mediul de provenienţă evidenţiază o frecvenţă a cazurilor din mediul rural de 32,6% valoare mică comparativ cu frecvenţa cazurilor din mediul urban (67,6%). Repartiţia anuală a cazurilor în funcţie de mediul de provenienţă duce la concluzia că pe toată perioada studiată au predominat cazurile din mediul urban, frecvenţa lor fiind de peste 51,6%.

Indicatorii statistici ai vârstei în funcţie de sexul pacienţilor evidenţiază valori mi-

nime de 19 ani atât la pacienţii de sex feminin cât şi la cei de sex masculin şi maxime de 86 ani, respectiv 87 ani pentru bărbaţi. Valoare mediană indică faptul că 50% dintre pacienţi au vârsta mai mică de 56 ani, aspect valabil pentru femei, iar pentru bărbaţi valoare mediană fiind 57 ani. Rezultatele testelor neparametrice de comparaţie şi corelaţie demonstrează pe de o parte diferenţa semnificativă a numărului de cazuri provenite din mediul urban comparativ cu cele provenite din mediul rural (χ^2=21,55, p=0,00303, 95%CI) şi tendinţa de creştere a numărului de cazuri din mediul urban în ultimii ani (r=0,539, p=0,0138). Aprecierile au fost făcute pentru un interval de încredere de 95%.

Compararea valorilor medii ale vârstei pentru cele două loturi analizate evidenţiază faptul vârsta medie a pacienţilor de sex feminin nu diferă semnificativ de vârsta pacienţilor de sex masculin. Vârsta medie a pacienţilor de sex masculin a fost de 63,7±12 cu valori minime de 19 şi maxime de 86 iar vârsta medie a pacienţilor de sex feminin a fost de 63,8±12,2 cu minim de 19 şi maxim de 87 ani.

Distribuţia pe segmente a cancerului colorectal (fig. 1) se prezintă astfel: rect 378 cazuri (58,70%), sigmoid si joncţiune rectosigmoidiană 88 cazuri (13.6%), colon descendent 23 (3,57%), colon transvers 80 cazuri (12,42%) şi colon ascendent 75 cazuri (11,65%).

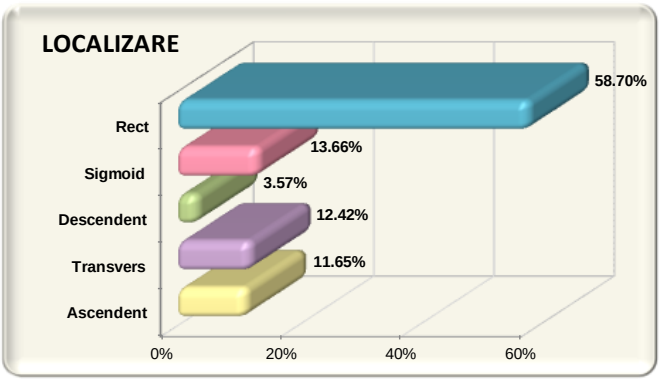

Fig. 1. Repartiţia cazurilor în funcţie de localizare

La nivelul colonului proximal (cec, colon ascendent, unghi hepatic, transvers şi unghi splenic) au fost identificate 24,07% cazuri de cancer colorectal, în timp ce la nivel distal am regăsit 75,9% din cazuri, valoare net superioară localizării proximale**.**

Analiza distribuţiei cazurilor în funcţie de localizare şi sexul pacienţilor evidenţiază prezenţa localizării pe colonul ascendent mai frecventă la sexul masculin 72% în timp ce la pacienţii de sex feminin această localizare este regăsită la 28% din cazuri. O frecvenţă similară, mai mare la pacienţii de sex masculin, o regăsim în cazul localizării carcinomului pe colonul transvers (67,5%) şi pe rect (58,99%). În cazul localizării pe sigmoid şi pe colonul descendent regăsim o frecvenţă uşor mai ridicată în rândul pacienţilor de sex feminin (52,27% respectiv 52,17%).

Studiul a demonstrat existenţa unei asocieri moderate între localizare şi grupa de vârstă a pacienţilor astfel, se remarcă faptul

că pe colonul descendent întâlnim numai pacienţi cu vârstă peste 50 ani în timp ce pe colonul transvers ponderea aparţine grupei de vârstă sub 50 ani (64%).

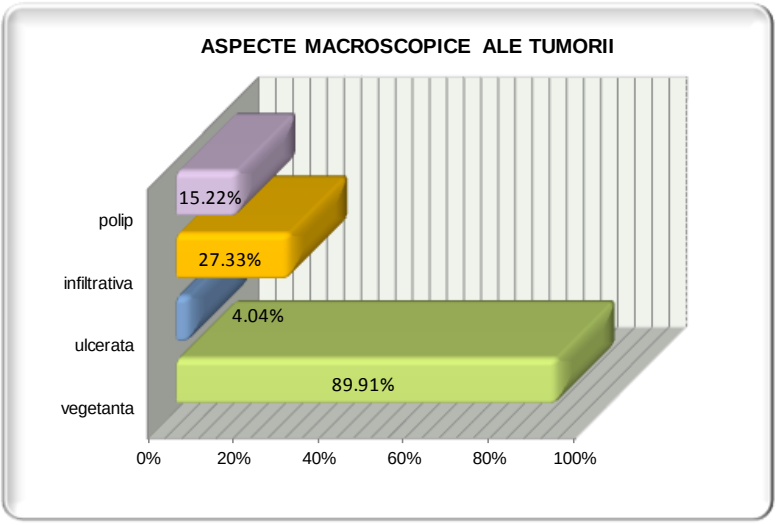

Fig. 2. Repartiţia cazurilor în funcţie de aspectele macroscopice

Analiza aspectelor macroscopice prezente la pacienţii lotului de studiu (fig. 2) a evidenţiat o frecvenţă mare a formelor vegetante (89,91%). Un procent semnificativ comparativ cu formele ulcerate (4,04%) îl prezintă polipii ce se regăsesc în proporţie de 15,22%. Formele infiltrative au fost prezente în proporţie de 27,33%.

În perioada analizată frecvenţa cazurilor în funcţie de aspectele macroscopice s-a schimbat semnificativ pentru formele infiltrative şi cele ulcerate evidenţiate colonoscopic. În cazul formelor infiltrative dacă în anul de început al studiului întâlneam 25,8% cazuri, procentul a crescut semnificativ atingând în anul 2004 un maxim de 44,7%, după care s-a înregistrat o uşoară scădere a numărului de cazuri. Pentru formele ulcerate se înregistrează o scădere bruscă a numărului de cazuri în ultimii 3 ani ai studiului frecvenţa ajungând la 0% (2005-2007). La începutul intervalului (2000) frecvenţa formelor ulcerate a fost de 16,1% iar în anul 2004 de 7,9%.

În tabelul I putem remarca faptul că dintr-un număr total de 76 cazuri de cancere colorectale prelevate din biopsii polipoide 64% erau formaţiuni polipoide, 28% polipi pediculaţi şi 8% polipi sesili.

TABELUL I

Reprezentarea grafică procentuală a tipurilor de polipi biopsiaţi

	2000	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**
Nr. total de polipi	2	3	7	8	9	12	15	20
Polipi pediculaţi	50 %	66,6 %	28,5 %	25 %	22,2 %	25 %	26,6 %	25 %
Polipi sesili	---	---	---	---	11,1 %	8,33 %	13,3 %	10 %
Formaţiuni polipoide	50 %	33,3 %	71,4 %	75 %	66,6 %	66,6 %	60 %	65 %

Studiul comparativ al vârstei pacienţilor în funcţie de dimensiunea polipilor colorectali evidenţiază faptul că pacienţii cu dimensiuni ale polipilor de peste 10mm au vârste semnificativ mai mari (vârsta medie 63,9 ani, p=0,00, 95%CI) comparativ cu vârsta pacienţilor prezintă dimensiuni mai mici de 10mm (55,5 ani – sub 5 mm respectiv 58,4 ani – 6÷10mm).

Din lotul studiat adenocarcinomul moderat diferenţiat reprezintă 46,43% , adenocarcinomul bine diferenţiat 42,24%, adenocarcinomul slab diferenţiat 7,14 % restul fiind reprezentat de adenocarcinomul mucinos, cel cu celule inel de pecete, adenoscuamos şi de carcinomul *in situ*.

Urmărind protocoalele operatorii a 279 pacienţi operaţi în Clinica nr.1 Chirurgie a Spitalului „Sf. Spiridon" Iasi în perioada 2008-2010 am efectuat un studiu privind rezultatele examenului anatomo-patologic a specimenelor tumorale rezecate chirurgical, a ganglionilor regionali precum şi a metastazelor la distanţă.

Stadializarea patologică a carcinoamelor conform criteriilor TNM (O.M.S.,2000) a fost urmatoarea:

- repartiţia cazurilor în funcţie de tumora primară: T1 12,18%, T2 18,27%, T3 59,19%, T4 10,39 %.
- repartiţia cazurilor în funcţie de ganglioni limfatici regionali: Nx 16,12%, N0 24,37%, N1 38,7%, N2 18,27%, N3 2,5%.
- repartiţia cazurilor în funcţie de metastazele la distanţă: Mx 69,17%, Mo 12,54% , M1 M1 18,27%.
- repartiţia cazurilor în funcţie de gradul histologic: G1 18,27% , G2 56,98% , G3 24,73%.
- repartiţia cazurilor în funcţie de stadiul CCR: Stadiul I 16.48%, Stadiul II 30.46%, Stadiul III 42.65%, Stadiul IV 10.39%.

DISCUŢII

Organizaţia Mondială a Sănătăţii (O.M.S., 2000) a estimat 875000 de cazuri de cancer colorectal în întreaga lume în 1996, reprezentând aproximativ 8,5% din toate cazurile noi de cancer. Acesta este cea mai frecventă cauză de deces prin cancer în ţările industrializate din Europa (58300 decese/an) şi SUA (156000 cazuri noi/an) (4). Deşi considerată multă vreme o tumoră a vârstei înaintate, carcinomul colorectal devine tot mai frecvent un diagnostic al adultului în plină activitate, formele grave (tipuri histologice slab diferenţiate şi nediferenţiate) apărând chiar sub 40 de ani.

În America, cancerul colorectal reprezintă a doua cauză de deces, numărul de noi cazuri anuale fiind aproximativ egal pentru bărbaţi si femei (5). Este principala boală în ţările din vestul Europei fiind a doua cauză de deces prin cancer;prevalenţa ei este în creştere (6).

În Anglia este principala boală, cu 30.000 noi cazuri diagnosticate în fiecare an şi aproximativ 17.000 de decese/an. Vârsta medie a incidenţei este în decada a şaptea fiind cea mai obişnuită cauză de deces prin cancer după cel pulmonar la bărbaţi Şi după cel de sân la femei. (6). Peste 90% din cazuri sunt sporadice şi cel mai frecvent tip, responsabil pentru 95% din cazuri, este adenocarcinomul.

În Australia, cancerul colorectal este a doua cauză de deces după cancerul pulmonar (7). Aproximativ 50% din cancere sunt atribuite factorilor alimentari şi aproximativ 15-20% factorilor genetici inclusiv sindroamele familiale cu risc înalt.

În Italia cancerul colorectal este al patrulea tip de cancer in funcţie de incidenţă şi a doua cauză de deces prin cancer (8).

Cu aproximativ 57.000 de noi cazuri în fiecare an, reprezintă cel mai frecvent tip

de cancer în Germania (9), fiind înaintea cancerului de sân (aproximativ 46.000 noi cazuri) şi a cancerului pulmonar (aproximativ 37.000). Cu toate ca incidenţa globală este in creştere, în Germania incidenţa este mai crescuta la bărbaţi. Rata mortalităţii (aproximativ 26.500 decese anual) este in declin la bărbaţi în ultimii 10 ani şi la femei în ultimii 20 de ani.

În România, incidenţa cancerului colorectal (CCR) este considerată medie (18,55/100.000 locuitori), constatându-se, în ultimii 10 ani, o creştere marcată a acesteia, de la 10,1 in 1989 la 18,55 în 1999. Incidenţa cancerului colorectal reprezintă 8% din incidenţa neoplaziei, în general. Atât la bărbaţi, cât şi la femei, este pe locul 3 în România după cancerul bronhopulmonar şi gastric şi, respectiv, sân şi uter (10).

Cancerul colorectal rămâne incontestabil o importantă problemă de sănătate publică.

CONCLUZII

Se observă o creştere a numărului de cazuri în ultimii ani ai perioadei studiate (2005, 2006, 2007) în raport cu anii de început (2000, 2001), ajungându-se în anul 2007 la 6,82% de cazuri cu cancer colorectal din numărul total de colonoscopii efectuate în timp ce în anul 2000 frecvenţa a fost de 1,52%.

Tendinţele în evoluţia incidenţei neoplasmului de colon ne arată că în anii marginali ai studiului (2000-2007) numărul cazurilor cu tumori slab diferenţiate a rămas relativ constant, în timp ce incidenţa neoplasmelor bine şi moderat diferenţiate a crescut de aproximativ 7 şi, respectiv, 4 ori. În studiul nostru se observă creşterea numărului de cazuri în ultimii 3 ani.

Nici o formă nu prezintă o predispoziţie semnificativă pentru pacienţii de un anumit sex, cu alte cuvinte forma macroscopică nu se asociază cu sexul pacienţilor. Carcinoamele colorectale prezintă o incidenţă crescută la pacienţii peste 60 ani.

Analiza aspectelor macroscopice prezente la pacienţii lotului de studiu a evidenţiat o frecvenţă mare a formelor vegetante (89,91%). Un procent semnificativ comparativ cu formele ulcerate (4,04%) îl prezintă polipii ce se regăsesc în proporţie de 15,22%. Formele infiltrative au fost prezente în proporţie de 27,33%.

Nu există o corelaţie semnificativă între localizarea tumorii şi aspectul macroscopic al acesteia.

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NOUTĂȚI

EFECTELE CONTAMINĂRII CU SALIVĂ ŞI SOLUȚIILE DE CURĂȚARE ASUPRA ADEZIUNII LA DENTINĂ A SISTEMELOR AUTOGRAVANTE

Contaminarea cu salivă reprezintă o problemă frecventă a stomatologiei restaurative, a cărei rezolvare a devenit şi mai importantă odată cu extinderea tehnicilor adezive. Contaminarea substratului aderent cu salivă, sânge sau fluid sulcular compromite sau diminuează semnificativ adeziunea materialului de restaurare, tehnicile de izolare sunt consumatoare de timp şi uneori ineficiente iar soluțiile utilizate pentru decontaminare interferă ele insele adeziunea. Studii recente par să indice faptul că unele sisteme adezive autogravante, deşi considerate mai puțin eficiente decât cele tradiționale cu gravaj total, de ar putea fi mai puțin sensibile la contaminare. Acest studiu a determinat efectul contaminării cu salivă şi al utilizării unor soluţii de curăţare asupră rezistenţei adeziunii asupra unor astfel de sisteme autogravante. Şaptezeci şi cinci de molari umani au fost secţionaţi pentru a expune dentina medie coronară şi au fost împărţiţi aleatoriu în cinci grupe: nici o contaminare, contaminarea saliva fără curăţare, contaminare cu salivă şi curăţare cu apă, contaminare cu salivă şi curăţare cu clorhexidină 2%, şi contaminare cu salivă şi curăţare cu hipoclorit de sodiu 5%. Câte o treime dintre eşantioanele din fiecare grup au fost tratate cu Adper Prompt L-Pop (3M ESPE), Adper Easy Bond (3M ESPE), respectiv Clearfil SE Bond (Kuraray). Probele au fost restaurate cu materiale compozite şi procesate pentru testarea rezistenţei adeziunii la microtensiune. Rezistenţele medii ale adeziunii au variat de la 17.3 MPa, pentru Adper Prompt L-Pop după curăţarea cu apă la 69.3 MPa pentru Clearfil SE Bond după curăţare cu apă. Pentru toate cele trei sisteme adezive, nu au existat diferenţe statistic semnificative între grupurile contaminate cu salivă, grupurile contaminate şi apoi curăţate, şi grupurile necontaminare. Rezultatele acestui studiu susţin ipoteza că performanţele sistemelor adezive testate nu sunt influenţate în mod semnificativ de contaminarea cu salivă sau de substanţele utilizate pentru curăţarea plăgii dentinare (Sheikh H , Heymann HO , EJ Jr Swift , TL Ziemiecki , AV Ritter . Effect of saliva contamination and cleansing solutions on the bond strengths of self-etch adhesives to dentin. *J Esthet Restor Dent* 2010; 22(6): 402-410).

Gianina Iovan

CERCETĂRI EXPERIMENTALE PRIVIND INFLUENȚA FACTORILOR DE STRES PE UN MODEL ANIMAL DE HIPERTENSIUNE ARTERIALĂ

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EXPERIMENTAL RESEARCH ON THE INFLUENCE OF STRESS FACTORS IN AN ANIMAL MODEL OF HYPERTENSION (Abstract): **Aim**: to investigate the influence of some stress factors in endothelin-1–induced hypertension. **Material and methods:** The experiment was carried out on Wistar rats, treated intraperitoneally, for 4 weeks, as follows: **Group 1:** saline solution (0.5ml/100g weight); **Group 2**: saline solution + stress; **Group 3**: Endotelin-1; **Group 4**: Endotelin-1 + stress; **Group 5**: Endotelin-1 + cholesterol diet (0,2g/kbw/day); **Group 6**: Endotelin-1 + cholesterol diet + stress. Endotelin-1 0.25nmol/kbw was initially administered, followed by 0.5nmol/kbw 15 min later, 2 times/week. Stress-inducing factors were immobilization and water immersion. In the 28^{-th} day of the experiment blood pressure was measured and blood samples were taken from the retro-orbitary plexus to assess plasma cortisol, blood count, phagocytic capacity of peripheral neutrophils, and serum complement activity. **Results and discussions**: Repeated administration of endotelin-1 determined an increase in blood pressure, statistically significant in stress conditions comparing to non-stressed animals. Our study proved a decrease of plasma cortisol, total leukocyte count, phagocytic capacity of peripheral neutrophils, without significant alterations in serum complement activity. **Conclusions**: Chronic exposure to complex stress conditions in rats with endothelin-1-induced hypertension determined a decrease of plasma cortisol levels, effect correlated with elevated blood pressure and decrease in the number and phagocytic function of peripheral neutrophils. **Key words**: STRESS, CORTISOL, BLOOD PRESSURE

Stresul reprezintă consecința lipsei de răspuns corespunzător a unui organism la agresiuni emoționale sau fizice. În general, stresul este determinat atât de factori interni, cât şi externi. Organismul uman răspunde la factorii stresori, prin activarea sistemului nervos şi a hormonilor specifici. În timpul unei reacții anxioase, printr-un lanț de stimulări ale sistemului nervos şi ale celui endocrin, sunt eliberați anumiți mediatori endogeni: catecolamine (adrenalină, noradrenalină), cortizol, vasopresină, ACTH, STH. Prima dintre substanțele eliberate este adrenalina, de către glandele suprarenale. Aceşti mesageri primari acționează asupra celulelor din diferite țesuturi şi organe, producând modificări funcționale, inițial, reversibile, apoi ireversibile. De exemplu, printre efectele rezultate în urma acțiunii catecolaminelor la nivelul inimii,

sunt: creşterea frecvenţei cardiace, a contractilităţii, a automatismului cardiac. La acelaşi nivel, cortizolul produce: creşterea debitului cardiac, creşterea răspunsului la catecolamine, hipervolemie (1,2).

Stresul eliberează catecolamine, care pot, indirect, să determine creşterea acizilor graşi şi a nivelului glucozei în sistemul circulator. Acestea pot fi convertite în lipide şi colesterol şi depozitate la nivelul pereţilor arteriali. Aceste depozite realizează o rezistenţă în fluxul circulator şi contribuie, astfel, la creşterea tensiunii arteriale. Stresul mental cronic creşte presiunea arterială, precum şi nivelul colesterolului, conducând, astfel, la hipertensiune arterială cronică şi dislipidemie. Studiile efectuate au pus în evidenţă existenţa unor corelaţii între diferitele tipuri de stres, reactivitatea organismului şi modificările presionale (3,4).

Scopul acestei cercetări a fost de a stabili influenţa unor factori de stres pe un model de hipertensiune arterială indusă experimental.

MATERIAL ŞI METODĂ

Au fost utilizate 6 loturi de câte 6 şobolani Wistar, cu greutate cuprinsă între 180-200g, cu repartiţie uniformă pe sexe, proveniţi de la Institutul de Sănătate Publică din Iaşi, trataţi intraperitoneal, timp de 28 de zile, începând cu ora 8 dimineaţa, conform schemei:

L1 (SF): Ser fiziologic (0,5 ml/100g c/zi);

L2 (SF+s): Ser fiziologic + stres (indus de imobilizare şi imersie în apă);

L3 (ET-1): Endotelină-1 (iniţial 0,25 nmol/kgc, iar după 15 min 0,5 nmol/kgc, 2 administrări pe săptămână);

L4 (ET-1+s): Endotelină-1 + stres;

L5 (ET-1+col): Endotelină-1 + dietă cu colesterol (0,2 g/kgc/zi);

L6 (ET-1+col+s): Endotelină-1 + dietă cu colesterol + stres.

După 28 de zile de tratament, s-a recoltat sânge din plexul retroorbitar (respectând condiţiile de etică a experimentului pe animale de laborator) şi au fost determinaţi următorii parametri: tensiunea arterială, nivelul cortizolului sanguin, formula leucocitară, capacitatea de fagocitoză a polimorfonuclearelor neutrofile (PMN) din sângele periferic, activitatea complementului seric.

REZULTATE ŞI DISCUŢII

Modelul experimental realizat poate fi folosit cu succes, ca o alternativă la achiziţionarea de şobolani modificaţi genetic, pentru a dezvolta hipertensiune arterială, ca şi pentru studierea implicării endotelinei în modelele de hipertensiune arterială indusă experimental la animale de laborator.

Stresul cronic realizat a antagonizat efectele imunomodulatoare ale endotelinei-1, de inducere a leucocitozei, cu creşterea absolută a PMN şi a funcţiilor fagocitare ale acestora.

Valorile absolute ale tensiunii arteriale au fost determinate cu sfigmomanometrul HAMEG (fig. 1).

Administrarea repetată de endotelină-1 determină creşterea valorilor tensiunii arteriale la şobolanii Wistar. Valorile tensiunii arteriale sunt semnificativ statistic crescute la animalele de laborator expuse la stres, comparativ cu valorile acestui parametru la animalele care nu au fost menţinute în aceste condiţii experimentale. Cele mai ridicate valori ale tensiunii arteriale au fost înregistrate la şobolanii Wistar cărora li s-a administrat endotelină-1, dietă cu colesterol şi au fost expuşi unui stres cronic.

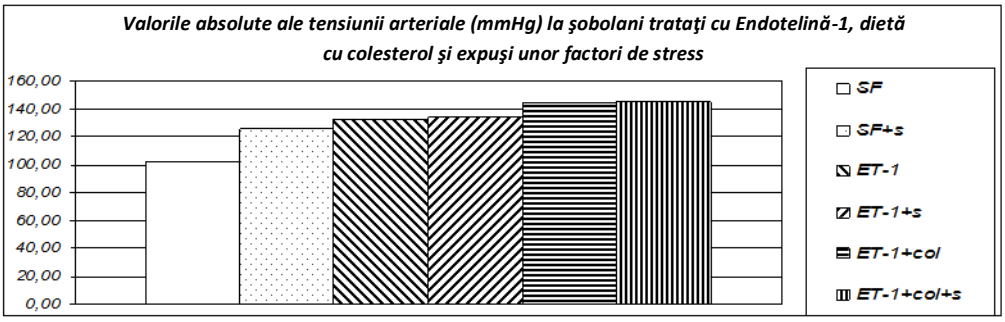

Fig. 1. Valorile absolute ale tensiunii arteriale după 28 de zile

În condițiile experimentale ale stresului cronic realizat, s-a constatat o scădere a valorilor cortizolului plasmatic (fig. 2), comparativ cu lotul martor, acest efect fiind semnificativ statistic mai puternic la animalele cu HTA indusă experimental, cu dietă cu colesterol plus menținerea în condiții de stres. Datele din literatura de specialitate demonstrează că, în cazul persoanelor stresate, valorile cortizolului se mențin crescute pe tot parcursul zilei (5). Tensiunea arterială, de asemenea, creşte. Când stresul devine cronic, rezervele de cortizol scad. Astfel, dacă, inițial, secreția hormonului este ridicată, în timp, ea devine inexistentă (2).

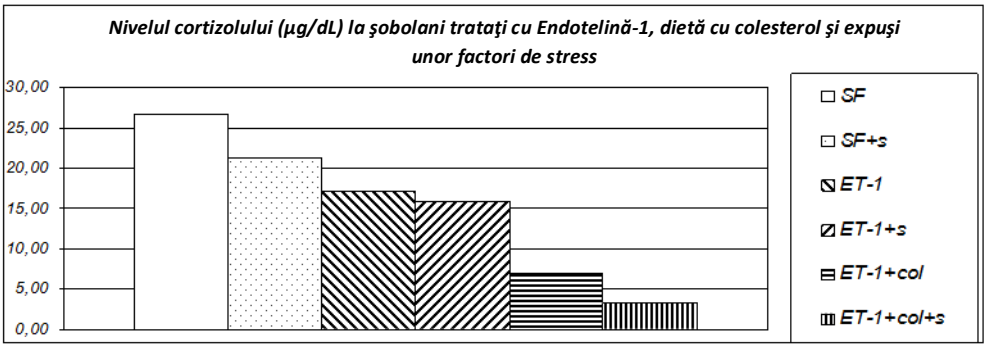

Fig. 2. Nivelurile cortizolului sanguin după 28 de zile

În condițiile experimentale realizate, s-a constatat o scădere a numărului total de leucocite (fig. 3), rezultată din scăderea PMN (fig. 4), precum şi o scădere a capacității de fagocitoză a PMN din sângele periferic (fig. 5), comparativ cu lotul martor. Astfel, stresul cronic a indus nu numai scăderea nivelului sanguin al cortizolului, ci şi o scădere semnificativă statistic a numărului şi funcției PMN. Administrarea repetată de endotelină, asociată sau nu cu dieta cu colesterol, nu a determinat modificări semnificative ale acestor parametri.

Nu s-au constatat modificări semnificative statistic ale activității complementului seric.

Condițiile de stres cronic în care au fost menținute animalele cu HTA indusă experimental cu endotelină-1 şi supuse unei diete cu colesterol au condus la creşterea presiunii arteriale şi la o scădere a nivelului cortizolului sanguin.

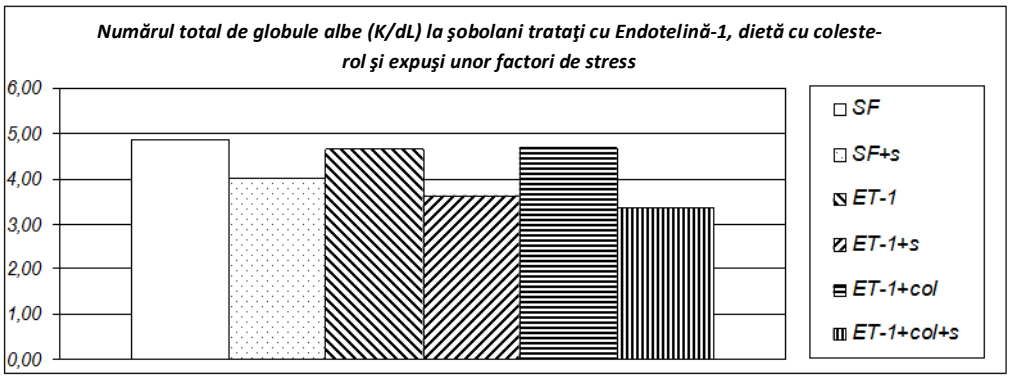

Fig. 3. Valorile leucocitelor după 28 de zile

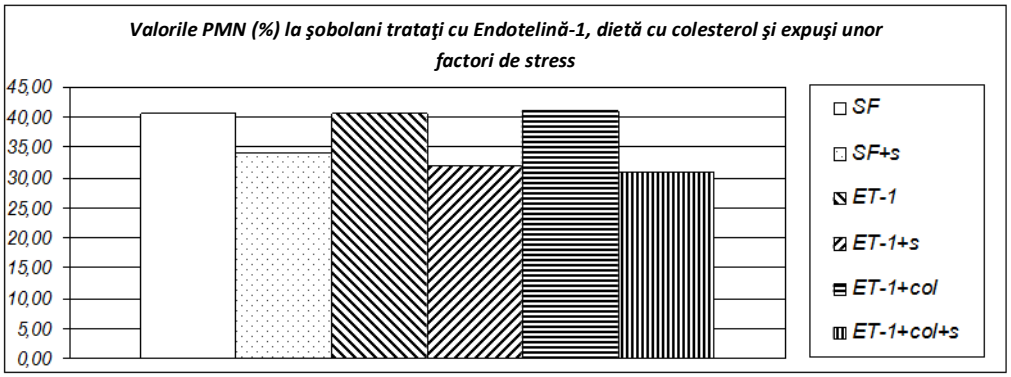

Fig. 4. Valorile PMN după 28 de zile

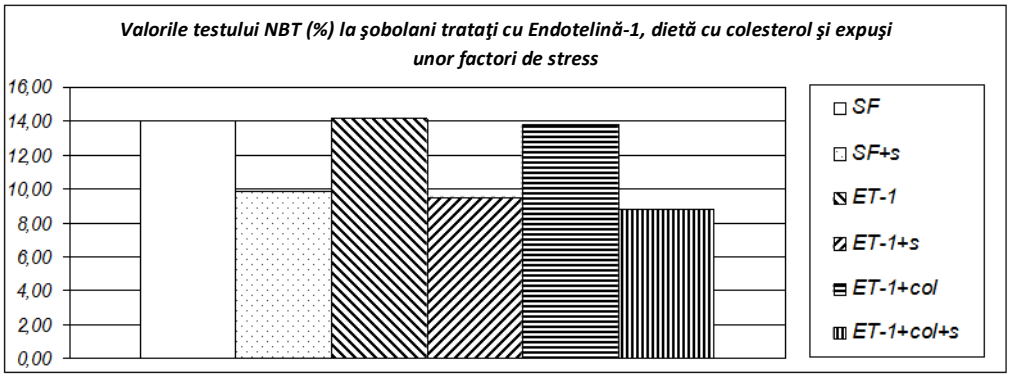

Fig. 5. Valorile testului NBT după 28 de zile

CONCLUZII

Administrarea repetată de endotelină-1 a determinat creşterea valorilor tensiunii arteriale la şobolanii Wistar. Valorile obținute au fost semnificativ statistic crescute la şobolanii expuşi la stres, comparativ cu nivelurile tensiunii arteriale la animalele care nu au fost menținute în aceste condiții experimentale. Cele mai ridicate valori ale tensiunii arteriale au fost înregistrate la

şobolanii Wistar cărora li s-a administrat endotelină-1, dietă cu colesterol şi au fost expuşi unui stres cronic.

În condițiile experimentale ale stresului cronic realizat, s-a constatat o scădere a valorilor cortizolului plasmatic, comparativ cu lotul martor, acest efect fiind semnificativ statistic mai puternic la animalele de laborator cu HTA indusă experimental, menținute în condiții de stres şi având, ca factor de risc, dieta cu colesterol.

În condițiile experimentale realizate, s-a constatat o scădere a numărului total de leucocite, rezultată din scăderea PMN, precum şi o scădere a capacității de fagocitoză a PMN din sângele periferic, comparativ cu lotul martor. Stresul cronic a indus, deci, scăderea nivelului sanguin al cortizolului, corelată cu o scădere semnificativă statistic a numărului şi funcției fagocitare a PMN. Condițiile experimentale de stres cronic realizate au menținut imunosupresia indusă, inițial, de cortizol, pentru ca, la finalul studiilor, să determine scăderea nivelului plasmatic al cortizolului, conducând la concluzia reducerii rezervelor din acest glucocorticosteroid. Administrarea repetată de endotelină, asociată sau nu cu dieta cu colesterol, nu a determinat modificări semnificative statistic ale acestor parametri. Nu s-au constatat modificări semnificative statistic ale activității complementului seric.

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PSYCHOLOGICAL APPROACH IN THE MANAGEMENT OF IRRITABLE BOWEL SYNDROME

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PSYCHOLOGICAL APPROACH IN THE MANAGEMENT OF IRRITABLE BOWEL SYNDROME (Abstract): Irritable bowel syndrome has a worldwide prevalence that affects up to 10-15% adults of all ages, being related with a significant health care and economic involvement. The factors that play a role in the alteration of the brain-gut axis include genetic predisposition, an intestinal infection prior to symptoms onset, or chronic stressful life events. People with IBS should discuss with the gastroenterologist in developing a management plan to address these issues effectively and improve quality of life. An important step for the diagnosis is to look for symptoms and signs that are suggestive for other conditions, like inflammatory bowel disease, or celiac disease. The treatment for IBS includes life style changes, medication, counseling with psychological and behavioral therapies. **Keywords:** IRRITABLE BOWEL SYNDROME, PSYCHOLOGICAL, MANAGEMENT.

INTRODUCTION. EPIDEMIOLOGICAL DATA

Irritable bowel syndrome (IBS) is a long-term recurrent disorder of gastrointestinal (GI) functioning with disturbances of gut motility. It is characterized by abdominal pain or discomfort, bloating and altered bowel habit (chronic or recurrent diarrhea, constipation, or both – either mixed or in alternation).

IBS has a significant worldwide prevalence that affects up to 10-15% of adults and between 25 and 45 million people in the United States. It involves persons of all ages and about 2 out of 3 IBS patients are females. This functional disease can also severely compromise a person's quality of life, being the second cause of absenteeism from work after the common cold (1).

Studies have shown that IBS patients have an increased number of outpatient health care visits, diagnostic tests, and even surgeries (2). The symptoms are the consequence of increased sensitivity to gas, food, or stool in the bowel and appear from disturbances in colonic motility. Finally, the bowel tends to be overly reactive to various factors (emotional stress, GI infections, eating, gaseous distension, or menstrual period) which can bring about or amplify the symptoms (3).

ETIOPATHOGENESIS

There are possible genetic factors and

prior adverse life experiences that can predispose to IBS, but the exact cause of it is not completely understood.

Brain-gut interactions

The altered patterns of colonic motility appear to be due to disruptions in the communication between the brain and gut, the interaction being known as the brain-gut axis. These bi-directional interactions respond to any potential disturbance or stressor and are important in maintaining normal bowel function. The factors that may play a role in the alteration of the brain-gut axis include: genetic predisposition, intestinal infection prior to symptom onset, chronic stressful life events, or other psychosocial factors.

PSYCHOLOGICAL FACTORS

Some people with IBS report symptoms such as anxiety, or depressed mood that occur mainly in persons with more severe symptoms and in patients seen in tertiary medical care referral centers. Psychological factors are not a primary cause of IBS, but they may influence how a person seeks to manage and deal with IBS (4).

Emotional distress may be associated with a worsening of IBS symptoms that may induce an anticipatory anxiety due to the severity, unpredictability, and negatively perceived consequences of having an "attack." This may result in continuing symptom occurrence and set up a vicious cycle between emotional distress, symptoms, and personal management strategies.

Many people experience abdominal pain and constipation or diarrhea without any evidence of anxiety, depression, or other psychological symptoms. This response in people with IBS is more pronounced on a recurrent or chronic basis and therefore, they are more likely to experience worse symptoms when they are exposed to a significant stressor.

Addressing these issues with an empathetic doctor, who can refer the patient for counseling, would improve IBS symptoms and daily function. In patients with IBS there is increased GI response to stress which basically represents anything that can stimulate the GI tract, including: diet, psychological stress, hormonal changes, physical activity that has been shown to increase motility and sensation of the colon to a greater degree in these patients.

Stress disturbs the balance between brain and body, with or without conscious feelings of anxiety, distress, or anger. It can be acute (short term) or chronic (more than three months), ranging from daily hassles to life-threatening events. People presenting IBS should work with their health care providers in developing a management plan to address these issues effectively, in order to decrease symptoms and improve, overall, quality of life (5).

DIAGNOSIS

The first step in making a positive diagnosis of IBS is for the doctor to identify if an individual has the symptoms of IBS. This is best determined by the use of the Rome Criteria, which is a collection of the most common symptoms that typify the disorder. This includes abdominal pain for several months that is associated with two of the following: the pain is relieved by defecation, the pain is associated with an increase or decrease in stool frequency, and/or the pain is associated with the stools becoming harder or softer in consistency (6).

The next important step is to look for signs and symptoms that are suggestive of a condition other than IBS, such as in-

flammatory bowel disease (IBD), or celiac disease which have been referred to as "alarm signs" or "red flags." They include: anemia and other abnormal blood tests, blood in the stool, unexplained weight loss, fever, new onset of symptoms at the age of 50 or older, family history of IBD, celiac disease, or colon cancer. When these symptoms and signs occur, they should be brought immediately to the attention of a doctor who may perform colonoscopy, upper endoscopy with small bowel biopsy, abdominal ultrasound and additional tests. Colonoscopy may also be done for younger people, particularly if the "red flags" or the doctor's judgment lead to the suspicion of other bowel diseases, such as IBD (7). It was noted that lymphocytic or microcytic colitis and nonspecific inflammation only occurred in patients with diarrhea-predominant IBS, and hence it was recommended that colonoscopy, when required, should include random colonic biopsies. Therefore, according to the American College of Gastroenterology guidelines, colonic investigation of diarrhea-predominant IBS and mixed diarrhea and constipation-type IBS subgroups with barium enema or CT colonography is not appropriate because of inability to perform random biopsies (8).

TREATMENT

Getting the IBS improvement means understanding that IBS is a sensory and a complex motility disorder which may have physical and stress-related dimensions.

A strong partnership between a knowledgeable patient and an empathetic doctor can control over symptoms and produces significant improvement for individuals with IBS.

The first line of treatment for IBS includes general measures such as: establishing an effective patient-physician relationship, obtaining education about IBS, and implementing lifestyle changes, which may be associated with symptoms.

Lifestyle changes

Lifestyle refers in evaluation any dietary or stress-related factors that could lead to symptoms and discuss these with the gastroenterologist. If certain foods set off or worsen symptoms, they must be reduced or avoid, also if the abdominal discomfort or pain occurs after eating, it may be helpful to eat smaller and more frequent meals.

Increased stress may result in the onset or worsening of IBS symptoms and associated non-bowel symptoms such as fatigue or low energy. Proper rest and exercise can help reduce stress levels and positively influence IBS.

Taking into consideration that IBS is also a stress, learning more about the disorder by self-care and communicating effectively with a gastroenterologist can reduce that stress.

Medication

A thorough evaluation by a physician is an important step toward selecting the treatment that is most appropriate for individual circumstances.

If lifestyle changes do not completely relieve IBS symptoms, a number of medications may be helpful. Antispasmodics: otilonium, pinaverium, dicyclomine, hyoscyamine, cimetropium may relieve abdominal pain or discomfort, particularly if the symptoms occur soon after eating. Anti-diarrheal agents: loperamide (Imodium), diphenoxylate (Lomotil) can be effective in preventing and relieving symptoms of diarrhea but may not be as helpful for the pain.

Laxatives (Linaclotide) can help treat symptoms of constipation and should be used under the supervision of a physician. Non-absorbable antibiotic rifaximin is recommended for reduction of IBS symptoms, as well as bloating in non-constipated patients. The use of prebiotics, synbiotics and probiotics that modify the gut microbiota are suggested for overall symptom improvement in IBS patients. Tricyclic antidepressant (TCAs) and selective serotonin reuptake inhibitors (SSRIs) medications are helpful for people with IBS and psychological distress (9). Individuals who have not responded to lifestyle changes and careful use of medications should be evaluated by a physician who specializes in functional GI and motility or stress-related GI disorders.

Counseling

Psychological and behavioral therapies may be indicated and effective in certain individuals.

Cognitive behavioral therapy (CBT) allows the individual to regain personal skills as well as mental techniques to better manage the symptoms (10). Hypnosis is useful to reduce painful discomfort mostly in treatment resistant patients. Psychological therapies like CBT, hypnotherapy, relaxation training is suggested for overall symptom improvement in IBS patients (11). Interpersonal psychotherapy addresses stressful responses to interactions with others. Behavioral therapy or skilled counseling may help with self-understanding and with identifying personal traits or problems that can be dealt with. Talking to a professional allows discussions of personal matters without fear of recrimination or blame (which is what can happen when talking to family, friends, or colleagues) and often helps to mutually develop a program for change. The effectiveness of complementary and alternative medicine therapies such as Chinese herbal therapy, acupuncture, acupressure, mindfulness meditation, and yoga are being evaluated in IBS patients. Acupuncture studies have demonstrated that a positive provider-patient interaction during acupuncture treatment sessions is associated with a beneficial effect in IBS (12).

CONCLUSIONS

IBS is a condition with well-defined clinical features and specific diagnostic criteria.

Worldwide it is estimated that 10-15% of the population has IBS and about 2 out of 3 patients are females.

Stress does not cause IBS, but it can worsen or trigger symptoms because of the connection between the brain and the gut.

IBS can only be diagnosed by a professional doctor and involves the exclusion of other organic diseases by colonoscopy.

Psychological and behavioral therapies are available to manage symptoms for IBS, being indicated and effective in certain individuals.

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NEWS

DIRECT ANTIVIRALS AND COAGULATION TO PATIENTS WITH HEPATITIS C VIRUS

The advent of direct-acting antivirals (DAAs) has been a major breakthrough in hepatology representing the therapeutic standard of care in patients with chronic hepatitis C virus infection over the past few years, providing safe and effective treatments for all patients with chronic HCV infection by increasing the sustained virological response rate to over 95%. A group of researchers from Italy (1) assessed the impact of DAA therapy on the coagulation parameters in viral cirrhosis C and sustained virological response. They investigated 28 patients on DAAs treatment and performed prothrombin-time, thrombin generation with and without thrombomodulin, whole-blood thromboelastometry, as well as the individual procoagulants (II, VIII, XIII, von Willebrand) and anticoagulants, antithrombin and protein-C. Patients had undetectable HCV-RNA at the end-of-treatment and at 12-weeks after end-of treatment. In conclusion, it has been demonstrated for the first time that AAD treatment in HCV positive patients and cirrhosis leads to improvement in pro- and anticoagulant status; does not substantially alter their balance but makes it more stable and less susceptible to disruption, as assumed before treatment (Tripodi A, D'Ambrosio R, Padovan L, *et al*. Evaluation of coagulation during treatment with directly acting antivirals in patients with hepatitis C virus related cirrhosis. *Liver Int* 2017; 37(9): 1295-1303).

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EVALUAREA CALITĂŢII VIEŢII ÎN CURSUL TRATAMENTULUI ADJUVANT CU TAMOXIFEN *VERSUS* INHIBITORI DE AROMATAZĂ LA PACIENTE CU CANCER MAMAR – UN TRIAL RANDOMIZAT

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ASSESMENT OF QUALITY OF LIFE OF THE ADJUVANT TREATMENT WITH TAMOXIFEN *VERSUS* AROMATASE INHIBITORS AT WOMEN WITH BREAST CANCER – A RANDOMIZED TRIAL (Abstract): **Purpose:** To evaluate and compare the impact on quality of life of tamoxifen and aromatase inhibitors (AIN's) in adjuvant settings, at women with breast cancer. **Methods**: From a total of 441 patients with breast cancer with hormonal treatment, were selected 177 of patients with adjuvant hormonal treatment (95 with tamoxifen treatment and 82 with AIN's treatment). These patients have completed the EORTC QLQ C-30 and EORTC QLQ BR23 questionnaires after 1 month of treatment (baseline), at 2 year of treatment with tamoxifen versus aromatase inhibitors (exemestane, anastrazole, letrozole). Mean change score from baseline and 2 years of treatment were compared in the both arms (tamoxifen vs. AIN's). **Results:** In the both arms, was observed a decreased QOL after 2 years comparative with the baseline. In the tamoxifen arm , global health status (GHS) and breast symptoms score (BRBS) were similar comparative with AIN's arm at baseline , with a similar status at 2 years measurement. **Conclusions:** The global health status (GHS) and breast symptoms score (BRBS) shows that there is no difference in the QOL at the women with breast cancer treated with tamoxifen , comparative with the women treated with AIN's . We conclude that AIN's did not have an adverse impact on overall QOL, comparative with tamoxifen, in adjuvant settings. Key words: QUALITY OF LIFE, TAMOXIFEN, BREAST CANCER, AROMATASE INHIBITORS

Tumorile maligne mamare sensibile la tratamentele hormonale care prezintă sau nu afectare a ganglionilor axilari sunt un grup heterogen de cancere. Ele au o diversitate prognostică, determinată şi de alţi factori prognostici, cum ar fi statusul HER2neu sau invazia vasculară. Tratamentul hormonal adjuvant în premenopauză este reprezentat de tamoxifen +/- supresia funcţiei ovariene. În postmenopauză, tratamentul hormonal adjuvant standard poate fi: tamoxifen 5 ani; inhibitori de aromatază 5 ani sau combinaţia tamoxifen 2 ani + inhibitori de aromatază 3 ani.

Tratamentul adjuvant cu tamoxifen timp de 5 ani de zile reduce riscul de recidivă a bolii şi de deces prin cancer mamar (1-5). Unul dintre efectele adverse majore ale terapiei cu tamoxifen este reprezentat de valurile de căldură (bufeuri), care duc deseori la discontinuarea tratamentului (6). Alte efecte secundare sunt evenimentele tromboembolice (7), atrofia mucoasei vaginale, sângerările vaginale, apariţia polipilor endometriali cu risc de transformare malignă (2,7%) (7). Efectele benefice ale

TABELUL I
Caracteristicile pacientelor cu cancer mamar in perioada 2001-2009

	Nr. total paciente	EP+;PR+ (71%)	ER;PR- (6%)	ER;PR (23%)	HER 2 Pozitiv.	HER2 Neg.	HER2 Necun..	Pre-menop.	Postmenop
STD. 0	7		1	6			7	7	
STD. I	50	29	5	16		18	32	29	21
STD. II	150	125	13	12	6	45	99	96	54
STD. III	317	270	8	39	29	149	139	131	186
STD. IV	105	24	10	71	9	20	76		105

TABELUL II
Caracteristicile pacientelor tratate hormonal

	Nr. total paciente	Tratamente hormonale				
		TMX 5 ani	LETROZOL 5 ani	ANASTRAZOL 5 ani	EXEMESTANE 5 ani	TMX 3 ani+ LETR2 ani
STD. 0	0					
STD. I	41	29	5	1	2	4
STD.II	150	96	2	8	8	12
STD. III	317	131		22	38	79
STD. IV	31		29		2	

tratamentului cu tamoxifen sunt creşterea densităţii osoase la pacientele în postmenopauză (8) şi scăderea LDL în cadrul metabolismului lipidic (9).

Inhibitorii de aromatază blochează conversia hormonilor androgeni în hormoni estrogeni şi reduc nivelurile de hormoni estrogeni în plasmă (10). A treia generaţie de inhibitori de aromatază sunt inhibitorii nesteroidieni : letrozol, anastrazol, exemestane.

Tratamentul adjuvant cu anastrazol sau letrozol reduce riscul de recidiva a bolii în postmenopauză, comparativ cu tratamentul cu tamoxifen (11,12). Terapia secvenţială cu tamoxifen 2-3 ani apoi exemestane sau anastrazol până la 5 ani, scade riscul de recurenţă comparativ cu tamoxifen 5 ani (13). În ceea ce priveşte efectele secundare ale inhibitorilor de aromatază, artralgiile şi mialgiile, împreuna cu o creştere a incidenţei fracturilor, reprezintă evenimentele adverse principale (14,15).

Unul dintre scopurile tratamentelor specifice în cancer este menţinerea unei calităţi a vieţii acceptabilă. În acest sens, s-a încercat în studiul actual o evaluare a calităţii vieţii în cursul tratamentului adjuvant la pacientele cu cancer de sân. S-a evaluat calitatea vieţii atât în cadrul tratamentului cu tamoxifen, cât şi în cursul tratamentului cu inhibitori de aromatază (letrozol, anastrazol, exemestane).

PACIENŢI ŞI METODE

În cadrul Centrului de Oncologie Medicală Iaşi, în perioada 2001- 2009, dintr-un total de 2998 pacienţi aflaţi în evidenţă cu cancer, 629 de paciente au fost în evidenţă cu cancer de sân (366 au fost în premenopauză şi 256 de paciente în postmenopauza) (tab. I). Marea majoritate dintre aceste paciente au urmat tratamente multimodale (chirurgie, radioterapie, chimioterapie, hormonoterapie). Tratamentele hormonale au fost administrate la un număr de 441 de paciente cu cancer mamar. După stadiul bolii (TNM), pacientele tratate hormonal au fost preponderente în stadiul II şi III. În stadiul IV au urmat tratament hormonal paciente care aveau contraindicaţie la chimioterapie. Tratamentul adjuvant a fost aplicat în premenopauză timp de 5 ani cu tamoxifen şi în postmenopauză timp de 5 ani cu tamoxifen sau inhibitor de aromatază. Dintre pacientele care au urmat tratament hormonal în postmenopauză, au existat paciente tratate în

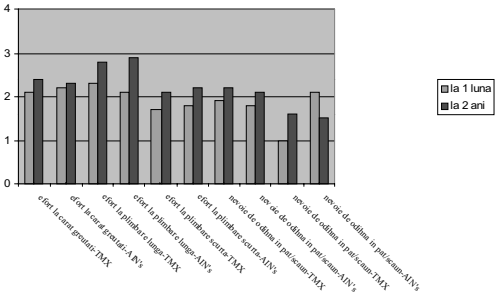

Fig. 1. Evaluarea efectelor adverse post-TMX/ AIN's

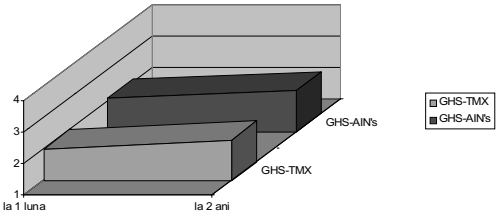

Fig. 2. Evaluarea GHS in terapia cu TMX/ AIN's

decursul celor 5 ani cu alternanţa tamoxifen – inhibitor de aromatază (tab. II).

Din totalul de 441 de paciente cu tratament hormonal, au fost selectate un număr de 177 de paciente (95 paciente în premenopauza şi 82 de paciente în postmenopauză) care au urmat tratament adjuvant cu tamoxifen sau/şi inhibitori de aromatază.

Lotul selectat, alcătuit din 177 de paciente, a fost format din paciente aflate în tratament hormonal astfel : premenopauză – 45 paciente stadiul II şi 56 paciente stadiul III, iar postmenopauză – 12 paciente în stadiul II şi 70 de paciente în stadiul III. Pacientele au avut, în momentul aplicării chestionarului de calitate a vieţii, vârste cuprinse între 29 şi 74 de ani.

Componenţa lotului evaluat din punct de vedere al calităţii vieţii a fost următoarea :

1. – bratul cu tratament cu tamoxifen - paciente care au urmat 5 ani adjuvanţa cu tamoxifen : stadiul II – 45 de paciente (31 în premenopauză şi 14 în postmenopauză) ; stadiul III – 50 de paciente (35 în premenopauză şi 14 în postmenopauză) - total 95 paciente

2. – bratul cu tratament cu AIN's - paciente care au urmat tratament cu inhibitori de aromatază (total 82 paciente) : stadiul II – 3 paciente care au primit exmestane 5 ani ; 2 paciente care au primit letrozol 5 ani ; 7 paciente care au primit 3 ani tamoxifen şi 2 ani letrozol (evaluarea a fost făcută în timpul tratamentului cu letrozol) ; stadiul III – 20 de paciente care au primit exemestane ; 14 paciente care au primit anastrazol ; 36 de paciente care au primit tamoxifel 3 ani şi apoi 2 ani letrozol (evaluarea s-a efectuat in timpul tratamentului cu letrozol)

La aceste paciente s-a aplicat, începand cu anul 2004 până în anul 2010, chestionarul EORTC-C30 şi chestionarul EORTC-BR23. În ceea ce priveşte EORTC-C30 s-a evaluat statusul sănătăţii globale (The global health status - GHS), iar în ceea ce priveşte chestionarul EORTC-BR23, specific cancerului mamar, s-a evaluat scorul simptomelor corelate cu cancerul de sân (breast symptoms score –BRBS).

Aplicarea chestionarelor EORTC-C30 si EORTC-BR23 a fost efectuată la 1 lună de la începerea tratamentului (cu tamoxifen sau AIN's) şi la 2 ani de zile de tratament cu medicaţia respectivă.

REZULTATE

În cazul chestionarului QLQ-C30, care evaluează global starea calităţii pacientelor cu cancer, s-a obţinut la o prima evaluare, un rezultat similar şi semnificativ statistic a evaluării simptomelor conţinute în chestionarul EORTC-C30 (fig. 1).

Scorul GHS la bratul care a primit tratament cu tamoxifen, comparativ cu bratul care a primit tratament cu AIN's, atât la 1 lună de la începerea tratamentului, cât şi la 2 ani de tratament a fost semnificativ statistic egal (fig. 2). S-au avut în vedere non--răspunsurile la chestionar.

Chestionarul EORTC-BR23 este specific simptomelor corelate cu cancerul mamar.

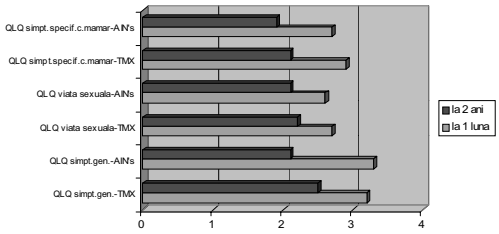

Fig. 3. Evaluarea simptomelor principale din chestionarul EORTC-BR23

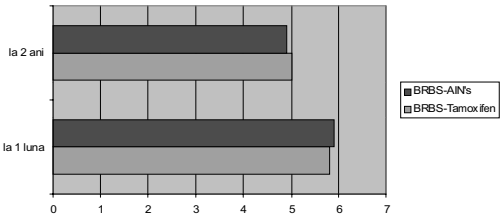

Fig. 4. Evaluarea BRBS – TMX/AIN's

S-a încercat evaluarea simptomelor dupa chestionarul EORTC-BR23. În ceea ce priveşte simptomele principale incluse în chestionarul EORTC-BR23, s-au obţinut rezultate similare semnificative statistic în ceea ce priveşte simptomele principale incluse în chestionar, la ambele brate (tratament cu TMX şi tratament cu AIN's). S-a avut în vedere de asemenea, în calculul statistic, non-răspunsurile la chestionare.

Chestionarul EORTC-BR23 a arătat rezultate statistice similare, demonstrând că la 1 lună şi la 2 ani de zile de tratament cu tamoxifen vs. AIN's, nu sunt diferenţe semnificative statistic între valorile scorului BRBS între cele 2 brate (paciente tratate cu tamoxifen vs. paciente tratate cu AIN's) (Fig.4).

Aplicarea chestionarelor EORTC-C30 si EORTC-BR23 a demonstrat de asemena şi faptul că la începutul tratamentului (1 lună), calitatea vieţii a suferit o scădere comparativ cu evaluarea la 2 ani de tratament în ambele brate – tamoxifen sau AIN's. Scăderea calităţii vieţii a fost vizibilă şi comparativ egala în ambele brate.

DISCUŢII

Rezultatele obţinute din studiul actual sugerează o calitate a vieţii asemănătoare în cadrul tratamentului cu AIN's, comparativ cu tratamentul cu tamoxifen. Ţinând cont de avantajele AINs în ceea ce priveşte supravieţuirea şi riscul mai scăzut de recidivă, se impune alegerea în postmenopauză a AIN's ca tratament de elecţie în adjuvantă, la pacientele în post-menopauză. Dacă avem în vedere studiile comparative între tratamentul adjuvant cu tamoxifen vs. letrozol (16), precum şi studiile comparative între tamoxifen vs. exemestane în adjuvanţă, utilizând chestionarul FACT-B (17) observam în literatura de specialitate o confirmare a non-inferiorităţii în ceea ce priveşte calitatea vieţii a tratamentului cu AIN's versus tratamentul cu tamoxifen. Într-adevar, tratamentul cu AIN's are marele dezavantaj al artralgiilor, dar faţă de tratametul cu tamoxifen, global, are acelaşi scor al calităţii vieţii. Studiul efectuat pentru evaluarea testelor cognitive, anxietate, depresie, astenie pentru a compara tratamentul cu exemestane vs. tamoxifen, au avut drept scop evaluarea simptomelor cu aceleaşi chestionare EORTC-C30 şi EORTC-B23, rezultatele fiind de non-inferioritate a exemestanului comparativ cu tamoxifenul în adjuvanţă, în ceea ce priveşte calitatea vieţii (18).

Rezultatele actuale demostrează non-inferioritatea tratamentului adjuvant cu AIN's comparativ cu adjuvanţă cu tamoxifen, în ambele categorii de vârstă (pre, post-menopauza, mai ales în postmenopauză).

O alta concluzie rezultată din studiul actual este scăderea calităţii vieţii la 2 ani comparativ cu nivelul iniţial, de la începerea tratamentului cu TMX sau AIN's, nivelul calităţii vieţii în ambele brate fiind egală.

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CONTRIBUTION OF IMMUNIZATION AGAINST STREPTOCOCCUS PNEUMONIAE AND HEMOPHILUS INFLUENZAE TYPE B IN REDUCING PEDIATRIC RESPIRATORY MORBIDITY

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CONTRIBUTION OF IMMUNIZATION AGAINST STREPTOCOCCUS PNEUMONIAE AND HEMOPHILUS INFLUENZAE TYPE B IN REDUCING PEDIATRIC RESPIRATORY MORBIDITY (Abstract). **Aim:** To highlights the peculiarities of acute respiratory infections and the possible complications in vaccinated children compared with unvaccinated children. **Material and methods:** This retrospective study included 346 patients aged 1 to 6 years, followed in a general practitioner center from an urban area. The cohort study was analyzed for immunizations and severe respiratory pathology requiring hospitalization or antibiotic therapy until the time of study. **Results**: Out of the 346 children surveyed, 20 (5.78%) presented ARI, according to the criteria we initially set for the selection, 21 (6.06%) presented otitis, 11 (3.17%) were hospitalized for respiratory tract infections, while 84.97% did not experience complicated respiratory pathology. In the age group 1-2 years old, from the 58 children vaccinated against Hib, but not against S. pneumoniae, 46 did not show severe respiratory pathology (79.31%), while the remaining 12 children (20.68%) recorded hospitalizations, otitis and respiratory infections that required antibiotic treatments. Of the 37 children aged 2-3 years, who were vaccinated against Hib, but not against S. pneumoniae, 32 did not have severe respiratory pathology (86.48%) and 5 children (13.51%) have a higher susceptibility. The distribution of 346 children according to respiratory pathology and vaccines showed that approximately 83% of non-vaccinated children developed respiratory pathology, while this pathology was present in less than 15% of the vaccinated children (p=0.001). **Conclusions**: We concluded that respiratory pathology was more prevalent in unvaccinated children comparing to vaccinated children. **Keywords**: VACCINATION, CHILDREN, RESPIRATORY DISEASE.

Acute respiratory infections are the most common cause of addressability in pediatric services. Vaccination is one of the most effective methods of prophylaxis, both in terms of effectiveness and cost, helping to reduce morbidity and mortality from ancient times. Each country has national vaccination programs established according to the epidemiological context of the region to which it belongs, with early childhood vaccination and the immunization of individuals at risk. The World Health Organization regularly develops strategies to increase vaccine coverage and

reduce the incidence of diseases that can be prevented.

Due to vaccine products availability to a large number of individuals, protection is extended to the entire community, through the phenomenon of "cohort immunity". When the majority of a population is vaccinated, even non-immunized or ineligible immunization (pregnant, immunocompromised) will be protected against the infectious agent because its spread is limited (1, 2).

Regarding vaccine-preventable diseases, the epidemiological situation in our country is similar to other countries in Europe, but there are also some exceptions. According to the dates in 2013, in Romania the incidence of tuberculosis is among the highest registered at European level, being reported cases of multidrug-resistant or antibiotics extensively resistant. Also, 1 out of 4 children who lose their life in their first year of birth die from respiratory illnesses, which are caused to a large extent by pneumococcus. Nowadays, among the preventive diseases by vaccination, pneumococcal disease is the main cause of global mortality in children under 5 years of age. Since February 2016, Romania has been experiencing a measles epidemic, with new cases still occurring, despite the strengthening of vaccination prevention measures (3).

In Romania, the national vaccination scheme, according to the Order of the Ministry of Health no. 386 of 31.03.2015 contains the following vaccines: tuberculosis vaccine (BCG - bacillus Calmette-Guérin - vaccination), HepB (hepatitis B), DTPa-IPV-Hib-HepB (diphtheria - tetanus - pertussis - poliomyelitis - *Hemophilus influenzae* type B - hepatitis B infection), ROR (rubella - mumps - measles) and PCV (pneumococcal vaccine). The optional vaccines currently available on the Romanian pharmaceutical market are those against: Rotavirus infection, varicella-zoster virus, hepatitis A or human papillomavirus (HPV) disease.

The aim of our study was to highlights the peculiarities of acute respiratory infections and the possible complications in vaccinated children compared with unvaccinated children.

MATERIAL AND METHODS

This retrospective study included 346 patients aged 1 to 6 years, followed in a general practitioner center from an urban area. The cohort study was analyzed for immunizations and severe respiratory pathology requiring hospitalization or antibiotic therapy until the time of study.

Inclusion criterion was age between 1 and 6 years of age in the course of the study (1st-31st December 2017).

Exclusion criterion were lack of appropriate data recorded in the observation sheets: new children in evidence or children who have not been present at the family medicine cabinet in the past year, lacking prophylactic mandatory controls and lacking accurate evidence and complete their pathological antecedents.

The cohort was divided into age groups. The diseases followed in the study were respiratory and otolaryngology (ENT) pathology. Moreover, the study cohort was divided into: "acute respiratory infections" (ARI), "Otitis", "Hospitalized", and "No serious respiratory diseases" groups. In the ARI group were included those children with more than three infectious episodes per year that required ambulatory antibiotic therapy, in the "Otitis" group children with at least three episodes of otitis media and in

the "Hospitalized" group those with at least one admission for acute respiratory tract infections. In those cases that have m*et al*l three conditions or two of them, we placed the children in the group with more numerous infectious episodes or higher severity (multiple days of hospitalization, antibiotic treatment extended over 10 days, use of two to three antibiotics per infectious episode).

RESULTS

The 346 cases of the study cohort were initially divided by gender and age groups, resulting in a homogeneous distribution (p = 0.583) (tab. I).

TABLE I

Distribution by age and gender

Age	Girls (n=162)		Boys (n=184)		All (n=346)	
	n	%	n	%	n	%
1-2 years	35	21.6	45	24.5	80	23.1
2-3 years	31	19.1	32	17.4	63	18.2
3-4 years	27	16.7	41	22.3	68	19.7
4-5 years	38	23.5	37	20.1	75	21.7
5-6 years	31	19.1	29	15.8	60	17.3

From the immunization point of view, the distribution of cases is as follows in table II. Vaccination against *Hemophilus influenzae* type b (Hib) was included in the national scheme for all children of the study group, while ant pneumococcal vaccination was part of the optional immunizations until October 2017, justifying the significantly lower number of cases vaccinated. Regarding the refusal of any vaccination, we did not record in any case religious or cultural reasons, only misinformation from sources other than scientifically validated data.

TABLE II

Distribution by age, gender and vaccinations

Age	Hib + Antipneumococic +		Hib + Antipneumococic -		Unvaccinated	
	Girls	Boys	Girls	Boys	Girls	Boys
1-2 years	12	8	23	35	0	2
	28.6%	19.5%	19.5%	25.2%	0.0%	50.0%
2-3 years	11	14	19	18	1	0
	26.2%	34.1%	16.1%	12.9%	50.0%	0.0%
3-4 years	8	10	18	29	1	2
	19.0%	24.4%	15.3%	20.9%	50.0%	50.0%
4-5 years	3	8	35	29	0	0
	7.1%	19.5%	29.7%	20.9%	0.0%	0.0%
5-6 years	8	1	23	28	0	0
	19.0%	2.4%	19.5%	20.1%	0.0%	0.0%
TOTAL	**42**	**41**	**118**	**139**	**2**	**4**

In the table above, children with immunization against *Hemophilus influenzae type* b and Streptococcus pneumoniae are marked "Hib +" and "Antipneumococcic +" and "Non-vaccinated" children those who have not been vaccinated, respectively. The latter include cases where the family refused by informed consent to carry out any vaccine, including those in the national scheme.

Hib +, Anti-pneumococcal vaccination + was predominates in girls aged 1 to 2 years (28.6%) and 2 to 3 years (26.2%), while Hib +, Antipneumococcic - vaccination in the age group 4 to 5 years (29.7%) ($p = 0.05$).

In boys, the proportion of Hib + Antipneumococcic + vaccinated predominates in the 2 to 3 years (34.1%) and 3 to 4 years (24.4%) groups, and the proportion of vaccinated and non-immunized children in the 1 to 2 years group (25.2 %); also, we find 4 unvaccinated children ($p = 0.004$).

Furthermore, we divided the 346 children according to the presence or absence of complicated respiratory or ENT pathology, in order to subsequently see how it correlates with immunizations. Thus, the distribution according to age and respiratory or ENT pathology requiring antibiotics or hospitalization is shown in the table III.

TABLE III

Distribution of children by age and respiratory or ENT pathology

Age	ARI	Otitis	Hospitalized	No serious respiratory diseases
1-2 years	6	4	5	65
2-3 years	4	4	1	54
3-4 years	3	5	1	59
4-5 years	5	4	2	64
5-6 years	2	4	2	52
TOTAL	**20**	**21**	**11**	**294**

Therefore, out of the 346 children surveyed, 20 (5.78%) presented ARI, according to the criteria we initially set for the selection, 21 (6.06%) presented otitis, 11 (3.17%) were hospitalized for respiratory tract infections, while 84.97% did not experience complicated respiratory pathology.

Next, children were analyzed for the above-mentioned pathology correlated with immunizations, to see their degree of impact on the reduction of respiratory morbidity. The analysis was performed for each age group.

Group 1 to 2 years old

In this age group, from the 58 children vaccinated against Hib, but not against *S. pneumoniae*, 46 did not show severe respiratory pathology (79.31%), while the remaining 12 children (20.68%) recorded hospitalizations, otitis and respiratory infections that required antibiotic treatments. There were no significant differences between the groups.

Regarding the 20 children vaccinated both Hib and antipneumococcic, 19 (95%) had no serious respiratory disease; there was only one case requiring medical care for severe respiratory tract illness.

Two unvaccinated children were also included in this age group; both of them had repetitive respiratory infections and hospitalizations requiring antibiotics until the time of the study (tab. IV).

TABLE IV

Distribution of children aged 1-2 years depending on immunizations and pathology

Pathology	Hib+ antipneumococcic+		Hib+ antipneumococcic-		Unvaccinated		p*
	G	B	G	B	G	B	
Total=80 children	**12**	**8**	**23**	**35**	**0**	**2**	**-**
ARI 6 children	1	0	2	2	0	1	0.250
Otitis 4 children	0	0	1	3	0	0	-
Hospitalized 5 children	0	0	2	2	0	1	0.276
No serious respiratory diseases 65 children	11	8	18	28	0	0	0.167
p*	0.304		0.864		-		

*Kruskal-Wallis test

Group 2 to 3 years old

Of the 37 children aged 2-3 years, who were vaccinated against Hib, but not against S. pneumoniae, 32 did not have severe respiratory pathology (86.48%) and 5 children (13.51%) have a higher susceptibility. Regarding the 25 children vaccinated both Hib and antipneumococcic, 22 (88%) did not have severe respiratory tract respiratory disorders (tab. V).

In this age group, there was only one child with all immunizations refused and which is evidenced by repeated otitis media.

The age group 3-4 years old

Of the 47 children 3-4 years old only vaccinated against Hib, 43 did not have severe respiratory pathology (91.48%), while of the 18 children vaccinated both Hib and Antipneumococcic, 15 (95%) were without respiratory problems (tab. VI).

Three children were denied immunizations, two of them registering until the time of study a number of repeated hospitalizations and otitis media.

The age group 4-5 years old

Of the 64 children vaccinated against Hib, 54 did not have severe respiratory pathology (84.37%), while 10 (90.90%) of the 11 Hib and antipneumococcic vaccinated children were without serious respiratory infections disease. There was no case of refusal of vaccinations at this age stage (tab. VII).

TABLE V

Distribution of children aged 2-3 years depending on immunizations and pathology

Pathology	Hib+ antipneumococcic+		Hib+ antipneumococcic-		Unvaccinated		p*
	G	B	G	B	G	B	
Total=63 children	**11**	**14**	**19**	**18**	**1**	**0**	**-**
ARI 4 children	1	1	1	1	0	0	1.000
Otitis 4 children	0	1	1	1	1	0	0.250
Hospitalized 1 child	0	0	1	0	0	0	-
No serious respiratory diseases 54 children	10	12	16	16	0	0	0.742
p*	0.547		0.665		-		

* Kruskal-Wallis test

TABLE VI

Distribution of children aged 3-4 years depending on the immunizations and pathology

Pathology	Hib+ antipneumococcic +		Hib+ antipneumococcic -		Unvaccinated		p*
	G	B	G	B	G	B	
Total=68 children	**8**	**10**	**18**	**29**	**1**	**2**	**-**
ARI 3 children	1	1	0	1	0	0	0.562
Otitis 5 children	0	1	1	2	0	1	0.387
Hospitalized 1 child	0	0	0	0	1	0	-
No serious respiratory diseases 59 children	7	8	17	26	0	1	0.435
p*	0.599		0.945		0.148		

* Kruskal-Wallis test

TABLE VII

Distribution of children aged 4-5 years depending on the immunizations and pathology

Pathology	Hib+ antipneumococcic+		Hib+ antipneumococcic-		Unvaccinated		p*
	G	B	G	B	G	B	
75 children	**3**	**8**	**35**	**29**	**0**	**0**	**-**
IACR = 5 children	0	1	3	1	0	0	0.135
Otitis = 4 children	0	0	2	2	0	0	-
Hospitalized= 2 children	0	0	1	1	0	0	-
No serious respiratory diseases= 64 children	/3	7	29	25	0	0	0.163
p*	0.412		0.854		-		

* Kruskal-Wallis test

Age group 5-6 years

Of the 51 children aged 5-6 years only vaccinated against Hib, 45 had no serious respiratory pathology (88.23%), and of the 9 children vaccinated with both Hib and antipneumococcic, 7 (77.77%) did not have had respiratory conditions requiring repeated antibiotic treatments. No cases of refusal of immunization have been reported in this age group (tab. VIII).

TABLE VIII

Distribution of children aged 5-6 years depending on the immunizations and pathology

Pathology	Hib+ Antipneumococic+		Hib+ Antipneumococic-		Unvaccinated		p*
	G	B	G	B	G	B	
60 children	**8**	**1**	**23**	**28**	**0**	**0**	**-**
IACR = 2 children	1	0	0	1	0	0	0.333
Otitis =4 children	1	0	1	2	0	0	0.189
Hospitalized=2 children	0	0	1	1	0	0	-
No serious respiratory diseases= 52 children	6	1	21	24	0	0	0.062
p*	0.764		0.240		-		

* Kruskal-Wallis test

The distribution of 346 children according to respiratory pathology and vaccines showed that 83% of non-vaccinated children developed respiratory pathology, while this pathology was present in less than 15% of the vaccinated children (p=0.001) (tab. IX).

TABLE IX

Distribution of the batch 1-6 years depending on the immunizations and pathology presented

TOTAL 346 children	Hib+ antipneumococcic -	Hib+ antipneumococcic+	Unvaccinated
With respiratory pathology	37 (14.4%)	10 (12.0%)	5 (83.3%)
Without respiratory pathology	220 (85.6%)	73 (88.0%)	1 (16.7%)

DISCUSSION

In the context of strategies to lower infant mortality rates, control over respiratory diseases must occupy an important place. Strategies for the prevention of acute respiratory infections in young children include vaccination in the first place. To this, from the prenatal period, it needs to be added the careful monitoring of pregnancy in order to reduce prematurity and low birth weight, promoting natural nutrition, prevention and correct treatment of debilitating diseases (iron deficiency anemia, obstructive rickets, malnutrition, zinc deficiency) (4, 5).

Acute respiratory infections represent a complex and heterogeneous group of diseases caused by a large number of etiological agents and affecting any part of the respiratory tract (6). Worldwide, the WHO has proposed Global Immunization Vision and Strategy (GIVs) so that, through a joint effort, national immunization programs promote anti-Hib, pertussis, antipneumococcal vaccination, the ultimate target being the decrease of morbidity and mortality thought respiratory disease in children (7).

Hemophilus influenzae type b, known as Hib, is a gram-negative bacterium that causes severe infections and invasive diseases such as meningitis, pneumonia, epiglottitis, otitis, cellulitis, septic arthritis, in children younger than 5 years of age. The pathway of penetration of the pathogen is nasopharynx, and the source of infection is, in most cases, the healthy carrier. The newborn is contaminated by aspiration of contaminated amniotic fluid or by contact with Hib-infected vaginal secretions. It should be noted that 2/3 of the Hib invasive infection cases were found under 18 months, with a peak incidence of 6-11 months.

One of the risk factors for the occurrence of systemic disease is viral infection or Mycoplasma pneumoniae prior to illness. These pathogens are the main etiological agents of the pneumonia of children attending gardens. Therefore, in the absence of vaccination of infants from families where there are older children going to collectivities, the risk of Hib infection and the occurrence of invasive disease with all its complications is very high. Also, in the absence of vaccination, the complications of these diseases, represented alongside death by neuromotor retardation, deafness, seriously affect the child's health and the quality of life of the whole family (8, 9).

Currently, two vaccines which contain the Hib capsular polysaccharide conjugated to different proteins are available for use in

infants and young children. Regardless of the type of vaccine chosen, the effectiveness of anti-Hib vaccination in the prevention of invasive disease (especially meningitis and pneumonia) was greater than 90%. Epidemiological studies conducted in the United States in the 1980s, prior to vaccination, in children under the age of 5, showed that there were approximately 20.000 cases of Hib infection annually compared to the period 2003-2010 where the incidence of the disease decreased to an average of 398 cases per year (10). Subsequently, between 2010-2011, 33% of children under 5 years of age were diagnosed with Hib invasive disease, all under the age of 6 months, without the complete vaccination schedule. The cause of the vaccination failure is not exactly known, but it is associated with a number of factors related to the child's early childhood environment, school-age brothers, families with many members, low socio-economic status, low education and information parents. Host factors include: lack of natural nutrition, chronic diseases (immunodeficiencies, hemolytic anemia, oncological diseases-especially during chemo-therapy) and possibly sex (there is an increased risk for the male sex) (11,12).

In Romania, prior to the introduction of vaccination, 60% of the invasive Hib disease occurred in children before 12 months, reaching a peak at the age of 6-11 months. In 2010, 16 new cases of Hib infection were reported, and in 2011 other 5 cases (13).

Streptococcus pneumoniae (*S. pneumoniae*) is another major cause of morbidity in children, and is the cause of most bacterial pneumonia, otitis media, septicemia and purulent meningitis. Prior to the large-scale use of Prevenar in the United States, 17,000 cases of pneumococcal infections occurred every year in children under 5, causing about 200 deaths. Furthermore, *S. pneumoniae* continues to pose a real challenge also for the 21st century medicine, because antibiotic resistance has increased in most countries in recent years (14).

In Romania, the study by Falup-Pecurariu *et al.*, 2013, before the introduction of routine antipneumococcic vaccination into the national immunization scheme showed that in two hundred and twelve children with AOM, 99 (46.6%) episodes occurred in patients <12 months of age, 114 pathogens were recovered: *S. pneumoniae* was the most common isolate (81 isolates, 70.3% of all culture-positive episodes), followed by non-typing *Hemophilus influenzae* (26, 20.7%), *Streptococcus pyogenes* (5, 4.5%) and *Moraxella catarrhalis* (2, 1.8%). The most common S. pneumoniae serotypes were 19F (14, 29.2%), 6B (8, 16.7%), 23F (8, 16.7%), and 14 (6, 12.5%). Non-PCV13 serotypes represented six (12.6%) of all serotypes (four of them non-susceptible to penicillin). Thirty-six (75.0%) isolates were potentially covered by PCV7, 37 (77.0%) by PCV10, and 42 (87.5%) by PCV13. In these patients, multidrug resistance was found in 33 (68.7%) isolates (13).

In our study, we observed of the 340 children vaccinated against *Hemophilus influenzae* type b, 47 had severe respiratory pathology - 13.82% and frequently required antibiotics or were hospitalized.

Although the distribution of cases by sex, associated pathologies and the presence of immunizations did not show statistically significant differences in each age group, however these differences were present in the whole cohort, demonstrating the effectiveness of immunization in reducing respiratory and severe ENT pathology.

CONCLUSIONS

The most common causes of invasive disease (pneumonia, meningitis) at young age are infections with Streptococcus pneumoniae and *Hemophilus influenza*. In our study, we concluded that respiratory pathology was present in 83% of unvaccinated children and in less than 15% of vaccinated children. Vaccination, as a strategy for decreasing the infant mortality rate, is the main method of preventing respiratory diseases in infants and children.

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PROFILUL CLINIC AL NODULILOR TIROIDIENI ÎN CLINICA DE ENDOCRINOLOGIE A SPITALULUI "SF. SPIRIDON" IAŞI ÎN PERIOADA 2005-2009

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CLINICAL PROFILE OF THYROID NODULES IN IAŞI, DEPARTMENT OF ENDOCRINOLOGY, ST. SPIRIDON HOSPITAL, BETWEEN 2005-2009 (Abstract): Thyroid nodules are common, with up to 50% of the adult population having palpable nodules. With the use of ultrasound, up to 10 times more nodules are likely to be detected. Given this we decided to evaluate by retrospective study the incidence of thyroid nodule in the North-Eastern Region of Romania (Moldavia) between 2005-2009. **Material and method**: We reviewed the records of patients admitted in the Department of Endocrinology, St. Spiridon Hospital Iaşi between 1.01.2005-31.12. 2009 (4712 patients) and with selected the cases with nodular goiter. **Results**: A total of 3105 cases of nodular goiter were identified. The women were most affected and highest prevalence was noticed for the patients with ages between 51-60 years. Most of the nodules were found in the right thyroidian lobe and the majority of the patients were euthyroid . The peak of incidence of nodular hyperthyroidism was different from that of diffuse hyperthyroidism. **Conclusions**: Thyroid nodules are the most common endocrine problem and the increasingly frequent detection of nonpalpable thyroid nodules in asymptomatic patients requires new approaches for their management. **Key words**: THYROID NODULE, INCIDENCE, ULTRASOUND

Nodulii tiroidieni reprezintă o patologie comună a glandei tiroide iar frecvenţa lor variază în funcţie de metoda de explorare aleasă. Astfel, prin palpare pot fi decelaţi la 4% -8% dintre adulţi, în timp ce prin evaluare ultrasonografică frecvenţa lor variază intre 13% -67%. La autopsie, aceştia au o incidenţă de aproximativ 50% (1,2).

Frecvenţa nodulilor tiroidieni creşte cu înaintarea în vârstă (3). De asemenea, există variaţii şi în funcţie de sex, femeile având de o prevalenţă mai mare decât bărbaţii.

Descoperirea clinică a unui nodul evocă întotdeauna posibilitatea unei tumori maligne, întrucât în 95% din cazuri cancerul tiroidian se prezintă sub această formă (4,5).

Patologia tiroidiană ocupă un loc important în afecţiunile endocrine din România şi, în special din Moldova, ca urmare a deficitului alimentar în iod şi a profilaxiei insuficiente (6).

OBIECTIVE

Studiul prezent şi-a propus evaluarea patologiei nodulare tiroidiene în Moldova , regiune caracterizată, anterior introducerii iodării universale a sării în 2004, drept o zonă cu un deficit moderat de iod, dar în acelaşi timp având şi un istoric particular din punct de vedere al expunerii populaţiei la radiaţiile ionizante (evenimentul de la Cernobîl din 26 aprilie 1986, când în urma

exploziei reactorului nuclear o cantitate mare de izotopi radioactivi, printre care şi I^{131}, au fost eliberată în mediu).

MATERIAL ŞI METODĂ

Studiul a fost retrospectiv, a vizat perioada 1.01.2005-31.12.2009 şi a cuprins cazuistica Clinicii de Endocrinologie din cadrul Spitalul Clinic Judeţean de Urgenţă "Sf. Spiridon" din Iaşi. Lotul de studiu a fost format din 3105 pacienţi, prezentând nodularizare tiroidiană. Criteriul de selecţie a pacienţilor a fost reprezentat de prezenţa la examenul clinic/ecografic a cel puţin unei formaţiuni nodulare în loja anterioară a gâtului.

Prelucrarea matematică a datelor experimentale

Datele au fost introduse într-un tabel de lucru în programul Excel, sortate şi filtrate după diferite criterii. Prezentarea datelor s-a realizat prin *reprezentări grafice de tip „coloană" şi „sectoriale".* Au fost determinate pentru parametrii cuantificabili din punct de vedere matematic următoarele valori caracteristice : valoarea medie, deviaţia standard a valorilor. Interpretarea datelor a fost realizată cu ajutorul programelor : *MS Excel* 2003, *SPSS 12.0.*

REZULTATE

În perioada menţionată, în Clinica de Endocrinologie Iaşi au fost internaţi 4712 pacienţi. Dintre aceştia 66% (3105 pacienţi) au prezentat guşi nodulare, restul de 34% (1607 pacienţi) prezentând alte afecţiuni endocrine. Din punct de vedere al incidenţei s-a constatat o creştere a frecvenţei odată cu înaintarea în vârstă, atingând un maxim în intervalul de vârstă 51-60 de ani, pentru ambele sexe, după care s-a înregistrat o scădere progresivă. Un număr de 384 de pacienţi au prezentat guşi micronodulare (diametru nodul sub un centimetru) diagnosticul fiind realizate doar pe baza ecografiei tiroidiene. Autoimunitatea tiroidiană a fost asociată cu boala nodulară tiroidiană într-un procent de 19% (579 de pacienţi). Din punct de vedere al prezentării clinice, majoritatea pacienţilor, 1834 (57%) au prezentat guşi uninodulare raportat la cei cu guşi multinodulare în număr de 1391 (43%). Raportat la lobul tiroidian stâng în lobul tiroidian drept s-a găsit o tendinţă crescută la nodularizare. Astfel din totalul pacienţilor cu guşi uninodulare 59% (1077 de pacienţi) au prezentat nodularizare în LTD în timp ce 41% (757 de pacienţi) au prezentat nodularizare în LTS. Raportat la sexe, femeile au avut o incidenţă mult crescută a patologiei tiroidiene nodulare raportată la bărbaţi. Din totalul pacienţilor luaţi în studiu 89% (2769 de pacienţi) a fost reprezentată de populaţia de sex feminin, în timp ce restul de 11% (338 de pacienţi) a fost reprezentată de populaţia de sex masculin.

Cea mai mare parte a nodulilor decelaţi nu au fost însoţiţi de o modificare a funcţiei tiroidiene. Astfel din totalul pacienţilor cuprinşi în studiu, 88% (2733 de pacienţi) au prezentat eutiroidie în timp ce 9% (284 de pacienţi) au prezentat hipertiroidie şi 3% (88 de pacienţi) au prezentat hipotiroidie. Din totalul pacienţilor cu hipertiroidie internaţi in perioada 2005-2009 (664 de pacienţi), guşile nodulare însoţite de hipertiroidie au reprezentat 42% din totalul hipiertiroidiilor dintre care cea mai mare parte, 33% (219 de pacienţi), au fost cauzate de adenoame toxice şi guşi multinodulare. Adenomul toxic şi guşa multinodulară toxică au prezentat un vârf din punct de vedere al vârstei diferit de cel al hipertiroidiilor difuze (neînsoţite de nodularizare). Astfel, pentru adenoamele toxice s-a decelat un vârf in jurul vârstei 51-60 de ani în timp ce pentru guşile multinodulare toxice peak-ul de vârsta a fost de 61-70 de ani, iar pentru hipertirodiile difuze vârsta cea mai afectată a fost situată între 41-50 de ani.

CONCLUZII

Boala nodulară tiroidiană reprezintă o problemă de interes major în practica medicală, atât datorită frecvenţei mari a nodulilor tiroidieni cât şi riscului de malignitate pentru o parte dintre noduli. Incidenţa bolii nodulare tiroidiene atinge un maxim în intervalul de vârstă 51-60 de ani pentru ambele sexe după care se înregistrează o scădere progresivă. Cea mai mare parte a nodulilor tiroidieni sunt însoţiţi de eutiroidie. Autoimunitatea este implicată într-o mare măsură în nodularizarea ţesutului tiroidian în prezentul studiu. Incidenţa nodulilor tiroidieni este mai mare la sexul feminin faţă de cel masculin. Incidenţa crescută a nodulilor tiroidieni la femei poate fi rezultatul faptului că terenul estrogenic este factor favorizant al procesului de nodularizare. Patologia nodulară tiroidiană îmbracă în Moldova forme polimorfe, majoritatea pacienţilor prezentând guşi uninodulare lobul tiroidian drept având o tendinţă crescută la nodularizare. Vârful de incidenţă al hipertiroidiilor nodulare este diferit de cel al hipertiroidiilor difuze.

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CURRENT ASPECTS REGARDING THE RELEVANCE OF INFLAMMATION IN MAJOR DEPRESSIVE DISORDER

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CURRENT ASPECTS REGARDING THE RELEVANCE OF INFLAMMATION IN MAJOR DEPRESSIVE DISORDER (Abstract): The World Health Organization predicts that depression, which already affects about 10% of the population in the United States, will be the world's leading medical condition by 2030. The relationship between inflammation and depression has a bidirectional potential, but the underlying mechanisms are still less knowledgeable. In this way, the theory that argues that the inflammatory process is an etiologic factor in depression associated increased levels of inflammatory markers with decreased levels of serotonin, a neurotransmitter important in the pathogenesis of depression. An increase in serum levels of cytokines may lead to decreased availability of serotonin and other neurotransmitters, activation of the hypothalamic-pituitary-adrenal axis and increased oxidative stress in the brain. Depressive symptoms can cause a slight inflammatory response by triggering certain hormonal disorders in the HPA axis that leads to increased susceptibility to infections. Thus, inflammatory mechanisms may provide new paths for intervention and prevention of depressive affective disorders. Also, inflammation could explain the fairly widespread association of somatic-type diseases (such as cardiovascular disease) and diabetes, as well as mental disorders such as depression or schizophrenia. Cytokines have been shown to be related to many depression symptoms to a variety of the population. Examination of gene expression profiles provides insight into how altered gene activity is linked with exposure to stress. The expression of a gene may increase or decrease, and it represents the first step in the production of proteins that communicate changes in cell functions, including those that are involved in the immune systems' response to the inflammation. **Keywords**: DEPRESSION, INFLAMMATION, COMORBIDITIES, GENETICS.

Depression is one of the most common diagnoses in psychiatric practice, having a major impact on patient functionality. The World Health Organization predicts that depression, which already affects about 10 percent of the population in the United States, will be the world's leading medical condition by 2030 (1). Research on the major depressive disorder is in continuous growth, given the scale of the phenomenon and its consequences both socially and financially (2). Currently, it is estimated that treating depression involves higher costs than the treatment of hypertension combined with that of diabetes (3). Also, given the high rate of relapse and suicidal risks attached to depression, new treatments, and in-depth studies are of course

needed in this area of research (4). Depression has a major impact regardless of social status, profession or age, for example, in a study published in 2017 on a sample of 89 students from the faculty of medicine, a series of scales and tests were applied and it was shown that 20% of first-year faculty students had a moderate to severe depressive episode, and those most likely to develop this disorder are those with a melancholic personality.

Numerous studies have highlighted biological abnormalities in patients with affective disorders and the inflammatory hypothesis has been lately under discussions (5). In this way, the inflammatory hypothesis of depression states that major depressive disorder is also associated with the activation of the innate immune system, increased proinflammatory cytokines and acute phase proteins (6). In support of this hypothesis, numerous studies have detected significantly increased levels of inflammatory markers in patients diagnosed with depression, as compared to healthy controls, especially in the case of interleukin IL-6, IL-1b, tumor necrosis factor alpha and C-reactive protein (7).

Empirical data previously indicated that stress is associated with high levels of inflammatory markers, particularly C-reactive protein and TNF-alpha (6). Experimental studies have also shown that stressful life situations may directly stimulate the production of pro-inflammatory cytokines (8). Inflammation also presents a risk factor for the development of cardiovascular diseases, diabetes, and neoplasia (9), while depressive symptoms have also been associated with the presence of inflammation (10).

However, the underlying mechanisms are not fully known. Several physiological mechanisms have been proposed, including changes in clotting factors, genetic factors that increase the risk of having depressive symptoms, as well as cardiovascular diseases and changes in glucose metabolism.

It cannot be said with certainty if depression leads to inflammation or inflammation to depression. It is not yet known due to the combination of the two, and it may be a reciprocal relationship through a positive feedback mechanism.

The relationship between inflammation and depression has a bidirectional potential, but the underlying mechanisms are still less knowledgeable. In this way, the theory that argues that the inflammatory process is an etiologic factor in depression associated increased levels of inflammatory markers with decreased levels of serotonin, a neurotransmitter important in the pathogenesis of depression (11). On the other hand, the depression could lead to inflammation. Psychological stress activates the sympathetic nervous system and releases hormones that in turn initiate acute phase responses, generating inflammation (12).

The psychological stress commonly associated with depression increases the number of inflammatory cytokines in both laboratory mice and in humans (13). Inflammation in patients with major depressive disorder is often associated with resistance to antidepressant therapy and it is believed that anti-inflammatory medication can increase the effectiveness of antidepressants (14).

Thus, in a study by Raison and Miller, numerous genetic changes were identified in patients with major depressive disorder involved with the inflammatory system. In rodents, administration of inflammatory cytokines or lipopolysaccharide-type in-

flammatory stimulants causes depressive behaviors that can be reduced by antidepressants (15). In this way, specific inflammatory cytokines that have been identified as promoters of depressive-like behavior in rodents are IL-6, TNF-a, and IL-1b, as well as C-reactive protein (16).

The mechanisms underlying the pathophysiology involves monoamines and contemporary drug therapy for depression and psychosis (17). However, the heterogeneity in appearance, evolution and response to treatment suggests the presence of additional mechanisms (18). Several observations support that the communication between the immune system and the brain is mediated by cytokines and that they are involved in the pathogenesis of these disorders (19). Animal models suggest that systemic inflammatory cytokines, such as IL-6, can communicate with the brain. An increase in serum levels of cytokines may lead to decreased availability of serotonin and other neurotransmitters, activation of the hypothalamic-pituitary-adrenal axis and increased oxidative stress in the brain (20).

Depressive symptoms can cause a slight inflammatory response by triggering certain hormonal disorders in the HPA axis that leads to increased susceptibility to infections (11).

Mechanistically speaking, chronic stress can trigger changes in the hypothalamic-pituitary-adrenal (HPA) axis and in the immune system, which in turn can trigger anxiety and depression (21). These paths are not independent because the HPA axis and the immune system are closely linked. While the HPA axis should normally moderate inflammatory reactions, its prolonged hyperactivity leads to abnormal anti-inflammatory responses that increase the inflammation. Also, hippocampal glucocorticoid receptors are inhibited by chronic neuroinflammation which exacerbates pro-inflammatory cytokine activity and aggravates chronic neuroinflammation. On the other hand, neuroinflammation causes an imbalance between oxidative stress and the anti-oxidant system, which is also associated with depression (22). It can also be hypothesized that the immunological changes associated with chronic diseases and aging could induce similar effects (23).

Since the HPA axis is regarded as the final common path for most depressive symptoms, recent studies have generated new hypotheses for the biological mechanisms of depression (24). For example, many studies have focused on cytokine-cytokine receptor interaction in the case of the inflammatory path. A meta-analysis comprising 24 studies that considered the measurement of serum cytokines in patients with major depressive disorder reported they had significantly higher concentrations of TNF-alpha and IL-6 (25).

Oxytocin, together with vasopressin, melatonin, insulin and other neuropeptides may influence mood and psychosocial functions. In a 2018-year study, for 8 days, oxytocin was injected intraperitoneally into adult mice, observing that compared to the control group, they experienced increased mood levels and reduced their stress and anxiety (26). Another study, referring to the implications of oxytocin in the mechanisms of mixed anxiety and depressive disorders, shows by a novel approach that intraperitoneal oxytocin injection for 9 days in a rat that was injected for 2 weeks with methionine revealed that levels anxiety, depression and stress have declined considerably (26).

Thus, inflammation is a physiological mechanism often mentioned in connection with the presence of depressive symptoms in the development of cardiovascular diseases (27).

Thus, inflammatory mechanisms may provide new paths for intervention and prevention of depressive affective disorders. Also, inflammation could explain the fairly widespread association of somatic-type diseases (such as cardiovascular disease) and diabetes, as well as mental disorders such as depression or schizophrenia (28).

In this way, we can state that the presence of depressive symptoms is not only a prognostic factor for patient with cardiovascular diseases but is also a risk factor for cardiovascular disease incidence in the case of healthy individuals (29).

During this time, the inflammatory response contributes to myocardial infarction by creating an instability at the atherosclerotic plaques level, which precipitates them to rupture or crack and leads to the formation of occlusive thrombus. This cascade of events could explain the high rate of morbidity and mortality in patients with myocardial infarction and associated depressive elements (30).

Cardiopathy represents a chronic inflammatory process involving an immune response to an injury on the vascular endothelium level (29). Moreover, in these patients, high levels of inflammatory markers (CRP, IL-6, and TNF-alpha) have been associated with increased cardiovascular events (31).

In this way, prospective epidemiological studies found that increased levels of inflammatory cytokines are a predictor for possible future cardiovascular disease in the initially healthy population. In addition, studies carried out on patients with depression, with or without cardiovascular disease, have found that the presence of depressive symptoms is associated with increased levels of inflammatory cytokines such as CRP, IL-6, IL-1 and TNF-alpha and that the associated antidepressant therapy appears to decrease the intensity of the inflammation (32).

Thus, the purpose of a study by Shang and his colleagues was to test the hypothesis that patients who suffered a heart attack also showed inflammation, indicated by elevated levels of inflammatory markers. A total of 280 patients were approached and 202 gave informed consent and completed baseline interview. 150 patients (77 females and 73 males with a mean age of 62,8 years old) were enrolled in the study and 75 met the criteria for major depression and heart attack and 75 served as control. They excluded patients suffering from chronic inflammatory diseases, other psychiatric pathology or a history of heart attack and the ones that were older than 80 and also the patient who refused to permit a blood sample to be drawn. Depression was evaluated using the BDI-II score (Beck depressive symptoms Inventory-II). Of the 77 females, 62.7% were depressed, while only 37.3% of the 73 males were depressed. Patients with depressive symptoms had significantly higher levels of TNF-α ($t = 2.070$, $p < 0.05$) compared with control group. Regarding CRP, IL-1β, and IL-6 levels no differences were found between the depressed and control participants. In conclusion, the results of the study demonstrated that patients with depressive symptoms had significantly higher levels of TNF-alfa in comparison with the control group (29).

Numerous studies have linked systemic inflammatory markers (IL-6, IL-1b, and CRP) with the risk of developing heart disease and diabetes, common comorbidities in depression and psychosis. A prospective study conducted by Golam and his colleagues came to support the hypothesis that high levels of IL-6 and CRP in childhood increases the risk of subsequently developing depression or psychosis (28).

Depressive and anxiety disorders are a category of psychiatric disorders with increased prevalence. There is much evidence that connects anxiety and cardiovascular risk factors such as atherosclerosis, metabolic syndrome, and coronary artery disease (33). Given that some degree of systemic inflammation is certainly involved in the etiology of these diseases, it was hypothesized that inflammation plays a role in the anxious-depressive pathology and may represent the link between anxiety and cardiovascular disorders (34).

Anxiety disorder is often associated with depression, which can be a sign of an immune function disorder. However, unlike with depression, very few studies have investigated the relationship between anxiety and inflammation (35). Recent studies have correlated anxiety with increased levels of cytokines and especially of C-reactive protein (36). In a study coordinated by Liukkonen the general population-based Northern Finland 1966 Birth Cohort was followed until age 31 (n=2,688 males and 2,837 females), when the highly sensitive CRP concentrations were measured. Anxiety and depressive symptoms were defined by Hopkins Symptom Checklist (37). After adjusting for confounders, logistic regression analyses showed that anxiety symptoms alone increased the probability for elevated CRP levels in men. Regarding anxiety disorders, research was primarily focused on post-traumatic stress disorder and increased levels of inflammatory markers were found (38).

Major Depressive Disorder, PTSD, and insomnia are associated with increased levels of inflammatory biomarkers as well as an increased risk of inflammatory comorbidities, such as metabolic syndrome, heart disease, obesity, and type 2 diabetes. (39) Chronic Insomnia, depression, and PTSD are associated with an increase in levels of inflammatory cytokines and C-reactive protein (40).

Depression is often correlated with suicidal behavior and there is now ample evidence that indicates that inflammation is associated with the severity of the depression since inflammatory molecules are increased in the blood and in the brains of depressive patients (post-mortem) (41).

For example, Mui *et al.* studied this issue in the case of US ethnic sub-groups and concluded that there is a greater association between the high degree of depression and level of inflammation in elderly Chinese immigrants, as compared to the older people of other ethnicities, and proposed further analysis that considers the stress caused by cultural shifts (42).

Another study by the Vogelzang group also followed the association between antidepressant medication and the characteristics of depressive disorders with the inflammation in a cohort of patients diagnosed with depression and a control group. It has been found that depressed men had elevated levels of CRP and IL-6, but not TNF-alpha. No associations of this type were found in women. A high level of inflammation was found in men that were

older at the onset of depression (CRP, TNF-alpha). Men with SNRI and TCA treatment had increased levels of CRP and IL-6, whereas patients with SSRI treatment had significantly lower values of IL-6. In conclusion, this study suggests a real correlation between depression and inflammation in men (43).

Regarding the genetical mechanisms behind this interaction, changes in gene expression lead to different protein activity in general. There are many mechanisms that help regulate protein production and availability, including epigenetic changes (37). Therefore, modifications of gene expression are one of the first investigations that may explain the molecular mechanisms that are related to changes in protein activities and offer new insights into the complex relationships between gene regulatory mechanisms.

Examination of gene expression profiles provides insight into how altered gene activity is linked with exposure to stress. The expression of a gene may increase or decrease, and it represents the first step in the production of proteins that communicate changes in cell functions, including those that are involved in the immune systems' response to the inflammation (44). Elements that disturb the balance of the body, such as physical and mental trauma, are correlated with increased concentrations of inflammatory proteins, which are most often the result of changes in gene expression. (37)

Epidemiological studies have linked infectious and autoimmune status of childhood psychosis to depression in adult life (45). Current antipsychotic and antidepressive treatments also have anti-inflammatory properties. A meta-analysis that involved increased cross-sectional studies have been reported and they had identified an increase in the serum levels of IL-6 and CRP in depression, first psychotic episode and recurrent acute psychotic disorders (46).

Also, The Avon Longitudinal Study of Parents and Children is a prospective cohort study which investigated the serum levels of IL-6 and CRP in children aged 9 years old and the risk of depression or psychosis at 18 years old. It was anticipated that the higher the initial values of IL-6 and CRP, the greater was the risk of developing depression or psychotic-type disorder. The study involved 4,500 children in England, and, at the end of the study, the initial hypothesis was confirmed (45).

Cytokines have been shown to be related to many depression symptoms to a variety of the population. For example, the allele of the rs IL4 2243248 gene has been associated with a high occurrence of depression in oncology (47), and polymorphisms of TNF-308 and IL6-174 were associated with fatigue and symptoms of depression in patients diagnosed with breast cancer (48). Also, IL-10 polymorphisms have been associated with depressive symptoms in patients with general infections or those with chronic kidney disease in the terminal stage (49). A high level of expression of the CXCL 11 gene (a gene that encodes cytokines) was identified in the case of a significant number of patients with Marfan syndrome and a low quality of life level (50).

Given that numerous genes likely to be involved in the mechanisms of depression were identified, but the results were not conclusive, Kao and his colleagues investigated this issue by reviewing the available data. 5055 genes were studied of whom 160

were identified as having an increased risk to be associated with depression. The first 7 genes are beta hydroxylase dopamine (DBH), the serotonin transporter SLC6A4, brain-derived neurotrophic factor (BDNF), nerve growth factor receptor (NGFR), tumor necrosis factor, glycogen synthase kinase 3 beta and the subunit gene that encodes the CHRNA7 receptor. Two of these genes support the theory of neurotransmitters. DBH dopamine catalyzes the biosynthesis of noradrenaline. A low DBH activity is a factor associated with depression. SLC6A4, belonging to serotonergic synapse pathways, is often associated with depressive symptoms. The neurotrophin signaling path is supported by the top 3 genes. BDNF is a neuroprotective protein that modifies the balance of neuroprotective and neurotoxic responses to stress. GSK3B is an enzyme involved in cell growth and neuronal energy metabolism and plays a key role in stabilizing the mood. NGRF modulates the activity of tyrosine-kinases for the neurotrophins family and may have a protective effect against developing depression. Kao and his collaborators concluded that neurotransmitters and the theory of neuroplasticity have the most evidence of association with depression (51).

Depression is a psychiatric disorder with a major impact on the patient's functionality, with implications in both the psychic sphere in the somatic one. Inflammatory hypothesis in depression is a current active topic, being supported by numerous studies and it represents a starting point for future research.

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RISK FACTORS FOR COLORECTAL POLYPS: FINDINGS FROM A GREEK CASE-CONTROL STUDY

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RISK FACTORS FOR COLORECTAL POLYPS: FINDINGS FROM A GREEK CASE--CONTROL STUDY (Abstract): There is a body of evidence showing that several lifestyle and dietary factors are associated with colorectal polyps' formation; however, the magnitude of this association in diverse populations remains unclear. The aim of this study was to investigate this association in a Greek sample. **Material and method**: The study comprised of 52 subjects with histologically confirmed advanced colorectal polyps and 52 healthy controls. Data concerning lifestyle and dietary factors were collected using a validated questionnaire. Logistic regression analysis was used to estimate odds ratios and 95% confidence intervals after adjustment for potential confounders. **Results**: Physical activity level (95% CI 0.032-0.953, P=0.044) and consumption of yoghurt (95% CI 0.969-0.996, P=0.024), cheese (95% CI 0.932-0.996, P=0.030), fish (95% CI 0.782-0.964, P=0.008), vegetables (95% CI 0.965-0.998, P=0.029), and garlic (95% CI 0.005-0.671, P=0.022) were inversely associated with colorectal polyps. Increasing age (95% CI 1.005-1.231, P=0.039) and central obesity (95% CI 1.001-1.019, P=0.025) were strongly associated with their presence. **Conclusion**: Our study indicates that a cluster of modifiable risk factors have significant impact on colorectal polyps' occurrence in Greek population. **Key words**: CASE-CONTROL STUDY, COLORECTAL POLYPS, DIET

It is well known that the prevalence of colorectal polyps (CRPs) varies among different ethnic and/or geographic regions. In average, their incidence among asymptomatic individuals ranges from 10% in sigmoidoscopic to 25% in colonoscopic studies. The possibility to find new polyps three years after an initial negative endoscopy seems to range from 7% in sigmoidoscopic to 27% in colonoscopic studies (1). Furthermore, the findings of a recently published study suggest that advanced colorectal adenomas' prevalence is 7% (2). What should be stressed at this point is that the incidence might be higher than estimated due to the fact that the so-called "flat colorectal adenomas" may, sometimes, not be recognized. What should also be taken into consideration is the fact that flat adenomas' prevalence could even exceed 10% of the total number of polyps found at colonoscopy (3). Recently developed methods, like chromoendoscopy, seem to considerably increase the detection rate (4).

CRPs are considered the most important precancerous lesions and it is important to note that hyperplastic and adenomatous polyps share many risk factors with each other as

well as with colorectal cancer (5). However, more likely to develop into colorectal cancer are advanced adenomas, which are either classified as ≥1cm in size or present ≥25% villous histology or exhibit high-grade dysplasia compared to smaller ones. Due to this, it is of great importance to try to reduce the formation of advanced CRPs and, of course, to remove them at the time of their discovery during colonoscopy.

Research on diet and CRPs has already focused on specific foods or nutrient components, such as fat (6,7,8,9,10,11), meat (12,13), dietary fiber (14,15,16), vitamins (17,18,19,20,21,22,23,24), fruits and vegetables (25,26,27,28) and dairy products (29,30). Furthermore, some research concerning the effect of smoking habits, alcohol consumption (31,32,33), obesity and physical activity (32,33,34,35,36,37,38,39,40,41,42) on CRPs' formation has also been carried out. So far, the findings of these studies remain either unclear or inconsistent.

Despite the fact that a large number of studies have investigated the role of dietary habits, physical activity level and certain anthropometric characteristics on colorectal carcinogenesis, a relatively small number of studies have examined simultaneously the association between the same variables and the presence of CRPs. Furthermore, it should be noted that in their majority these studies examine this association independently of CRPs' size. The present study was specifically designed to investigate the effect of anthropometric characteristics and lifestyle factors, such as dietary habits, smoking, alcohol consumption and physical activity level on CRPs' formation in a cohort of Greek patients with advanced CRPs (as, to our knowledge, no other study has examined this association in a Greek population).

PATIENTS AND METHODS

The study population consisted of 52 cases (32 males, 20 females), aged 30-77 years, with histologically confirmed advanced (diameter >1cm) CRPs, who underwent full colonoscopy at Gastroenterology Department of Saint Panteleimon Hospital, Greece during the period from January 2008 to June 2009. An equal number of healthy subjects matched for age (±3 years) and sex were used as controls. The latter were participants of a colorectal cancer screening program held by the same Department of the same Hospital and had also underwent full colonoscopy during the same period. In more details, 52 subjects with negative colonoscopy, of the similar distribution by sex (32 males, 20 females) and age (range 33-80, mean age of controls vs cases: 60±11 vs 57±10.5 years, $P=0.11$), served as controls. At this point, it is worthy to mention that in the present study cases and controls were matched one to one.

Data were collected through a face-to-face interview guided by a standardized questionnaire and each interview lasted approximately 60 min. The questionnaire included information on socio-demographic characteristics and lifestyle habits, such as smoking habits and physical activity level.

In order to assess physical activity level, a questionnaire based on the validated Cambridge Physical Activity Index, as described by Wareham et al. (43), was used. According to this, subjects were asked about the type of their occupation (sedentary, standing, manual or heavy manual) as well as the time they used to spend weekly physically active during their leisure time (no time, 3.5 hours or less per week, from 3.5 to 7.0 hours per week). After answering these questions, subjects' physical activity was divided into three levels. In level 1 were inactive subjects (those with sedentary work and with no time spent at physical activity during leisure time). In level 2 were moderately active subjects (those with sedentary work who spent 3.5 hours or less per week physically active as well as those with standing

TABLE I
Differences in the distribution of potentially risk factors for CRPs between cases and controls (results of univariate analysis)

Variables	Cases (n=52)	Controls (n=52)	P-value
Smoking (Yes)	34.6	30.8	0.676
Physical activity			
Level 1	61.5	34.6	0.006
>Level 1	38.5	65.4	
BMI (kg/m^2)			
<25	36.4	63.6	0.354
25-29.99	53.6	46.4	
≥30	53.8	46.2	
Waist circumference	103.2±13.3	98.3±12.8	0.061
Body Fat (%)	32.1±8.1	30.1±7.3	0.189
Body Fat (Kg)	25.8±8.2	24.1±8.5	0.327
Hypercholesterolemia (Yes)	65.4	34.6	0.002
Water	1.2±0.55	1.2±0.6	0.867
Alcohol	286.1±538.7	98.7±101.8	0.015
Milk	154.0±231.5	130.0±149.8	0.532
Yoghurt	39.3±45.4	116.7±103.9	0.000
Cheese	30.6±56.5	89.3±70.4	0.024
Poultry	31.9±19.1	34.1±12.8	0.503
Red meat	46.5±32.6	35.7±12.5	0.027
Sausages	13.2±22.3	11.4±13.5	0.622
Fish	29.2±16.2	41.8±18.7	0.000
Eggs	8.7±16.3	9.1±8.9	0.877
Fruits	267.5±168.5	300.8±170.5	0.318
Vegetables	252.8±129.7	319.0±112.4	0.006
Legumes	43.1±24.3	43.3±22.7	0.970
Onion	34.6±24.8	35.1±29.6	0.930
Garlic	0.32±0.33	0.49±0.28	0.006

Data are mean (SD) and percent for continuous and categorical variables, respectively

work who spent no time at physical activity during leisure time). Finally, in level 3 were active subjects (those with sedentary work who spent from 3.5 to 7.0 hours per week of their leisure time physically active, those with standing work who spent 3.5 hours or less per week of their leisure time physically active, as well as those with manual and heavy manual work – independent of the time they used to spend per week at physical activity during their leisure time) (44).

A medical history was also recorded and participants were classified as presenting hypercholesterolemia if a doctor had told them that blood cholesterol was high, before being prescribed or not a cholesterol-lowering drug.

Height and weight were measured in all case and control subjects by using standard procedures. In particular, body weight was measured by the researchers to the nearest of 0.1Kg and in light clothes, while height was also measured in standing position. Body Mass Index (BMI, in Kg/m^2) was calculated as weight in kilograms divided by height in meters squared. A body fat analyzer (BF 902, OMRON) was used in order to determine body composition by measuring bioelectrical impedance. At this point, it should be mentioned that this method of estimating body composition is based on the ability of the tissues to conduct an electric current and is generally thought to be a good pre-

dictor of body fat, especially when it comes to epidemiologic studies (45,46). Finally, subjects' central obesity was estimated by measuring waist circumference (in cm) while they were in a standing position.

Finally, in order to assess subjects' habitual diet, a validated Food Frequency Questionnaire (FFQ) was used (47). The latter consisted of 79 questions and was used to assess the average daily frequency of consumption of specific foods during the 2 years prior to the survey. In more details, with its use it was able to estimate each subject's average daily consumption of milk, yoghurt, yellow and white cheese, margarine, butter, cream, mayonnaise, oranges, apples, pears, tangerines, peaches, apricots, bananas, melon, watermelon, grapes, strawberries, cherries, broccoli, cauliflower, cabbage, carrots, corn, lettuce, spinach, pea, lentils, beans, tomatoes, poultry, processed meat, veal, lamb, pork, fish, seafood, eggs, bread, cereals, biscuits, cakes, chocolate, potatoes, rice, spaghetti, nuts and seeds, coffee, tea, alcohol, sugar and honey. The supplementary use of nutrients, the type of fat used in the preparation of the food, as well as the consumption of organic foods was also investigated.

STATISTICAL ANALYSIS

Pearson chi-squared statistics and t-tests were used to evaluate case-control differences in the distribution of potentially confounding factors. Odds ratios (ORs) were used to measure the association of CRPs' presence with risk factors. Logistic regression analysis was used to estimate ORs and 95% confidence intervals (CIs), after adjustment for potential confounders. Variables that were statistically associated in the univariate analysis were included in the analysis. Furthermore, risk factors identified previously (including age, sex, nutritional status and smoking) as having an independent association with CRPs were controlled in the logistic regression model. A P value <0.05 was considered statistically significant and all statistical tests were based on two-sided probabilities using SPSS, version 16.0.

RESULTS

Univariate analysis

As shown in table I statistically significant differences between cases and controls were found for physical activity level, alcohol, fish, yoghurt, cheese, red meat, vegetables and garlic consumption. Furthermore, it should be mentioned that marginally significant differences were found concerning waist circumference. One interesting finding of the present study concerns the effect of hypercholesterolemia on CRPs' development. In more details, the presence of hypercholesterolemia was found to be significantly associated with higher percentage of CRPs. Finally, no significant differences between cases and controls were found concerning smoking habits as well as consumption of water and other foods such as milk, eggs, poultry and legumes.

Multivariate analysis

After performing multivariate analysis it was found that physical activity and consumption of yoghurt, cheese, fish, vegetables, and garlic were negatively associated with CRPs (table II). On the other hand, age and waist circumference were found to be positively associated with CRPs. Finally, a trend for significant difference on alcohol consumption between cases and controls was also found, but the difference did not actually reach statistical significance (OR= 1.006, 95% CI 1.000-1.013, $P=0.066$).

DISCUSSION

This case-control study examined the possible associations between lifestyle and dietary factors and CRPs, which are thought to be precursors of colorectal cancer. It was conducted in a sample of Greek population, which ranked among the populations with

TABLE II
Factors significantly associated with presence of colorectal polyps (results of multivariate analysis)

Risk factor	Odds ratio†	95% Confidence Intervals	P-value
Age	1.11	1.005-1.231	0.039
Physical activity			
Level 1*	1.00		
>Level 1	0.18	0.032-0.953	0.044
Waist circumference	1.27	1.052-1.542	0.013
Yoghurt	0.98	0.969-0.996	0.024
Cheese	0.96	0.932-0.996	0.030
Fish	0.87	0.782-0.964	0.008
Vegetables	0.98	0.965-0.998	0.029
Garlic	0.06	0.005-0.671	0.022

† Adjusted for age, sex, smoking, physical activity, BMI, waist circumference, hypercholesterolemia, alcohol, yoghurt, cheese, red meat, fish, fruits, vegetables and garlic
** Reference group*

the lowest colorectal cancer incidence in Europe (48). It has been hypothesized that the latter observation is attributed to the protective effect exerted by favorable combinations of the Mediterranean diet's components, which is generally characterized by frequent consumption of fruits, vegetables, pulses and fish, moderate consumption of milk and dairy products and low consumption of meat and cheese, while one of the Mediterranean's diet major characteristics is the almost exclusive use of olive oil as seasoning (49).

The findings of the present study suggest that yoghurt and cheese consumption seems to exert a significant protective action against CRPs, which is thought to be due to their high content in Ca. This hypothesis is supported by the finding of a recent study, according to which supplementary intake of 1000-2000mg of Ca daily seemed to reduce adenoma recurrence (29). However, dairy products' high content in Ca might not be the sole underlying mechanism of the observed association, as they are rich sources of other potential chemopreventive agents, such as vitamin D, sphingolipids, conjugated linoleic acid, butyric acid and probiotic bacteria. In fact, the findings of a previous trial cohort suggest that Ca and vitamin D intakes may be inversely associated with adenoma recurrence risk (22).

One interesting finding of the present study was the one concerning hypercholesterolemia. As the univariate analysis showed, the presence of hypercholesterolemia was found to be associated with increased CRPs' risk, but the association was not confirmed after performing multivariate analysis. This might be due to the possible interaction between the presence of hypercholesterolemia and yoghurt and cheese consumption. Therefore, this finding highlights the need that, in clinical practice, patients should be advised to consume dairy products as part of a balanced diet.

We also found an inverse association between garlic consumption and CRPs, which remained statistically significant after adjusting for other dietary factors. To our knowledge, few studies have investigated the association between garlic consumption and CRPs' risk (50,51,52). Garlic contains components such as diallyl sulphide and allyl methyl trisulfide that might be responsible for the observed protective effect of garlic consumption against carcinogenesis. In particular, garlic's sulphur components have been reported to suppress experimentally induced tumor incidence in several organs, including the colon. In preclinical studies, both water- and lipid-soluble allyl

sulfur compounds arising from processed garlic inhibited aberrant crypt foci, which are proposed to be early preneoplastic lesions of adenoma-carcinoma in humans (53).

The protective effect of fish consumption on CRPs' development has also been observed in a previous quantitative review of eleven case-control and two cohort studies (30). Moreover, it has been found that higher fish consumption seems to be associated with lower risk of adenoma recurrence (13). This favorable effect of fish consumption is thought to be due to its high content in n-3 fatty acids, which have been found to reduce the progression of small adenomas to large ones (10). Moreover, when trying to identify the plausible mechanisms of the observed association, it should seriously be taken into consideration the fact that fishes are rich sources of selenium, as it has already been found that higher selenium levels seem to be associated with decreased risk of both colorectal adenoma and colorectal cancer (24).

A similar association has also been found concerning vegetables. More specifically, vegetables consumption seems to protect against CRPs. The findings of previous studies that have examined the association between vegetables intake and the risk of CRPs' development remain equivocal. However, the risk estimates have, generally, been in the protective direction (25,27,28). When trying to explain the findings of these studies, it should seriously be taken into consideration that it is a very complex group, involving food items that might exert protective action, others that could exert this action to a lesser extent, while there might also be vegetables that do not protect against CRPs at all. So, it might be a good idea to examine those foods in particular and not as a whole.

In the present study waist circumference was an independent risk factor, as it was found to be significantly associated with CRPs' occurrence. The finding supports the findings of previous studies (36,37,38). Moreover, it has already been proposed that obesity, especially central obesity, could also be viewed as a promoting factor in colorectal adenomas' growth as well as that it could increase colorectal cancer risk (37, 38,54). Finally, at this point it is important to mention that obesity has been related to colorectal cancer through its association with elevated insulin and glucose levels (41). However, the underlying mechanisms of the observed association remain unclear.

Finally, in our study, increased physical activity was found to protect against advanced CRPs' formation in the Greek population studied. Since advanced CRPs are thought to be precancerous lesions, it should be mentioned that the underlying biological mechanisms of the observed association could be similar to the ones explaining the association that has consistently been found between physical activity and colorectal cancer. In more details, sedentary lifestyle has generally been associated with hyperinsulinaemia, since both the insulin-like growth factor family and insulin have been associated with enhanced tumor growth and antiapoptosis. Individuals with higher circulating levels of insulin, its secretion's marker, C-peptide and inslulin-like growth factor-1 seem to be at increased risk for colon cancer (55). Apart from that, it has been proposed that increased physical activity protects against colorectal cancer by helping to maintain the energy balance and, thus, to maintain a healthy body weight, as well as by enhancing the immune system (56,57). Furthermore, increased physical activity is associated with increased gut motility, which leads to decreased gastrointestinal transit time and, thus, decreased exposure of the intestine mucosa to carcinogens. Finally, increased physical activity leads to a favorable ratio of prostaglandins, as it seems to

increase prostaglandin F, which increases gut motility and inhibits colonic cell proliferation, while, at the same time, it does not increase prostaglandin E2, which affects colonic cell proliferation opposite to the way that prostaglandin F does (58).

CONCLUSION

Our findings confirm that yoghurt, cheese, fish, vegetables and garlic consumption provides protection against advanced CRPs' formation. The same protection seems to be provided by increased physical activity. On the other hand, age and central obesity were found to be independent aggravating factors, as they significantly increased CRPs' development risk. Our findings also support the hypothesis that some of the environmental and nutritional factors involved in colorectal carcinogenesis are also involved in the genesis of colorectal polyps in Greek population.

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NEURO-PSYCHIATRIC ONSET OF GRAVES' DISEASE IN CHILDREN. CASE REPORT AND LITERATURE REVIEW

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NEURO-PSYCHIATRIC ONSET OF GRAVES' DISEASE IN CHILDREN. CASE REPORT AND LITERATURE REVIEW (Abstract): Children with thyrotoxicosis are usually hyperactive with behavioral problems, difficulty in concentration and attention. Neuropsychiatric disturbances in children with Graves' disease (GD) can be mistaken for "true" psychiatric or neurologic disorder delaying the diagnosis and the correct therapeutic approach. We present the case of an 8 years old with important height and weight gain in the last two years. The patient had a medical history of ADHD. Despite the psychotherapy the behavior has not improved. Physical examination revealed increased weight and height, exophthalmia and lid retraction, diffuse goiter, important tremor, hyper talkativeness, warm and moist palms and tachycardia. The diagnosis of GD was confirmed by the values of thyroid hormones and the presence in high titers of thyroid stimulating hormone receptor antibodies (TRAb). After several months of treatment with ATDs only, we have switched "on block and replace" therapy. During the 3 years surveillance, the height and weight velocity rates remained increased with an advanced bone age. Once euthyroidism achieved and maintained, also the neuropsychiatric abnormalities, including irritability, anxiety, sleep disturbances and mood changes improved. The school performances improve to, and the inability to concentrate disappeared after several months. At the moment the patient is still on a minimal dose of ATD with normal hormonal levels. Neuro-psychiatric manifestations can be the first sign of thyrotoxicosis in children and adolescents. A close collaboration with parents is required in order to have the desired results: euthyroidism and disappearance of neuropsychiatric dysfunctions. **Keywords**: THYROTOXICOSIS, CHILDREN, MOOD DISORDERS, HYPERACTIVITY.

Thyroid dysfunction may cause, or be associated with, a wide range of psychiatric and or neurological disorders in children and adolescents. Graves' disease (GD) is the most common cause of thyrotoxicosis in pediatric population with a prevalence of 1 in 10,000 children (1). GD account for 10-15% of childhood thyroid diseases and its incidence rises from 0.1/100,000 in young children to 3/100,000 in adolescents (2). GD is more common in girls than in boys (3). The classic clinical picture includes diffuse goiter, exophthalmia, increase appetite, weight loss, palpitations, excessive physical activity, nervousness, thermo phobia, tremor, accelerated linear growth (1). Children with thyrotoxicosis are usually hyperactive with behavioral problems such as: difficulty in concentration and attention, disturbances which may lead to decreased cognitive performance (4).

Neuro-psychiatric disturbances in pediatric GD are rare and can be mistaken for "true" psychiatric or neurologic disorder delaying the diagnosis and so, the correct therapeutic approach. Hyperthyroidism can present as a hypomania or maniac-like state, anxiety, depression, mixed mood disorders, emotional lability and irritability (5). It is believed that hyperthyroidism affect the activities of neurotransmitters (serotonin and dopamine) in the central nervous system and possibly account for the neuropsychiatric abnormalities. Thyroid hormones are responsible for the modulation of the beta-adrenergic response to catecholamines in the central nervous system and so, may contribute to psychotic behaviors in thyrotoxic patients (6). GD is associated with, or may cause, a spectrum of central and/or peripheral neurological dysfunction and a considerable variation in clinical phenotype is described (7). There is a connection between the effects of thyroid hormones and several frequent psychiatric disorders. Minor changes in thyroid hormone concentrations, may have important effects on cerebral function. This may manifest as alterations in mood, behavior and cognition (8).

The diagnostic of GD may be very challenging in children due to clinical manifestations which may mimic a neuropsychiatric disorder. It takes sometimes a long period of time until the positive diagnosis of GD is done. This will interfere with growth and development of the child and also may lead to serious cardio-vascular complications. Here we report a girl with GD who was initially diagnosed with attention deficit hyperactivity disorder (ADHD) due to important cognitive deterioration, difficulties with attention and hyperactivity.

CASE REPORT

We present the case of an 8 years old girl admitted in our department for important height and weight gain in the last two years. The patient had 18 months history of increasing irritability, insomnia, mood swings, and impaired concentration. Her mother said that she become unfriendly with colleagues at school and the anxiety, nervousness, restlessness, diminished concentration and hyperkinesia lead to her poor school performance. Due to difficulties with concentration and attention and hyperactivity she was diagnosed with ADHD and underwent psychological counseling. Despite the psychotherapy the behavior has not improved. Moreover, she has developed an uncontrolled appetite which leads to a rapid increase in weight.

Physical examination revealed increased weight and height (both at +2 SD), exophthalmia and lid retraction (fig. 1). At palpation thyroid was soft, diffuse enlarged, smooth, and non-tender (fig.1). The patient presented hyperkinesia, important tremor, hyper talkativeness, warm and moist palms, tachycardia (110 beats/minute) and normal blood pressure.

The thyroid ultrasound examination revealed a 40 mL thyroid volume (normal value according WHO (9) for age and sex is 6.9 mL), intense hypoechogenic structure and an increased vascularization in Doppler mode (fig. 2).

Fig. 1. Lid retraction and diffuse goiter at clinical examination.

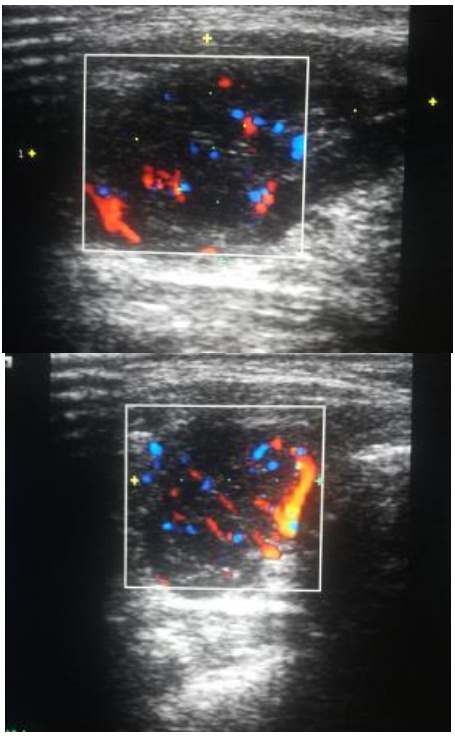

Fig. 2. Thyroid with increased volume, important hypoechogenic structure and increased vascularization on neck ultrasound.

All these data were specific for autoimmune thyroid disorder. The hormonal profile was in accordance with the clinical picture of hyperthyroidism (thyrotoxicosis): TSH (thyroid stimulating hormone) = 0.022 µIU/mL (normal values 0.35-5.6), fT4 (free thyroxin) = 1.44 ng/dL (normal values 0.89-1.76), fT3 (free triiodothyronine) =10.5 pg/mL (normal values 2.4-8.6). The presence in high titers of antithyroid antibodies: anti-peroxidase (TPOAb) > 1,000 IU/mL (normal values 0-35) and anti-thyroglobulin (TGAb) > 3,000 IU/mL (normal values 0-40) was also recorded. Considering the presence of thyrotoxicosis with ophthalmopathy, suggestive for GD, we have measured the level of thyroid stimulating hormone receptor antibodies (TRAb) which showed very high values 37.4 UI/L (positive> 1.5).

The treatment was started with antithyroid drug (ATD) Thiamazole 15 mg/day with caution for side effects such as: agranulocytosis, thrombocytopenia, hepatitis, skin rash, and urticaria. Also, a ß blocker (propranolol) was added in order to manage the tachycardia and also to improve the metabolism of T4 in T3 at cellular level.

After two months the patient was retested for thyroid function and the physical examination was performed. The girl's mother said that it was very difficult for her to persuade her daughter to take the medication. Indeed, the hormonal picture was slightly improved (TSH still low, fT4 normal and fT3 8.9 *vs.* 10.5 pg/mL (before ATD). The tremor was present, blood pressure was normal and heart rate= 90 beats/minute. The patient was seen every 3 months for a period of 3 years. This strict follow-up was necessary due to unpredictable evolution with ATD treatment (tab. I).

It was very difficult to set a dosage able to achieve euthyroidism. Even slight increase or decrease in daily dosage of Thiamazole (according with fT4 and fT3) were succeeded by important variations of TSH, fT4 and fT3 values. After several months of treatment with ATD only, we have switched "on block and replace" therapy (high dose of ATD with levothyroxine in order to achieve euthyroidism). During the 3 years surveillance, the height and weight velocity rates remained increased with an advanced bone age. Once euthyroidism achieved and maintained, also the neuropsychiatric abnormalities, including irritability, anxiety, sleep disturbances and mood changes improved. The school performances improve to, and the inability to concentrate (major complain at the first visit) disappeared after several months. Regarding the ophthalmopathy, the evolution was positive with no aggravation of exophthalmia. At the moment the patient is still on a minimal dose of ATD with normal hormonal levels.

TABLE I
Evolution of biologic parameters during the ATDs therapy

DATE	TSH (0.35-5.6 µIU/mL)	fT4 (0.89-1.76 ng/dL)	fT3 (2.4-8.6 pg/mL)	TPOAb 0-35 IU/mL	TGAb 0-40 IU/mL	TRAb >1.5
08. 2015	0.022	1.44	10.5	>1,000	>3,000	37.8
10. 2015	0.026	1.50	8.9	-	-	-
03.2016	0.004	1.22	-	>1,000	-	-
06. 2016	**0.018**	**2.03**	-		-	36.4
11. 2016	**0.019**	**0.450**	-	-	-	-
07. 2017	0.007	1.37	-	-	-	-
12. 2017	**24**	**0.3**	-	-	-	-
05. 2018	0.043	1.96	10.5	-	-	-
11. 2018	0.023	0.82	5.79	>1,000	-	-
02. 2019	0.260	1.06	7.38	>1,000	-	-

DISCUSSION

Our case illustrates the diagnostic difficulties in pediatric GD. The intensity of psychological disturbances overlapped the classical manifestation of thyrotoxicosis. The delay in diagnosis and treatment of GD in this 8 years old girl lead to interference in growth and development including increased height velocity rate. Moreover, due to behavioral problems, the compliance on medical treatment was very challenging. The improvement of the biological picture has been very difficult to achieve to. Long-term suppressed TSH along with fluctuations of fT4 and fT3 (which is more important as treatment efficacy marker in pediatric GD) were present throughout several months. The presence of anti-thyroid antibodies (TPOAb and TGAb) with stimulatory or inhibitory effect, in high titers can explain the irregular pattern of fT4 and fT3.

The literature indicates that remission rates in children are less than 25% following many years of ATD treatment. Puberty, TRAb levels and thyroid volume influence remission rates (1). Another particularity of this case was the weight of this girl that has been increased over the entire follow-up period. Commonly the children with GD present poor weight gain (4) due to hyper metabolism and hyperactivity. In our case the patient was overweight all the time despite the prolonged, uncontrolled thyrotoxicosis.

Pediatric Graves' disease is an autoimmune disorder due to thyroid stimulation by thyroid receptor antibodies (TRAb) (1). The pathophysiology of GD is not fully understood but it speculates the role of selenium (Se) in the lymphocytic chronic thyroiditis. Se supplementation has a stimulatory effect on glutathione peroxidases and thioreductases and inhibits the Human Leukocyte Antigen – DR isotype (HLA-DR) molecule (10, 11).

Beside the medical treatment with ATD in pediatric GD, the surgical option remains for cases which do not respond to ATD or develop serious adverse events (leucopenia, agranulocytosis). Total thyroidectomy is recommended in order to avoid the recurrence on the remaining thyroid tissue but the

post-operative complications such as hypoparathyroidism or recurrent laryngeal nerve injury should be considered (12, 13). The radioactive iodine use in pediatric GD represents an alternative to ATD and surgery (1).

Thyroid hormones have direct and important effect on brain function (8). Thyrotoxicosis in children is associated with neurological manifestations such as tremor, seen in the hands, face, and legs. Tremor is more commonly seen in pubertal/post pubertal than in prepubertal children (7) and is thought to be secondary to stimulation of the beta-adrenergic system and it is seen both at rest and with intention (7).

Other rare neurological manifestations in pediatric GD are: intermittent encephalopathy (14), acute confusional state (followed by thyroid storm) (15), choreiform movements (7). Seizures also can occur and vary from generalized tonic-clonic to complex partial, or more focal findings (7).

Hyperthyroid state can associate with cerebral ischemic events, and in most cases, patients recover from their neurologic symptoms after achieving euthyroidism. One possible explanation might be a hemodynamic compromise, induced by an increase in the cerebral metabolism and oxygen expenditure (16). Thyroid hormones may increase vascular sensitivity to the sympathetic nervous system and induce changes in the arterial walls. Vasculitis induced by antithyroid drugs may cause changes in the intracranial arteries. Cerebrovascular hemodynamic changes induced by thyrotoxicosis were considered to be responsible for the cerebral ischemic events. In addition, thyrotoxicosis-induced hypercoagulability may influence ischemic events (17, 18, 19). ADHD and autism spectrum disorder can mimic the onset of GD in children, so is very important always to test the thyroid function in children or adolescents with hyperactivity or comportment disorders (20, 21). Psychiatric disorders, loss of consciousness and movement disorders may all be manifestations of pediatric GD (8).

CONCLUSIONS

Neuro-psychiatric manifestations can be the first sign of thyrotoxicosis in children and adolescents. This may delay the diagnostic and proper treatment of thyrotoxicosis leading to important complications in pediatric GD. It is very important to assess the thyroid function in children with mild behavioral, mood disorders or neurological/ neuromuscular disturbances. A close collaboration with parents is required in order to have the desired results: euthyroidism and disappearance of neuro-psychiatric dysfunctions.

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THE IMPACT OF SUBCLINICAL ATHEROSCLEROSIS TO THE PATIENTS WITH STROKE AND DIABETES

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THE IMPACT OF SUBCLINICAL ATHEROSCLEROSIS TO THE PATIENTS WITH STROKE AND DIABETES (Abstract): **Aim** Atherosclerosis is a multifactorial disease, modulated by numerous risk factors and the basis for multiple micro and macro angiopathic pathologies. The main risk factor that triggers atherosclerosis and thickening of IMT is the increased cholesterol level. Smoking, obesity, arterial hypertension and increased glycemia are also risk factors for atherosclerosis. **Material and methods**: This paper aims to perform a retrospective study including patients presenting with a history of ischemic stroke diagnosed clinical and by native cerebral computer tomography, in which intima-media thickness (IMT) was evaluated at bilateral carotid level as an independent risk factor in relation to cholesterol, glucose and body mass index. **Results**: The statistical analysis showed a significant difference between high cholesterol and glycemia values, but also for IMT in common carotid artery and glycemic values. Although mean IMT was increased in stroke patients, statistical data analysis revealed that there was no significant difference between the IMT at the common and internal carotid arteries and the cholesterol levels. In our study, the patients with hypercholesterolemia had a normal IMT (80.9%). 19.1% of patients with stroke and hypercholesterolemia show a linear decrease of IMT with lowering cholesterol levels, similar to the trend observed at ACC level. **Conclusions**: The stroke patients who also had diabetes mellitus have an increased value of IMT, so it is once again confirmed that diabetes, along with stroke, represents an important risk factor in the process of atherosclerosis. **Keywords**: ATHEROSCLEROSIS, INTIMA MEDIA THICKNESS, CHOLESTEROL.

Atherosclerosis is a multifactorial disease, modulated by numerous risk factors and the basis for multiple micro and macro angiopathic pathologies. It is considered to be an infiltrative and degenerative process that associates both lipid deposits in the intima and a fibrous reaction (1, 2). The first lesions of atherosclerosis occur in the second decade of life, but this does not become symptomatic until after the sixth decade of age (3). It can be detected in a subclinical stage through intima-media thickness measurements at the carotid level (IMT), ankle-branch index, arterial calcification score and arterial stiffness (4, 5).

The main risk factor that triggers atherosclerosis and thickening of IMT is the increased cholesterol level. Smoking, obesity,

arterial hypertension and increased glycemia are also risk factors for atherosclerosis (6, 7). Diabetes mellitus is an independent risk factor for cardiovascular and cerebrovascular disease in both women and men. The risk of hyperglycemia is also present in people with obesity and metabolic syndrome (8, 9). Cardio and cerebrovascular diseases are the main cause of mortality and morbidity among people with diabetes, where their prevalence is up to 55% (10).

Stroke represents one of the most important cause of long-term disability of the middle and elderly age population. Approximately 4.4 million people die from the consequences of stroke each year, which accounts for about 9% of all deaths worldwide, 88% of these in people over 65 years old (11, 12). Risk factors for atherosclerotic stroke seem to be important: age, gender, hypertension, tobacco, obesity, hyperlipidemia, diabetes, alcohol consumption, oral contraception and family history (13). It appears that a major role lies in the hyperlipidemic risk factor further investigated in experimental and clinical studies. In some cases, an established stroke is preceded by a TIA (transient ischemic accident), when the neurological deficit occurs suddenly, in full health and is of short duration. The most common cause of TIA is carotid artery and vertebral artery atherosclerosis or thrombosis. The association of stroke with type 2 diabetes increases mortality by approximately 55% (14). Since both pathologies result from the interaction between genetic predisposition and vascular risk factors, identifying common elements could lead to the development of primary and secondary prevention recommendations.

Atherosclerosis is a process underpinning multiple vascular and non-vascular comorbidities and recent studies offer promising results on the role of IMT both as an independent risk factor for stroke and type 2 diabetes mellitus, but also as a prognostic factor. According to the current guidelines in all patients who have a history of stroke, it is recommended to use statins as a prognostic element, as on the one hand they lower the cholesterol value and on the other hand stabilize the already formed atherosclerotic plaque (15, 16, 17).

MATERIAL AND METHODS

This paper aims to perform a retrospective study including patients presenting with a history of ischemic stroke diagnosed clinical and by native cerebral computer tomography, in which IMT was evaluated at bilateral carotid level as an independent risk factor in relation to cholesterol, glucose and body mass index (BMI).

The main exclusion criteria involved any embolic conditions, atheroma plaques, stenosis or occlusion in the carotids or a value of the IMT more than 1.5 mm. In the present study, all subjects before being examined were informed of the research method and signed an informed consent. The batch was examined by the same person, with the Siemens Accuson X300 system using a 7.5 MHz linear probe via a standardized method, in mode B, on a longitudinal section, at the level of both common (CCA) and internal (ICA) carotid arteries, bilaterally (18). The IMT measurement was performed at the distal wall of the CCA and ICA, at 10 mm of the bifurcation, in the area without any atheroma plaque (19). The value used for the subsequent statistical analysis was the average of the three measurements on each side. Medium IMT values greater than 1 mm were considered elevated. No values greater than or equal to 1.5 mm were considered (these representing the formation of the atherosclerotic plaque). We also measured:

weight, height, total cholesterol and glycemia levels. Based on these anthropometric parameters, the body mass index (BMI) was calculated according to the formula: G (kg)/T^2 (m^2). BMI is the most used surrogate parameter to estimate excess weight. A level of cholesterol above 200 mg/dL was considered increased and for glycemia above 100 mg/dL.

Statistical analysis

In interpreting the statistical results, the reference value of significant values p = 0.005 was considered, which corresponds to a confidence interval of 95%. Continuous type variables were presented as mean ± standard deviation. The statistics was performed in *SPSS v.18.0*

RESULTS

We gathered 47 patients in our study distributed evenly, the total gender distribution included 22 females (46.80 %) and 25 males (53.20 %). The mean age was 52.7 years old and the average BMI was 26.5 kg/m^2.

In our study, there is a direct proportional relationship between cholesterol and IMT at common carotid artery (CCA) level in patients with previous stroke. The mean value of 300 mg/dL is the mean value at which the average intima thickness is maximal. Regardless of the increase in cholesterol, the average intima thickness is no longer altered. If cholesterol is between 200 and 300 mg/dL, IMT is maintained as high as in patients with cholesterol levels of 300 mg/dL or above. This is also confirmed in the statistical analysis, with no statistically significant difference (fig. 1). The usefulness of hypolipidemic medication results in the stabilization of the atheromatous plaque without influencing IMT.

The same tendency is observed between cholesterol values and mean intima media thickness at the left CCA. This time, there is a decrease in IMT with decreasing cholesterol. Thus, although there is no statistically significant difference between the studied parameters, a hypolipidemic medication is required to diminish the IMT and help stabilize any existing cholesterol plaques (fig. 2).

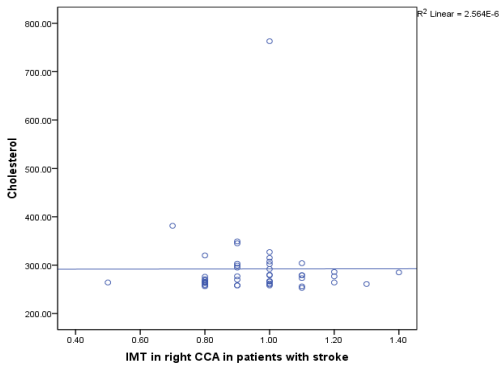

Fig. 1. Correlation of IMT at the right CCA with cholesterol

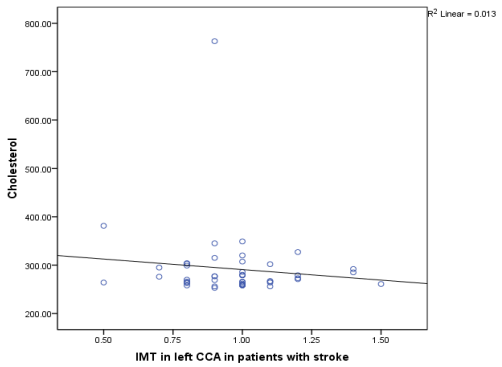

Fig. 2. Correlation of IMT at the left CCA with cholesterol

There is no statistically significant difference between the three groups of patients, but the decrease in cholesterol in patients with hypercholesterolemia is inversely proportional to increases in glycemic values. Thus, most patients with ele-

vated cholesterol levels have normal glycemia, and those with hypocholesterolemia have elevated glycemic levels. Also, the statistical analysis objected to a significant difference between high cholesterol and glycemia (p = 0.000) (fig. 3). This fact is inconsistent with the data from the literature to confirm once again the potency of these parameters in the development of the atherosclerotic process.

Another parameter monitored in the study is the body mass index (BMI). Most stroke patients had grade I obesity. Statistical data analysis revealed that there was no statistically significant difference between cholesterol and body weight (fig. 4).

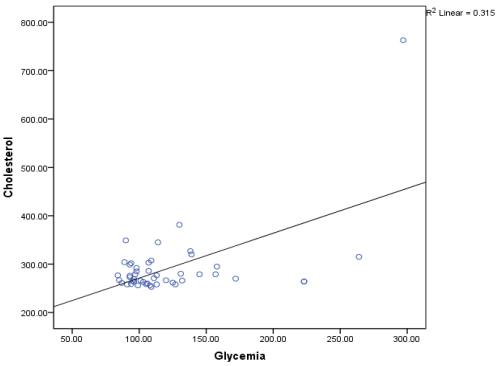

Fig. 3. Correlation of glycemia with cholesterol

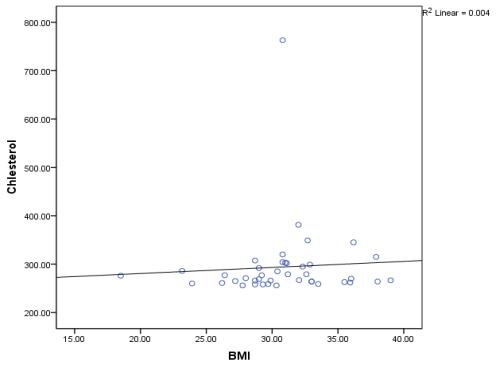

Fig. 4. Correlation of BMI with cholesterol

Increasing the mean interstitial thickness would be partly due to changes in the parietal level due to the slightly higher flux in the left carotid system than the right one in the right patients, and because of the changes associated with the preexisting comorbidities: diabetes, hypertension, obesity, hypercholesterolemia (fig. 5).

In our study we find that 14.89% of diabetic patients have an increased value of IMT, a statistically significant percentage (p = 0.047), so it is once again confirmed that diabetes represents an important risk factor in the process of atherosclerosis (fig. 6).

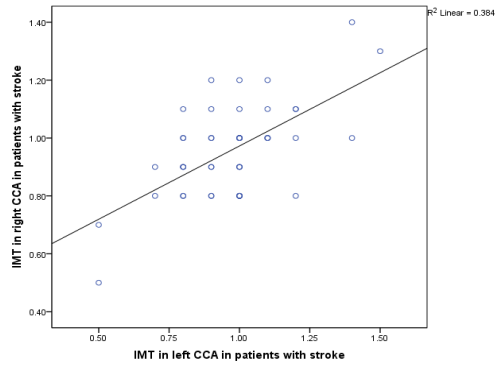

Fig. 5. Correlation of IMT at the left and right CCA

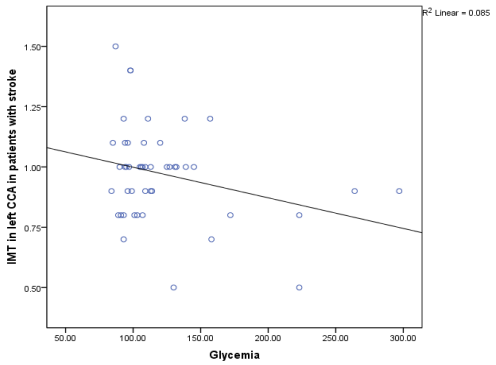

Fig. 6. Correlation of IMT at the left CCA and glycemia

DISCUSSION

In our study, the patients with hypercholesterolemia had a normal IMT (80.9%). The data obtained by us at the level of the right internal carotid artery (ICA) are in contradiction with the data obtained at the bilateral ACC and with the literature.19.1% of patients with stroke and hypercholesterolemia show a linear decrease of IMT with lowering cholesterol levels, similar to the trend observed at ACC level. So, we can once again say that hypolipidemic medication causes IMT to decrease but this decrease is not statistically significant. Liping *et al.* in a recent study had also a decrease of the IMT after 6 months of treatment with rosuvastatin or atorvastatin, but with a statistical significance compared to the values before treatment ($p<0.05$) (20).

The same trend is observed between cholesterol and IMT, respectively, at the level of the left ACI. Although insignificantly statistically from the previous group, only 25.5% of patients with hypercholesterolemia exhibit changes in IMT at the left ACI level. The decrease in IMT with decreasing cholesterol is less steep, maintaining almost constant although cholesterol values range between 253 and 763 mg/dL. And this time, the results are not in line with those in the literature and there is no statistically significant difference between cholesterol and IMT. Guideline recommendations on the usefulness of hypolipidemic medication are only reflected in the stabilization of the atheroma plate without affecting the value of the cholesterol (15).

The main risk factors in the atherosclerotic process are diabetes mellitus and hypercholesterolemia. We have tried to see if there is any relationship between the presence of hypercholesterolemia and the amount of glucose. The highest blood glucose level was observed in 29.79% of patients with hypercholesterolemia, a decrease in glucose tolerance was seen in 31.91% of patients and 38.3% had normal blood glucose.

It is known that overweight and obese patients often have elevated cholesterol levels. This can also be observed in our study. Thus, 41.5% of patients with hypercholesterolemia were obese grade I, 17.1% had grade II obesity, 34.1% were overweight and only 7.3% had a normal weight. So, there is no direct proportional relationship between cholesterol and weight.

In the studied group a statistically significant difference between IMT at right and left level ($p = 0.000$) was observed. This is consistent with literature data knowing that most of the right-handed patients have a slightly higher carotid IMT value physiologically.

There is also a direct proportional relationship between modified blood glucose and IMT. Thus, we note that 19.15% of patients with modified basal blood glucose have increased IMT and 12.77% have normal IMT value. In patients with stroke and diabetes, the highest thickness of IMT is observed. Thus, once again we can say that the main cause of stroke is carotid atheromatosis, to which vascular intraparietal changes are added due to the prolonged presence of diabetes. According to the current guidelines, patients with stroke and diabetes should be given hypoglycemic medication, but also hypolipidemic with the desire to stabilize the plaque and to reduce the average intima media thickness (15).

CONCLUSIONS

In our study, the mean IMT was increased in stroke patients, but statistical

data analysis revealed that there was no statistically significant difference between the IMT at the level of common and internal carotid arteries and the cholesterol levels. Patients with stroke and increased cholesterol levels showed a linear decrease of IMT with lowering cholesterol levels also at the ICA but with no significant difference. Also, no statistically significant difference was found between cholesterol and BMI. Most patients with elevated cholesterol levels have normal glycemia, and those with hypocholesterolemia have elevated glycemic levels.

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NEWS

HYPERSENSITIVITY PNEUMONITIS. RADIOLOGIC PHENOTYPES ARE ASSOCIATED WITH DISTINCT SURVIVAL TIME AND PULMONARY FUNCTION TRAJECTORY

Hypersensitivity pneumonitis (HP) is an interstitial lung disease with a better prognosis, on average, than idiopathic pulmonary fibrosis (IPF). We compare survival time and pulmonary function trajectory in patients with HP and IPF by radiologic phenotype. HP (n = 117) was diagnosed if surgical/transbronchial lung biopsy, BAL, and exposure history results suggested this diagnosis. IPF (n = 152) was clinically and histopathological diagnosed. All participants had a baseline high-resolution CT (HRCT) scan and FVC % predicted. Three thoracic radiologists documented radiologic features. Survival time is from HRCT scan to death or lung transplant. Cox proportional hazards models identify variables associated with survival time. Linear mixed models compare post-HRCT scan FVC % predicted trajectories. Subjects were grouped by clinical diagnosis and three mutually exclusive radiologic phenotypes: honeycomb present, non-honeycomb fibrosis (traction bronchiectasis and reticulation) present, and nonfibrotic. Nonfibrotic HP had the longest event-free median survival (> 14.73 years) and improving FVC% predicted (1.92%; 95% CI, 0.49-3.35; p = .009). HP with non-honeycomb fibrosis had longer survival than IPF (> 7.95 *vs.* 5.20 years), and both groups experienced a significant decline in FVC % predicted. Subjects with HP and IPF with honeycombing had poor survival (2.76 and 2.81 years, respectively) and significant decline in FVC % predicted. Three prognostically distinct, radiologically defined phenotypes are identified among patients with HP. The importance of pursuing a specific diagnosis (e.g., HP *vs.* IPF) among patients with non-honeycomb fibrosis is highlighted. When radiologic honeycombing is present, invasive diagnostic testing directed at determining the diagnosis may be of limited value given a uniformly poor prognosis (Margaret L. Salisbury, Tian Gu, Susan Murray, Barry H. Gross *et al. CHEST* 2019; 155(4): 699-711).

REAL-LIFE PERCEPTION OF ACLF IN ROMANIA: RESULTS OF A SURVEY COMPLETED BY PRACTICIANS

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REAL-LIFE PERCEPTION OF ACLF IN ROMANIA: RESULTS OF A SURVEY COMPLETED BY PRACTICIANS (Abstract): Acute-on-chronic liver failure (ACLF) is a relatively new concept introduced in order to better assess the risk of death in patients with acute decompensation of chronic liver disease. The diagnostic criteria are currently still under debate, but the most recent definition is based on the presence of acute decompensation of cirrhosis associated with organ failure according to the CLIF Consortium ACLF score (CLIF-C ACLF score). **Aim:** To establish the real-life perception of ACLF among Romanian practitioners. **Material and methods:** We assessed current diagnosis patterns for ACLF, and we developed a dichotomous question survey concerning the current knowledge on ACLF. The survey was sent by e-mail to the members of the Romanian Society of Gastroenterology and Hepatology (SRGH); a printed survey was completed by intensive care practitioners from "Sf. Spiridon" County Clinical Emergency Hospital Iasi, Romania. We evaluated the specialty of the practitioners included, the professional degree, and the participant's clinical and diagnostic abilities concerning the assessment of ACLF. **Results:** 263 practitioners responded to the survey: 69% gastroenterologists, 22% internal medicine consultants and 9% intensive care specialists; 31% of responders were trainees, 35% consultants and 34% senior consultants, most of them working in a university hospital (66%). The majority of the participants had heard of ACLF (94%). 73% of responders considered that there were unanimously accepted criteria for the diagnosis of ACLF. Almost all responders (98%) considered the liver to be the most common organ failure in ACLF patients. Most participants agreed that the younger and alcoholic patients had a higher risk for developing ACLF (84%) and that ascites degree was not relevant for the diagnosis (65%). **Conclusions:** The results show that knowledge of the diagnosis and characteristics of ACLF among practitioners in Romania are according with the current ACLF published criteria. However, the percent of practitioners that applied the ACLF criteria in clinical practice was not determined and could represent a subject for future research. **Keywords:** ACUTE-ON-CHRONIC LIVER FAILURE, LIVER CIRRHOSIS, ACUTE DECOMPENSATION, CLIF-C ACLF SCORE, SURVEY.

Liver cirrhosis (LC) is a chronic disease generally characterized by a long asymptomatic evolution. However, the diagnosis is frequently established in the setting of decompensation and/or complications such as variceal bleeding (VB), ascites, hepatic

encephalopathy (HE), acute kidney injury (AKI), and bacterial infections which associate a poor prognosis (1, 2). Moreover, the practitioners have been often surprised by rapid impairment of liver, kidney or circulatory function, in patients with known, yet stable liver cirrhosis. These observations suggested the existence of a distinct syndrome, different from the "mere" decompensation of liver cirrhosis, namely Acute-on-chronic liver failure (ACLF). Jalan R *et al*. were among the first to describe ACLF in 2002 (3), but since then many definitions have been proposed, most of them lacking solid clinical proof, which led to a discrepancy between the prevalence and clinical outcomes of ACLF reported by different authors (4, 5). Moreau *et al*. (6) conducted the first large prospective multicentric study, carried on in Europe (the CANONIC study) in which the authors defined ACLF as acute decompensation of liver cirrhosis associated with organ failure and high short-term mortality rates. The authors established specific criteria for the diagnosis of the syndrome. ACLF grade 1 was diagnosed in the setting of kidney failure or other single organ failure (OF) with a serum creatinine level between 1.5-1.9 mg/dL and/or Grade I-II HE. The diagnostic of ACLF grade 2 was set in the presence of 2 OFs and ACLF grade 3 in patients with 3 or more OFs. Moreau *et al*. defined liver failure as total bilirubin levels of more than 12 mg/dL, kidney failure as an increase of serum creatinine $\geq$ 2 mg/dL or the use of renal replacement therapy, cerebral failure as grade III or IV West Haven HE, coagulation failure as an INR value over 2.5 and/or platelet count $\leq$ 20 x 109/L, respiratory insufficiency as a partial pressure of arterial oxygen to $FiO_2 \leq 200$ or an SpO_2 to FiO_2 ratio $\leq$ 200, and circulatory failure in the setting of vasoactive drug therapy (6). The authors diagnosed ACLF in 30.9% of the participants included. Moreover, three grades of ACLF have been validated, each associating a different 28- and 90-day outcome (22.1% and 40.7% mortality rate for grade 1, 32% and 52.3% for grade 2, 76.7% and 79.1% for grade 3). In order to define organ failure and to assess disease severity several scores have been proposed, namely the Chronic Liver Failure Consortium Organ Failure (CLIF-C OF) score, a simplified version of the CLIF-C ACLF score (1, 7) (tab. I).

TABLE I

CLIF-C ACLF score

Organ System	Score=1	Score=2	Score=3
Liver (mg/dL)	Bilirubin<6	6 ≤ bilirubin ≤ 12	Bilirubin > 12
Kidney (mg/dL)	Creatinine <2	Creatinine ≥ 2 <3.5	Creatinine ≥ 3.5/ renal replacement
Brain (West Haven)	Grad 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥ 70 mm Hg	MAP < 70 mm Hg	Vasopressor therapy
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214 - ≤ 357	≤ 200 ≤ 214

Abbreviations: PaO_2, partial pressure of arterial oxygen; FiO_2, fraction of inspired oxygen; SpO_2, pulse oximetric saturation; MAP, mean arterial pressure; INR, international normalized ratio.

Certain precipitating factors of ACLF have been identified, such as active drinking and bacterial infection which were the most common but also gastrointestinal bleeding, therapeutic paracentesis without administration of intravenous albumin, placement of trans jugular intrahepatic portosystemic shunt, major surgery, viral hepatitis, and alcoholic hepatitis. Surprisingly, in 40.3% of the cases no precipitating event was identified, and the outcome was not influenced by the precipitating factor but rather by the number of organ failures. The most commonly identified failing organ in ACLF is the kidney; also, studies indicate that ACLF is associated with younger age and alcohol abuse and patients without any history of decompensation developed a more severe syndrome (6).

MATERIAL AND METHODS

The aim of our study was to assess the patterns of practice, disease awareness and recognition of ACLF among Romanian practitioners.

General information regarding the participants	
1. Please choose your specialitv	☐ gastroenterology ☐ intensive care ☐ internal medicine
2. Please choose your professional degree	☐ intern ☐ specialist ☐ consultant
3. Are you currently working in a university hospital?	☐ yes ☐ no
Knowledge of ACLF	
1. Have you heard of ACLF?	☐ yes ☐ no
2. In your opininon do you consider ACLF to be different from the acute decompensation of liver cirrhosis?	☐ yes* ☐ no
Diagnostic criteria for ACLF	
1. Do you consider that currently there are unanimously accepted criteria for the diagnostic of ACLF?	☐ yes ☐ no*
2. When you suspect the presence of ACLF, do you find the CLIF-OF score useful in establishing the diagnosis?	☐ yes* ☐ no
3. Is liver failure mandatory for the diagnosis of ACLF?	☐ yes ☐ no*
4. Is the degree of ascites relevant for the diagnosis of ACLF?	☐ yes ☐ no*
Particularities of ACLF	
1. Is liver failure the most commonly found organ dysfunction in ACLF patients?	☐ yes ☐ no*
2. Do younger or alcoholic patients pose a higher risk of developing ACLF than the others?	☐ yes* ☐ no
3. Is ACLF evolution more severe in patients with previous acute decompensation?	☐ yes ☐ no*
4. Is mortality in patients with ACLF similar or higher with those associating acute decompensation of liver cirrhosis?	☐ yes* ☐ no

*correct answer, ACLF=acute-on-chronic liver failure

Fig. 1. The survey used for the evaluation of the practitioners

We composed a survey based on the results of the CANONIC study (6) and consisting of questions regarding the current knowledge on ACLF (fig. 1). Most questions were dichotomous, and the questionnaire was designed to be as short and as little time consuming as possible.

The survey was sent by e-mail to the contact list of members of the Romanian Society of Gastroenterology and Hepatology (SRGH); additional printed survey were sent to intensive care practitioners from "Sf. Spiridon" County Clinical Emergency Hospital Iași, Romania. The questionnaire was divided into several sections; the first section included questions regarding to participant information (age, sex, place of practice, the professional degree, the affiliation to a university hospital). We emphasized gathering information regarding the participant's clinical and diagnostic abilities concerning the assessment of ACLF; thus, we distributed the questions according to the following criteria: knowledge of ACLF, diagnostic criteria of ACLF, and particularities in patients with ACLF. The survey was sent to the participants in March 2017. The responses were received from April to September 2017. The results of survey were expressed as percentage.

We were also interested in comparing our results with unpublished data presented by Trifan, *et al*. on 4th UpDate on Hepatology Course, April 6th-7th 2017, Bucharest, Romania (fig. 2). We were also interested in observing if the presentations held on ACLF during the meeting had an effect on the general concepts of Romania practitioners.

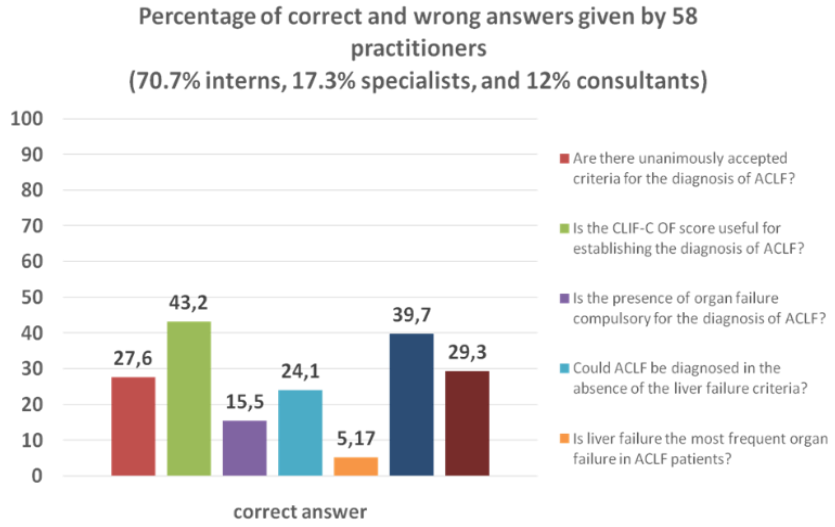

Fig. 2. Real-life perception of ACLF in Romania: results of a survey completed by practicians in a northeastern Romanian university tertiary care center

RESULTS

Out of 317 gastroenterology practitioners, members of SRGH, 181 (57%) responded. All of the 24 intensive care specialists that received the printed version of the survey responded. Out of 72 internal medicine consultants 58 (80%) responded. The total number of respondents was 263, and included 181

(69%) gastroenterologists, 58 (22%) internal medicine specialists and 24 (9%) intensive care specialists. Regarding the professional degree, 82 (31%) of responders were trainees, 92 (35%) were consultants and 89 (34%) were senior consultants.

Most practitioners said that they worked in a university hospital (66%).

The majority of the respondents said that they had heard of ACLF (94%) and seventy eight percent of them considered ACLF to be different from the "simple" acute decompensation of liver cirrhosis. 191 (73%) of responders considered that there were unanimously accepted criteria for the diagnosis of ACLF.

TABLE II
Results of the survey conducted

Question	Yes (%)	No (%)
1. Have you heard of ACLF?	94	6
2. Do you consider ACLF to be different from the acute decompensation of liver cirrhosis?	78	22
3. Do you consider that currently there are unanimously accepted criteria for the diagnosis of ACLF?	73	27
4. When you suspect the presence of ACLF, do you find the CLIF-OF score useful in establishing the diagnosis?	70	30
5. Is liver failure mandatory for the diagnosis of ACLF?	59	41
6. Is the degree of ascites relevant for the diagnosis of ACLF?	35	65
7. Is liver failure the most commonly found organ dysfunction in ACLF patients?	98	2
8. Do younger or alcoholic patients pose a higher risk of developing ACLF that the others?	84	16
9. Is ACLF evolution more severe in patients with previous acute decompensation?	94	16
10. Is mortality in patients with ACLF similar or higher with those associating acute decompensation of liver cirrhosis?	76	24

183 (70%) of responders considered the CLIF-OF score is useful in establishing the diagnosis of ACLF. When asked what organ dysfunction was the most common among patients with ACLF, almost all of them (98%) found liver failure to be the most commonly organ insufficiency. However, 41% of them stated that severe liver failure was not mandatory for the diagnosis of this syndrome. The degree of ascites was not considered relevant for the diagnosis of ACLF by more than half of the practitioners (65%) and a great proportion agreed that the younger and alcoholic patients had a higher risk for developing ACLF (84%). Data obtained from the survey is illustrated in table II.

DISCUSSION

ACLF was firstly mentioned by Sarin(8) and for a long time an evidence-based definition of ACLF was not established. The syndrome was characterized based on expert opinions and not on objective data. In 2014 more than13 definitions of ACLF were accepted (9).

According to WGO Consensus Definition ACLF is a syndrome distinct from acute liver failure(ALF) or decompensated cirrhosis in patients with chronic liver disease (with or without known cirrhosis), characterized by acute hepatic decompen-

sation (liver failure and one or more organ failures), associated with high mortality within 28 days and up to 3 months from onset (10).

ACLF is a newly defined concept, but our survey confirms that the majority of the Romanian practitioners have heard about this entity (94%) without significance depending on the specialty. Unfortunately, although having heard of ACLF most of practitioners do not know whether there are unanimously accepted criteria for the diagnosis of ACLF. Trifan A *et al*., on The 4th UpDate on Hepatology Course, 6th-7th April 2017, Bucharest, Romania reported that 27.6% of practitioners in a North-eastern Romanian university tertiary center were aware of unanimously accepted criteria for the diagnosis of ACLF. Our survey revealed that 73% of responders were confident with the diagnosis criteria. Diagnosis criteria for ACLF represent a subject still under debate and the answers of our responders show relative uncertainty, in accordance with the lack of unanimously accepted diagnostic criteria.

ACLF is defined as acute decompensation of liver cirrhosis associated with organ failure and high short-term mortality rates (6). The diagnosis is made in the setting of organ failure(s), as defined by the CLIF-C OF, a score developed by Moreau *et al*. by adapting the Sequential organ failure assessment (SOFA) score (used by all intensive care practitioners) in accordance with the particularities of cirrhotic patients. The SOFA score includes the evaluation of six types of organ failure: liver, renal, coagulation, cerebral, respiratory, and circulatory. One hundred and eighty-three of the responders (70%) considered the CLIF-C ACLF score useful in establishing the diagnosis of ACLF. The percent of correct responses is significantly higher compared to the percent reported by Trifan *et al*. in April 2017 during the 4th Update on Hepatology Course Bucharest, Romania. Unfortunately, we have no data of current evaluation of CLIF-C OF score (except for intensive care units) in daily clinical practice.

ACLF should be assessed in all patients with acute decompensation of cirrhosis: ascites grade 2 to 3 (according definition of International Ascites Club) within less than 2 weeks (first/new episode), acute hepatic encephalopathy in patients with previous normal consciousness (first/new episode), acute upper and/or lower gastrointestinal hemorrhage of any etiology and bacterial infections (any type of acute bacterial infection) in patients with liver cirrhosis. ACLF should not be assessed in patients with refractory ascites or chronic hepatic encephalopathy.

Although many of us do not use this score we emphasize that it is not very time consuming (easy access http://www.efclif.com/ scientific-activity/score-calculators/clif-c-aclf) and not more time consuming than calculating the CHILD or MELD scores, requiring no special equipment and therefore could find its place in clinical practice.

CONCLUSIONS

The prevalence of ACLF in Romania is currently unknown, but the mortality rate of patients with liver cirrhosis is known to be high, as liver cirrhosis has been considered the fourth cause for death in a recent WHO report with a mortality rate of 47.8/100,000 (11). ACLF is a newly introduced syndrome that benefits from increased attention; thus, we conducted a survey in a representative population of Romanian practitioners in order to assess

the current knowledge regarding this syndrome and to set the grounds for future prospective studies in our country. As Moreau *et al*. were the first to conduct a large prospective trial and the definition of ACLF was based on clinically relevant facts we chose to consider the criteria proposed by the authors in our survey. The results show that knowledge of the diagnosis and characteristics of ACLF among practitioners in Romania are according with the current ACLF published criteria. However, future research is needed in order to evaluate the percent of practitioners that apply the ACLF criteria in clinical practice.

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EFECTUL TERAPIEI CPAP ASUPRA CALITĂŢII SOMNULUI LA PACIENŢII CU SAOS EVALUATĂ PRIN SLEEP DISTURBANCES INDEX (SDI)

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EFFECT OF CPAP THERAPY ON SLEEP QUALITY IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA SYNDROME (Abstract): For an adequate analysis of sleep quality was assessed a new composed index wich objectively approached variate polisomnographic parameters. We used this new concept – sleep disturbances index-SDI in patients with obstructive sleep apnea(OSAS), before and after the beginning of CPAP therapy. **Material and method**: 41 patients were evaluated and for each one we calculated SDI score. All patients were split in two groups according with apnea-hipopnea index (AHI), and we correlated SDI with AHI as marker of severity of the disease and with Epworth Sleepiness Scale's scores, as an subjective evaluation. After 1 month of CPAP therapy, SDI was reevaluated. **Results**: SDI is correlating with the severity of the disease: for group 1, with $5 < AHI < 15$, SDI= 2.24 ± 0.88, $r = 0.53$, $p < 0.01$ and for group 2, with $AHI > 15$, SDI= 3.23 ± 0.69, $p < 0.01$, $r = 0.46$. There was no significant correlation between SDI and ESS scores ($r = 0.23$, $p < 0.01$). SDI lowers significant during CPAP therapy: SDI= 1.48 ± 0.60, respectiv SDI= 1.90 ± 1.23. **Conclusion**: The severity of OSAS is correlated with increased SDI, but can be seen interindividual variability. The effect of CPAP therapy is reflecting also in sleep quality. **Key words**: SDI, OSAS, SLEEP QUALITY

AGREEMENT RATE AMONG ONCOLOGISTS FOR SYSTEMIC THERAPIES IN THE REGIONAL INSTITUTE OF ONCOLOGY IASI

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AGREEMENT RATE AMONG ONCOLOGISTS FOR SYSTEMIC THERAPIES IN THE REGIONAL INSTITUTE OF ONCOLOGY IASI (Abstract): In recent years, cancer treatment has become more complex due to the increasing involvement of other specialists in the multimodal approach and to the constant innovations and changes brought on by the discovery and approval of new drugs. As such, the multidisciplinary team (MDT) management model has been implemented in most European countries, including Romania. Material and method: We performed a retrospective analysis of all cases eligible for systemic therapy that were discussed in the MDT of the Regional Institute of Oncology Iasi between January 2017 and December 2018. For each case, we recorded several parameters, together with data regarding the agreement rate between the proposed treatment and the final MDT decision. Results: We identified 723 cases that respected the inclusion criteria. Patients most often discussed in the MDT had been diagnosed with colorectal (29%), breast (22.7%) and lung cancer (18.7%). The most common reason why the cases were discussed was the initiation of biological therapies and immunotherapy. Agreement rate between the proposed treatment and the final MDT decision was 92.1%, with changes being suggested by the MDT most often in the case of breast (26.3%), colorectal (22.8%), prostate (17.5%), and lung cancer (7%). Conclusions: MDTs have become an essential part of cancer care. Although in Romania, they do not yet have legal responsibility, they offer an opportunity for medical professionals to interact and together decide the best outcome for patients diagnosed with solid tumors. **Keywords**: CANCER, MULTIDISCIPLINARY TEAM, SYSTEMIC ANTICANCER TREATMENT.

The multidisciplinary team (MDT) has brought many benefits to patients in all medical fields, especially in oncology, where its importance derives from the complexity of cancer disease (1) and from the need for collaboration between clinical and paraclinical specialties, highlighting the importance of the holistic approach in cancer treatment. Although MDT benefits are almost self-evident, demonstrating the efficacy of this type of collaboration is abstract and the barriers against it are more difficult to perceive. Participating in regular meetings of the MDT requires significant time and financial costs, as well as effective coordination (2-5).

The legal frame by which MDTs operate in Romania was set by Order 1202 of

2017 on the establishment, organization and functioning of the specialized committees and subcommittees of the Ministry of Health. The oncological committees are an advisory board with mediation responsibilities and do not assume legal responsibilities regarding the patient rights and malpractice issues (6). Consequently, the attending physician is the one that has the medico-legal responsibility and the MDT's decision is not definitive. Within the Regional Institute of Oncology (IRO) Iasi, a multidisciplinary team has been implemented in order to help physicians establish the diagnosis, to identify optimal therapeutic solutions, to integrate palliative medicine and end-patient care, improving interdisciplinary communication and stimulating clinical research.

The purpose of this retrospective study was to identify the efficacy of MDT for systemic treatment decisions in IRO Iasi, the utility of this approach in clinical routine and the agreement rate among oncologists.

MATERIAL AND METHOD

In the Regional Institute of Oncology, Iasi, the multidisciplinary team meetings are held weekly with the participation of medical oncologists, pathologists, surgeons, radiotherapists and radiologists. The result of the discussions is recorded by the secretary of the commission and signed by all members. It should also be noted that there is a custom that the discussions go on until everybody agrees and a unanimous decision is reached. When the members disagree on a topic, the case is discussed again after more patient data is available.

We performed a retrospective study of all cases presented in the MDT that were not eligible for local curative therapies (such as surgery or radiotherapy) and were referred by their oncologist for systemic therapy. To investigate the efficacy of these committee meetings and the agreement rate, we have considered data like diagnosis, cancer stage, committee decision and type of treatment. Consecutive patients who were presented to the committee between January 2017 and December 2018 that respected the selection criteria were included. Data were analyzed using *SPSS* software (IBM *SPSS Statistics for Windows, Version 21.0*. Armonk, NY: IBM Corp.).

RESULTS

From 5946 new cases presented in our institution for treatment decision or second opinion we identified 723 consecutive cases presented to the MDT in the specified time frame. Clinical and pathological characteristics of patients are summarized in first table.

Most frequently discussed by the committee were patients diagnosed with colorectal (29%), breast (22.7%) and lung cancer (18.7%) (tab. II). In women, the most common cancers were breast and colorectal, while for men it was colorectal, followed by lung cancer. The most common reason why the cases were discussed was the initiation of biological therapies and immunotherapy such as bevacizumab (22.5%), trastuzumab (13.3%) and nivolumab (9.5%) (tab. III).

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immunotherapy such as bevacizumab (22.5%), trastuzumab (13.3%) and nivolumab (9.5%) (tab. III).

Most patients were recommended combined systemic treatment: chemotherapy and biological treatment (40.5%), only biological treatment (28.2%), or only chemotherapy (9.7%). Radiotherapy was recommended in 8 cases (1.1%) and surgery in 4 cases (0.6%), in contradiction with the treatment initially proposed. Of the patients presented, 30.6% were referred by the same oncologist; 79% of the cases were discussed only once in the committee, 20.3% twice and 0.7% three times during the course of their disease.

TABLE I

Clinical and histological characteristics

Variable	Patients, n (%)	Variable	Patients, n (%)
Age (years)		***Gender***	
Medium	62	Male	367 (50.8%)
Range	19-87	Female	356 (49.2%)
Stage		***Histology***	
I	20 (2.8%)	carcinoma	663 (91.6%)
II	46 (6.4%)	adeno	618 (85.5%)
III	89 (12.3%)	squamous	34 (4.7%)
IV	568 (78.6%)	neuroendocrine	11 (1.5%)
ECOG PS		melanoma	20 (2.8%)
0	19 (2.6%)	sarcoma	36 (5%)
1	628 (86.9%)	germ cell tumors	2 (0.3%)
2	73 (10.1%)	borderline tumors	2 (0.3%)
3	2 (0.3%)		
4	1 (0.1%)		
4	1 (0.1%)		

TABLE II

Tumor sites

Type of cancer	Patients, n (%)	Type of cancer	Patients, n (%)
Colorectal	210 (29%)	Sarcoma	9 (1.2%)
Breast	164 (22.7%)	Pancreas	8 (1.1%)
Lung	135 (18.7%)	Cervix	3 (0.4%)
Prostate	30 (4.1%)	Urothelial	3 (0.4%)
GIST	29 (4%)	Neuroendocrine	3 (0.4%)
Liver	27 (3.7%)	Thyroid	2 (0.3%)
Kidney	25 (3.5%)	Mesothelioma	2 (0.3%)
Malignant melanoma	22 (3%)	Esophageal	2 (0.3%)
Ovarian	21 (2.9%)	Cholangiocarcinoma	1 (0.1%)
Gastric	13 (1.8%)	Brain	1 (0.1%)
Head and neck	13 (1.8%)		

TABLE III

Therapies indicated following the discussion of the case in committee

Drug	Patients, n (%)	Drug	Patients, n (%)
Bevacizumab	163 (22.5%)	Olaparib	6 (0.8%)
Trastuzumab	96 (13.3%)	Gefitinib	6 (0.8%)
Nivolumab	69 (9.5%)	Erlotinib	6 (0.8%)
Chemotherapy	66 (9.1%)	Dabrafenib/Trametinib	6 (0.8%)
Cetuximab	43 (5.9%)	Crizotinib	5 (0.7%)
Pemetrexed	39 (5.4%)	Aflibercept	4 (0.6%)
Sorafenib	28 (3.9%)	Pazopanib	4 (0.6%)
Imatinib	25 (3.5%)	Axitinib	4 (0.6%)
Palbociclib	24 (3.3%)	Trabectedin	3 (0.4%)
Trastuzumab emtansine	23 (3.2%)	Lapatinib	3 (0.4%)
Abiraterone	17 (2.4%)	Palliative care	2 (0.3%)
Sunitinib	16 (2.2%)	Tamoxifen	2 (0.3%)
Afatinib	11 (1.5%)	Osimertinib	1 (0.1%)
Enzalutamide	11 (1.5%)	Olaratumab	1 (0.1%)
Panitumumab	9 (1.2%)	Pertuzumab	1 (0.1%)
Local treatment	7 (1%)	Aromatase inhibitor	1 (0.1%)
GnRH analogue	7 (1%)	Methotrexate	1 (0.1%)
Ramucirumab	6 (0.8%)	Vemurafenib	1 (0.1%)
Octreotide	6 (0.8%)		

Following the discussion, the treatment proposed by the committee agreed with the choice of the attending physician in 92.1% of the cases. The treatment was changed in 7.9% of cases (57 patients), either the physician chose another treatment, or the committee changed the decision of the attending physician. In this group, the patients were followed up instead of the active treatment in 52.6% of the cases, received hormonal therapy (instead of chemotherapy or biologics) in 36.8% of cases and symptomatic treatment in 5.2%. These discrepancies were found in cases of breast (26.3%), colorectal (22.8%), prostate (17.5%), and lung cancer (7%).

DISCUSSION

The purpose of this study was to quantify the effect of discussing patients with different neoplasms within a multidisciplinary committee and to analyze the rate of agreement between the decision of the committee and the attending physician. This is especially relevant for Romania, because, although this country has reimbursed most modern oncological drugs, it is a country with small to medium resources, and the regulatory bodies limit the prescription of these drugs by strict criteria protocols. As such, the MDT's decision is mostly consultative, as it is not entitled to modify or override the provisions of these protocols. Furthermore, the attending physician might still choose to reside on his/her own skills and experience, instead of the MDT's opinion, when selecting the treatment.

A retrospective review by Greer (7) and his collaborators including patients with gynecological neoplasms showed that their management was changed in 20% of cases after being discussed in an MDT. Another study, which included 75 breast cancer patients, concluded that MDT members did not agree with the attending physician's

decision in 43% of cases (8). According to a cohort study, the introduction of multidisciplinary teams in the management of breast cancer led to a decrease in mortality by 18% (9).

In our analysis, 29% of the patients subject to discussion were diagnosed with colorectal cancer. Available data from similar research sets show an improvement in 5-year survival for colorectal cancer patients presented in a multidisciplinary committee: 52.3% *vs.* 33.6% ($p < 0.00001$). However, for patients with incipient disease, the committee did not have a significant effect on survival. This suggests that the committee's main contribution may be to coordinate management of patients with a complex pathology such as potentially resectable liver metastases or borderline resectable tumors (10).

The results of a study involving patients with rectal cancer showed that the existence of a multidisciplinary committee and the possibility of discussing the cases led to the modification of the treatment plan in 29% of cases, with 42% of the changes being attributed to the reinterpretation of the imaging assessments (11), which is especially relevant since there are several new types of imaging techniques being investigated in digestive cancers (12). However, the work of Ryan and colleagues (13) showed that not all patients with colorectal neoplasm benefit from the input of a multidisciplinary committee, with only 3.4% of early colorectal cancer patients having their management plan changed. In contrast, management changed in 50% of complex cases: patients with associated pathology, preoperative rectal neoplasm management, and disease recurrence or resection criteria. The author of the study proposes a two-tier approach of the committee: all patients should be presented during the committee meeting but only cases with a complex pathology and non-standard therapeutic indications should be discussed in detail. This approach to the committee is also the one used in our institution.

In the present study, lung cancer was the third most common neoplasia (18.7%). Modern bronchopulmonary neoplasm management should be multidisciplinary, given the multiple treatment options and the potential long-term side-effects such as neuropathic pain (14). A study in France evaluating the efficacy of a committee in the management of lung cancer showed that the discrepancy between planned and actual treatment was 4.4% (15). This group of patients had lower survival but the difference between the two groups was not statistically significant. Forrest and his collaborators (16) have studied the management of patients with inoperable lung cancer before and after the cases were discussed in a committee; they have seen an increase in the use of chemotherapy in the group of patients discussed in committee (23% *vs.* 7%) but also an increase in median survival (6.6 months *vs.* 3.2 months). Similar data was reported by another study (17). In the case of elderly lung cancer patients discussed in a committee, an increase in the rate of curative or palliative radiotherapy and an improvement in 1-year survival rate (23.5% *vs.* 18.3%) have been observed. However, the data is controversial. Boxer *et al*. showed in his study that presenting lung cancer cases in a multidisciplinary tumor board was associated with a higher probability of radiotherapy and chemotherapy administration, with a potential impact on the quality of life of patients but without influencing their survival (18). Another study in Australia, which included 98 patients with inoperable lung cancer, concluded that there was no difference in

treatment for patients who were or were not discussed in a multidisciplinary committee but there was a difference in survival (237 *vs.* 208 days) in favor of the patients discussed in the committee. It is worth mentioning that the study focused on the evaluation of treatment and survival, but there are other goals such as waiting time, satisfaction or quality of life, which can be equally important (19).

A retrospective analysis showed that changes in treatment recommendations occurred in more than one-third of patients with gastrointestinal malignancies after a multidisciplinary team meeting. Change in management was recommended in 36%; 72% were major and 28% were minor. Agreement rate between the committee's decision and the treatment followed by the patient was 77% (20).

Regarding lung cancer, multidisciplinary team had an 80% agreement on risk factors, biopsy, staging, treatment and follow up of patients (21). Another survey among the multidisciplinary lung cancer team showed a 95% agreement rate regarding staging, 99% regarding treatment plan and 74% regarding the follow up (22).

The introduction of the multidisciplinary team in the present study led to a 92.1% agreement rate among oncologists for systemic therapies. These data support multidisciplinary communication and approach within a team dedicated to the management of oncological patients, which is extremely necessary given the complexity of pathology, the profile of side effects and their comorbidities. Optimal collaboration between oncologist, radiotherapist, surgeon, pathologist, and radiologist should improve the results of multimodal treatment. Additionally, patient preference and adequate informed consent should also be an important factor when deciding on optimal management and/or including the patient in a clinical study.

The present study has some limitations which may affect data interpretation. First, although it included a large number of cases, it is still a retrospective analysis and case selection bias must be considered. Second, it is likely that not all patient data was recorded in a unitary fashion, which is why some information might be missing. However, despite these limitations, the results clearly indicate that the MDT can change treatment course in some patients and its implementation is beneficial.

CONCLUSIONS

The organization of the multidisciplinary committee is mandatory in all oncology institutions. In addition to the benefits for patients, this way of managing cases leads to an interdisciplinary communication which will also improve the activity within the institution, from both the professional and the social/collaborative perspectives. In the end, we have to underline the pressure imposed by the insurance company on all oncologists to which the MDT might be a solution to share the financial responsibility.

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REDUCTION OF SPLENIC VOLUME AFTER DIRECT ACTING ANTIVIRALS TREATMENT IN PATIENTS WITH HCV COMPENSATED CIRRHOSIS

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REDUCTION OF SPLENIC VOLUME AFTER DIRECT ACTING ANTIVIRALS TREATMENT IN PATIENTS WITH HCV COMPENSATED CIRRHOSIS (Abstract): The **aim** of this study was to evaluate the impact of direct-acting antivirals (DAAs) treatment on spleen size and portal circulation after the achievement of sustained virological response (SVR) in patients with hepatitis C virus (HCV)-related cirrhosis. **Material and methods:** CT examination was performed in 60 patients with HCV-related compensated cirrhosis before and within 6-18 months after the treatment with Ombitasvir/ Paritaprevir/ ritonavir + Dasabuvir. The CT changes of spleen were assessed by measuring spleen volume. Portal trunk, splenic and superior mesenteric vein diameters have been assessed as part of portal hypertension. **Results:** Splenic volume was considerably higher before treatment (median: 564.79 mm^3, IQR: 342.54) than after (median: 474.45 mm^3, IQR: 330.00), z=-2.500, p=0.012. The portal vessels diameters showed no significant changes. **Conclusions:** There is a statistically significant reduction in size of spleen in patients with HCV-related compensated cirrhosis who achieved SVR after treatment with DAAs and this can be used as a sensitive imaging marker to assess improvement in cirrhotic patient's condition. **Keywords:** SPLENIC VOLUME, CIRRHOSIS, HEPATITIS C VIRUS, COMPUTED TOMOGRAPHY, DIRECT-ACTING ANTIVIRALS.

Spleen is the largest lymphoid organ in the body with elaborate tissue and vascular architecture. It plays an important role in the modulation of the immune system and filtration of blood from antigenic particles and from aged or abnormal cells. There is more than one reason to consider that spleen and liver are strongly connected. Both organs are important anatomic components of portal circulation. From histological perspective, spleen and liver have a similar reticuloendothelial structure and participate to cellular migration and substance exchange. Last, but not least, both have an essential role in immune hemostasis and pathogen clearance (1). Due to

these tight and variate connections, the concept of "liver-spleen axis" has been used in various conditions (2, 3).

This interconnection is responsible on the one hand for the morphological changes of the spleen in different liver pathologies, as well as for the influence exerted by the abnormal spleen on the liver and the portal venous system. This both-ways influence is also visible in HCV-related cirrhosis where splenomegaly is one of the most common abnormalities that accompany liver cirrhosis.

Splenomegaly is associated with poor prognostic in liver cirrhosis and is used as an index on sectional imaging techniques such as ultrasound, computed tomography, and magnetic resonance for non-invasive assessment of esophageal varices and risk of hemorrhage (4).

Hypersplenism, present in parallel with the increase in the volume of the spleen, is considered the main cause of blood dyscrasia such as cytopenia and thrombopenia in cirrhotic patients.

Patients with cirrhosis and splenomegaly have splenic blood flow increased, and portal congestion is considered the initial cause of splenic enlargement during liver cirrhosis (5). In the end splenic tissue hyperplasia stands as the main cause of increased spleen size instead of portal congestion (6). Moreover, the spleen actively congests the portal system participating in the maintenance of portal hypertension (7).

Spleen also influences the hepatic immune microenvironment both by cell migration and the secretion of splenic soluble factors via portal blood flow (1). Splenic red pulp macrophages are a major source of transforming growth factor β1 and are significantly correlated with the progression of liver fibrosis via liver-spleen cross-talk (8).

Spleen modulates also liver regeneration, aspect highlighted by studies who have demonstrated that splenectomy improved liver regeneration in cirrhotic patients (9).

Advances in understanding the of HCV molecular biology have led to the development of direct-acting antivirals (DAAs) for treatment. Currently chronic hepatitis C is the only chronic viral infection that can be cured with antiviral therapy (10,11).

The elimination of the causative agent and the interruption of the perpetuation of the inflammatory process after the antiviral treatment can also have an effect on the portal circulation with the improvement of the blood flow (12). The spleen as an integral part of the portal venous system may react to changes at this level secondary to improved liver condition. The purpose of this study was to evaluate the impact of DAAs treatment on spleen size and portal circulation after the achievement of sustained virological response (SVR) in HCV related cirrhotic patients.

MATERIAL AND METHODS

Study design. This is a prospective study on patients with genotype 1 HCV-related compensated cirrhosis who have reached SVR after treatment with DAAs (Ombitasvir/Paritaprevir/ritonavir + Dasabuvirum. Information from 60 cases with virus C related cirrhosis who underwent treatment with DAAs was collected within a 5-year period from January 2015 to December 2019.

Each patient had a CT scan before treatment to rule out liver malignancy and to assess both morphological and dimensional changes in the liver. In addition, the scan evaluates the manifestations of portal

hypertension such as dilation of the portal system, portosystemic collateral circulation, and splenomegaly. A second CT examination was performed after the treatment, within 6 and 18 months following SVR achievement.

The local Ethical Committee approved this study. Written informed consent was obtained from each patient and the study was conducted according to the Declaration of Helsinki.

Scanning protocols. We used a Siemens Sensation® 16 slice configuration CT scanner (Siemens AG Medical Solution) available in our institution. Our scanning protocol includes non-enhanced scan followed by i.v. administration of iodine-based contrast medium with tri-phase liver scan (arterial, portal and equilibrium phase) in order to exclude liver malignancy.

To evaluate the dilation of the portal system, a consequence of portal hypertension, the average diameters (reported in millimeters) of other portal trunk, splenic veins and superior mesenteric vein were measured.

The splenomegaly was quantified using the index value (product of cranio-caudal dimension, maximum size in axial plane and maximum thickness in axial plane) and the splenic volume (index value x 0.58 + 30) in milliliters (13). Since both, the splenic index, and the splenic volume, are interrelated, we selected for evaluation only the splenic volume.

RESULTS

Statistical evaluation began with the inspection of continuous variables to assess the normality of distributions. Since most of the differences did not meet the normality requirement, comparisons between the two sets of measurements, to see whether there were any changes in parameter levels, were performed with the Wilcoxon signed-rank test. A value of $p < 0.05$ was considered statistically significant. All results are summarized in first table and the boxplots of variables is shown in figure 1.

The volumes of the spleen before the treatment (*median: 564.79 mm^3, IQR: 342.54*) were significantly higher than the volumes determined after the treatment (*median: 474.45 mm^3, IQR: 330.00*), *z=-2.500, p=0.012*. An example of a reduction in the volume of the spleen after antiviral treatment is shown in figure 2.

In our study there were no significant differences before and after treatment between the average diameters of the main vessels of the portal system. This aspect was found in both the portal trunk (*p = 0.831*), the splenic vein (*p = 0.536*) and the superior mesenteric vein (*p = 0.624*).

TABLE I

Pre and post treatment comparation of splenic volume and portal vessels diameters

	Before treatment		After treatment		Z (Wilcoxon)	p
	median	IQR	median	IQR		
Splenic volume (mm^3)	*564.8*	*342.54*	*474.5*	*330.00*	*-2.500*	*0.012**
Portal trunk diameter (mm)	13.73	2.70	13.78	2.00	-0.214	0.831
Superior mesentery vein diameter (mm)	12.7	2.90	12.2	2.70	-0.491	0.624
Splenic vein diameter (mm)	9.85	3.30	10.2	3.73	-0.618	0.536

IQR - interquartile range

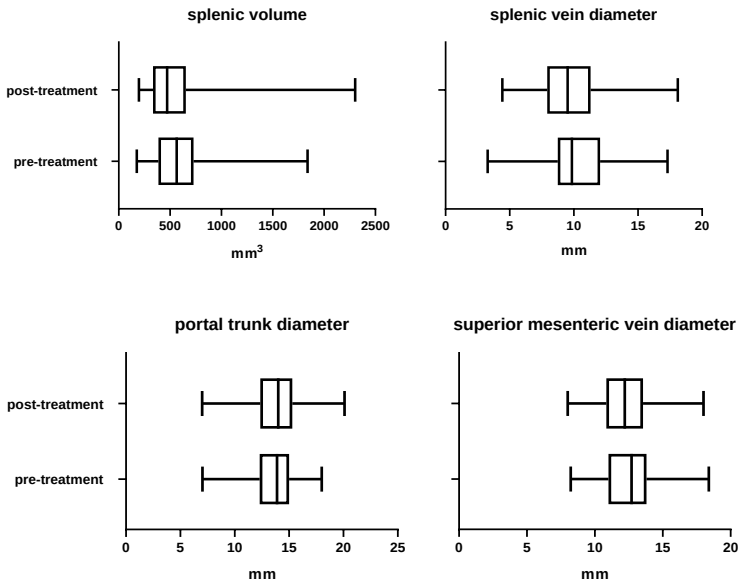

Fig. 1. Boxplots before and after treatment of splenic volume

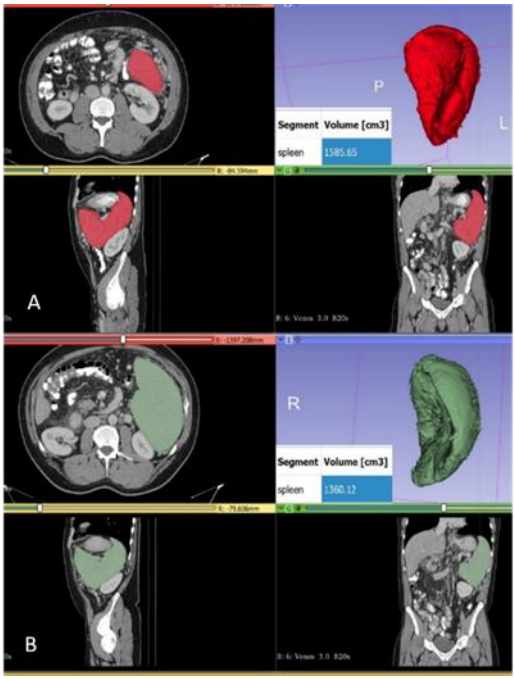

Fig. 2. Splenic volume before (A) and after treatment (B)
showing important reduction of spleen volume after DAAs treatment.
Note: the 3D segmentation of spleen was performed using *3D Slicer software v. 4.11.0*.

DISCUSSION

In the evaluation of the portal system vessel diameter variations we observe no statistically significant changes. This absence of modifications may reflect a balance between two opposite influences: one tendency is to reduce diameter of vessel to compensate blood flow reduction and the second tendency is to enlarge due to portal hypertension. The same situation was observed in other studies that assessed the vasculature of the cirrhotic patients (14, 15).

The improvement of the portal circulation is indicated by the decrease of the splenic volume. This reduction in volume may be the combined result of decreased portal hypertension and general inflammatory status.

This leads to the conclusion that this reduction in splenic volume could be a sensitive marker for the overall improvement of hepatic status and, consequently, of portal circulation, but further study is needed.

CONCLUSIONS

There is a statistically significant reduction in spleen size in patients with HCV-related compensated cirrhosis who obtained SVR after treatment with DAAs and this can be used as a sensitive imaging marker to assess improvement in cirrhotic patient's condition.

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NEWS

THE ENDOMETRIOSIS EFFECT ON OVARIAN MALIGNANCY AND ITS IMPLICATION IN GRANULOSA CELL TUMORS

Even if endometriosis is considered a benign pathology, it may play an essential role in ovarian cancer developing. There are data about the endometriosis effect on ovarian cancer but it is a lack regarding its involvement in granulosa cell tumor. Ashari et al. (2019) applied steroid-depleted serum or whole serum from endometriosis patients to the granulosa cells obtained from tubal or infertile women due to a male factor and used flow cytometry and quantitative real-time polymerase chain reaction in order to establish the expression level of inflammatory and apoptotic genes and also the apoptosis level of granulosa cells. The study shown that the granulosa cells which went under treatment with steroid depleted serum from patients with endometriosis presented a considerable increase expression of NFkB/RelA and AKT compared with control group. On the other hand, the genes related to apoptosis had a lower expression in treatment group compared with the control but also the synthesis of saturated fatty acids whereas the levels of unsaturated fatty acids were increased. The implication of steroids on endometriosis is contradictory and the sex hormones and cortisol levels may be modified due to this pathology, leading to alteration of the condition progression. Thus, decreased level of sex hormones in endometriosis females may be considered as a decisive risk factor for granulosa cell tumor (Asghari S, Dizajeykan AV, Ahmadi M et al. Evaluation of ovarian cancer risk in granulosa cell treated with steroid-depleted endometriosis serum: role of NF-kB/RelA and AKT. *J Cell Physiol* 2019; 234(7): 12011-12018).

MODIFICATION OF BIOCHEMICAL AND BIOLOGICAL PARAMETERS IN URINARY TRACT INFECTIONS IN ELDERLY

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MODIFICATION OF BIOCHEMICAL AND BIOLOGICAL PARAMETERS IN URINARY TRACT INFECTIONS IN ELDERLY (Abstract): **Aim** To determinate the biochemical and microbiological tools for diagnosing urinary tract infections in elderly patients hospitalized at the "Sf. Parascheva" Clinical Hospital of Infectious Diseases, in Iasi, from January 2018 to June 2019. **Material and methods:** A retrospective analysis of the hospital - based medical records of the patients aged over 65 years diagnosed with urinary tract infection who presented signs and symptoms of UTI and urocultures were positive. The parameters followed were age, sex, place of residence, presence of comorbidities, duration of hospitalization, laboratory data. **Results:** We included in our study 387 patients over 65 years old which presented clinical manifestations of UTI. The highest frequency was in the age group 65-74 years. *Escherichia coli* was the most isolated pathogen (63%) followed by others as follows *Proteus, Klebsiella, Enterobacter, Pseudomonas*. **Conclusions:** Antibiotic resistance of uropathogenic strains isolated revealed that the highest resistance was found to Ampicillin followed by Fluoroquinolones and Cephalosporins**. Keywords**: BIOCHEMICAL MARKERS, AGING, UROCULTURES, ELDERLY, LABORATORY DIAGNOSIS.

Frequently found in adults age 65 and over, urinary tract infection (UTI) represent an important fragment of the amount of work in clinical microbiology laboratories (1). UTIs are the most frequent community and nosocomial acquired infections, accounting for considerable morbidity and health-care expenditure (2, 3).

The effects of aging make UTIs in the elderly hard to detect and hard to cure. The classic symptoms of a UTI are burning pain at urination associated frequent urination, but in elderly we may not found these symptoms. Instead, the aged population, mostly those presenting dementia, may encounter behavioral symptoms which can include confusion (4).

Seniors are susceptible to UTIs for many reasons. For example, their immune system is usually weak and can`t fight rapidly multiplying bacteria (5), and that, increases susceptibility to any infection. In addition, impaired cognitive skills and lower energy levels are concerns that determinate decreased self-care capacity and development of bacteria in elderly patients too (6). Many other medical conditions can increase the susceptibility to urinary tract infection in people over 65 years old, like diabetes, immobility, complications from medicines, and

problems emptying the bladder completely (7). Also, the elderly may experience more than one episode of UTI. Recurrent UTI have been liked as a potential cause of primary biliary cirrhosis (8, 9).

As a result of physiologic modifications corelated to aging and associated pathology, asymptomatic bacteriuria (ASB) is a common manifestation in older ones (10). This for, the diagnosis of UTI is challenging and may require particular diagnostic criteria than the standard ones. As a consequence of this factors doctors often depend on a few laboratory tests to sustain clinical suspicions and to determinate the cause of the infection and/or to provide isolates for antimicrobial susceptibility tests. Therefore, it is not surprising that the laboratory investigation of urine samples represents a substantial proportion of the workload in laboratories (1, 10).

Mainly, the cases of UTI have as etiological agent Gram-negative bacteria, including *Escherichia coli, Proteus mirabilis, Klebsiella spp., and Enterobacter* species (11). Fungal UTIs are not very common compared with the bacterial one . The risk of a fungal infection increases in patients with indwelling catheters, those that are suffering from diabetes, or who received recent antibiotic treatment (12).

A global threat is represented by the rising incidence of resistant or multi drug resistant uropathogens that require rapid diagnosis and initiation of antibiotic therapy with caution (12). Antimicrobial resistance can appear by mutation of the bacteria or by horizontal gene transfer. We can take as example the resistance that is provided by the naturally evolved β-lactamase genes which grant extensive resistance to β-lactams such as penicillins and cephalosporins (13).

MATERIAL AND METHODS

Studied patients

This is a retrospective study on biochemical and microbiological tools for diagnosing urinary tract infections in the elderly, performed at the "Sf. Parascheva" Infectious Diseases Clinical Hospital, in Iasi, from January 2018 to June 2019. The inclusion criteria for the present study were patients aged over 65 years admitted to the clinic, who presented signs and symptoms of UTI and urocultures were positive (bacterial confirmation $\geq 10^5$ CFU / mL).

Data collection

The parameters followed were age, sex, place of residence, presence of comorbidities, duration of hospitalization, laboratory data, including the etiological spectrum of the disease. The data were collected from the observation sheets. Given the fact that is a retrospective research, the consent was not necessary as long as the anonymity of the participants was respected (name, document, medical chart).

The urinalysis and the urine culture were performed in the hospital's microbiology laboratory. Urinalysis is performed on a sample of urine, test that find and/or quantify several elements in the urine sample, such as byproducts of normal and abnormal metabolism, cells, cellular fragments, and bacteria. For urine culture, the urine sample is cultured on agar plates for growth, concentration, identification, and isolation. The diagnosis of urinary tract infection requires the presence of bacteria $\geqslant 10^5$ CFU per milliliter of urine.

The diffusimetric method was used on the Mueller Hinton agar, used for antibiotic susceptibility testing. After 18 hours, the sensitivity of the germ can be assessed, depending on the diameter of the inhibition zone that appears around the antibiotic tablet. The correlation between the diameters of the inhibition zones and the sensitivity of the germ was made by comparison with the existing data in the tables of EUCAST (European Committee on Antimicrobial Susceptibility Testing) standards. And the results are expressed in sensitive,

resistant, and intermediate (14, 15).

RESULTS

During the above-mentioned period were hospitalized in the clinic 4,075 patients age 65 and over, 833 (20.44%) presenting clinical manifestations of UTI, either at the first examination or during hospitalization for another medical problem. Of these, 387 patients were enrolled for the current study. The highest frequency was in the age group 65 - 74 years (49.6%), with a predominance of cases in women (66.9%) and at those in the urban area (58.4%).

TABLE I

Characteristics of patients

	N	%
Mean age (years)	73 ± 5	
Sex (M/F)	128 / 259	
Rural/urban area of residence	226 / 161	
History of previous UTI	32	8.26
Presence of comorbidities	345	89.1
Healthcare facility-onset (HO) UTI	159	41.08

The duration of hospitalization was significantly longer (9 days *vs*. 5 days; p <0.05) compared to patients with urinary tract infection but unidentified pathogen.

Regarding clinical diagnosis, in our studied group only 39 patients had asymptomatic bacteriuria, the diagnosis being made after routine analysis. The other patients showed signs and symptoms of urinary tract infection, 189 patients at admission in the clinic and 159 patients during their hospital admission for other medical problem (tab. I).

The presence of nitrites in the urine also indicates a UTI which was found in 197 (50.9%) patients (tab. II) .

TABLE II

Analyzed laboratory variables

	N	%
Pyuria	326	84.2
Nitrites	197	50.9
$>10^4$ CFU	387	100

In the present study the prognosis of the disease was affected by the presence of comorbidities (n = 345; 89.1%) -diabetes (23,4%), hypertension (22,6%), acute kidney failure(20%), benign prostate hyperplasia (5,4%), chronic kidney disease (4,6%) and relapses (n = 32; 8.26%).

In our study group, laboratory investigations revealed plurimicrobial flora (82 urine cultures detect concomitantly infection with two different bacteria, 28 cultures with three types of bacteria). Statistically significant data were obtained for gram negative bacteria (77%). *Escherichia coli* was the most isolated pathogen (n = 244; 63%). Other uropathogens: *Proteus, Klebsiella, Enterobacter, Pseudomonas.* As causative pathogen, were identified also *Enterococcus spp* in 64 (16.5%) patients. and *Candida spp* in 25 (6.4%) patients. Urinary infections caused by *Candida spp.* are considered to be caused by frequent and long-term use of antibiotics. A number of 43 strains of *Enterobacteriaceae* were beta-lactamase releasing (28 *E. coli* and 15 *K. pneumoniae*).

Antibiotic resistance of uropathogenic strains isolated revealed that the highest resistance was found to Ampicillin (74.1%), followed by Fluoroquinolones (51.1%) and Cephalosporins (34.1%). Uropathogenic *E. coli* showed low resistance levels to Nitrofurantoin, Amikacin, and Gentamycin.

DISCUSSION

It is well known that on people over 65 years may be associated comorbidities, such hypertension, diabetes, or other pathologies in the urological sphere. Men with UTI are suffering from low urinary tract conditions, such as benign prostate hyperplasia, prostate cancer, urethral strictures and in female patients, pathology such as urinary stress incontinence, pelvic organ prolapse it is frequently encountered (16). Also, UTI can be encountered in patients that suffers from different other pathologies (e.g., leptospirosis) which can require urinary catheterization (17,18)

In order to establish a positive diagnosis for UTI we need to check out for the presence of clinical symptoms and then the next step is represented by laboratory examination. We start by collecting urine samples for biochemical examination, looking out for nitrites and leukocyte esterase, in order to detect the possible presence of bacteriuria and pyuria. But, in case of decreased urine concentration, or in the case of non-nitrite-producing bacterium (*Enterococcus spp.* and *Staphylococcus spp*. for example), the colorimetric dipstick may give us false-negative results (14, 15, 19, 20). Because of this reason in the majority of health-care settings, urine dipstick tests are no longer utilized the urine analysis at the point of care but in the clinical laboratory, where standardized readouts and expended microscopic urinalysis can be performed (21). The clinical and paraclinical data described above should be completed with urine culture. This test is performed in the microbiology laboratory where the urine sample is cultivated on agar-agar plates for growth, concentration, identification, and isolation. Are considered positive, urine cultures containing bacteria that exceed the threshold of $\geq 10^4$ CFU/mL whether we are talking about a single isolated bacterium or several (22). If the bacterial flora is dominated by non-*E. coli Enterobacteriaceae* species, for the identification of uropathogenic strains an additional phenotypic characterization of the isolated bacteria is needed. This investigation is regularly performed with automated systems based in the clinical microbiology laboratory. The results of a urine culture take between 18 to 30 hours (23, 24).

The bacterium was isolated and identified. What is next? The antibiogram. In order to determinate the microbial sensitivity. This investigation takes 24 to 48 hours more. Antimicrobial susceptibility testing (AST) is a procedure conducted as a phenotypic assay used to measure the pathogen growth in the presence of a specific or a group of antimicrobial agents. Once the results of antimicrobial susceptibility are available, they are divided into three categories: sensitive (S), intermediate (I), or resistant (R). According to standardized guidelines (25, 26) .

The clinical diagnosis of UTI is essentially based on medical history (includes an evaluation of urinary tract symptoms, history of UTI or other urinary tract problems, family history and sexual history). Urine tests (urinalysis - visual exam, microscopic exam, dipstick test) are the second important element in diagnostic testing. The

gold standard for positive diagnosis in UTI is urine culture, with identification and isolation of the pathogen, completed by testing for drug sensitivity (7).

Urinalysis represents a valuable diagnostic tool, a qualitative and semi-quantitative test. It is always used for the evaluation of a potential UTI case. This test can detect bacteriuria and pyuria which are marks of inflammation (27). Pyuria, is defined as the presence of white blood cells (WBC >10 elements) or positive leukocyte esterase (28). In our study, it was found at a rate of 84.2%.

The definite diagnosis of a UTI and the initiation of treatment require further investigation. Performing a urine culture is essential and we must also consider the patient's medical history.

Regarding the treatment, for patients with uncomplicated urinary tract infection Nitrofurantoin was selected, due to high concentrations in the urinary tract, minimal impact on gastrointestinal flora and low propensity to resistance. For those with complicated UTI or with multiresistant Gram-negative bacteria we chose Carbapenems which have good penetrability and are broad-spectrum antibiotics that can be used as empirical treatment for severe sepsis that may have as a couse AmpC or ESBL-producing bacteria.

Old age is a complicated process and involves a number of changes. In the elderly, self-esteem drops to alarming levels. This may be due to repeated urinary tract infections or the presence of urinary catheters, which exacerbates depression, which any older person struggles. In those cases, the psychologist can help increase self-esteem and regain self-confidence (29).

CONCLUSIONS

In this study the main tool for the diagnostic of UTI was the uroculture. In most of the cases, the etiology of UTIs was microbial; sometimes even plurimicrobial, in certain circumstances co-infection with two or more bacteria. *Escherichia coli* remains the most commonly isolated uropathogens, followed by *Klebsiella spp., Pseudomonas aeriuginosa, Enterobacter spp., Proteus mirabilis and Enterococcus spp*. Urinary tract infections caused by *Candida spp.* are considered to be caused by frequent and long-term use of antibiotics.

A urinary tract infection in seniors can become quite serious and ultimately can lead to sepsis, which can become life-threatening. An unexplained alteration of the general condition, digestive disorders or an isolated fever in an elderly person draws attention to the disease. Therefore, it is important to treat UTIs in the elderly as soon as the symptoms appear. Prompt diagnosis and targeted therapy can prevent complications and shorten the hospitalization.

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NO EVIDENCE OF A MORE AGGRESSIVE PATTERN OF HEPATOCELLULAR CARCINOMA AFTER DIRECT ACTING ANTIVIRALS -RESULTS FROM A SINGLE CENTER OBSERVATIONAL STUDY

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NO EVIDENCE OF A MORE AGGRESSIVE PATTERN OF HEPATOCELLULAR CARCINOMA AFTER DIRECT ACTING ANTIVIRALS-RESULTS FROM A SINGLE CENTER OBSERVATIONAL STUDY (Abstract): After great concerns were raised regarding a high incidence of hepatocellular carcinoma (HCC) after direct acting antivirals (DAAs) therapy in cirrhotic HCV patients, an avalanche of studies that debated this issue emerged, whose results highlighted also a more aggressive HCC pattern after DAAs. We aimed to assess the pattern and imaging features of HCC in patients with HCV-related liver cirrhosis after DAAs. **Material and methods:** This is a single-center case-controlled retrospective study in which we included consecutive patients with HCC and HCV-related liver cirrhosis admitted in our clinic between January 1st, 2016 and May 1st, 2018. **Results:** Sixty-nine patients with HCV-related cirrhosis and HCC were divided into 2 groups depending on the DAAs-treated or untreated status: group I included 15 patients that were previously treated with DAAs and group II included 54 naïve patients. Regarding the distribution of HCC, there were no significant differences between the two groups (73.3% of group I patients and 55.5% of group II had unicentric lesion, p=0.215). The tumor size was significantly higher in group II compared to group I, with 66.6% of patients in group I and only 29.6% of patients in group II that have a tumor size less than 3 cm (p=0.009). Supportive treatment without ablation was more frequent in group II (33.3%) than in group I (6.6%) (p=0.047). **Conclusions:** Our study found no evidence of a more aggressive behavior of HCC after DAAs treatment compared to naïve patients. Furthermore, the pattern of HCC after DAAs did not affect the access to curative treatment. **Keywords:** HEPATOCELLULAR CARCINOMA, DIRECT ACTING ANTI-VIRALS, AGGRESSIVE PATTERN.

Hepatocellular carcinoma (HCC) is the most frequent primary liver malignancy ranked as the second leading cause of cancer-related death, with increasing incidence rates worldwide (1). The developments from the past 10 years considerably improved the therapeutic management of HCC leading to extended access to thera-

peutic options that increase the life expectancy of patients with HCC, regardless of the evolutionary stage. However, HCC still remains an aggressive malignant tumor with a high rate of cumulative recurrence over 5 years (70%) after curative therapies (2). The prognosis of HCC is estimated based on imaging features of the tumor such as the number and size of the tumors and the presence of vascular invasion.

The risk for HCC in patients with chronic infection with hepatitis C (HCV) infection and liver cirrhosis ranges between 2% and 8% per year (2). According to WHO's Global Hepatitis Report, about 1% of the world population is infected with HCV and the subsequent liver disease due to chronic HCV infection represents a favorable environment for the development of hepatocarcinogenesis (3,4). Previous studies from the IFN era demonstrated a significant decline in HCC incidence in patients who achieved SVR, thus prospects regarding the ability of the new DAAs to prevent HCC occurrence after HCV clearance were promising (5). However, great concerns were raised when several studies published in the beginning of the DAA era reported a high occurrence and recurrence rate along with a more aggressive behavior of HCC after DAA therapy in cirrhotic HCV patients (6, 7). This led to an avalanche of studies that debated this issue, whose results tend to a reduced incidence rate of both de novo and recurrent HCC after achieving SVR with DAAs therapy (8-10). Still, a worrying aspect is the identification of a more aggressive HCC pattern after DAAs therapy which several authors have previously reported, but data concerning this issue is scarce. Recent studies which assessed the impact of DAAs on HCC, provided evidence of high tumor aggressiveness comprising an advanced Barcelona Clinic Liver Cancer (BCLC) stage and early vascular invasion (11, 12). These tumor features are associated with limited access to ablation therapy and thus, have a negative impact on survival rate.

We aimed to identify and characterize the changes in the pattern and imaging features of HCC developed after DAA therapy in patients with chronic HCV infection and liver cirrhosis, as well as the impact on HCC treatment decisions, compared to patients with HCC and HCV-related liver cirrhosis without previous antiviral treatment.

MATERIAL AND METHODS

This is a single-center case-controlled retrospective study in which consecutive patients with HCC and HCV-related liver cirrhosis admitted in a tertiary gastroenterology referral center from North-Eastern Romania, between January 1st, 2016 and May 1st, 2018, were included. All patients who presented during the same period were divided into 2 groups depending on the DAA-treated (group I) or untreated status (group II). All patients with other concomitant causes of liver disease such as autoimmune hepatitis, thesaurismosis, non-alcoholic fatty liver disease, HCV/HBV and HCV/HIV co-infection were excluded. The regimens used in treated patients were: Paritaprevir/Ritonavir, Ombitasvir and Dasabuvir (PrOD) in patients with HCV-related compensated liver cirrhosis and Ledipasvir/Sofosbuvir (LED/SOF) + Ribavirin (RBV) for 12 weeks, in patients with decompensated liver cirrhosis.

The HCC tumor pattern was evaluated in all patients and consisted of the quantification of the largest tumor dimension, HCC multiplicity, the presence of malignant portal vein thrombosis (PVT), and the al-

pha-fetoprotein (AFP) levels.

Statistical analysis

Data collected was statistically analyzed using *SPSS 20.0* (Chicago, IL, SUA). All tests were two-tailed with p-value <0.05 considered statistically significant. Continuous variables were expressed as mean ±standard deviation for normally distributed continuous data, and as median for non-normally distributed continuous data. Groups were compared using Chi square test or Fisher's exact test for categorical variables and using independent *t*-Student test or Mann-Whitney *U* test for continuous variables (depending on data distribution).

RESULTS

This study included 69 patients diagnosed with HCC and HCV-related liver cirrhosis, mean age 67.5±12.2 years, mostly males 53.6%. Thirty-eight (55.1%) patients had comorbidities such as arterial hypertension (42%), diabetes (18.8%) and obesity (11.6%). Most patients had decompensated liver cirrhosis (55.1%), with a mean CPT score of 7.4±0.25.

All study patients were divided into 2 groups, depending on the DAAs-treated or untreated status, as follows: group I included 15 patients with HCC and HCV-related liver cirrhosis who were previously treated with DAAs, whereas group II consisted of 54 patients who were admitted in the same period with HCC and HCV-related liver cirrhosis without evidence of previous antiviral treatment.

The majority of patients from group I were females (53.4%), with mean age of 65.1±9.8 years. The majority had compensated liver cirrhosis; 86.6% had Child-Pugh A class, whereas 13.4% only were decompensated (1 patient had Child-Pugh B and 1 patient Child-Pugh C) (tab. I).

TABLE I

Demographics and clinical characteristics in the study groups

Parameter	DAAs treated N=15 (Group I)	No DAAs N=54 (Group II)	p
Age (yrs.), mean ± SD	65.1±9.8	68.1±12.8	0.397
Male, n (%)	7 (46.6)	30 (55.5)	0.541
Child-Pugh class, n (%)			**0.001**
A	13 (86.6)	18 (33.3)	
B	1 (6.6)	22 (40.7)	
C	1 (6.6)	14 (25.9)	
Child-Pugh score, mean ± SD	5.9±1.4	7.8±2.1	**0.002**
FIB4, mean ± SD	3.4±2.3	33.5±25.1	0.536
APRI, mean ± SD	0.84±0.5	2.4±2.1	**0.004**
Comorbidities, n (%)	8 (53.3)	30 (55.5)	0.878
Arterial hypertension	7 (46.6)	22 (40.7)	0.681
Diabetes mellitus	3 (20)	11 (20.3)	0.538
Obesity	3 (20)	5 (9.2)	0.250
ALT (UI/L), mean ± SD	41.6±19.2	112.2±13.2	**0.049**
AST (UI/L), mean ± SD	37.1±25.1	70.4±13.5	**0.028**

Abbreviations: IQR, interquartile range; SD, standard deviation;
FIB4, Fibrosis-4 Index for Liver Fibrosis; APRI, AST to Platelet Ratio Index.

Eleven (73.4%) patients were previously treated with PrOD and 4 (26.6%) patients received LED/SOF+RBV. SVR rate in the studied patients in group I was 100% and was defined by undetectable HCV-RNA at 12 weeks after end of treatment (EOT).

Patients in group II were predominantly males (55.5%), with similar mean age as group I (68.1±12.8), but with a higher percentage of decompensated liver cirrhosis than group I (Child-Pugh score was 5.9±1.4 in group I *vs.* 7.8±2.1 in group II, p=0.002). With respect to liver function, hepatocytolysis was more significant in group II compared to group I (ALT: 112.2 IU in group II *vs.* 41.6 IU in group I, p= 0.049; AST 70.4 IU in group II *vs.* 37.1 IU in group I, p=0.028). Also, APRI score was higher in group II compared to group I (p=0.004).

Regarding the distribution of hepatic focal lesions, there were no significant differences between the two groups (73.3% of group I patients and 55.5% of group II had unicentric lesion, p=0.215) (tab. II). The tumor size was significantly higher in group II compared to group I, with 66.6% of patients in group I and only 29.6% of patients in group II that have a tumor size less than 3 cm (p=0.009). AFP level was higher but not statistically significant in group II with a median (IQR) value of 78 ng/mL (1617.4) compared with a median (IQR) value of 8.4 ng/mL (32.7) in group I (p=0.059).

TABLE III

Tumor characteristics in the studied patients

Parameter	DAAs treated N=15 (Group I)	No DAAs N=54 (Group II)	p
AFP, mean ± SD	163.1±42.1	602.8±84.1	0.059
BCLC stage, n (%)			
A	5 (33.3)	15 (27.7)	0.481
B	6 (40)	13 (24.1)	
C	2 (13.3)	14 (25.9)	
D	2 (13.3)	12 (22.2)	
Within Milan criteria, n (%)	10 (66.6)	19 (35.2)	**0.029**
Unicentric, n (%)	11 (73.3)	30 (55.5)	0.215
Tumor size < 3cm, n (%)	10 (66.6)	16 (29.6)	**0.009**
PVT, n (%)	2 (13.3)	24 (44.4)	**0.028**
Lymph node metastasis, n (%)	1 (6.66)	13 (24%)	**0.031**

Abbreviations: IQR, interquartile range; SD, standard deviation; PVT, portal vein thrombosis.

An aggressive tumor pattern was established based on the identification of malignant adenopathy in 1 patient and malignant PVT in 2 patients from group I, and in 13 and 24 patients, respectively in group II, with a statistically significant difference between the two groups (p=0.031 and p=0.028, respectively). None of the studied patients developed another distant metastasis. According to BCLC classification, the majority of patients in group I were in stage A (33.3%) and B (40%), whereas patients in group II were approximately equally distributed in all stages (A-27.7%, B-24.1%, C-25.9%, and D-22.2%). With respect to Milan criteria, 6.6% of patients from group I and only 35.2% of patients from group II were within the criteria

(p=0.029).

Aggressive pattern of HCC had its impact on therapeutic decision. Supportive treatment without ablation was more common in group II (33.3%) than group I (6.6%) (p=0.047). Otherwise, TACE was the most frequent choice followed by surgery in both groups. One patient (1.8%) from group II had liver transplant.

DISCUSSION

Although there is a high amount of data regarding HCC occurrence and recurrence after DAAs, few reports assessed the behavior of HCC and the access to curative therapy after DAAs treatment. Data from our study emphasis important findings regarding the behavior of HCC after DAAs therapy compared to naïve patients. The incidence rates of HCC in patients without previous antiviral therapy seems to be related to the severity of cirrhosis considering that the mean APRI score was lower among DAA-treated patients with HCC than in naïve HCC patients. Furthermore, patients from group I had a higher percentage of compensated cirrhosis than those from group II (86.6% *vs.* 33.3%, p=0.001).

Recent studies which evaluated the behavior of HCC after DAAs, have found more aggressive features of HCC. For instance, a recent article by Fayoume *et al.* found that multiplicity and infiltrative patterns were more common in DAAs-treated HCC patients compared to naïve HCC patients (13). Another study revealed that malignant PVT and lymphadenopathy were significantly more frequent in DAAs-treated HCC patients (14). On the contrary, we found a more aggressive behavior of HCC among untreated patients from group II compared to group I. Although the lesions were predominantly unicentric in both groups, we found that malignant PVT and lymphadenopathy were more frequent in group II than in group I. Furthermore, patients from group I met the Milan criteria more frequently and had smaller sizes of tumor lesions than those from group II. According to BCLC classification, we found no significant differences between the two groups. Similarly, Fatima *et al.* reported that the pattern of HCC did not differ between patients treated with IFN-based therapy and those DAAs-treated, considering BCLC stages, multiplicity, malignant PVT and lymphadenopathy (15).

The best supportive care was the only recommended line of treatment for 6.6% patients from group I and for 33.3% patients from group II. The significantly higher percentage of patients from group II that received only best supportive care reflects the limited access to therapy based on BCLC classification. Access to curative therapy by surgical ablation was better in group I *vs.* group II, although TACE and sorafenib were the next most frequent options in both groups.

CONCLUSIONS

Our study found no evidence of a more aggressive behavior of HCC after DAAs treatment compared to naïve patients. On the contrary, we found a better access to HCC curative therapy in this group compared to untreated patients. Based on these findings, the screening of HCC in patients with HCV-related liver cirrhosis remains necessary after achieving viral clearance with DAAs therapy. According to current guidelines, abdominal ultrasound and AFP monitoring every 6 months in HCV-related liver cirrhosis seems the most appropriate strategy for these patients.

CONFLICT OF INTERESTS AND FUNDING

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IMPACT OF OBESITY ON SKIN DISEASES

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IMPACT OF OBESITY ON SKIN DISEASES (Abstract): Obesity is a chronic disease recognized as a global epidemic; however, the impact of obesity on the skin has received minimal attention. The aim of the study was to evaluate the skin diseases of aggravated by overweight. **Material and methods**: We conducted a retrospective study of 100 patients admitted in 2019 to the Dermatology Clinic of the Iasi "Sf. Spiridon" County Clinical Emergency Hospital. Of these, 79 adults had BMI above the healthy range. Moreover, the obese patients were divided into 4 BMI classes: overweight (28%), grade 1 obesity (27%), grade 2 obesity (16%) and morbid obesity (8%). **Results**: All participants underwent dermatological examination for the detection of skin diseases. This article describes the most common skin diseases associated with obesity: skin infections (43%), superinfected skin ulcers (37%), xerosis (23%), plantar hyperkeratosis (10%), worsening of psoriasis (9%), atopic dermatitis (6%), rosacea (4%) and acne (1%). **Conclusions**: Our study confirm that there is a high prevalence of different skin pathologies in overweight and obese patients. **Keywords:** OVERWEIGHT, OBESITY, BODY MASS INDEX, SKIN DERMATITIS, SKIN INFECTIONS.

Obesity, a serious global health problem, is nowadays the most common nutritional disorder in Romania, with a continuous rise in incidence (1).

The impact obesity on the skin and sebaceous and sweat glands is alarming, it being responsible for circulatory and lymphatic changes. Common obesity-related skin manifestations are acanthosis nigricans, keratosis pilaris, plantar hyperkeratosis, decreased skin mechanical strength, and increased sebum production. Also, obesity is associated with an increased incidence of bacterial and *Candida* skin infections, as well as with onychomycosis, inflammatory skin diseases, psoriasis, and rosacea (2).

The global epidemic of obesity results from a combination of genetic susceptibility, increased availability of high-energy foods and decreased need for physical activity in modern society. Obesity should no longer be regarded just as a cosmetic problem that affects certain individuals, but as an epidem-

ic that threatens global wellbeing (3).

In this article we will focus on obesity-associated skin, metabolic and physiologic changes.

MATERIAL AND METHODS

We conducted a retrospective observational study which included 100 patients with various diseases admitted to the Dermatology Clinic of the "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, in 2019. For each of these cases included in the study the following data were collected: sex, weight, height, body mass index, cholesterol, triglyceride and blood sugar levels, blood pressure and associated diseases.

RESULTS

Obesity, defined as a BMI (weight divided by squared height) higher than or at least equal to 30, influences both morbidity and mortality in the society. In the first table is presented the distribution of all the studied patients, according to the BMI - related categories, respectively 51% of the studied patients were obese.

Of the total number of obese patients admitted to our Dermatology clinic during 2019, 55% were women.

TABLE I

Distribution of study patients according to BMI

Category	BMI, kg/m^2	%
Normal weight	18.5 – 24.9	21
Overweight	25.0 – 29.9	28
Grade 1 obesity	30.0 – 34.9	27
Grade 2 obesity	35.0 – 39.9	16
Morbid obesity	> 40	8

Formula: BMI=kg/m^2 / BMI= body mass index

TABLE II

Distribution of patients with skin diseases according to BMI

Skin disease	Overweight (%)	Grade I obesity (%)	Grade II obesity (%)	Morbid obesity (%)
Skin infections	9	40	14	37
Ulcer	38	28	24	10
Xerosis	41	35	18	6
Plantar hyperkeratosis	0	30	30	40
Psoriasis	29	21	29	21
Atopic dermatitis	33	67	0	0
Rosacea	0	50	50	0
Acne	0	100	0	0

A strong correlation was found between obesity and severity of psoriasis. As shown in first figure, 9% of the overweight and obese study patients had moderate to severe psoriasis vulgaris, as well as psoriatic arthritis. In these patients the outcome was unfavorable on both topical, systemic, as well as biological therapies measured by

quality-of-life questionnaire.

Of the total overweight and obese study patients approximately 23% were diagnosed in addition to other conditions with xerosis cutis. Of these 35% had obesity grade I, 18% grade II, 6% morbid obesity, and the remaining 41% were overweight.

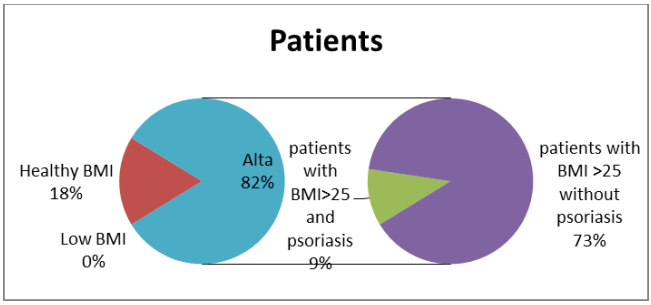

Fig. 1. Patents with obesity and psoriasis. Patients, Healthy BMI, Low BMI, patients with BMI > 25 and psoriasis, patients with BMI > 25 without psoriasis

DISCUSSION

Obesity causes various changes that affect skin barrier integrity, such as xerosis and increased *Trans Epidermal Water Loss* (TEWL). Xerosis is a common finding in patients with high BMI because of the change in *stratum corneum* hydration (4, 5).

In a study in which overweight and obese children were clinically assessed and TEWL was calculated, Massimiliano *et al.* (6) demonstrated that TEWL was significantly higher in those with high BMI. Obesity increases the TEWL rate, suggesting that due to the thick layers of subcutaneous fat obese patients sweat more profusely (7).

Another study conducted in 2017 (8) examines the influence of excess weight on skin hydration. The results suggest that overweight and obesity could promote water loss at the skin level, reflecting a particular skin adaptation to weight gain.

Of the 79 patients with high BMI included in the study, 37% presented on admission superinfected ulcers of different causes and with different locations.

Obesity is associated with altered collagen structure and impaired wound healing. In obesity, collagen deposition does not match the increase in skin surface, resulting in decreased skin mechanical strength. Leptin promotes wound healing, and obesity is associated with increased leptin resistance, thus contributing to the pathological wound healing process (9).

The main cause of superinfected ulcers was chronic venous insufficiency (CVI). Of the 79 patients with high BMI included in this study, most of them (41/52%) had this disease. Also, on admission the majority presented lipodermatosclerosis besides CVI and ulcer.

Obesity appears to be a major cause of microvascular dysfunction, contributing to the development of microangiopathy and hypertension, and this medical condition could adversely affect the clinical severity of chronic venous disease. Chronic venous insufficiency can result in varicose eczema and ulcerations. In addition, in resting state, the skin capillaries of obese patients are maximally recruited, with no functional capillary reserve, contributing to the development of ulcers (10).

A study conducted in 2002 by Danielsson *et al*. (11) investigated the impact of excess weight in patients with CVI and aimed at determining whether the effect can be explained only by the increased venous reflux. Patients with chronic venous disease were classified according to the clinical, etiological, anatomical and pathophysiologic system and BMI index. Most CVI patients were overweight (61%). It was demonstrated that patients with high BMI were more likely to present skin changes and ulcers ($p < 0.001$) than patients with a BMI < 25 kg/m^2, despite similar values for total reflux time ($p = 0.92$) and peak reverse flow velocity ($p = 0.98$). Overweight seems to be an independent risk factor for the increased severity of skin changes in patients with chronic venous disease (11).

The prevalence of plantar hyperkeratosis in the obese patients included in the study was 10%. Although this percentage is not significantly high, this is one of the most common dermatological conditions encountered in people with high BMI, as demonstrated by many other articles. Palmoplantar keratodermas are a group of disorders characterized by abnormal thickening of the skin on the palms and soles. Plantar hyperkeratosis in a horseshoe pattern affects the heel, arch of the foot and toe and is associated with obesity. Excess weight changes the anatomy of the foot and transfers the high pressure on the bony projections of the sole, causing mechanical trauma (12, 13).

In 1999, Garcia-Hidalgo *et al.* (14) conducted a study in a dermatology clinic in Mexico and aimed at determining the prevalence of skin diseases in 156 obese patients. Plantar hyperkeratosis was the most common finding in obese patients. Excess weight carried by people with BMI > 25kg/m^2 determines the transfer of weight during the gait cycle to other foot areas, abnormal foot posture, increased pressure and friction over bony prominences, thus generating keratotic disorders. Thus, plantar hyperkeratosis should be considered a cutaneous stigma of severe obesity. This is probably a result of pressure directly related to overweight.

Obesity increases the risk of infectious diseases in general, skin infections included (15). Possible mechanisms for this predisposition are the association of obesity with a proinflammatory state, decreased cellular immunity, and pharmacological interactions with antimicrobial agents (12, 13).

The incidence of candidiasis, intertrigo, folliculitis, furunculosis, erysipelas, cellulitis, erythroses, onychomycosis and tinea pedis and corporis is increased in patients with BMI > 25 kg/m^2.

In this article we demonstrated the increased frequency of intertrigo among patients with obesity, this condition being detected in 6% of the study patients who associated increased BMI. Intertrigo is an inflammatory skin condition characterized by well-defined erythematous plaques, maceration and linear cracks associated with moderate itching located in the skin folds (genitocrural, submamar, axillary, intergluteal, retro auricular, interdigital).

Obese patients have larger skin folds and sweat more profusely, thus increasing friction and moisture and leading to maceration and inflammation (16, 17).

A study conducted in 2010 had shown that intertrigo is among the most common skin dermatoses associated with obesity (22.2%) (18). Another study on intertrigo in diabetic patients found that skin surface pH is higher in the inguinal folds of women with a BMI> 25 compared to women with a

BMI < 25 kg/m^2 (19).

The association between obesity and severe lower limb cellulitis was also reported in 4% of our study patients. The literature specifies that obesity, in addition to being an independent risk factor for erysipelas and its complications, also prolongs length of hospital stay, favors the recurrence of erysipelas after prophylactic treatment with penicillin (20).

Erythrasma, a superficial localized skin infection caused by *Corynebacterium minutissimum*, and a relatively a common finding in obesity, was present only in one study patient.

Obesity is also an important risk factor for onychomycosis. This disease is quite common among patients with increased BMI, in our study patients it being present in a significant percentage, namely 20% of those with overweight and obesity.

In another 2002 study conducted on 1000 patients, obesity was found to be one of the most frequent predisposing factors in people with onychomycosis (9). In addition, in 2011, researchers at a pediatric clinic aimed at determining the frequency of nail onychomycosis in diabetics and found a strong association with obesity (21).

There is a linear trend between severity of obesity and *tinea pedis*.

The skin dermatosis most commonly found in our study patients with increased BMI was the tinea pedis, both intertriginous and hyperkeratotic, which with an incidence of 43% far exceeded the presence other conditions.

A 2008 study aimed at determining the prevalence of tinea pedis in diabetic and nondiabetic population. Cultures have shown a prevalence of 40% in the diabetics, as well as the fact that diabetic patients may be susceptible to interdigital fungal infections at a younger age than those with normal blood sugar (22). Other authors showed that tinea pedis is one of the first five obesity-associated skin diseases, being favored by increased humidity and profuse sweating (23).

The severity of psoriasis was closely correlated with obesity. Systemic and biological therapy used in the treatment of moderate to severe psoriasis appears to be less effective in patients with higher BMI. This finding could be associated with the higher doses needed to treat these patients. There is evidence that progressive weight loss reduces the severity of psoriasis (9).

In 2009, Perez *et al.* (24) conducted a study to evaluate the effect of weight loss in obese patients with psoriasis. Significant improvements in psoriasis were found after weight loss, and the positive changes were maintained for 64 weeks. Also, in 2012 Gleison *et al.* (25) reached a similar conclusion in their study, highlighting a direct correlation between obesity measured by different parameters and severity of psoriasis.

Rosacea is a chronic facial skin disease, increasingly common nowadays. In our study patients we found that 4% presented on admission to the Iasi Dermatology Clinic erythematotelangiectatic rosacea and BMI > 30 kg/m^2.

Although no direct correlation between obesity and rosacea was found in many studies, Suyum Li *et al.* managed to demonstrate in 2017 in a series of 5249 patients with this condition that the risk of rosacea increases with the increase in waist and hip circumference, and the associations appear to be independent of BMI (26).

Of the 51 obese patients admitted tour clinic in September 2019, 6% presented and atopic dermatitis besides atopic stigmata. Although further research is needed to determine the temporal relationship be-

tween obesity and atopic dermatitis and to clarify causality, there are several studies in the literature that strengthen the relationship between this disease and weight gain.

A 2012 retrospective case-control study of 2090 adults concluded that obesity in people over 21 years was associated with atopic dermatitis and increased atopic asthma, but not with of nonatopic dermatitis, nonatopic asthma, atopic or nonatopic rhino conjunctivitis and food allergies (27). Also, the same authors had conducted in 2011 another retrospective study on a series of 414 patients with atopic dermatitis aged 1-21 years. They found that overweight in children is associated with an increased incidence of atopic dermatitis and that the disease was more severe when obesity occurred at age 5 and was present for over a 2.5-year period (28).

Of the total number of obese patients, only one patient had papulopustular acne lesions. However, there are studies that suggest that increased BMI is associated with a higher risk of acne compared to normal weight population. This suggestion is based on the fact that adipose tissue synthesizes testosterone, thus contributing to the hyperandrogenism observed in obesity. Furthermore, weight gain is associated with a decrease in female sex hormones and increase in circulating androgen blood level or vitamin D deficiency. In women, obesity is associated with polycystic ovary syndrome, which can cause hirsutism, menstrual disorders, hyper seborrhea and acne (9, 29, 30).

CONCLUSIONS

Obesity is responsible for changes in skin barrier function, sweat glands, lymphatics, sebum production, collagen function and structure, wound healing, macrocirculation and microcirculation.

Thus, in this article we have highlighted the association of obesity with the increased incidence of several skin diseases: neurosis cutis, difficult wound healing, chronic venous insufficiency, presence of ulcers, ochre and purple dermatitis, plantar hyperkeratosis, skin infection, worsening of psoriasis, and in fewer cases with rosacea, atopic dermatitis and acne.

CONFLICT OF INTEREST AND FUNDING

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SHORT-TERM CHANGES OF LIVER FIBROSIS IN PATIENTS WITH HCV GENOTYPE 1b - RELATED COMPENSATED CIRRHOSIS AFTER SUSTAINED VIROLOGIC RESPONSE

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SHORT-TERM ASSESSEMENT OF LIVER FIBROSIS REGRESSION IN PATIENTS WITH HCV GENOTYPE 1b - RELATED CIRRHOSIS AFTER SUSTAINED VIROLOGIC RESPONSE (Abstract): Several studies suggested a regression of liver fibrosis (LF) over time after successful therapy in HCV infected patients. The aim of this study was to assess the changes of fibrosis occurring after successful direct acting antiviral (DAA) treatment using transient elastography (TE) and FIB-4 score in patients with HCV genotype 1 b compensated cirrhosis. **Material and methods**: LF were evaluated by TE and FIB-4 at baseline, sustained virological response (SVR) and 12 months after end of treatment (EOT). LF regression was defined as an improvement of ≥ 30% in Fibroscan® score at follow-up compared to baseline. **Results:** There were 98 patients with HCV-related compensated liver cirrhosis who achieved SVR after DAAs, most female (65.3%), aged 60.64 ± 9.56 years. Overall, LF had a significant improvement with a median (IQR) regression of 4.5 (1.8-7.8) from baseline to SVR and 5.1 (2.5-8.65) from baseline to 12 months after EOT (p <0.001). Also, the FIB-4 score significantly decreased at SVR and 12 months after EOT, similar to LSM values [median (IQR) at SVR 2.27 (1.55-3.26) and 2.21 (1.51-3.28) at 12 months after EOT (p <0.001)]. **Conclusions:** We demonstrated that achieving SVR with DAA-based regimens was associated with significant improvement of liver stiffness measured by TE, and liver function monitored by FIB-4 index, but the regression of fibrosis must be constantly evaluated in dynamic thus further studies are warranted to confirm these findings in other populations. **Keywords:** CHRONIC HEPATITIS C, VIBRATION-CONTROLLED TRANSIENT ELASTOGRAPHY, FIBROSIS 4 INDEX.

Chronic hepatitis C virus (HCV) infection is a major health concern being the leading cause of liver cirrhosis with its complication, including hepatocellular carcinoma (HCC) (1). Data from WHO's Global Hepatitis Report shows that 1% of the world population is infected with HCV (2). Among all HCV genotypes, genotype 1 is the most common, accounting for 60% to 70% of the cases, while genotype 1b is

predominant in Europe, especially in Romania, with an estimated prevalence of 92.6% (3, 4).

After decades of suboptimal treatment of chronic HCV infection with interferon (IFN) - based therapies, with high rates of adverse events and approximately 50% rate of sustained virological response (SVR), the advent of direct acting antivirals (DAAs) represented a revolutionary breakthrough in the management of HCV infection, with good safety profile and SVR rates in real-life practice that exceed 95% (5-7).

Previously, some studies demonstrated the near-term and long-term histological benefit of a SVR after treatment for chronic HCV infection with IFN-based regimen (8, 9). However, as DAA has been applied just since 2011, the short-time usage of DAA limited our knowledge regarding the histological outcomes after SVR with DAA therapy and few related studies drew inconsistent conclusions (10, 11).

Given the insidious onset and progression of fibrosis which secondary leads to architectural impairment of the liver parenchyma with high morbidity and mortality rates, the assessment of the stage of liver fibrosis (LF) is mandatory in the management of chronic HCV infection, in terms of prognosis and therapeutic strategies. Evaluation of LF was initially performed by liver biopsy (LB) which was considered as the gold standard procedure for the assessment of fibrosis. However, LB is an invasive procedure with serious complications in rare cases, limited diagnostic accuracy that depends on the specimen's size, sampling error and intra- and interobserver variability (12). Therefore, a great number of methods for noninvasive assessment (serological or imaging) for diagnostic, surveillance and treatment monitoring methods to determinate LF, that avoid the risks of invasive procedures, have been developed (13).

Transient elastography (TE) is a highly accurate ultrasound - based technique, most widely used and accepted for the assessment of LF, with good sensitivity and specificity (14). TE uses ultrasound acquisitions to measure the speed of a mechanical shear velocity propagating wave through the liver which is directly related to hepatic tissue stiffness and is subsequently converted in kilopascals as a unit measure (15). This was the first ultrasound - based technique to measure the liver stiffness (LS) used in clinical practice and is currently recommended by the international guidelines for the evaluation of patients with chronic liver disease (16).

The fibrosis index based on 4 factors (FIB-4) is one of the many noninvasive serological tests developed to detect LF in patients with chronic HCV infection. The FIB-4 index is a publicly available formula consisting of age, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and platelets, which has a higher accuracy in predicting advanced fibrosis or cirrhosis when compared to LB (17,18). Initially, FIB-4 was developed for patients who were coinfected with human immunodeficiency virus (HIV) and HCV, to predict cost-effective significant fibrosis. Vallet-Pichard *et al.* validated FIB-4 for predicting significant fibrosis in HCV patients using cut-off values of ≤ 1.45 and ≥ 3.25 with an NPV of 94.7% and a PPV of 82.1% to predict the presence of significant fibrosis (19, 20). Two recent studies which assessed the predictive value of noninvasive serological tests and elastography compared to LB, reported FIB-4 to be better

than other scores such as BARD, AST-to platelet ratio index (APRI), AST/ALT ratio, and as good as MRE for predicting advanced fibrosis in patients with NAFLD (21, 22).

The aim of this study was to assess the changes of fibrosis occurring after successful DAA treatment with noninvasive methods by analyzing LF measurements obtained with TE and FIB-4 index in patients with HCV genotype 1 b compensated cirrhosis, and to identify the risk factors associated with persistent elevated biomarker score and Fibroscan® related values after obtaining SVR.

MATERIAL AND METHODS

This study was a single center, prospective observational cohort study which included 98 consecutive patients with HCV genotype 1b compensated cirrhosis who achieved SVR after DAA therapy with paritaprevir/ritonavir, ombitasvir and dasabuvir (PrOD) for 12 weeks in the Institute of Gastroenterology and Hepatology of "Sf. Spiridon" County Clinical Emergency Hospital Iasi, from December 2016 to July 2017. For each patient, baseline demographics, clinical and laboratory data were recorded, and further data was collected during patient's visits at baseline, SVR and 12 months after the end of treatment (EOT).

The diagnosis of cirrhosis was based on clinical, biochemical, imagistic features of liver cirrhosis, a liver stiffness ≥ 12.5 kPa measured with Fibroscan® and a FIB-4 index ≥ 3.25. Exclusion criteria were co-infection with HIV or HVB, the presence of decompensated liver cirrhosis.

FIB-4 score was calculated following Sterling's formula (17): FIB-4 = Age (years) x AST (U/L)/platelet count ($x10^9$/L) x ALT $(U/L)^{1/2}$ (21). LF was categorized as nonsignificant, significant (≥F2 to ≤ F4) and cirrhosis (F4) as follows: <1.45, >1.45 and <3.25 and ≥ 3.25 respectively. A significant improvement of serological test represents a decrease by ≥1-degree METAVIR score class (23).

Liver stiffness measurement (LSM) was performed by using TE (Fibroscan®, EchoSens, Paris, France) at baseline, SVR and 12 months after EOT. For a valid interpretation, at least 10 valid measurements were required with an interquartile range median (IQR/M) <30% and a successful rate > 60% (24). The median of LSM was expressed in kilopascals (kPa) and cut-off values for fibrosis were as follows: (1) less than 7.1kPa for < F2, (2) from at least 7.1kPa to less than 9.5 kPa for ≥ F2, and (3) at least 9.5 kPa for ≥ F3, and at least 12.5 kPa for F4. More than 30% regression in LF, at SVR and 12 months after EOT, was considered a significant improvement (25).

This study was conducted in compliance with the Helsinki Declaration and was approved by the institutional review board. Written inform consent was obtained from all patients, at the start of the study.

Statistical analysis

Data were expressed as mean ± standard deviation, if normally distributed, and as median and interquartile-range (IQR), if not normally distributed. Friedman's test and Wilcoxon signed rank test were carried out to detect longitudinal differences between noninvasive fibrosis assessment and clinical characteristics during follow-up. A *p* value less than 0.005 was judged to be statistically significant. All the analyses were performed by using *IBM SPSS 19.0* statistical software package (IBM corp., Armonk, NY, USA).

RESULTS

Patient characteristics

We included 98 patients with HCV-related cirrhosis with DAAs-associated SVR. The baseline characteristics of all patients are summarized in first table. The mean age of the studied patients was 60.64±9.56 years with a female predominance (65.3%) and a body mass index [BMI (kg/m^2)] of 28.16±4.28. Thirty-four (34.5%) patients were IFN-based regimens experimented, 18 (18.36%) had diabetes and 55 (59.12%) had hypertension. Liver stiffness at baseline was 21.30 (16.9-27.1) kPa.

TABLE I

Patient characteristics

Variable	Baseline	SVR	12-month post-treatment
Age in years	60.64 ± 9.56	-	-
Female, *n* (%)	64 (65.3)	-	-
BMI (kg/m^2)	28.16 ± 4.28	-	-
HCV treatment - experienced, *n* (%)	64 (65.3)	-	-
Diabetes, *n* (%)	18 (18.4)	-	-
Hypertension, *n* (%)	55 (56.1)	-	-
Esophageal varices, *n* (%)	27 (27.6)	-	-
BMI (kg/m^2)	28.16 ± 4.28	28.00 ± 3.90	28.77 ± 3.85
Platelet (1000/μL)	155.74 ± 69.01	154.92 ± 62.47	171.96 ± 76.35
ALT (IU/L)	100.6 ± 75.96	39.62 ± 9.44	26.39 ± 16.31
AST (IU/L)	91.83 ± 61.88	34.14 ± 6.96	29.85 ± 14.35
GGT (IU/L)	87.46 ± 58.24	53.42 ± 9.33	43. 45 ± 49.77
Fasting sugar (mg/dL)	112.43 ± 31.73	99.60 ± 11.18	112.08 ± 29.01
Urea (mg/dL)	32.50 ± 9.94	39.20 ± 8.35	35. 81 ± 10.77
Direct bilirubin (mg/dL)	0.50 ± 0.30	0.35 ± 0.17	0.33 ± 0.17
INR	1.09 ± 0.10	1.08 ± 0.09	1.06 ± 0.07
Fibrinogen (mg/dL)	303.56 ± 66.36	311.09 ± 52.68	355.46 ± 42.37
Total cholesterol (mg/dL)	157.84 ± 36.84	174.09 ± 18.1	190.82 ± 38.55
LDL cholesterol (mg/dL)	97.09 ± 17.95	129.98 ± 25.88	125.40 ± 30.01
HDL cholesterol (mg/dL)	36.82 ± 5.68	41.38 ± 7.99	29.01 ± 6.67
Triglycerides (mg/dL)	106.79 ± 37.23	96.97 ± 22.63	121.13 ± 53
AFP (ng/mL)	15.82 ± 21.20	6.20 ± 5.89	5.46 ± 4.11
Albumin (g/dL)	4.12 ± 0.52	4.46 ± 0.39	4.50 ± 0.41
FIB-4	3.69 (2.19-6.02)	2.27 (1.55-3.26)	2.21 (1.51-3.28)
LSM (kPa)	21.30 (16.9-27.1)	16.45 (13.8-23.7)	15.65 (11.3-22.9)

Data are shown as mean ± SD, but LSM are expressed as median (interquartile range); BMI, body mass index; HCV, hepatitis C virus; ALT, alanine aminotransaminase; AST, aspartate aminotransaminase; GGT, gamma-glutamyl transferase; INR, international normalized ratio; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AFP, α-fetoprotein; LSM, liver stiffness measurement

Laboratory data at SVR and 12 months after EOT

All patients showed a statistically significant decline in AST, ALT, GGT and AFP levels after treatment (tab. II). Albumin, total cholesterol and low-density lipo-

protein cholesterol levels significantly increased overall, while high density lipoprotein cholesterol level increased at SVR and recorded a significantly reduction at 12 months after EOT. Significant differences were recorded between baseline *vs.* SVR, SVR *vs.* 12 months after EOT, and baseline *vs.* 12 months after EOT (tab. II). Triglycerides and INR levels showed no significant improvement.

TABLE II

P values for continuous clinical data

Variable	P value		
	Baseline *vs.* SVR[1]	SVR *vs.* 12-month post - treatment[1]	Baseline *vs.* 12-month post -treatment[2]
BMI (kg/m^2)	0.400	< 0.001	< 0.001
Platelet (1000/µL)	0.918	0.020	< 0.001
ALT (IU/L)	< 0.001	< 0.001	< 0.001
AST (IU/L)	< 0.001	< 0.001	< 0.001
GGT (IU/L)	< 0.001	< 0.001	< 0.001
Fasting sugar (mg/dL)	0.003	0.001	0.608
Total bilirubin (mg/dL)	0.002	< 0.001	< 0.001
Direct bilirubin (mg/dL)	< 0.001	0.050	< 0.001
INR	0.008	0.084	0.001
Fibrinogen (mg/dL)	0.523	< 0.001	< 0.001
Total cholesterol (mg/dL)	< 0.001	0.001	< 0.001
LDL cholesterol (mg/dL)	< 0.001	0.265	< 0.001
HDL cholesterol (mg/dL)	< 0.001	< 0.001	< 0.001
Triglycerides (mg/dL)	0.077	0.001	0.351
AFP (ng/mL)	< 0.001	0.013	< 0.001
Albumin (g/dL)	< 0.001	0.104	< 0.001
FIB-4	< 0.001	< 0.001	< 0.001
LSM (kPa)	< 0.001	0.021	< 0.001

BMI, body mass index; HCV, hepatitis C virus; ALT, alanine aminotransaminase; AST, aspartate aminotransaminase; GGT, gamma-glutamyl transferase; INR, international normalized ratio; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AFP, α-fetoprotein; LSM, liver stiffness measurement; [1] Wilcoxon signed-rank test was applied [2] Friedman's test was applied

Fibrosis evaluation assessed by TE and FIB-4 score

Overall baseline median (IQR) of LSM was 21.3 kPa (16.97-27.1) using Fibroscan® (tab. I). At SVR, the median TE measurement was 16.45 kPa (13.8-23.7) and 15.65 kPa (11.3-22.9) at 12 months after EOT, with an overall median (IQR) regression of 4.5 (1.8-7.8) from baseline to SVR and 5.1 (2.5-8.65) from baseline to 12 months after EOT (p <0.001). Moreover, the LSM improvement is maintaining from SVR to 12-month post - treatment (p =0.021). LSM regression was found in 83 (84,6%) patients at 12 months after EOT evaluation. Subsequently, the primary outcome was obtained in 26 patients (26,53%) (p <0.001), which had more than 30% LSM decrease. Distribution of LSM over the time are showed in figure 1.

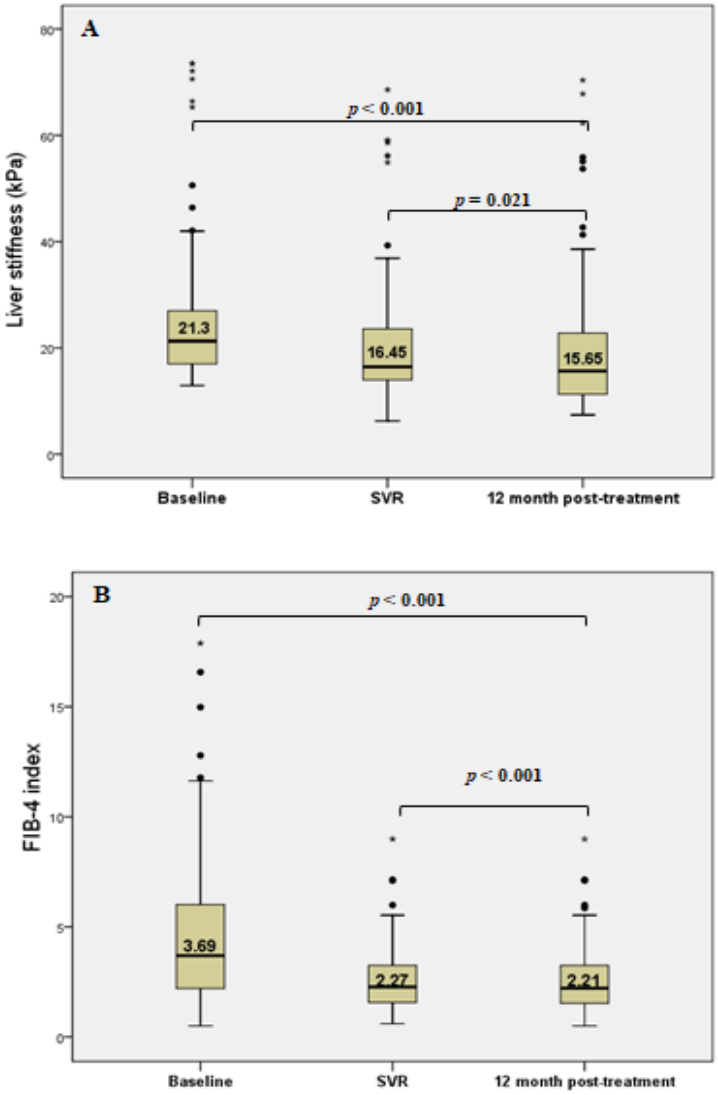

Fig. 1. Distribution of LSM (A) and FIB-4 index (B) over the time. The lines through the boxes represent the median score. The upper and lower bottom of each box represent the 25th and 75th percentiles, representing the interquartile range (IQR). The rest of the outliner observations are plotted as dots beyond the whiskers. Each bottom error bar ends at 1.5*(IQR) < Q1, while each top error bar ends at 1.5*(IQR) > Q3.

The FIB-4 score significantly decreased at SVR and 12 months after EOT, similar to LSM values (tab. II). At baseline, median (IQR) FIB-4 was 3.69 (2.19-6.02), while at SVR, the median (IQR) value reached 2.27 (1.55-3.26) with a decrease of 1.52

(0.37-2.75) (p <0.001). At 12 months after EOT, the median (IQR) was 2.21 (1.51-3.28), with a decrease of 1.52 (0.45-2.72) from baseline. The improvement of liver function expressed in FIB-4 score correlates with Fibroscan® METAVIR staging fibrosis. At 12 months after EOT, 73 (74.79%) patients had reached a downgrade of fibrosis stage from F4 (98) to F3 - F2 [(50)- significant fibrosis], < F2[(23)-nonsignificant fibrosis], while 25 remained at the same stage (F4).

In 13 patients there was a significant increase value of LSM median from 16.3 to 22.8 kPa while at 2 patients a stationary TE was reported. In this subgroup of patients median FIB-4 score was 2.43 from 4.3 initially, mean age 65 years.

DISCUSSION

Several previous studies conducted in the IFN era have shown a significant improvement of LF in patients with SVR (26-29). Given that DAAs have recently been introduced into the management of patients with chronic HCV infection, conclusive data on the LF dynamics after SVR are still scarce.

This study seeks to enrich the current literature by prospectively evaluating short-term LF changes after obtaining SVR with DAA-based therapies. The key finding of our study consists of the short-term improvement in liver function demonstrated by a significant improvement of LSM and FIB-4 score. In our study, at one year of follow-up, 83 (84,6%) patients achieved a significant LSM decrease from baseline while 26 (26.53%) had a significant improvement of LSM (more than 30% regression in LF). A similar rate of improving LSM and FIB4 score after SVR was reported by Mohammed *et al.*, with a 46% reduction of LSM assessed by Fibroscan® and a > 60% decrease of FIB-4 from baseline to one year (30). Another study which included patients treated with sofosbuvir-based DAA therapy, found a 31% reduction of liver stiffness after one year since EOT compared to baseline (31). In a prospective study, Chan *et al.* found a significant reduction in LSM after EOT in 49% of enrolled patients and, furthermore, a significant decline in LSM score with a median value of 1.4 kPa in patients with advanced LF (>9.5 kPa) (32).

Even if LB remains the gold standard for estimating LF, in the last decade, non-invasive methods for evaluating the degree of LF are tempting in clinical practice. Their repeatability and cost-effectiveness, besides patient convenience, represent sustainable arguments for abandoning LB in long-term evaluation of HCV cirrhotic patients. A partial regression of fibrosis ranging from 24% to 100% in patients with HCV-related liver cirrhosis who obtained SVR with IFN-based regimens was reported in literature (33). Accordingly, in a cross-sectional study by Hendenstierna *et al.* in which patients with SVR obtained with IFN-based regimens were followed-up through LSM for a period ranging from 5 to 10 years, concluded that the improvement of LF after viral clearance is significant in patients with pretreatment advanced fibrosis or cirrhosis, but with respect to long-term dynamics of fibrosis, regression becomes a slow process which at one point is stabilized (34). Therefore, the patients with more than 9.5kPa cut-off baseline value from their cohort had an improvement of LF. Only 21% patients remained with more than 9.5kPa at 10 years of fol-

low-up. Also, the authors reported a progression of fibrosis in 5% of the patients, correlated with an age ≥ 55 years and a BMI ≥ 25 kg/m^2. In our study, we found an increase of TE score in 13 patients, while 2 patients had a stationary value. Of these subgroups, the mean values of age, BMI and FIB-4 index after DAA treatment were 65 years, 30.9 kg/m^2 and 2.43, respectively. We tend to conclude that age and BMI values after treatment are risk factors for fibrosis progression, this data being in accordance with those reported by Hedenstierna *et al.* (34). In a retrospective study from Italy, the authors compared changes in LS during a long follow-up period of 5 years in 153 HCV patients treated with IFN-based therapy or IFN-free regimens, from which 36 had cirrhosis with baseline values of TE >12.5 kPa (35). First, the authors observed a significant regression in LS at SVR in patients who followed DAAs *vs.* IFN-based regimens. Secondly, a considerable decrease of fibrosis represented by a reduced proportion of cirrhotic patients compared to baseline was observed at EOT (20.5%), with a continuous declining trend over time (the proportion of patients with F4 was 9.8% at 1 year, 7.1% after 2 years, and < 5% after 4 years). Both these longitudinal studies concluded that after antiviral treatment, a rapidly regression of hepatic fibrosis is reached with improved liver metabolic function, but over time, especially after 1 year of follow-up, fibrosis regression tends to plateau.

Data from a retrospective study by Tada *et al.* showed an increase of total cholesterol, low density lipoprotein cholesterol, and triglyceride levels in patients with chronic HCV infection who achieved SVR with IFN-based regimens (36). Likewise, Hashimoto *et al.* reported similar changes in lipid levels in HCV patients treated with DAAs, with a much greater and rapid increase in serum low density lipoprotein cholesterol levels associated with regimen type and decreased HCV core protein levels (37). In our study, total cholesterol levels, high-density lipoprotein cholesterol levels, and LDL cholesterol levels changed significantly overall, thus concluding that viral clearance is closely related to lipid metabolism in patients treated with antivirals (37-39).

Our paper has some limitations. First, the small number of patients enrolled, secondly the lack of a LSM at EOT in accordance with FIB-4 score with variation range of TE (± 3 months). Thirdly, the short follow-up period represents a limitation because, for a true diminishing of liver stiffness, a longer follow-up period is required with a well-defined reevaluation-time. Fourthly, factors that are not HCV-related and might influence LSM such as alcohol consumption, steatogenic medication and lifestyle were not recorded.

CONCLUSIONS

TE was validated for assessing liver stiffness in patients with HCV-related liver cirrhosis, but long-term follow-up study based only on DAA based regimes treated patients are still limited. A rapid regression of fibrosis suggested by the improvement of LSM is secondary to the elimination of the hepatic inflammation after EOT. However, a dynamic assessment of LSM shows a tendency to settle on a constant value. In our patients, achieving SVR with DAA-based regimens was associated with significant improvement of liver stiffness measured by TE, and liver function monitored by FIB-4 index. A true regression of fibro-

sis must be constantly evaluated in dynamic, reason for which we intend to continue the TE follow-up of our cohort. Further studies are warranted to confirm these findings in other populations.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to acquisition of data, or analysis, conception and design, and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

SILENT SINUS SYNDROME

The silent sinus syndrome represents maxillary sinus atelectasis that results in painless enophthalmos, hypoglobus and facial asymmetry and usually presents in the 3rd to 5th decade, without a gender predilection. Chronic occlusion of the maxillary sinus ostium results in gradual resorption of the air, negative pressure is generated within the sinus and gradual inward bowing of all four maxillary walls: roof, medial, posterolateral and anterior. Orbital volume increases with resultant enophthalmos and variable flattening of the malar eminence. Although the diagnosis is usually made clinically, imaging of the sinuses confirms the findings. CT demonstrates changes in the sinus and the adjacent structures. The sinus is fully opacified and reduced in volume with inward bowing of all four walls. The uncinate process is usually superiorly and laterally displaced, in direct contact with the inferomedial wall of the orbit and the ostiomeatal complex is occluded. MRI is not required for the diagnosis; it will demonstrate the anatomical changes and a sinus with thick, enhancing mucosa and secretions of variable intensity. Differential diagnosis includes: congenital maxillary sinus hypoplasia (which does not present with a developing facial deformity), post-traumatic maxillary sinus deformity, mucocele (expansion of sinus due to secretions), chronic sinusitis withmucoperiosteal thickening. (J. Jones. Silent sinus syndrome. https://radiopaedia.org/articles/silent-sinus-syndrome?lang=gb)

THE INCIDENCE OF PEDIATRIC EYE INJURIES – BEFORE AND DURING THE SARS-CoV-2 PANDEMIC

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THE INCIDENCE OF PEDIATRIC EYE INJURIES-BEFORE AND DURING THE SARS-CoV-2 PANDEMIC (Abstract): **The aim** of our study is to evaluate the incidence of the pediatric eye injuries during the non-pandemic and pandemic period and its impact on the patients' quality of life. **Material and methods**: The study included children who presented to the emergency department from January 2016 to December 2020. The following data were analyzed: age, sex, place of origin, type of injury therapeutic management, and hospitalization period and expenses. All patients were examined in detail including complete history and ocular examination. **Results:** 117 patients with eye injuries were enrolled in the study, being examined at the Ophthalmology Clinic of "Sf. Spiridon" County Clinical Emergency Hospital, Iasi between 2016 and 2020. Of the total study group, 32 children (27.4%) were under 5 noted as Group I, the remaining 85 (72.6%) were between the ages of 5-18, noted as Group II. According to the BETTS classification, an estimated risk for the following types of injury was identified for the age group 5-18 years vs 1-5 years: GD - penetrating 45.9% vs 40.6% and GI - contusion 28.2% *vs*. 18.8%. Associated systemic / ocular pathologies were significantly more common in the 5-18 age group. Depending on the eye injury, the length of hospital stay was indirectly correlated with visual acuity at discharge. The strongest correlation, although statistically insignificant, was between the length of hospital stay and the type of injury with lamellar laceration. In 69.1% of complex cases, the higher scores of costs were significantly correlated with the high length of hospital stay, and in 35.8% of cases without increased complexity. During the pandemic in 47.7% of complex cases, higher cost values correlated with the high length of hospital stay. In 67.2% of complex cases, the higher scores of costs were significantly correlated with the high length of hospital stay before the pandemic (2016-2019). In over 30% of children, the higher risk score was associated with the higher length of hospital stay. In over 26% of children, higher visual acuity at discharge was associated with a lower risk score. **Conclusions:** Severe pediatric eye injuries are a major cause of declining quality of life and one of the most common causes of ocular morbidity. Young children are more prone to injuries because of their natural curiosity to explore and their inability to avoid danger. **Keywords:** COVID-19, SARS-Cov-2, PEDIATRIC EYE INJURIES, OPEN GLOBE INJURY, BLUNT EYE INJURIES, CSIO, GLOBE RUPTURE, POST-TRAUMATIC QUALITY OF LIFE.

Pediatric eye injuries represent a serious concern for ophthalmologists, and it is estimated that between 20% and 50% of ocular injuries presenting to emergency occur in children (1). Most patients who have suffered eye injuries are blind to one eye, accounting for 8-14% of all injuries suffered by the child. Children are more prone to injury due to their inability to avoid danger. In general, men are more commonly reported to have eye injuries compared to women (2). Eye injuries have a significant impact on the patient's future quality of life, with children being at greater risk of amblyopia. SARS-CoV-2 is a highly transmissible and pathogenic coronavirus that was discovered in December 2019 in Wuhan, China. This virus has caused a pandemic of acute respiratory disease called COVID-19, which poses a threat to human health and public safety (3). The number of eye injuries reported in studies during the pandemic was contradictory. According to Pellegrini *et al.* there was a significant decrease in the number of eye injuries by 68.4% compared to the same period of the previous year in Italy. The authors found that daily activities changed during quarantine and were associated with a minimal risk of eye injuries (4). Another study by Hamroush *et al.* concluded that there is a higher risk of eye injuries occurring at home (5). According to these studies, traumatic eye injuries are largely accidental from an etiological point of view. Babies and toddlers suffer fewer injuries due to close supervision while older children injure themselves with toy tips, pencils, arrows, needles, thorns and stones (6, 7). The state of emergency was declared in Romania in March 2020, when the Ophthalmology Clinic of St. Spiridon Hospital was the only center in Moldova that was open and solved all the juries that were addressed to both surgically and medically. This paper analyzed both the number and type of injuries during the pandemic and before this period, as well as the functional prognosis related to the complexity of the case and the length of hospital stay.

MATERIAL AND METHODS

The Ophthalmology Clinic at "Sf. Spiridon" County Clinical Emergency Hospital, Iasi was the only ophthalmological clinic that hospitalized and treated all the ocular emergencies from Moldova region during COVID-19 pandemic. Our study included the cases that were addressed to the clinic between January 2016 and December 2020, including all children who presented with ocular emergencies. Inclusion criteria were all children aged 1 to 18 years, and all types of injuries (according to the BETTS classification). Patients who refused medical or surgical treatment, those over 18 and who did not show up for follow-up or did not cooperate in the evaluation were excluded from the study. For a better analysis and due to the low compliance of some categories, the children were divided into two age groups: Group I included the 1-5 years age group and group II the 5-18 years age group. The following data were analyzed: age, sex, place of origin, type of injury (according to the BETTS classification), associated pathologies, visual acuity, bio-microscopy of the anterior segment, therapeutic management, and hospitalization length and expenses. Those who met the criteria were considered complex: penetrating injuries, with or without CSIO (intraocular foreign body) and other associated systemic / ocular pathologies. In order to better appreciate the evolution of visual

acuity and the functional prognosis, a risk score that considered the type of injury, with or without CSIO, sex, age and environment was calculated. The highest score (i.e., 6) was given to the contusions (most frequent) and the lowest score (respectively 1) to the place of origin. This risk score was correlated with the complexity of the cases, the length of hospital stays and the visual acuity at discharge (tab. I).

Ethical Approval. During the study, the principles of the Helsinki Declaration were observed. Patients have signed an informed consent, which was attached to the observation sheets.

Statistical processing. The database was built in EXCEL and was statistically processed with the *SPSS 18.0*. These represent software for statistical and graphical data analysis, with an interface that helps the user to solve any need for experimental data processing. The statistical analysis of the data was performed with SPSS 18.0 and consisted of applying the following tests: ANOVA, Student t-test, F-test, ROC curve, linear regression analysis.

RESULTS

The study group consisted of 117 children with eye injuries, examined in the Ophthalmology Clinic of "Sf. Spiridon" County Clinical Emergency Hospital, Iasi between 2016 and 2020, the distribution by pandemic and non-pandemic years being similar in all five years of follow-up (figure 1).

The children's age ranged from 1 to 17 years. Of the total study group, 32 children (27.4%) were under 5 marked as Group I, the remaining 85 (72.6%) were aged between 5-18 years, noted as Group II. Of the total study group, 83 were male (70.9%), mainly in the 5-18 age group (56.3% vs 76.5%; p = 0.036).

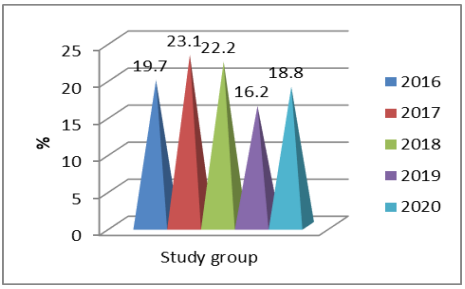


Fig. 1. Group structure by years of study

According to the results, 69 came from rural areas (59%), with no significant differences by age groups (53.1% vs 61.2%; p = 0.432). Associated systemic pathologies were significantly more common in the 5-18 age group (23.5 vs 6.3%; p = 0.02). Figure 2 shows the distribution of cases by sex and age groups and the place of origin.

The age of 5-18 years compared to 1-5 years was an estimated risk for the following types of injury: the prevalence of penetrating (45.9%) and contusive (28.2%) injuries. In the 1-5 age group, the higher frequency of GD-rupture was noted 6.3% vs 1.2% (OR = 2.53; 95% CI: 1.08-6.0; p = 0.150), but the result cannot be extrapolated to the general population. Depending on the eye injuries, the length of hospital stay was indirectly correlated with visual acuity at discharge as follows: in the case of GI contusion, the correlation was moderate, statistically significant (r = -0.405; r = 0,); in the case of GD CSIO, the correlation was moderate, statistically significant (r = -0.472; r = 0.038); in the other types of injury the correlation was moderate but statistically insignificant (figure 3). In 69.1% of complex cases, the higher scores of costs were significantly correlated with the high length of hospital stay (r = +0.691; p = 0.001), a significant aspect and in 35.8% of cases without increased complexity (r = + 0.358; p = 0.013).

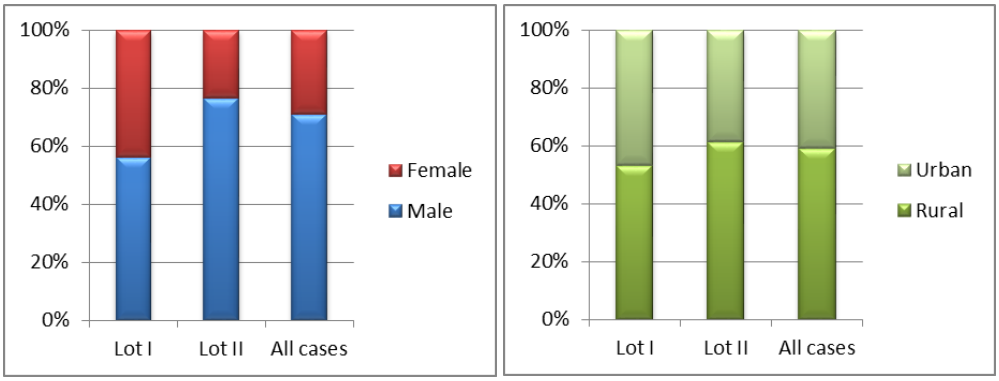


Fig. 2. a) Group structure by sex and age groups;
b) Group structure by place of origin and age groups

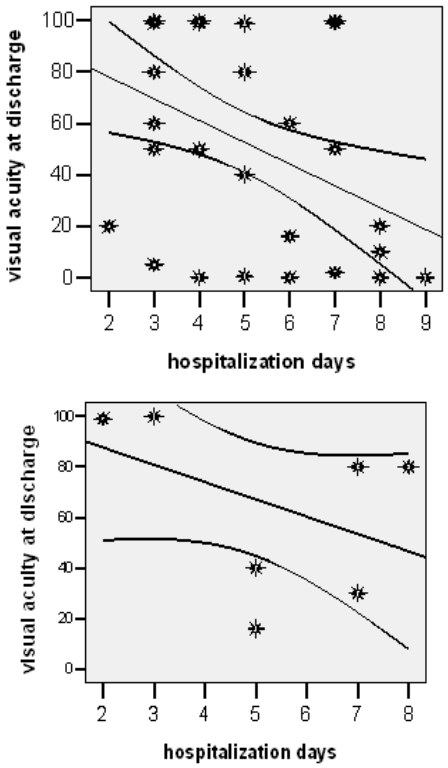


Fig. 3. The correlation of the length of hospitalization days with VA at discharge based on eye injuries

By analyzing the evolution of visual acuity according to eye injuries in group II (5-18 years age group) an increase in visual acuity at discharge vs. hospitalization in case of contusions and penetrating injuries was found. Since in group 1 (1-5 years age group) it was not possible to quantify the value of visual acuity, the calculation of a risk score that considered the type of injury, the presence or not of CSIO, sex, age

and place of origin was performed for both groups. The highest score (i.e., 6) was given to the contusions (most frequent) and the lowest score (respectively 1) to the place of origin. This risk score was correlated with the complexity of the cases, the length of hospital stays and the visual acuity at discharge. It was found from the statistics that males under 5 from the rural area, under medical treatment had an increased risk for a low functional prognosis (high risk score) (tab. I and fig. 1).

TABLE I

Descriptive risk score

Risk factor	Score
contusion	6
penetrating	5
CSIO	4
break	3
lamellar laceration	2
attached trauma	1
pliable	1
<6 y	1
Rural	1

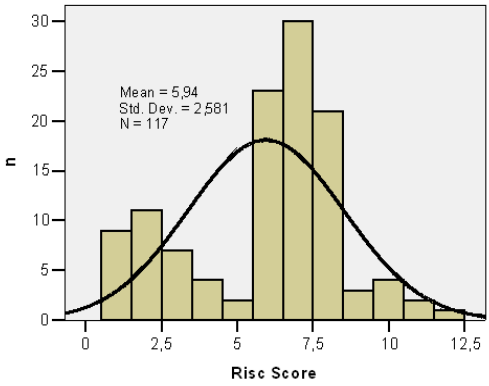


Fig. 4. Data and risk score histogram

According to statistics, a higher risk score was found in male children, in the 1-5 years age group, from rural areas and who were treated with medication *vs.* surgery. In over 30% of children, the higher risk score was associated with the higher number of hospitalization days (length of hospital stay) ($r = +0.308$; $p = 0.001$). The correlation between the risk score and the costs was direct, low in intensity, but statistically significant ($r = +0.247$; $p = 0.007$) (fig. 5).

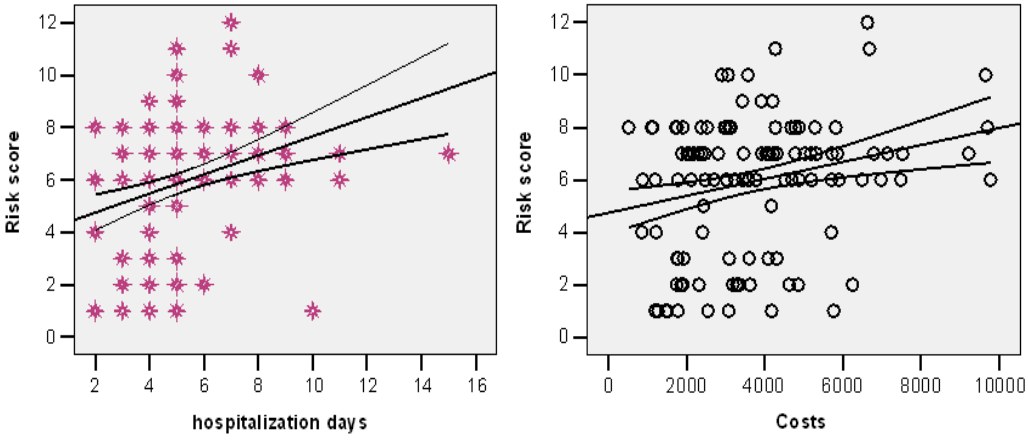


Fig. 5. The correlation between the number of hospitalization days (length of hospital stay), the costs and the risk score

In over 26% of children, higher visual acuity at discharge was associated with a lower risk score (r = -0.263; p = 0.004) (fig. 6).

Analyzing the ROC curve, age was an accurate predictor of an unfavorable prognosis (AUC = 0.703; IC95: 0.610-0.796; p = 0.001), while the age under 6 has a sensitivity of 83% and a specificity of 48% in predicting an unfavorable prognosis (fig. 7). Multiple linear regression analysis confirmed that no models that included significant dependence on eye injuries were found.

An analysis of the number of hospitalized and resolved cases during the non-pandemic years (2016-2019) vs. the pandemic year 2020 found that most cases were aged in the 5-18 range in a higher percentage, which was also noticed between 2016 and 2019 (81.8% *vs*. 70.5%; p = 0.268). During the pandemic (2020), in 47.7% of complex cases, the higher scores of costs were correlated with the large number of hospitalization days (high length of hospital stay) (r = +0.477; p = 0.164), an aspect noted in 37.6% of cases without high complexity (r = +0.376; p = 0.228). Before the pandemic (2016-2019), , the higher scores of costs were significantly correlated with the large number of hospitalization days (high length of hospital stay) (r = +0.672; p = 0.001) in 67.2% of complex cases, an aspect noted in 52.2 % of cases without high complexity (r = +0.522; p = 0.001).

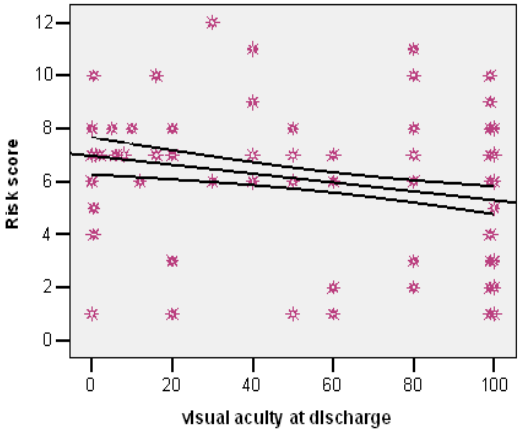
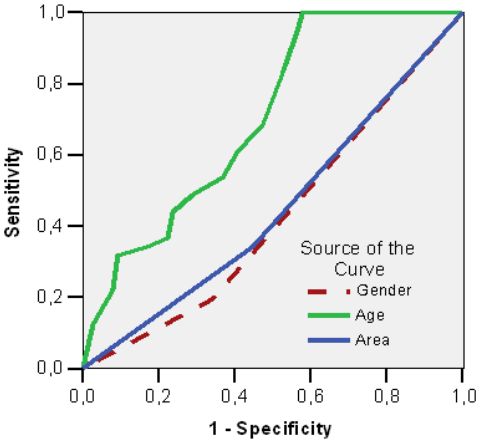


Fig. 6. The correlation between visual acuity at discharge and risk score



Area Under the Curve

Test Result Variable	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Gender	,427	,054	,191	,320	,533
Age	,703	,047	,000	,610	,796
Area	,447	,055	,346	,338	,556

The test result variable(s): Gender, Age, Area has at least one tie between the pos group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Fig. 7. ROC curve. Demographic characteristics predicting an unfavorable prognosis

DISCUSSION

One of the common causes of pediatric blindness is eye injuries (8). Compared to adults, children are more exposed to traumatic events due to their immature fine motor skills and curiosity, therefore adult supervision is a key factor in preventing eye injuries (9). The field of pediatric eye injuries assessment is quite broad and involves all practitioners who care for children, including the pediatrician, emergency physician, general and pediatric ophthalmologists. The American Academy of Ophthalmology (AAO) annually reports the results of the survey on the causes of eye injuries. These findings show common causes for injuries including missiles (20%), blunt objects (10%), body parts (i.e., fingers, fist) (12%), sharp objects (9%), and motor vehicle accidents (4%) etc. Other reports report unusual causes such as a rooster attack, pencil sharpening, microwave egg blasting, door handles, chopsticks, carbonated drinks and dishwashing detergent, (10). The assessment of an injured child should include the following steps: the extent of the injury should be determined quickly, without risking further damage to the eye, and the need for further examination with a subspecialist; the assessment of visual acuity should be tried and documented if the child is verbal and conscious; if the globe or eyelid is suspected to be affected, eye dressing, analgesic medication, tetanus immunization, and then the ophthalmologist contacted for further surgery (2, 6). Children over 5 are examined with Snellen's diagram and E diagram, the examination of the anterior segment is performed with the help of a direct ophthalmoscope, slit lamp; and the fundus with the direct ophthalmoscope or the biomicroscopy of the fundus (2).

SARS-CoV-2 is a highly transmissible and pathogenic coronavirus that was discovered in December 2019 in Wuhan, China. This virus has caused a pandemic of acute respiratory disease called COVID-19, which poses a threat to human health and public safety (3).

Strict isolation at home and other measures of social distancing have been applied to reduce the pressure on the health system. Although the Romanian government issued national isolation orders, the emergency ophthalmology department of our hospital continued to provide emergency ophthalmology consultations and care, due to the fact that it was the only hospital in Moldova that operated at the appropriate parameters during this period.

Pandemics can have an influence on the behavior of patients in seeking emergency medical treatment. The Emergency Department Syndromic Surveillance System: England (EDSSS) and the Royal College of Emergency Medicine, UK reported a decrease in the volume of patients during the first week of lockdown in the UK (11). In a study conducted by Pellegrini *et al*, a significant decrease in the number of ocular injuries by 68.4% compared to the same period of time of the previous year was discovered in Italy. The authors concluded that daily activities changed during the quarantine and were associated with a minimal risk of ophthalmological injuries. Because of the school closing, a decrease in the number of eye injuries was reported in children (4). Contrariwise, in a study conducted in the United Kingdom it was concluded that there was a higher risk for ocular injuries occurring at home (5).

In our study analyzing the cases managed during the non-pandemic (2016-2019) and the pandemic period (2020), we found a

similar number of hospitalized and resolved cases, most of the male cases from rural areas in the 5-18 years age group. According to the classification of injuries (BETTS), penetrating injuries were prevalent (52 cases-40.2%), followed by the contusive ones (30-18.8%), eyeball rupture, the presence of CSIO and other associated injury (28% - craniofacial injuries, eyelid wounds, post-traumatic cataract, secondary intraocular hypertension, retinal detachment). There has been a significant increase in visual acuity statistic at discharge vs. hospitalization (length of hospital stay). The number of hospitalization days was statistically significantly indirectly correlated with the visual acuity at discharge and hospitalization costs. In 47.7% of complex cases, the higher cost scores were correlated with the high length of hospital stay during the pandemic (2020). Although the number of cases during the COVID 19 pandemic was similar to previous years (2016, 2017, 2018 and 2019), health costs were higher in 2020 to prevent occupational exposure.

In order to better appreciate the evolution of visual acuity and the functional prognosis, the risk score achieved considered the type of injuries, the presence or lack of CSIO, sex, age, place of origin. It was statistically found that males under 5 from rural areas and under medical treatment had an increased risk for a low functional prognosis. In over 30% of children, the higher risk score was associated with the higher number of days in hospital. In over 26% of children, higher visual acuity at discharge was associated with a lower risk score. Similar studies used the ocular trauma score and prognosis of visual acuity (OTS) of Kuhn, ranging from 1 (worst injury and worst prognosis at 6 months of follow-up) to 5 (least severe injury) and the least severe prognosis at 6 months. Each score is associated with a set of predicted post-injury visual acuity. It has a predictive accuracy of about 80%, which means that the OTS will be accurate 4 out of 5 times. It shows the prognosis, but due to the impossibility of assessing visual acuity in some cases (non-compliant, and under 5), it cannot be used every time (12). Other studies that looked at the epidemiological features of the injury also showed a prevalence of male injuries, for the 6-11 age groups from rural areas. The increased hospitalization costs are directly associated with the complexity of the cases and the length of hospital stay (13, 14, 15).

In a recent study conducted at the same hospital for the pandemic period (March-May 2020), the analysis of the cases included in the study found that most were surgical (82%) and different types of injuries (wounds, contusions, foreign bodies), with a hospitalization period of approximately 6, 3 days and a duration of surgery of 45 minutes. The number of cases that were addressed was lower than 5.6 times for general hospitalizations and 77.6 times for 1 day of hospitalization, due to the restrictions regarding elective hospitalizations (cataracts, strabismus, etc.), practically during the emergency state only the pathology that needed to be surgically or medically resolved immediately was hospitalized (16).

STUDY LIMITATIONS

There are a number of limitations in this study. There is a retrospective study on a small number of patients. The first worth mentioning is the impossibility to quantify the visual acuity of the 1-5 years age group. There are not enough data on the evolution of visual acuity at 3, 6 months or one year after

the injury.

CONCLUSIONS

Severe pediatric eye trauma is a major cause of declining quality of life and one of the most common causes of eye blindness. Young children are more prone to trauma because of their natural curiosity to explore and their inability to avoid danger, but open injuries were prevalent in both age groups; Age under 5 years with a higher sensitivity and was a good predictor in the determinism of unfavorable functional prognosis. The low-risk score was associated with increased visual acuity and low hospitalization. The complexity of the cases has led to an increase in hospital days, costs and a reduction in patients' quality of life. Most cases during the pandemic and before the pandemic were surgical. Usually, the current management is based on the surgeons' professional expertise and will continue to be based on a retrospective review of the data gathered by the personal preferences of the attending ophthalmologist. The psychological burden of the associated visual impairment is of major importance, as it is known that one in ten children will have a permanent visual impairment.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

CRYPTORCHIDISM AND TESTICULAR CANCER - A LOOK IN THE PACIENTS FUTURE

Cryptorchidism is one of the most common congenital anomalies affecting new borns, and as a singular pathology or associated with other dysgenesis syndromes is one of the main factors of risk for the testicular tumors. Although a great number of cases of undescended testicles are diagnosed and treated during the first 6-12 months of life, there are rare cases of adults who are undiagnosed and untreated, with a high risk of malignancy. The article presents the case of a 50-year-old male with palpable tumor in right iliac fossa, with right scrotal sac empty. Clinical, imaging and laboratory findings suggests a seminoma in right undescended testis. Intra-abdominal mass in context of empty scrotum should alert for intra-abdominal testicular tumor. Adult with intraabdominal testicular tumor usually present at advance stage compared to intra-scrotal testicular tumor. Cryptorchidism needs to be managed preferably at 6 months for prevention/early detection of cancer. (Parajuli P, Pradhan M M, Chapagain S, *et al*. Intra-abdominal mass with empty scrotum in adult male. *Urol Case Rep* 2020; 34: 101451).

INVERTED PAPILLOMAS - UNCOMMON SINONASAL TUMORS

Inverted papillomas are rare (0.5-4.0%), benign sinonasal tumors, sometimes with malignant transformation, which affects middle-aged men (most frequently are seen in patients 40-60 years of age). The presentation can be similar to other sinonasal masses, with nasal obstruction, sinus pain and epistaxis. The lesions most commonly occur on the lateral wall of the nasal cavity, related to the middle turbinate, middle meatus and maxillary ostium. Radiography no longer plays a significant role in the assessment of sinonasal tumors. Computed tomography depicts a soft tissue density mass with enhancement; when the tumor enlarges, bone destruction and extension into the maxillary antrum, intralesional calcifications (residual bone fragments) are present in ~40% of cases; the location of the mass on the lateral wall of the nasal cavity is a clue for the correct diagnosis. On magnetic resonance imaging (T2 and contrast-enhanced T1 weighted images) the lesion has a convoluted cerebriform pattern. Differential diagnosis includes: sinonasal carcinoma (imaging does not distinguish between the two lesions), polyps, juvenile nasopharyngeal angiofibroma, olfactory neuroblastoma, paranasal sinus mucocele. (B Layton, F Gaillard, et al. Inverted papilloma. https:// radiopaedia. org/articles/ inverted-papilloma?lang=us)

INSIGHTS ON ADIPOKINES AND PHYSICAL ACTIVITY IN OBESITY PHENOTYPES

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INSIGHTS ON ADIPOKINES AND PHYSICAL ACTIVITY IN OBESITY PHENOTYPES (Abstract): Obesity activates multiple pathways of metabolic syndrome (MetS), therefore the balance between adiponectin and leptin, hormones that are secreted by adipocytes, is of great interest in better managing and understanding this complex disease. The aim of our study was to characterize obesity phenotypes by circulating levels of adipokines and insulin, followed by a better understanding of the influence a lifestyle modification, like physical activity, may have upon the values of these biomarkers. **Material and methods:** Our cross-sectional study included 104 patients organized in 4 groups, based on BMI and metabolic syndrome (MetS): metabolically healthy obese (MHO), metabolically unhealthy obese (MUO), metabolically healthy non-obese (MHNO), metabolically unhealthy non-obese (MUNO). Unhealthy phenotypes were characterized by the presence of MetS and obesity by a BMI $\geq$ 30 kg/m^2. All patients underwent insulin, adiponectin and leptin measurements, as well as MetS criteria investigations. After completion of the International Physical Activity Questionnaire, participants were graded with a low, moderate or high activity level. **Results**: All obesity parameters are significantly different between obese and non-obese patients, with leptin-adiponectin ratio (LAR) having the highest statistical power ($p<0.001$, $\eta^2=0.15$). Adiponectin, insulin, and HOMA-IR were statistically different between MHO and MUO, with no significance between MHNO and MUNO. Adiponectin levels are not significantly associated with a class that describes a specific physical activity level, whereas leptin levels are decreased in non-obese patients once they were more physically active ($p=0.042$). Relationship between physical activity level and obesity markers in obesity phenotypes did not conclude with any significant results. **Conclusions**: Adiponectin levels are higher in MHO phenotype than in MUO phenotype even though both groups present similar BMI means, therefore we can consider adiponectin a possible biomarker for metabolic syndrome in obese patients. Also, adiponectin, LAR, insulin, and HOMA-IR levels do not change in physically active patients, whereas leptin values decrease significantly in non-obese subjects that engage in physical activity. **Keywords:** ADIPOKINES, PHYSICAL ACTIVITY, OBESITY, METABOLIC SYNDROME, PHENOTYPES.

Metabolic syndrome (MetS) is a constellation of risk factors specific for cardiometabolic diseases, including abdominal obesity, atherogenic dyslipidemia, insulin

resistance, hypertension (1, 2). The pathophysiology of the MetS is not yet fully explained in the literature and it is still controversial whether each different criteria for the MetS stands for a specific pathology or they all fall under the same pathogenic process (2).

Obesity and even more important, abdominal obesity, activates multiple pathways of MetS, therefore adipose tissue serves as a key factor in the development of MetS (3, 4). The recently discovered endocrine function of the adipose tissue may explain some of the processes that occur in the MetS (5-7), emphasizing on the adipokines released that include hormones (e.g., leptin, adiponectin), peptides (e.g., apelin, resistin), inflammatory cytokines (e.g., interleukin-6, visfatin, omentin) (8).

Adiponectin and leptin are important adipocytokines secreted by fat containing adipocytes, which expresses contrasting effects. Leptin is reported to be directly proportional to obesity and body fat levels (9), reducing food intake and engaging energy expenditure while controlling insulin sensitivity and glucose levels (10). At the same time, leptin is associated with a higher proinflammatory immune response and higher risk of cardiovascular disease (11). In contrast, adiponectin is an anti-inflammatory, anti-atherogenic and anti-diabetic adipokine (12, 13), several studies reporting low levels in coronary heart disease, diabetes, hypertension (14-16). Therefore, the balance between these 2 adipokines is of great interest in better managing and understanding of obesity.

In the first part of our study, we analyzed circulating levels of adipokines and insulin in order to see if these may characterize a specific obesity phenotype and therefore explain pathogenic pathways in MetS. In the second part, we wanted to understand if lifestyle modifications may represent a therapeutic intervention in switching between these phenotypes, therefore we studied the influence of physical activity on adiponectin, leptin and insulin levels.

MATERIAL AND METHODS

Our cross-sectional study was conducted over a period of two years and included 104 patients organized in four groups: metabolically healthy obese (MHO), metabolically unhealthy obese (MUHO), metabolically healthy non-obese (MHNO), metabolically unhealthy non-obese (MUNO). Participants included in the study had no known chronic disease or had not followed treatment in the last 6 months for any cardiovascular or metabolic disorder and had no antecedent acute atherosclerotic pathology. The study was approved by the University Ethics Committee, number 1/27.07.2020 and all participants signed an informed consent before entering the study.

Baseline characteristics of the population

The following parameters were assessed for each patient: height, weight, abdominal circumference (AC), blood pressure, age, gender, residence, estimated visceral activity index (eVAI), along with the obesity and metabolic syndrome biochemical markers.

Blood pressure was measured after a 15-minute rest, with no prior caffeine consumption or no prior physical exercise engaged. A validated automated device and cuffs of 3 sizes were used, according to

arm circumference. Values were registered twice, and a mean value was considered.

Visceral adiposity index was calculated with previous validated equations (17):

$$\text{Men: eVAI} = \left(\frac{\text{AC}}{39.68 + (1.88 \times \text{BMI})}\right) \times \left(\frac{\text{TG}}{1.03}\right) \times \left(\frac{1.31}{\text{HDL} - \text{chol}}\right)$$

$$\text{Women: eVAI} = \left(\frac{\text{AC}}{36.58 + (1.89 \times \text{BMI})}\right) \times \left(\frac{\text{TG}}{0.81}\right) \times \left(\frac{1.52}{\text{HDL} - \text{chol}}\right)$$

A value of 1 is attributed to healthy subjects without obesity, with normal adipose tissue distribution, normal triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-chol) levels.

Biochemical markers for obesity and MetS

All biochemical markers were measured in the same laboratory, using the same equipment and the same method during the entire study. Blood samples were collected by trained personnel after a 12 hrs. overnight fast in serum clot activator tubes and centrifuged in maximum 2 hrs, at 4^0 C and 4500 x g, for 10 minutes. Hemolyzed samples were excluded. For adiponectin, leptin and insulin measurements serum was stored in separate tubes at -20^0C. A specific requirement was addressed for insulin and prior to storage, blood samples were centrifuged in maximum 15 minutes after collection.

HDL-chol was measured using the direct method (elimination/peroxidase), TG using the glycerol phosphate oxidase method and glucose using the glucose-6-phosphate dehydrogenase method.

Quantitative determination of the adiponectin and leptin levels was performed using enzyme-linked immunosorbent assay (ELISA) kits supplied by Biovendor-Laboratorni medicina a.s., Brno, Czeck Republic: Adiponectin Human ELISA Competitive kit, CE-IVD, limit of detection 26 ng/mL, catalog number RD195023100 and Leptin Human ELISA, CE-IVD, limit of detection 0.2 ng/mL, catalog number RD191001100. Afterwards, leptin to adiponectin ratio (LAR) was calculated.

Serum total insulin concentration was measured using a solid-phase, enzyme-labeled chemiluminescent immunometric assay kit, Immulite 2000 Insulin, provided by Siemens (catalog number L2KIN2).

The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated according to the report by Matthews *et al*. (18), using the formula: HOMA-IR = [insulin (μU/mL) x glucose (mg/dL)] / 405.

Obesity phenotypes

The four obesity phenotypes were established based on the metabolic and obesity status. Obesity was assessed at a BMI ≥ 30 kg/m^2 and metabolic syndrome criteria were as follows: glucose ≥ 100 mg/dL, HDL < 40 mg/dL (men) / < 50 mg/dL (women), TG ≥ 150 mg/dL, SBP/DBP ≥ 130/85 mmHg (19). Metabolically healthy status included participants with AC > 88cm (women) / 104 cm (men) and maximum 1 abnormal component from the metabolic syndrome criteria, while unhealthy status was established if patients presented a higher-than-normal AC and the metabolic syndrome (≥ 2 abnormal components).

Physical activity questionnaire

All participants in the study completed the International Physical Activity Questionnaire (IPAQ) in order to assess a physical activity level. IPAQ is a standardized self-report measurement tool that provides an estimate of physical activity and sedentary behavior for adults across a range of socio-economic settings (20, 21). Results were reported in categories (low/moderate/high activity levels) depending on the MET (metabolic equivalent of task), which represents the amount of energy expended carrying out physical activity. Activities reported by participants were quantified in METs according to the 2000 compendium of physical activities (22).

High activity was considered when any of the 2 criteria was met: (a) vigorous-intensity activity on at least 3 days and accumulating at least 1500 MET-minutes/week or (b) 7 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum of at least 3000 MET-minutes/week. Moderate activity was assessed if any of the 3 criteria was met: (a) 3 or more days of vigorous-activity of at least 20 minutes/day or (b) 5 or more days of moderate-intensity activity or walking of at least 30 minutes/day or (c) 5 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum of at least 600 MET-minutes/week. Individuals that do not meet criteria for moderate or high categories were considered inactive or with a low activity level (21).

Statistical analysis

Data analysis was performed using the *SPSS version 23.0* (IBM Corporation, USA) and Microsoft Excel 2003 (Microsoft Corporation, USA). Continuous variables were expressed as mean ± standard deviation (SD) and categorical values were expressed as frequencies (percentages). Continuous values between the obese and non-obese groups were compared using the t-test for independent samples. The same comparison between the 4 phenotypes was performed with one-way *ANOVA* test. Chi-square test was used for categorical comparisons. Eta squared (η^2) reports statistical power and a p value < 0.05 is considered statistically significant.

RESULTS

Baseline characteristics for the obese and non-obese groups are reported in Table I. Our study included more women than males per group, but the difference between groups was not significant, therefore excluding a possible bias to our research. Mean age was 57.86±8.21 for obese participants and 62.26±7.47 for non-obese participants. Biochemical markers involved in obesity that were measured in our study are all statistically significant between the 2 groups. The highest statistical power is observed for leptin-adiponectin ratio (LAR), suggesting that 15% variability in LAR is accounted for by the obese or non-obese category the patient is in.

We further analyzed the metabolic syndrome criteria, biochemical markers for obesity, eVAI and physical activity score for the 4 obesity phenotypes, according to metabolic health and obesity (Tab. II). Blood pressure, glucose, TG, HDL-chol were all statistically significant between phenotypes as expected since metabolic syndrome is one of the criteria for stratifying these phenotypes. At the

same time, we expected to see no statistically significant difference of obesity biomarkers between sub-groups of obese and non-obese, respectively, since BMI is very similar in the MHO and MUO groups and also in the MHNO and MUNO groups. Adiponectin, insulin and HOMA-IR were statistically different between MHO and MUO, with no significance between MHNO and MUNO. Adiponectin may be used as a biomarker for metabolic syndrome only in obese patients, reporting an 8% variability in adiponectin values that is accounted for by the metabolic syndrome status.

Our study also involved analyzing the relationship between physical activity and obesity biomarkers (Tab. III). Results report a significant decrease in leptin ($p=0.042$, $\eta^2=0.16$) as the person has a higher engagement in physical activities, only for non-obese patients (Fig. 1). We also observe a decrease in adiponectin levels for non-obese, but it is not statistically significant. In contrast, obese patients show slighter higher results for adiponectin, leptin, LAR (not statistically significant) when they perform moderate physical activity, followed by a decrease in a more active individual.

In addition, relationship between physical activity level and obesity markers in obesity phenotypes did not conclude with any significant results.

TABLE I.
Baseline characteristics of the study population

	Obese patients (n=65)			Non-obese patients (n=39)				
	Mean±SD	Min value	Max value	Mean±SD	Min value	Max value	p	η^2
BMI (kg/m^2)	34.66±4.55	30.04	53.31	26.99±2.24	21.91	29.87	<0.001	0.49
AC (cm)	112.97±12.21	93	160	99.15±7.05	89	118	<0.001	0.29
Age (years)	57.86±8.21	39	72	62.26±7.47	43	75	0.007	0.07
Gender							0.057	-
Females	67.69% (44)	-	-	84.61% (33)	-	-		
Males	32.31% (21)	-	-	15.39% (6)	-	-		
Residence							0.247	-
Urban	67.69% (44)	-	-	56.41% (22)	-	-		
Rural	32.31% (21)	-	-	43.59% (17)	-	-		
Adiponectin (µg/mL)	43.15±17.16	15.10	89.70	51.99±17.36	16.30	95.10	0.013	0.06
Leptin (ng/mL)	28.15±15.03	5.80	58.20	18.69±12.77	2.60	47.60	0.001	0.10
LAR	0.71±0.43	0.07	2.27	0.38±0.27	0.06	1.16	<0.001	0.15
Insulin (µU/mL)	20.76±12.02	3.72	51.10	13.26±8.66	2.13	52	0.001	0.10
HOMA-IR	6.22±5.15	0.81	27.25	3.76±2.90	0.49	16.31	0.008	0.07
eVAI	6.96±5.64	1.42	33.66	5.35±3.41	1.38	19.25	0.109	-

TABLE II.

Metabolic health parameters and physical activity in obesity phenotypes

	Obese patients				Non-obese patients			
	MHO (n=20)	MUO (n=45)	p	η^2	MHNO (n=16)	MUNO (n=23)	p	η^2
BMI (kg/m^2)	34±3.68	34.96±4.89	0.437	-	26.11±2.36	27.61±1.98	0.039	0.11
SBP (mmHg)	129.05±18.81	143.73±17.92	0.004	0.12	135.12±28.09	135.87±16.47	0.918	-
DBP (mmHg)	80.95±10.94	89.11±10.12	0.005	0.12	84.69±15.33	84.96±12.78	0.953	-
Glucose (mg/dL)	94.45±11.29	120.67±34.92	0.002	0.15	99.69±19.33	121.09±50.27	0.115	-
TG (mg/dL)	97.65±26.46	195.71±95.81	<0.001	0.24	99.69±30.42	163.13±54.60	<0.001	0.32
HDL-chol (mg/dL)	56.9±11.31	46.87±13.34	0.005	0.12	62.25±12.94	53.52±11.76	0.035	0.11
Adiponectin (µg/mL)	50.49±19.75	39.89±14.98	0.02	0.08	54.51±18.59	50.26±16.65	0.459	-
Leptin (ng/mL)	28.14±14.06	28.15±15.59	0.999	-	19.11±14.60	18.40±11.67	0.866	-
LAR	0.65±0.43	0.74±0.42	0.446	-	0.38±0.32	0.38±0.24	0.944	-
Insulin (µU/mL)	13.44±8.07	24.01±12.13	0.001	0.17	10.96±6.21	14.86±9.83	0.170	-
HOMA-IR	3.10±1.75	7.61±5.56	0.001	0.16	2.87±2.15	4.39±3.23	0.109	-
eVAI	3.36±1.15	8.57±3.10	<0.001	0.18	3.40±1.81	6.71±3.63	0.002	0.23
IPAQ score			0.027	-			0.571	-
Low	35% (7)	31.1% (14)			50% (8)	47.8% (11)		
Moderate	20% (4)	51.1% (23)			43.8% (7)	34.8% (8)		
High	45% (9)	17.8% (8)			6.3% (1)	17.4% (4)		

TABLE III.

Relationship between physical activity and obesity biomarkers

	IPAQ score category for obese patients				IPAQ score category for non-obese patients			
	Low (n=21)	Moderate (n=27)	High (n=17)	p	Low (n=19)	Moderate (n=15)	High (n=5)	p
Adiponectin (µg/mL)	43.08±17.97	43.18±16.97	43.15±17.16	0.999	55.56±13.48	49.29±17.37	46.60±29.42	0.450
Leptin (ng/mL)	25.52±15.49	32.41±14.74	24.61±14.04	0.153	22.49±14.60	17.87±9.66	6.72±3.57	**0.042**
LAR	0.64±0.44	0.84±0.46	0.59±0.31	0.105	0.44±0.33	0.39±0.20	0.16±0.05	0.128
Insulin (µU/mL)	22.06±13.61	21.03±10.37	18.74±12.87	0.698	11.93±5.71	13.12±6.98	18.71±18.70	0.303
HOMA-IR	6.96±5.68	5.95±3.64	5.75±6.58	0.731	3.45±2.02	3.61±2.39	5.44±6.14	0.392
eVAI	8.55±8.17	6.45±3.65	5.82±4.14	0.280	4.56±2.64	6.63±4.31	4.49±2.04	0.180

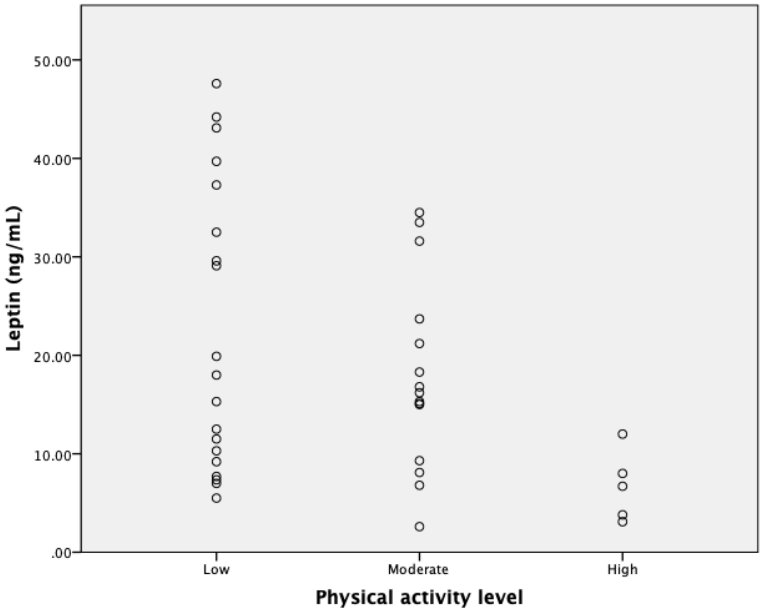

Fig. 1. Relationship between leptin levels and physical activity in non-obese patients

DISCUSSION

As more studies on a large scale are conducted, obesity phenotypes still represent a scientific controversial. MHO phenotype may be considered healthy, or it may just be one step before the unhealthy phenotype, therefore it is not clear if MHO remain healthy later in life or not (23). A recent study performed on 381,363 UK Biobank participants confirmed the healthier status of non-obese compared to MHO concerning heart failure and respiratory disease, but not concerning fatal atherosclerotic cardiovascular disease (24). Even if MHO phenotype is a transitory state, further investigating pathogenic processes engaged in these patients is essential to explain, prevent or even treat adiposopathy.

Our main finding in this study is that MHO have higher levels of adiponectin than MUO ($p=0.02$) even though both phenotypes report a very similar BMI mean (34 ± 3.68 vs. 34.96 ± 4.89, $p=0.437$). Other studies involving non-European descent individuals confirm the paradoxical hyperadiponectinemia in MHO phenotype (25-27) reported in our study, suggesting a protective role adiponectin might have in preventing metabolic syndrome. Interestingly, comparing MHO adiponectin levels with the ones for MHNO, which is considered a reference, the mean level of adiponectin slightly decreases in MHO, remaining at a very similar value to the one for MUNO ($p>0.05$). This proves that the secretion of adipokines is not affected as long as only one factor (obesity or metabolic syndrome) is present. Considering adiponectin's' anti-inflammatory effect, we can say that MHO have reduced inflammation compared to their MUO counterparts. Contrary to the above data, there are few scientific studies that report no difference between adiponectin levels among MHO and MUO individuals (28, 29).

The literature data concerning the inter-

connection between adiponectin and physical activity show contradictory results. A recent review stressed the importance of all variables that need to be considered for a more efficient interpretation of results: type, duration and intensity of exercise, but also age, gender, BMI and health status (30). There are studies that analyzed also genetic predisposition in the adiponectin gene as a possible contributor to a change in these values, even though no significant correlation was found (31). Plasma adiponectin levels seem to be negatively correlated with body fat percentage in males, but not in females (32), suggesting the presence of a possible gender-specific mechanism. Moreover, several studies involving aerobic interventions reported that adiponectin levels change independently from body composition changes, therefore changes in fat mass may be only one way to influence adiponectin levels and other mechanisms can link exercise to adiponectin levels (33-35). Our results noted no significant difference in adiponectin values between low, moderate or high levels of physical activity in obese or non-obese groups and not even among obesity phenotypes. Interestingly, the mean for adiponectin levels was almost identical in the obese group among low, moderate of high levels of physical activity ($p=0.999$), whereas in the non-obese group the values are lower once the subjects engage in more intense physical activity ($p=0.450$). We can conclude that for our population adiponectin levels are not significantly associated with a class that describes a specific physical activity level, but we do not rule out that there may be a change in values while performing acute physical training. Further studies need to clarify this aspect.

There was no significant difference among obese and non-obese concerning eVAI values, but the significance was present with high statistical power when we compared these values between MHO and MUO ($\eta^2=0.18$) or MHNO and MUNO ($\eta^2=0.23$). These results strengthen other findings in the literature that consider VAI a biomarker for MetS (36-38).

Leptin and LAR show no difference between the MHO and MUO, suggesting no association between these biomarkers and metabolic syndrome in our population. Our result may be explained by the development of "leptin resistance" in obesity, which includes mechanisms like negative regulation of ObRb (the long form of leptin receptor in mice) signaling in the hypothalamus, increased proinflammatory cytokine signaling in the brain or reduced transport of leptin across the blood-brain barrier (8). At the same time, of all the obesity biomarkers analyzed between obese and non-obese, the most significant difference was established by LAR, with 15% of variance in the value of this biomarker being accounted for by the inclusion in one of the 2 groups. The obese patients showed almost 2 times higher values than non-obese ones, confirming results from other studies (39-41).

A recent review extracted 107 effects from 72 articles regarding the relationship between leptin and physical activity and reported that chronic exercise training can effectively reduce leptin levels. There was no difference concerning plasma leptin decrease and the type of exercise (aerobic, resistance, concurrent exercise), intensity, frequency or duration. Even though body fat percentage is strongly associated with low leptin levels, chronic exercise training independently shows a decrease in leptin values, regardless of change in adiposity (42). Our results reported a significant

decrease in leptin levels in non-obese participants once they were physically more active. Interestingly, obese patients did not present changes in plasma leptin levels that were statistically significant. These results support the findings of a recent study that analyzed the influence of a 12-week physical activity program on leptin resistance in metabolic syndrome, which brought changes in most metabolic syndrome parameters, but not in leptins' plasma levels (43). On the contrary, previous studies showed a 22% reduction in leptin levels associated with a 7% decrease in body weight (44) or a 45% reduction in leptin levels associated with a decrease of 2.1 kg/m^2 in BMI (45). The small sample size or the subjective evaluation of participants' own physical activity through a questionnaire and not a specific program that is prescribed and followed up by trained specialists, may represent the reasons for not reporting a significant change in leptin levels in obese patients.

CONCLUSIONS

Adiponectin levels are higher in MHO phenotype than in MUO phenotype even though both groups present similar BMI means, therefore we can consider adiponectin a possible biomarker for metabolic syndrome in obese patients. Also, adiponectin, LAR, insulin and HOMA-IR levels do not change in physically active patients, whereas leptin values decrease significantly in non-obese subjects that engage in physical activity.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding the scientific research.

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THE IMPACT OF ESBL-PRODUCING ENTEROBACTERALES: AN OLD STORY WITH NEW PERSPECTIVES

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THE IMPACT OF ESBL-PRODUCING ENTEROBACTERALES: AN OLD STORY WITH NEW PERSPECTIVES (Abstract): Antibiotic resistance represents a global crisis, one of the biggest challenges that humanity is facing. In addition to the SARS-Cov-2 pandemic, we are currently witnessing another, more devastating pandemic of multidrug-resistant microorganisms, not only in hospitals but also in the community. **Material and methods:** we aimed to analyze the incidence and implications of urinary isolated, extended-spectrum β-lactamase-producing (ESBL+) *Enterobacterale*s, from patients admitted between 01/01/2018 and 31/12/2018. **Results:** the main identified pathogen was *Escherichia coli*, accounting for 67.3% of all cases; the ESBL-production rate was 26.5%, with *E. coli* and *Klebsiella pneumoniae* emerging as leading etiologies. Mean age was significantly higher for patients with ESBL+ isolates (57.6 *vs.* 65.1 years, $p<0.0001$), while urinary catheterization was more frequently associated with ESBL+ strains (21.8% ESBL+ *vs.* 6.2% ESBL-, $p<0.0001$). As anticipated, antibiotic susceptibility was significantly influenced both by ESBL production and urinary catheterization, except for nitrofurantoin and fosfomycin**. Conclusions:** comprehensive approach of the local antibiotic susceptibility profile can facilitate the choice of a proper therapeutic management, based on regional particularities of an East European country. **Keywords**: ENTEROBACTERALES, ESBL, ANTIBIOTIC-RESISTANCE, UTIs.

Extended-spectrum β-lactamase (ESBL) producing *Enterobacterales* have become a significant clinical and epidemiological concern. Those strains are associated with adverse patient outcomes, such as prolonged length of hospitalization and a higher risk for therapeutic failure. In 2017 alone, only in the United States were recorded more than 197,000 cases of infections caused by an ESBL-producing (ESBL+) microorganisms (1).

ESBLs are capable of hydrolyzing most broad-spectrum β-lactam antibiotics. Compared to infections caused by non-ESBL-producing (ESBL-) microorganisms, those caused by ESBL-producing strains tend to be more severe, with higher morbidity and mortality rates (2). However, these enzymes have a rather reduced capacity to hydrolyze carbapenems, thus they are typically required for the treatment of these infections, but carbapenem resistance is

also on an upward trend (3).

ESBL production is the most important mechanism that induces β-lactam resistance, with over 200 such enzymes described to date (4). The spread of ESBL-producing isolates is alarming, especially concerning the strains of *Escherichia coli* and *Klebsiella pneumoniae*; however, other members of the *Enterobacterales* family that produce such enzymes have also been identified (5).

Urinary tract infections (UTIs), whether community - acquired or healthcare-associated, are the most common infections caused by *Enterobacterales* (6). In the context of increasing antibiotic resistance among *Enterobacterales* isolates, the management of these infections has become increasingly challenging There are multiple studies analyzing the risk factors for UTIs with ESBL-producing *Enterobacterales* (3, 4). The most commonly incriminated risk factors are previous antibiotic use (within the last 3 months), especially third-generation cephalosporins (7), history of urinary tract infections, previous hospitalizations (within 3-6 months before the admission), urinary catheterization, age, prostatic hypertrophy, male gender; the presence of congenital abnormalities, urolithiasis, history of invasive urological procedures, or associated comorbidities such as diabetes mellitus. However, those risk factors are highly variable, not only at individual level, but also geographically influenced, therefore the outlining of a regional high-risk pattern is of utmost importance.

MATERIAL AND METHODS

We conducted a retrospective study, in which we performed an analysis of the incidence of positive urine cultures determined by an *Enterobacterales* strain, from 01/01/2018 to 31/12/2018.

Inclusion criteria were isolation of an *Enterobacterales* strain (*E. coli, K. pneumoniae, Enterobacter* spp. *Proteus* spp., *Serratia* spp., *Providencia* spp. or *Morganella* spp.) in urine cultures ($\geq 10^5$ colony-forming units/mL). Confirmation of ESBL production was achieved by the double-disc synergy method, which showed synergy between ceftazidime, ceftriaxone, cefotaxime, and amoxicillin discs with clavulanic acid on Mueller-Hinton agar plates.

Antibiotic susceptibility testing was performed using the Kirby Bauer disk diffusion method, using the EUCAST clinical breakpoints table v 8.1, for interpretation of minimum inhibitory concentrations (MICs) and zone diameter breakpoints (mm).

Cases *of Enterobacterales* colonization and those with CFU counts below the threshold value of 10^5/mL were excluded.

For data collection we used the records from the archive of the Microbiology Laboratory of the Infectious Diseases Hospital "Sf. Parascheva" from Iasi, from where we were able to extract data on certain demographic characteristics of the patients (age, sex), the presence of a permanent urinary catheter, and, most important, the etiological agent, the antibiotic susceptibility profile with the identification of ESBL-producing strains.

Statistical analysis was performed by using *SPSS Software version 23.0* (IBM, Armonk, VA, SUA) and *Microsoft Excel version 2013* (Microsoft Corporation, Redmond, WA, USA). A *p*-value of 0.05 was considered statistically significant.

RESULTS

During the analyzed period, we identified 742 positive urine cultures with an *Enterobacterales* strain. Of those, we found

a total of 197 (26.5%) urine cultures with ESBL-producing (ESBL+) *Enterobacterales*.

The most frequent etiological agent identified was *E. coli*, responsible for 500 cases, followed by *Klebsiella* spp. - 157 cases (of which 147 *K. pneumoniae*, 8 *K. oxytoca* and 2 *K. aerogenes*), *Proteus* spp. - 53 cases (*P. mirabilis* - 51 cases, *P. vulgaris* - 2 cases), *Enterobacter* spp. - 19 cases (*E. cloacae* - 4 cases, *Enterobacter* sp. - 15 cases), *Providencia* spp. - 7 cases (*P. stuartii* - 6 cases, *Providencia* spp. - 1 case), *Serratia* spp. - 4 cases *(S. marcescens* - 3 cases, *Serratia* sp. - 1 case) and *Morganella morganii* - 2 cases.

As expected, the most commonly ESBL+ isolate was *E. coli*, identified in 83 (42.3%) cases; the second position was occupied by *Klebsiella* spp., accounting for 76 (38.5%) cases, followed by *Enterobacter* spp. (14 cases – 7.1%), *Proteus* spp. (13 cases – 6.6%), *Providencia* spp. (7 cases – 3.5%), *Serratia* spp. (3 cases 1.5%) and *Morganella morganii* (one case – 0.5%).

We identified an association between patient age and infection with an ESBL+ strain, with 85.3% of the total number of patients aged over 50 years, while almost half, 43.2%, were aged over 70 years, with no significant differences by year (tab. I).

TABLE I

Age distribution

Age	ESBL + 197	ESBL - 545	*p*
≤ 30 years	10 (5%)	87 (15.9%)	0.0001
31 – 50 years	19 (9.7%)	85 (15.5%)	0.03
51 – 70 years	83 (42.1%)	206 (37.7%)	0.28
> 70 years	85 (43.2%)	167 (30.6%)	0.001
Mean age (years)	65.1	57.6	<0.0001

We identified an overall predominance of female patients, 512 (69%) *vs.* only 230 (31%) male patients. When assessing the gender of patients with an ESBL + isolate, we identified a milder difference, with only 104 (52.8%) females and 93 (47.2%) males, compared to 408 (74.9%) females and 137 (25.1%) males with an ESBL – strain (tab. II).

We identified 77 (10.3%) patients with an indwelling urinary catheter; of those 43 had a ESBL + strain (tab. III).

We also observed some variability concerning the etiology in patients with urinary catheter. Thus, the main identified agent for those patients was still *E. coli*, identified in 33 (42.8%) cases, followed by *K. pneumoniae* in 28 (36.3%) cases, *Proteus* spp. – 8 (10.4%) cases, *Providencia* spp. – 6 (7.8%) cases, *Enterobacter* sp. and *Morganella morganii*, one (1.3%) case each (tab. IV).

TABLE II

Gender distribution

Gender	ESBL + 197	ESBL - 545	Total	*p*	OR (CI)
Female gender	104 (52.8%)	408 (74.9%)	512	**<0.0001**	0.3755 (0.2672-0.5276)
Male gender	93 (47.2%)	137 (25.1%)	230		

TABLE III

Urinary catheterization

	ESBL + 197	ESBL - 545	Total	*p*	OR (CI)
Urinary catheterization	43 (21.8%)	34 (6.2%)	77 (10.3%)	**<0.0001**	4.1965 (2.5849-6.8128)

TABLE IV

The microorganisms isolated, in urinary catheterized patients

	ESBL + 43	ESBL -34	Total 77	*p*	OR (CI)
E. coli	9 (20.9%)	24 (70.6%)	33 (42.8%)	<0.0001	0.1103 (0.0389-0.3124)
K. pneumoniae	23 (53.4%)	5 (14.7%)	28 (36.3%)	0.0004	6.6700 (2.1710-20.4927)
Proteus spp.	5 (11.6%)	3 (8.8%)	8 (10.4%)	0.688	1.3596 (0.3010-6.1421
Providencia spp./ *Enterobacter* spp./ *Morganella morganii*	6 (13.9%)	2 (5.9%)	8 (10.4%)	0.249	2.5946 (0.4890-13.7666)

When analyzing ESBL + strains, we identified a worrisome susceptibility rate to aminopenicillins in combination with beta-lactamase inhibitors, the lowest being for *Klebsiella* spp. isolates (3.9% to amoxicillin+clavulanic acid and 6.5% to ampicillin + sulbactam).

]The most active beta-lactam identified (excluding carbapenems) was cefoxitin, with 63.4% of all strains sensitive to this antibiotic, with the highest percentage found in *E. coli* isolates (97.5%). We also identified high resistance for other antibiotic classes, notably fluoroquinolones, with only 11.1% sensitivity to ciprofloxacin, the lowest among *Klebsiella* spp. strains (6.5%). Sensitivity to aminoglycosides is preserved, especially for amikacin (58.8%), with the lowest percentages for tobramycin (22.8%) (tab. V).

TABLE V

Antibiotic susceptibility of ESBL+ strains

	E. coli	*Enterobacter* spp.	*Klebsiella* spp.	*Proteus* spp.	*Providencia* spp.	*Morganella morganii*	*Serratia marcescens*	Total
AMC	21.6%	RI	3.9%	15.3%	RI	RI	RI	11.6%
SAM	26.5%	RI	6.5%	15.3%	RI	RI	RI	12.1%
TMT - SMX	38.5%	21.4%	19.7%	23%	0	0	66.6%	27.9%
CXM	1.2%	21.4%	0	0	0	0	RI	2%
CAZ	2.4%	0	0	0	0	0	0	1%
FEP	6%	0	2.6%	30.7%	0	0	0	5.5%
FOX	97.5%	21.4%	43.4%	30.7%	0	0	RI	63.4%
GM	49.3%	21.4%	13.1%	69.2%	0	0	33.3%	32.4%
TOB	38.5%	14.2%	9.2%	23%	0	0	33.3%	22.8%
AK	80.7%	71.4%	32.8%	92.3%	0	0	66.6%	58.8%
CIP	15.6%	21.4%	6.5%	7.7%	0	0	0	11.1%

	E. coli	*Enterobacter* spp.	*Klebsiella* spp.	*Proteus* spp.	*Providencia* spp.	*Morganella morganii*	*Serratia marcescens*	Total
TZP	69.8%	21.4%	17.1%	92.3%	14.2%	0	33.3%	44.6%
IMI	98.7%	71.4%	61.8%	69.2%	14.2%	0	33.3%	76.1%
MEM	100%	71.4%	63.1%	100%	42.8%	0	33.3%	80.2%
ETP	93.9%	42.8%	42.1%	92.3%	28.5%	0	33.3%	66.4%

AMC=amoxicillin+clavulanic acid; SAM=ampicillin sulbactam; TMT-SMX=trimethoprim+ sulfamethoxazole; CXM=cefuroxime; CAZ=ceftazidime; FEP=cefepime; FOX=cefoxitin; GM=gentamicin; TOB=tobramycin; AK=amikacin; CIP=ciprofloxacin; TZP=piperacillin+ tazobactam; IMI=imipenem; MEM=meropenem; ETP=ertapenem.

In contrast, for ESBL- isolates, we identified significantly higher sensitivity rates, not only to beta-lactams, but also to all antibiotic classes (tab. VI).

TABLE VI

Antibiotic susceptibility of ESBL- strains

	E. coli	*Enterobacter* spp.	*Klebsiella* spp.	*Proteus* spp.	*Morganella morganii*	*Serratia marcescens*	Total
AMP	46.7%	RI	RI	35.8%	RI	RI	45.7%
AMC	75.9%	RI	57.3%	66.6%	RI	RI	72.4%
SAM	80.5%	RI	63.4%	69.2%	RI	RI	77.1%
TMT -SMX	69.3%	80%	89%	38.4%	0	0	70%
CXM	96.1%	100%	85.3%	69.2%	0	RI	92.2%
CFM	98.8%	100%	87.8%	74.3%	100%	100%	95.4%
CTX	99%	100%	91.4%	74.3%	100%	100%	96.1%
CAZ	98.8%	100%	85.3%	74.3%	100%	100%	95%
FEP	97.3%	100%	90.2%	89.7%	100%	100%	96.3%
FOX	98.3%	80%	89%	84.6%	0	RI	96.5%
GM	95.6%	100%	91.4%	64.1%	0	100%	92.6%
TOB	94.7%	100%	86.5%	58.9%	0	0	90.6%
AK	99.7%	100%	95.1%	94.7%	100%	100%	98.7%
CIP	82.7%	60%	80.4%	48.7%	0	0	79.4%
TZP	92.3%	100%	75.3%	91.6%	100%	0	89.1%
IMI	100%	100%	98.7%	94.7%	100%	100%	99.6%
MEM	100%	100%	98.7%	94.7%	100%	100%	99.6%
ETP	99.5%	100%	92.6%	97.4%	100%	100%	98.3%

AMP= ampicillin; AMC=amoxicillin+clavulanic acid; SAM=ampicillin sulbactam; TMT-SMX=trimethoprim+ sulfamethoxazole; CXM=cefuroxime; CFM=cefixime; CTX=cefotaxime; CAZ=ceftazidime; FEP=cefepime; FOX=cefoxitin; GM=gentamicin; TOB=tobramycin; AK=amikacin; CIP=ciprofloxacin; TZP=piperacillin+ tazobactam; IMI=imipenem; MEM=meropenem; ETP=ertapenem.

Sensitivity to nitrofurantoin and fosfomycin was routinely determined only for *E. coli* strains, observing high susceptibility rates, but without notable differences between ESBL+/- isolates; For ESBL(-) strains we identified a 98.5% and 99.2% sensitivity to nitrofurantoin and fosfomycin, respectively. Concerning ESBL (+) isolates, we observed that 87.9 and 100% of them were sensitive to nitrofurantoin and fosfomycin, respectively.

In addition, we aimed to perform a comparative analysis of the sensitivity profile, according to the presence of an indwelling urinary catheter. Thus, we noticed that urinary catheterization was associated with significantly lower susceptibility rates, especially among *Klebsiella* spp. and *Proteus* spp. isolates (tab. VII).

TABLE VII

Antibiotic susceptibility for urinary catheterized patients

	E. coli	*Enterobacter* spp.	*Klebsiella* spp.	*Proteus* spp.	*Providencia* spp.	*Serratia marcescens*	Total
AMP	33.3%	RI	RI	12.5%	RI	RI	19.5%
AMC	48.4%	RI	14.2%	25%	RI	RI	31.8%
SAM	60.6%	RI	10.7%	25%	RI	RI	21.7%
TMT -SMX	57.5%	0	28.5%	25%	16.6%	0	38.9%
CXM	57.5%	100%	14.2%	37.5%	0	RI	36.3%
CFM	63.6%	100%	14.2%	37.5%	0	100%	38.9%
CTX	66.6%	100%	14.2%	37.5%	0	100%	41.5%
CAZ	63.6%	100%	14.2%	37.5%	0	100%	38.9%
FEP	63.6%	100%	14.2%	62.5%	0	100%	41.5%
FOX	90.9%	100%	39.2%	50%	0	RI	59.7%
GM	78.7%	100%	17.8%	25%	0	100%	46.7%
TOB	75.7%	100%	17.8%	25%	0	0	42.8%
AK	93.9%	100%	64.2%	75%	0	100%	74%
CIP	51.5%	100%	14.2%	25%	0	0	31.1%
TZP	93.5%	100%	22.2%	75%	0	0	58.4%
IMI	100%	100%	43.4%	100%	0	100%	77.9%
MEM	100%	100%	43.4	87.5	0	100	75.3
ETP	100%	100%	39.2	100	0	100	72.7

AMP= ampicillin; AMC=amoxicillin+clavulanic acid; SAM=ampicillin sulbactam; TMT-SMX=trimethoprim+ sulfamethoxazole; CXM=cefuroxime; CFM=cefixime; CTX=cefotaxime; CAZ=ceftazidime; FEP=cefepime; FOX=cefoxitin; GM=gentamicin; TOB=tobramycin; AK=amikacin; CIP=ciprofloxacin; TZP=piperacillin+ tazobactam; IMI=imipenem; MEM=meropenem; ETP=ertapenem.

DISCUSSION

ESBL-producing *Enterobacterales* represent a significant clinical issue, as it is very epidemiologically challenging. These strains are associated with adverse outcomes such as prolonged length of hospitalization, therapeutic failure, and increased mortality. Consequently, broad-spectrum antibiotics, (e.g., carbapenems), are typically required for the treatment of these

infections, but carbapenem resistance is also, on an upward trend, an aspect which was highlighted by our present study and previous work (8, 9).

The most commonly isolated etiological agent was *E. coli*, for both ESBL+ and ESBL- isolates, followed by *Klebsiella* spp. (the vast majority, 93.6%, is represented by *K. pneumoniae*). These findings are consistent with the majority of studies, which classify *E. coli* as the main etiology for both pediatric, adult, and geriatric UTIs (10-12).

We have identified a relatively small percentage of ESBL+ *E. coli* isolates, 16.6% (83 strains). Most studies have reported much lower rates (13), although we identified even more dramatic reports, generally from India, where incidence reaches up to 45% (14-16). This "borderline" pattern may be due to some socioeconomic particularities, Romania (and especially its Northeast region) exhibiting certain particularities from both developed and developing countries (17). A very recent study provided additional evidence concerning the relationship between low income or poor education and the increased risk of UTI (18).

Of course, the incidence of ESBL+ *E. coli* causing community UTIs differs notably, with a significant geographical variability; in China, for example, it ranges from 45.2 to 68.2% (19, 20), in Canada from 9.1 to 14.1%, and in the US from 6.5% in 2010 to 16.0% in 2014 (21, 22). For *Klebsiella* spp. we have identified a more dramatic ESBL production rate, 48.4%, with the highest carbapenem resistance associated.

Overall and somehow expected we identified notable prevalence of women, accounting for 69% of all patients, even though the percentage was significantly lower in ESBL + isolates (52.8% ESBL + *vs.*74.9% ESBL -, $p<0.0001$). This can be explained by the fact that the female gender, due to its associated anatomical particularities, is considered an additional, non-modifiable risk factor for the development of UTIs, regardless of the etiological agent, but especially among urinary catheterized patients (23, 24).

UTIs represent the fourth most common healthcare-associated infection (25). Although morbidity and mortality from UTI in urinary catheterized patients are considered relatively low compared to other healthcare-associated infections, the high prevalence of urinary catheterization use leads to a significant burden on the public health systems. It is estimated that between 15% and 25% of hospitalized patients are being urinary catheterized (26, 27), and, unfortunately, in many of these cases, the catheters are placed after an inappropriate indication (28, 29). In addition, for every additional day a patient has an indwelling urinary catheter, there is a 3-7% increased risk of developing a caUTI (1, 30, 31).

In our study, we observed significant differences while analyzing the microorganisms identified among patients with urinary catheterization. Thus, the main etiology is still represented by *E. coli*, responsible for 42.8% of the cases, most of those being ESBL-strains (70.6%), while the leading etiology of ESBL + caUTI is *K. pneumoniae*, accounting for 53.4% cases, *vs.* only 20.9% for *E. coli*. Most studies classify *E. coli* as the main etiologic agent of caUTI (24, 32), although we also found studies that reported *K. pneumoniae* as the most common *Enterobacterales* isolated (33, 34). In European countries, the incidence of caUTIs caused by ESBL + *E. coli* is generally less than 5% (7,35) but can be as high as 23.6% in Spain or 38.2% in Turkey (22).

A study conducted by Albaramki et al., which examined the incidence and impact of UTIs caused by ESBL+ *Enterobacterales* in the pediatric population, also reported increased rates of resistance to amoxicillin + clavulanic acid (94. 8%), third-generation cephalosporins (98.7%), fluoroquinolones (54.5%) or gentamicin (54.5%), while resistance to amikacin (32.5%) and especially to carbapenems (1.3%) remained at significantly lower percentages (36). Also, Vachvanichsanong *et al.* reported increased rates of resistance to cephalosporins (99-100%, except for cefoxitin, to which only 19% of isolates were resistant) or aminopenicillins (99%), but with much lower rates of resistance to fluoroquinolones (44%), piperacillin+tazobactam (22%) or amikacin (11%), while none of the isolates were resistant to carbapenems (37).

Another recent study from China, which assessed the antibiotic susceptibility profile of ESBL+ *E. coli* strains isolated from patients with community-acquired UTIs, showed increased resistance rates to common cephalosporins (93.4% resistance to cefotaxime, 93.1% to ceftriaxone, 76.8% to cefepime), while to cefmetazole they identified only 2.4% resistance or 8.1% resistance to the combination of cefoperazone and sulbactam. In addition, they reported very low resistance rates to amikacin (3%), nitrofurantoin (2.7%), fosfomycin (8.4%), or carbapenems (0.3% resistance to imipenem and 0.6% to ertapenem) (22). As in our study, resistance to fluoroquinolones (72.3%) or trimethoprim+sulfamethoxazole (68.7%) was identified in increased percentages (22).

This polymorphic and dynamic pattern of resistance and the resulting therapeutic challenges represent a solid argument for the constant, further research of UTIs in this area of Europe, already characterized by high rates of resistance.

CONCLUSIONS

The incidence of UTIs caused by ESBL+ *Enterobacterales* in the Northeast region of Romania is continuously increasing, especially among elderly and female patients. We have identified significant antibiotic resistance rates, especially when compared to ESBL- isolates, the levels observed for some antibiotics representing a serious epidemiological alert.

Antibiotic resistance caused by the production of ESBLs is limiting the currently available treatment options for UTIs caused by these pathogens. Given the lack of new antibiotic molecules, there is a valid rationale for sensitivity testing of older antibiotics that retain some activity against ESBL+*Enterobacterales*. Of these, fosfomycin or nitrofurantoin emerge as promising options due to their broad spectrum of activity against both gram-positive and gram-negative bacteria. Microbiological data seem to support this possibility, as susceptibility rates for strains of *E. coli*, the main etiological agent of UTI, are very high. However, further research is needed to ascertain these findings.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding the scientific research.

ETHICAL APPROVAL

The study was approved by the Ethics Committee of "Sf. Parascheva" Clinical Hospital of Infectious Diseases, Iasi, Romania.

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POSTPARTUM HEALTH-RELATED QUALITY OF LIFE AND ASSOCIATED PERINATAL FACTORS AMONG WOMEN IN NORTH-EAST ROMANIA

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POSTPARTUM HEALTH-RELATED QUALITY OF LIFE AND ASSOCIATED PERINATAL FACTORS AMONG WOMEN IN NORTH-EAST ROMANIA (Abstract): The aim of this cross-sectional study was conducted to assess the health-related quality of life and its affecting factors among women from North-East Romania at six weeks postpartum. **Material and methods:** The study was conducted in a tertiary care hospital, at the "Cuza-Vodă" University Hospital of Obstetrics and Gynecology in Iași (Romania), from May to December 2021. The validated short-form (SF-36) questionnaire was used to assess health-related quality of life (HRQoL) of 321 women at six weeks postpartum. **Results:** The mean Physical Component Summary and Mental Component Summary scores, two summary measures that aggregate the eight most important domains of health status, were both reported higher than 50% (59.05 ± 19.48, 55.08 ± 25.17, respectively). Highest median scores were reported in body pain (69.25 ± 28.87) and physical functioning domains (65.42 ± 14.78). Lowest median scores were reported in vitality (44.57± 20.20) and general health perceptions (48.73 ± 25.99). Factors associated with higher overall HRQoL were maternal age under 30 and multiparity. Number of days of peripartum hospitalization was inversely proportional to all scales of the SF-36 questionnaire. **Conclusions:** In this study, we highlight the need for additional studies on ways to predict the onset of labor, in order to shorten the period of peripartum hospitalization of pregnant women in North-East Romania, and implicitly to improve their quality of life**.** **Keywords:** POSTPARTUM, HEALTH-RELATED QUALITY OF LIFE, SF-36.

Nowadays, the increase in life expectancy has drawn attention to the concept of quality of life. Quality of life is a complex and subjective concept, a parameter considered by the World Health Organization(WHO) as a fundamental indicator of health. In the field of health, quality of life is a multidimensional concept that addresses issues such as the physical, mental, emotional, and social functioning of an individual (1). More specifically, the quality of life in medicine means the ability of pa-

tients to perform their usual tasks in their daily lives.

Women's subjective perception of their health-related quality of life (HRQoL) is essential for measuring the quality and effectiveness of maternal and child health programs. Although pregnancy, childbirth and puerperium are considered to be physiological conditions, they involve multiple changes on the physical, emotional and social level of the woman. There is also the risk of a series of comorbidities and complications, changing the woman's quality of life. As a result, health interventions are increasingly focusing on prevention and treatment. In addition, to reduce the incidence of comorbidities and complications, maternal and fetal health professionals strive to provide quality healthcare and a satisfying experience to patients during preconception, conception, pregnancy, childbirth, and postpartum, with the ultimate goal of improving women's HRQoL.

Traditional indicators used in statistics on health and quality of life associated with pregnancy and childbirth remain essential, but mortality and morbidity are considered inappropriate and defining, as the health associated with these periods should be assessed not only on the basis of saving lives, but also in terms of improving the quality of life (2).

The following instruments have been developed to measure the quality of life of women in postpartum period: Mother-Generated Index (MGI) developed by Symon *et al. (*2003); Maternal Postpartum Quality of Life (MAPPQOL); Postnatal Morbidity Index (PNMI), Maternal Adjustments and Maternal Attitude (MAMA) (3, 4). These questionnaires include different areas, such as: socioeconomic, psychological-spiritual, physical appearance, sexuality, relationship with partner/newborn/family and symptoms. Unfortunately, none of these measuring instruments have been translated, validated and used in patient studies in Romania. In the absence of such specific instruments, researchers use generic measuring instruments. The most widely used tool for measuring the quality of life of women in pregnancy and postpartum is SF-36. SF-36 includes multi-point scales measuring the eight most important domains of health status: physical functioning (PF); physical role limitations (RP); body pain (BP); general health perceptions (GH); energy/vitality (VT); social functioning (SF); emotional role limitations (RE) and mental health (MH). These domains can be aggregated into two summary measures: the Physical(PCS) and Mental(MCS) Component Summary scores.

There are studies in literature that aimed to define the factors associated with the mode of birth and the medical care provided to the patient at birth that may influence the quality of life of women (5). Also, socio-demographic, obstetric and psychosocial factors have been identified to be strongly associated with HRQoL postpartum (2, 6, 7). However, most research has been conducted in the United States and other developed countries. In addition, quality of life is a concept affected by culture and social systems. Therefore, the results of studies in other countries are not always applicable in Eastern Europe countries. Furthermore, for our knowledge, there are no studies on how prolonged perinatal hospitalization may affect postpartum HRQoL of women.

The objective of the current study was to assess the HRQoL in women from Northeast Romania and to determine the

influence of age, number of days of perinatal hospitalization and parity, on the HRQoL of women at six-weeks postpartum. The results of this study can be useful in improving the quality of life of postpartum women in Northeast Romania.

MATERIAL AND METHODS

Designing and selecting subjects for the study

This was a cross-sectional study of health-related quality of life in women who presented themselves to the gynecologist at six weeks postpartum for the indicated postnatal gynecological examination at the Cuza-Voda University Hospital in Iasi, Romania, from May to December 2021. The inclusion criteria were age over 18 years, singleton, term birth, uncomplicated pregnancy. Exclusion criteria included: a)women with severe obstetric or medical conditions, b) fetal anomalies, c) perinatal fetal death, d) a positive SARS-CoV-2 test in the last 2 months. Eligible women were informed about the purpose of the study and signed informed consent before participation. This study was approved by the local institutional ethics board (no. 74/26.04.2021, "Grigore T. Popa" University of Medicine and Pharmacy from Iasi).

Information sources

The dependent variable for this study is HRQoL, assessed through the validated SF-36 instrument, whereas the independent variables include socio-demographic characteristics such as age, and obstetrics factors such as number of days of peripartum hospitalization and parity.

Women were informed about how to complete the questionnaire in their own time.

The SF-36 questionnaire evaluates both the physical and mental components of HRQoL, and consists of 36 question, eight domains, and two component summaries. Scores for each domain range from 0 to 100, where higher scores represent better QOL. Socio-demographic and obstetric information was extracted from the medical records of the women.

Statistical analysis

The statistical analysis was performed in *SPSS 27.0.* For the qualitative data (whose values represent codes), frequency distributions were made, calculating the absolute and percentage frequencies, and the quantitative data were characterized by calculating the mean value, the standard deviation and the median value. The differences between the scales were characterized using the non-parametric Mann-Whitney test, after previously checking whether the value distributions of the scales comply with the law of normal distribution, using the Kolmogorov-Smirnov fitting test. To investigate the association between scales and quantitative variables, we calculated the Pearson linear correlation coefficient. The significance coefficient $p < 0.05$ was considered statistically significant, and $p < 0.01$ was considered statistically high significant.

RESULTS

Socio-demographic characteristics of the group

The study group consisted of 321 patients, with a mean age of 28.17 ± 6.45 years, with the youngest patient of 18 years old and the oldest of 43. Distribution of patients by age categories was relatively balanced, in the sense that 54.2% of patients were under 30 years of age and the remaining 45.8% were of 30 years of age or older; also, the group registered a slight prevalence of the rural environment

(58.9%) compared to the urban environment (41.1%).

Most patients (60.7%) gave birth naturally, and there is a significant percentage (39.3%) of patients who gave birth by Cesarean section; just over a third (38.3%) of patients have one child, the remaining 61.7% having two to six children. In general, mothers with two (29.9%) or three children (24.3%) predominate in the group. The mean number of days of hospitalization before birth was 3.81 ± 3.21, with a maximum of 14 days, and the mean number of days of hospitalization after birth was similar, with a mean of 3.84 ± 1.49 days, a minimum of 3 days and a maximum of 10 days.

Two other significant elements for the study were the exclusive breastfeeding, practiced by most patients (60.7%) and their social status – the presence / absence of a stable relationship – also the vast majority of patients (80.4%) had stated that they were in a stable relationship.

Almost a quarter of patients, 23.4%, respectively had a family history of depression. Depressive episodes were identified in 51 cases, representing 15.9% of the total number of patients; also 36 patients (11.2%) had previous hospital admissions.

Quality of life of postpartum women at 6 weeks after birth:

It can be seen from first table that the highest values are recorded for the somatic pain score, with an average of 69.25 ± 28.87 and a range of variation between 0 and 100, followed by the physical activity function of patients, calculated at a mean value of 65.42 ± 14.78, with a range of variation between 50 and 100, and the scale of social function, with an average value of 63.31 ± 29.80 and a range of variation between 0 and 100. But on the other hand, the lowest scores are observed for vitality, with an average value of 44.57 ± 20.20 and a range of variation between 0 and 95 and for the general health scale, with an average of 48.73 ± 25.99 and a range of variation between 0 and 100.

TABLE I.

Scores of postpartum women in SF-36 domains

	Mean	Standard error of mean	Standard deviation	Median	Min	Max
Physical Function (PF)	65.42	0.82	14.78	60.00	50.00	100.00
Physical Role (PR)	52.80	2.31	41.40	50.00	.00	100.00
Somatic Pain (SM)	69.25	1.61	28.87	67.50	.00	100.00
General Health (GH)	48.73	1.45	25.99	55.00	.00	100.00
Vitality (VT)	44.57	1.12	20.20	45.00	.00	95.00
Social Function (SF)	63.31	1.66	29.80	62.50	.00	100.00
Emotional Role (ER)	54.51	2.28	40.92	66.67	.00	100.00
Mental Health (MH)	57.90	1.17	21.13	56.00	.00	100.00
Physical Health (PCS)	59.05	1.08	19.48	57.50	27.50	90.00
Mental Health (MCS)	55.08	1.40	25.17	50.45	.00	97.75

The concept of Physical Health, which aggregates the first four scales, has an average value of 59.05 ± 19.48, with a range of variation between 27.50 and 90.00. In its turn, the concept of Mental Health has an average value of 55.08 ± 25.17, with a range of variation between 0 and 97.75. Therefore, both concepts investigated have

values located in the upper half of the range of possible variation, which can be appreciated as a positive fact.

Factors influencing the quality of life of postpartum women:

The age under 30 of the patients does not significantly improve their scores from a statistical viewpoint for any of the categories investigated. However, it is found that postpartum women under 30 years of age have slightly higher scores than those over 30 years of age in terms of physical function, vitality, but also somatic pain, social function, respectively; postpartum women over 30 years of age have slightly higher scores in terms of physical role and mental health. The global concepts of physical and mental health, calculated on the basis of the 8 scores, are also slightly higher in the under-30s compared to the others, but, again, the discrepancies observed are very small (tab. II).

TABLE II.

Differences in HRQoL scores according to age (Mann-Whitney U-test)

	Age < 30 years (n = 174)		Age >= 30 years (n = 147)		p-Value†
	Mean ± SD	Median	Mean ± SD	Median	
Physical Function (PF)	66.07 ± 16.12	60.00	64.64 ± 13.02	62.50	.772
Physical Role (PR)	51.72 ± 42.12	50.00	54.08 ± 40.65	50.00	.623
Somatic Pain (SM)	69.87± 27.65	67.50	68.52 ± 30.32	67.50	.845
General Health (GH)	48.96 ± 25.46	50.00	48.46 ± 26.68	55.00	.609
Vitality (VT)	45.86 ± 23.37	45.00	43.06 ± 15.59	45.00	.145
Social Function (SF)	64.22 ± 29.02	62.50	62.24 ± 30.77	62.50	.761
Emotional Role (ER)	54.59 ± 42.42	50.00	54.42 ± 39.22	66.67	.941
Mental Health (MH)	56.89 ± 24.54	52.00	59.10 ± 16.18	60.00	.635
Physical Health (PCS)	59.15 ± 18.03	54.06	58.92 ± 21.13	57.50	.557
Mental Health (MCS)	55.39 ± 27.27	46.14	54.70 ± 25.51	56.29	.550

The Pearson correlation coefficient between patients' age and SF-36 scales/ concepts also does not show significant associations; most of the values observed are very low, almost zero, with only 2 exceptions: the general health score and the mental health score, for which there are directly proportional but weak correlations with the age of the postpartum women (tab. III).

TABLE III.

Pearson correlation coefficient between SF-36 items and maternal age

	Age			Age	
	r	p		r	p
Physical Function (PF)	.006	.913	Social Function (SF)	.033	.551
Physical Role (PR)	.080	.154	Emotional Role (ER)	.068	.224
Somatic Pain (SM)	.029	.607	Mental Health (MH)	.170**	.002
General Health (GH)	.129*	.021	Physical Health (PCS)	.097	.082
Vitality (VT)	.098	.079	Mental Health (MCS)	.093	.096

Not the same can be observed in the case of parity; only the physical function has similar values in the postpartum women with one child, as compared to those with several children, for all the other scores investigated, large and highly statistically significant differences between the 2 categories of postpartum women were observed (tab. IV). Thus, postpartum women with more than one child have much higher scores, sometimes even double, than those with only one child, for all functions investigated. The largest discrepancies are found for the physical and emotional role (almost double values), and the lowest discrepancies are observed in the case of the score of vitality and that of mental health. Both the general concept of physical health and that of mental health are also significantly increased, by approx. 25%, for postpartum women with several children compared to those with only one child.

The number of days of hospitalization before birth is inversely correlated with all the scales investigated, and statistically significant in all cases with one exception (general health scale) – which means that as the number of days of hospitalization before birth increases, the values of the scales investigated decrease. The highest correlation coefficients are found with the scale of vitality and that of mental health, but also with the global concept of mental health, and the lowest correlations are observed with the scales of somatic pain and social function, but also with the global concept. physical health (tab. V).

TABLE IV.

Differences in HRQoL scores according to parity (Mann-Whitney U-test)

	Parity: 1 child (n = 123)		Parity: > 1 child (n = 198)		p-Value
	Mean ± SD	Median	Mean ± SD	Median	†
Physical Function (PF)	65.54 ± 12.46	62.50	65.34 ± 16.08	60.00	.174
Physical Role (PR)	34.75 ± 39.54	25.00	64.01 ± 38.56	75.00	<.001
Somatic Pain (SM)	55.91 ± 29.36	55.00	77.53 ± 25.30	90.00	<.001
General Health (GH)	38.78 ± 22.55	40.00	54.92 ± 26.11	60.00	<.001
Vitality (VT)	40.73 ± 20.06	45.00	46.96 ± 19.97	47.50	.009
Social Function (SF)	49.08± 31.04	50.00	72.15 ± 25.32	68.75	<.001
Emotional Role (ER)	36.58 ± 38.27	33.33	65.65 ± 38.57	66.67	<.001
Mental Health (MH)	50.82 ± 21.22	52.00	62.30 ± 19.89	60.00	<.001
Physical Health (PCS)	48.75 ± 18.64	38.75	65.45 ± 17.13	69.06	<.001
Mental Health (MCS)	44.30 ± 25.40	43.45	61.77 ± 22.63	65.00	<.001

TABLE V.

Pearson correlation coefficient between SF-36 items and number of days of hospitalization

	No. of days of hospitalization before birth			No. of days of hospitalization before birth	
	r	p		r	p
Physical Function (PF)	.238**	<.001	Social Function (SF)	-.180**	.001
Physical Role (PR)	-.295**	<.001	Emotional Role (ER)	-.290**	<.001
Somatic Pain (SM)	-.141*	.012	Mental Health (MH)	-.325**	<.001
General Health (GH)	-.101	.071	Physical Health (PCS)	-.197**	<.001
Vitality (VT)	-.358**	<.001	Mental Health (MCS)	-.311**	<.001

DISCUSSION

Assessment of quality of life of women in postpartum period is essential for global healthcare, however only a few studies have monitored these women's HRQoL. Most studies focused on how a particular pre-existing or pregnancy-associated pathology affects a woman's quality of life during pregnancy. Studies suggest that HRQoL in pregnant women depends on socioeconomic, medical, and psychological factors(2,6,7). Low HRQoL during pregnancy is associated with an increased risk of preterm birth and restricted intrauterine growth. Among the articles on quality of life during pregnancy with associated pathologies), we observe a wide range of studied pathologies, such as depression (8), urinary tract disorders (9), diabetes (10), hypertension (11) and obesity (12). There is, however, a predominance of quality-of-life articles in pregnant women in relation to depression, representing an interest that is twice as high as for any other pathologies associated with pregnancy (6, 13, 14).

Maternal and child health professionals focus on prenatal care, while the postpartum period remains neglected, although research has been conducted in recent decades to study various aspects of postnatal care and its related QoL(15). It is therefore not surprising that the care given to the postpartum women has been criticized by them, being inadequate in meeting the needs of these women. For example, in the UK, the postpartum follow-up of the patient six weeks after birth involves gynecological examination and discussion about family planning, neglecting the patient's mental health(3). In Romania, the recommendations for the woman who has just given birth are to come as soon as possible after discharge to the medical dispensary in the territory of her home for both the patient and the newborn to be registered, and to perform a gynecological exam in six weeks postpartum. Unfortunately, due to financial problems or long distance from the maternity ward, just a few patients return to a gynecological exam.

Numerous postpartum morbidities have been reported to influence HRQoL. Asthenia or fatigue is the most common postpartum physical health problem, found in over 50% of women (16). Fatigue is associated with postpartum depression and breastfeeding problems. Another common symptom among women in postpartum period is pain located in different areas of the body. Urinary incontinence, hemorrhoids, constipation, sleep disturbances and a variety of emotional changes, such as depressive symptoms, are examples of other conditions that affect the physical and mental health of new mothers. These health problems affect not only the health of the baby, but also the wellbeing of the newborn. Therefore, inadequate postpartum supervision of the women affects the quality of life of both mothers and newborns.

Women who have just given birth face many changes with repercussions on their physical and mental status. Statistics show that in the first two months after birth, 86% of these women have physical problems and 10-12% suffer from postpartum depression (17).

The results of our study reported the mean PCS and MCS scores both higher than 50% (59.05 ± 19.48, 55.08 ± 25.17, respectively). The results are comparable with a study conducted in Kuwait, with the mean PCS and MCS scores 54.5 and 52.9 respectively but are lower than studies conducted in Spain where overall HRQoL mean score at less than one year since last

birth is 71.94 ± 17.48 (5,18). Also, the results of a study conducted in Ethiopia on 409 randomly selected post-partum women showed that the PCS Mean±SD score was higher in Mean±SD score (49.5±9.3) when compared with the MCS Mean±SD score of 40.79±10.90, but both scores were lower than our study results(19). The socioeconomic discrepancy of the study participants may be the reason for the difference between the results of these studies. A higher quality of life is favored by a higher socioeconomic standing. A further variation could be brought on by the differences in the study population. In this study we included women at six weeks postpartum, whereas the study conducted in Spain included women who gave birth in the year before and the Ethiopian study and the one conducted in Kuwait included women who gave birth in the last 6 months.

Postpartum women from Northeast Romania scored higher in physical functioning, bodily pain and social functioning. The lowest scores were observed in the vitality and general health perceptions dimensions. Our findings are similar to Spanish study results, where the score of vitality dimension was the lowest of the scores of the eight dimensions and the score of physical functioning was the higher of the scores.

There are studies that have tried to define the factors associated with the birth and health care of peripartum women, which may influence the quality of life of these women in postpartum period. Therefore, one of the factors that affects the quality of life after childbirth is the delivery method(5). According to the results of the Spanish study, Cesarean section is a factor that negatively influences postpartum QoL.

Based on findings from similar research that were considered as particular predictors of HRQoL in postpartum women, we used several simple predictors as the input variables. Thus, maternal age was shown to have an association with HRQoL; the mean PCS and MCS scores are higher in women under 30 years of age. However, the results are contrary to the Ethiopian study according to which women who were in the age group 17-24 were more likely to have lower level HRQoL. This may be due to the tendency for women to become pregnant later in their reproductive life (20). Also, in the current finding maternal age was directly proportional to general health perceptions and mental health.

Another putative factor studied in this research was parity. Our results showed that multiparity positively influences scores. The more children the woman has, the higher the scores. In our study, the PCS and MCS scores were at least 25% higher in multiparous women. Other studies found that multiparity was negatively related to mental health (21, 22).

Number of days of peripartum hospitalization is another factor that showed a statistical association with postpartum HRQoL so such the longer the duration of peripartum hospitalization, the more negatively affected the postpartum HRQoL. In our study the number of days of hospitalization was inversely proportional to all scales of the SF-36 questionnaire. The most affected domains were vitality and mental health. The minimum length of peripartum hospitalization is controversial. In the USA and Western countries, the pregnant woman is hospitalized when the labour is onset, when the patient has regular painful uterine contractions associated with progressive changes of the cervix and dilation. But on the other hand, considering the fact that many of the

pregnant women in Romania come from rural areas, from localities far from a maternity hospital, where they cannot benefit from specialized medical help in case the birth is imminent, the decision not to hospitalize a patient with irregular uterine contractions involves many risks assumed by the obstetrician. He cannot predict how the patient's contractions will progress, as well as the moment of labour onset. Thus, many of the patients with a diagnosis of prelabour, "false labour" or latent phase of labour, remain hospitalized for long periods, until birth, increasing the costs of maternity, and the risk of their quality of life being affected in postpartum period.

Also, in the USA, the mother and her newborn are discharged at 24-48 hours postpartum, regardless of the mode of birth (vaginal delivery or C-section), while in Romanian maternities, protocols suggest discharging them at minimum 72 hours from birth. The hospitalization is extended until the medical team that attended the birth considers the mother and the newborn out of any danger of medical complication. Thus, peripartum, women in Romania spend more time hospitalized compared to women in other countries, and the implications on the quality of life of these women cannot be overlooked. Again, we emphasize that the results of studies on the quality of life of women from other countries in postpartum period, do not define the quality of life of Romanian women in postpartum period.

The existing literature demonstrates the association between prolonged hospitalization and a low quality of life after discharge in various categories of patients. For example, studies showed that women with a high-risk pregnancy who are hospitalized for a long-time during pregnancy have a higher risk of impaired mental and physical health compared to pregnant women hospitalized peripartum for the minimum period of time required for childbirth and recovery (23). Indeed, the fact that the patient's pregnancy is classified as a high-risk pregnancy may affect the woman's mental health (24). HRQoL after discharge was also investigated in elderly patients who required prolonged hospitalization. Among them, a low quality of life was found as compared to people of the same age who did not need hospitalization(25). Of course, these categories of patients have been hospitalized for a long period of time (weeks or even months), while after birth, women in Romania spend an average of 3 days in hospital. However, every day away from the family, in a new, unknown environment, affects the quality of life of these women.

CONCLUSIONS

This study identified maternal age under 30 and multiparity to be associated with higher postpartum HRQoL of women from Northeast Romania. Also, the longer the peripartum hospitalization period, the lower the mental and physical health at six weeks postpartum.

In this regard, we highlight the need for additional studies on ways to predict the onset of labour, in order to delay the admission of term pregnant women and shorten the hospitalization period of those hospitalized (26). Implicitly, the use of biomarkers with a predictive role in determining the moment of the onset of labour would have as repercussions the increase of the quality of life of Romanian women in postpartum period.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

GUT MUCOSA MICROBIOTA IN ULCERATIVE COLITIS: RELAPSE AND TREATMENT RESPONSE IN ADULTS

A longitudinal study in UC investigates whether the mucosal gut microbiota is associated with relapse events and treatment response, by means of RNA-based 16S rRNA analysis which detects not only live and active microbes but also dead and dormant microbes. 51 adult patients (age>20) were included in this study with mean age of 45, between 2012 and 2017 and followed up through 2020; patients who had taken antibiotics within 3 months before biopsies and with primary sclerosis cholangitis were not enrolled in this study; no patients enrolled developed colorectal cancer during the follow-up period. Colon mucosal biopsy were obtained at enrollment and 16S rRNA sequencing was performed. 24 patients were in remission of which 7 developed relapses during follow-up and 27 in active UC which were classified into response group (n=6), refractory group (n=13) and non-response group (n=8) according to their treatment response in 6 months. Results**:** no difference in the gut microbiota composition between rectum and colon; the proportion of Bacteroides decreased as the severity of mucosal inflammation increased; unexpectedly Bacteroides was significantly lower in the non-relapse group than in the relapse group; secondly, the proportion of Enterobacteriaceae increased as the mucosal inflammation increased; in the active period a high proportion of Enterobacteriaceae was resistance to any treatments; the proportion of Clostridiales was higher in the non-relapse group whereas Prevotella was higher in the response group. In conclusion, gut microbiota composition can predict treatments strategies and clinical course of UC in terms of the relapse rate during the remission period and intractability during the active phase. (Nishihara Y, Ogino H, Tanaka M, *et al.* Mucosa-associated gut microbiota reflects clinical course of ulcerative colitis. *Sci Rep* 2021; 11: 13743 / https://doi.org/10.1038/s41598-021-92870-0).

VIRAL B AND C HEPATITIS IN RHEUMATIC PATIENTS UNDER BIOLOGICAL THERAPY

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VIRAL B AND C HEPATITIS IN RHEUMATIC PATIENTS UNDER BIOLOGICAL THERAPY (Abstract): Both rheumatic immune-inflammatory diseases and viral hepatitis represent important health problems worldwide. Modern therapies with biological and targeted synthetic disease-modifying antirheumatic drugs (b/tsDMARDs) have revolutionized the management of immune-inflammatory diseases. However, these therapies have a strong immunosuppressive impact, patients being at risk of reactivation of viral hepatitis. The aim of this study was to assess the prevalence and the risk of reactivation of B and C hepatitis (HBV, HCV) in rheumatic patients who are treated with b/tsDMARDs for rheumatoid arthritis (RA) and ankylosing spondylitis (AS). **Material and methods:** We retrospectively analyzed all patients with RA and AS receiving b/tsDMARDs therapies in the Rheumatology department over a one-year period. Serological screening for HBV infection (HBsAg, anti-HBc, anti-HBs) and HCV antibodies was performed in all patients. For each patient we reviewed the diagnosis, demographic data, type of the treatment, HBV and HCV serology, viral infection treatment. **Results:** There were 132 patients diagnosed with RA and 90 patients diagnosed with AS receiving therapy with bDMARDs or tsDMARDs. 65 of the patients with RA (49.24%) and 32 of those with AS (35.55%) presented changes in viral markers. Both in the group of patients with RA and those with AS, approximately 10% of patients showed previous B vaccination. Only 2.27% of the patients with RA and 1.11% of those with AS presented chronic B infection. Most of the patients (36.36% RA, 24.44% AS) had resolved B infection. Inactive HCV infection was found in 2.25% of total number of patients. A percentage of 12.12% among those with RA and 3.33% of those with AS received treatment with Entecavir/Tenofovir. There were no cases of hepatitis reactivation. **Conclusions:** Viral B infection has a high prevalence in patients with RA and AS. Screening for chronic viral B and C infections should be done in all patients with autoimmune rheumatic diseases. Treatment with nucleotide/nucleoside analogs is effective in preventing HBV flare regardless of the type of b/tsDMARDs. All patients with viral C infection should be treated with direct-acting antivirals. **Keywords:** VIRAL HEPATITIS, RHEUMATOID ARTHRITIS, ANKYLOSING SPONDILITIS, BIOLOGICAL THERAPY.

Both rheumatic immune-inflammatory diseases and viral hepatitis represent important health problems worldwide. Chronic hepatitis B (HBV) and C virus (HCV) infection are major causes of morbidity and mortality, leading to cirrhosis, hepatocellular carcinoma, and liver transplantation (1). The prevalence of hepatitis varies between populations, and even in areas with low endemic levels it is necessary to identify those whose current or previous infection could influence their clinical outcome (2). It is estimated that approximately 1 in 3 individuals in this world may have been exposed to HBV infection (3,4), and 110 million persons are HCV antibody-positive, with 80 million viremic HCV infection (5).

The treatment of patients with rheumatoid arthritis (RA) and ankylosing spondylitis (AS) is aimed at relieving symptoms, improving physical function and quality of life, and preventing structural damage (6). Pharmacological options include (6,7):

- conventional synthetic (cs) disease-modifying antirheumatic drugs (DMARDs) such as Methotrexate, Leflunomide, Sulfasalazine;
- biological (b) DMARDs, including tumor necrosis factor (TNF) inhibitors (Adalimumab, Etanercept, Infliximab, Certolizumab, Golimumab), T-cell stimulation modulators (Abatacept), interleukin-6 (IL-6) receptor antagonists (Tocilizumab), interleukin-1 (IL-1) inhibitors (Anakinra) , interleukin-17 (IL-17) inhibitors (Secukinumab) and anti-CD20 antibodies (Rituximab);
- targeted synthetic (ts) DMARDs, such as Janus kinase (JAK) inhibitors (Tofacitinib, Baricitinib and Upadacitinib).

The new therapies (b/ts DMARDs) have revolutionized the management of rheumatic immunoinflammatory diseases. However, these therapies have a strong immunosuppressive impact. Patients receiving this treatment have the risk of reactivation of viral hepatitis - especially reactivation of HBV infection (HBVr) (8, 9). Recent guidelines advice to perform a complete hepatitis status screening before starting biologic therapy (2, 8, 10). This involves testing for four serological components of hepatitis infection: hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), hepatitis B core antibody (anti-HBc) and hepatitis C antibody (HCV) (2). HBsAg indicates the presence of acute or chronic hepatitis B infection, anti-HBc antibodies may be present in acute, chronic or resolved infection, anti-HBs antibody may indicate resolved infection, or vaccination if found alone (2,11). Before the initiation of biological therapy, antiviral therapy is recommended for positive HbsAg patients, while for negative HbsAg, positive anti-HBc patients recommendations of the guidelines are not uniform, varying between monitoring and treatment. Anti-HBV drugs with a high resistance barrier, such as entecavir or tenofovir, should be preferred over low-barrier agents such as lamivudine (12).

Although all rheumatologists should be aware that the immunosuppression associated with b/tsDMARDs can provoke HBVr, with a significant morbidity and mortality of susceptible patients, screening and treatment practices for B and C viral infections are extremely varied worldwide (13). In addition, the risk of reactivation of viral infections under new b/tsDMARDs other than anti-TNF agents is incompletely known.

The aim of this study was to assess the prevalence and the risk of reactivation of B and C hepatitis in rheumatic patients who are treated with b/tsDMARDs for RA and AS.

MATERIAL AND METHODS

Study design and patients

We retrospectively analyzed all patients with RA and AS receiving b/tsDMARDs therapies in the Rheumatology department (a tertiary referral care center), over a one-year period, between 1st September 2021 and 1st September 2022. Data among these patients were screened from the first b/tsDMARDs administration to the last visit to our hospital. Data were collected from the Romanian Registry of Rheumatic Diseases. This study was performed in accordance with the ethical principles of the Declaration of Helsinki. All patients have signed inform consent before therapy initiation.

Diagnosis of RA and AS

Diagnosis of chronic rheumatic diseases was established according to criteria recommended by international guidelines. The diagnosis of RA was based on the 1987 American College of Rheumatology criteria (14) or 2010 American College of Rheumatology/European League Against Rheumatism collaborative initiative (ACR/EULAR) classification criteria (15). The diagnosis of AS was established according to the modified New York 1984 criteria (16).

Diagnosis of HBV and HCV infections

Serological screening for HBV infection (HBsAg, anti-HBc, anti-HBs) and HCV antibodies was performed in all patients. Based on the results of the screening tests, patients were categorized to the following groups:

1. Non-modified serological markers (HBsAg-, anti-HBc-, anti-HBs-, anti-HCV);
2. Previous B vaccination (HBsAg-, anti-HBc-, anti-HBs+);
3. Chronic B infection (HBsAg+, anti-HBc+, anti-HBs-);
4. Resolved HBV infection (HBsAg-, anti-HBc+, anti-HBs±);
5. HCV infection (anti-HCV+).

According to the Romanian protocol for the treatment of viral B infection in patients under immunosuppressive treatment (17), all patients with positive HBsAg, as well as those with negative HBsAg, positive anti-HBc and anti-HBs <10 mIU/mL received prophylactic treatment with nucleoside/nucleotide analogues (Entecavir/ Tenofovir).

Data collection. For each patient we reviewed the diagnosis, demographic data, type of the treatment, HBV and HCV serology, viral infection treatment.

RESULTS

Baseline characteristics of the patients

There were 132 patients diagnosed with RA and 90 patients diagnosed with AS receiving therapy with bDMARDs or tsDMARDs. In the group of patients with RA, the majority were female, and the average age was 62.68 years. In the group of patients with AS, the majority were male, the average age being lower than in the group of patients with RA. We note that patients with AS mostly received anti-TNFα treatment (according to the Romanian treatment protocol), only 4.44% being treated with anti-IL-17. In the group of patients with RA, the majority received anti-IL-6 treatment, followed by those with anti-TNFα, but also with JAK-kinase inhibitors in a close percentage (tab. I).

TABLE I.
Baseline characteristics of the study group

Parameter	RA (n=132)	AS (n=90)	Total (n=222)
Sex M	34 (25.75%)	66 (73.33%)	100 (45.04%)
F	98 (74.24%)	24 (26.66%)	122 (54.95%)
Medium age	62.68±9.42	51.15±14.31	58.84±12.53
Treatment			
Anti-TNF	41 (31.06%)	86 (95.55%)	127 (57.20%)
Infliximab	4 (3.03%)	9 (10%)	13 (5.85%)
Adalimumab	15 (11.36%)	33 (36.66%)	48 (21.62%)
Etanercept	16 (12.12%)	28 (31.11%)	44 (19.81%)
Certolizumab	2 (1.51%)	5 (5.55%)	7 (3.15%)
Golimumab	4 (3.03%)	11 (12.22%)	15 (6.75%)
Anti-interleukins	47 (35.60%)	4 (4.44%)	51 (22.97%)
Tocilizumab	47 (35.60%)	-	47 (21.17%)
Secukinumab	-	4 (4.44%)	4 (1.80%)
JAK inhibitors	38(28.78%)		38 (17.11%)
Tofacitinib	17 (12.87%)	-	17 (7.65%)
Baricitinib	16 (12.12%)	-	16 (7.20%)
Upadacitinib	5 (3.78%)	-	5 (2.25%)
Anti-B-cell agents			
Rituximab	6 (4.54%)	-	6 (2.70%)

The presence of markers of B and C viral infections

65 of the patients with RA (49.24%) and 32 of those with AS (35.55%) presented changes in viral markers. Both in the group of patients with RA and those with AS, more than half did not show any change in viral markers. Regarding vaccination, both in the group of patients with RA and those with AS, approximately 10% of patients (13 of those with RA and 9 of those with AS) showed positive anti-HBs antibodies, these being considered subjects immunized by vaccination.

Most of those who presented changes in the serological markers were those with resolved HBV infection (48 patients with RA and 22 patients with AS). Among them, in the case of patients with RA, 35 subjects (26.51% of total number of patients) presented anti-HBc+ and anti-HBs+, meaning spontaneous seroconversion. They did not require prophylactic treatment. 13 patients (9.84% of total number of patients) presented only anti-HBc+, without protective titer of anti-HBs antibodies. All these patients received treatment with Entecavir/ Tenofovir. In the case of patients with AS, approximately 20% of total number of subjects presented spontaneous seroconversion, with protective titer of anti-HBs. Two patients showed anti-HBc+, anti-HBs- and they also received treatment with Entecavir.

Only two of the patients with RA and one patient with AS presented HBsAg+, anti-HBc+, anti-HBs-, these being classified as patients with chronic B infection. These patients also received antiviral treatment with NAs resulting a total of 16 patients among those with RA (12.12%) and 3 (3.33%) of those with AS being treated with Entecavir / Tenofovir.

Among the patients with RA, 4 subjects

showed positive anti-HCV, 2 of them associated with resolved HBV infection, one with chronic B infection, and one with no changes in serology for hepatitis B. Also, one patient with AS associated inactive HBV + HCV infection. All five patients had sustained virologic response to HCV treatment (tab. II).

TABLE II.

The presence of markers of B and C viral infections

Type of viral infection	RA	AS	Total
Non-modified serological markers (HBsAg-, anti-HBc-, anti-HBs-, anti-HCV-)	67 (50.75%)	58 (64.44%)	125 (56.30%)
Previous B vaccination (HBsAg-, anti-HBc-, anti-HBs+)	13 (9.84%)	9 (10%)	22(9.90%)
Chronic B infection (HBsAg+, anti-HBc+, anti-HBs-)	3 (2.27%)	1(1.11%)	4 (1.80%)
Resolved HBV infection (HBsAg-, anti-HBc+, anti-HBs±)	48 (36.36%)	22 (24.44%)	70 (31.53%)
HCV infection (anti-HCV+)	4 (3.03%)	1 (1.11%)	5 (2.25%)

Viral infection reactivation

Among the patients with chronic B infection, half were treated with anti-TNFα and half with JAK-inhibitors. Most of the patients with resolved HBV infection were treated with anti-TNFα (47.14%), followed by anti-IL-6 treatment (34.28%) and JAK-inhibitors treatment (12.85%). Among the patients with HCV infection, 80% were under anti-TNFα treatment and one patient under JAK-inhibitors treatment (tab. III).

There was no case of hepatitis reactivation during the follow-up period.

TABLE III.

Relationship between the type of treatment followed and the B and C infection markers.

Type of treatment	Chronic B infection	Resolved HBV infection	HCV infection
Anti-TNFα	2 (50%)	33 (47.14%)	4 (80%)
Anti-IL-6	0 (0%)	24 (34.28%)	0 (0%)
Anti IL-17	0 (0%)	1 (1.42%)	0 (0%)
JAK-inhibitors	2 (50%)	9 (12.85%)	1 (20%)
Anti-CD20 antibodies	0 (0%)	3 (4.28%)	0 (0%)

DISCUSSION

Chronic viral hepatitis B and C represent a real public health problem. Despite the fact that they can be asymptomatic for many years, they have the potential to progress to liver cirrhosis, hepatocarcinoma and death. In immune rheumatic diseases, such as RA and AS, there is an increased risk of activation of viral infections as a result of immunosuppressive treatment.

It is considered that HBV and HCV infection have the same prevalence in rheumatic diseases patients as in the general population. In these patients the prevalence of positive HBsAg is estimated at 3-3.5%, past HBV infection between 13-50% and anti-HCV positive 1.2% (18,19). The prevalence data from our study (HBsAg was found in 1.80% of the patients, past HBV infection in 31.53% and anti-HCV positive in 2.25% of the patients) are similar to the data from the literature. In our study, we recorded a higher prevalence of HBV infection markers in patients with RA com-

pared to those with AS, a fact also reported in other studies in the literature (20). Most patients had the profile of negative HBsAg/positive anti-HBc/positive anti-HBs; only 4 patients had positive HBsAg. It should be mentioned that in Romania the complete screening of HBV infection (HBsAg, anti-HBc, anti-HBs) is mandatory for all patients who initiate b/tsDMARDs. In the USA, for example, only 1/3 of the patients of the national Rheumatology Informatics System for Effectiveness (RISE) registry have the full screening done (21). In Mohareb's study (22), only 29% of the patients who initiated tocilizumab and 24% of those who initiated tofacitinib had complete screening for HBV infection.

There is evidence regarding the risk of reactivation of viral B infection under biological therapy (23,24). The reactivation rate of HBV infection in patients with rheumatological diseases seems to be lower compared to data from oncology or hematology: only 1.4% in the meta-analysis performed by Moghoofei (25). However, in patients with B viral infection treated with anti-TNF agents, who did not receive prophylactic antiviral therapy, reactivation of HBV infection occurred in up to 75% of patients (19). The risk of reactivation of HBV infection is much lower in HBsAg negative/anti-HBc positive patients: 1.7% in Lee meta-analysis (26).

In Romania, the protocol of the National Health Insurance House (17) stipulates that all patients who initiate immunosuppressive therapy or chemotherapy should be screened for markers of HBV infection. All HBsAg - positive or anti-HBc positive subjects (without positive anti-HBs) will initiate therapy with nucleotide/nucleoside analogues during immunosuppressive therapy and 6 months after stopping it. Patients with negative HBsAg, positive anti-HBc and positive anti-HBs (> 10 mIU/mL) will be closely monitored at 3 months. Following the national protocol, all HBsAg positive or anti-HBc positive patients (without anti-HBs positive antibodies) benefited from prophylactic antiviral treatment with Entecavir or Tenofovir before starting b/tsDMARDs. During the studied period, we did not register a single case of reactivation of the B viral infection. Many authors have shown that the prophylactic treatment with analogues is effective for preventing HBV flare both in HBsAg positive and in anti-HBc positive patients (23). The lack of reactivation of viral B infection in our study is explained by the prophylactic treatment of all patients with HBV markers, including those with negative HBsAg, which is different from most other countries. Current practice in most countries involves - in the case of patients with resolved HBV infection - only monitoring without initiation of antiviral treatment (27).

Data from the literature regarding the risk of reactivation of HBV infection under the new biological and targeted synthetic therapies are poor, given that HBV positive patients were excluded from the pivotal studies. Rituximab, an anti-CD20 agent, is known from oncology-hematology for the increased risk of reactivation of viral B infection. In rheumatic diseases this risk seems to be lower (2%) (19). The data on Abatacept, interleukin inhibitors and oral targeted therapies (JAK inhibitors) regarding the risk of HBV reactivation come from small, retrospective studies or case reports. Lin's meta-analysis (28) showed the same risk of reactivation of HBV infection regardless of the type of b/tsDMARDs used: anti-TNF, other biological, non-biological therapies. It is generally accepted that the risk and management of patients is similar in the new b/tsDMARDs therapies to that of anti-

TNF agents. A significant proportion of patients included in our study were treated with non-anti-TNF biological agents and JAK inhibitors. Our study showed that prophylaxis with nucleoside/nucleotide agents is also effective in the case of these new therapies in RA and AS.

According to the Romanian protocol, patients with anti-HBs over 10 mIU/mL did not initiate prophylactic treatment with nucleotide/nucleoside analogs. Indeed, data from the literature reveal a low risk of HBV reactivation in patients with positive anti-HBs, directly proportional to their titer (29,30). However, Tien's study (31) demonstrated that there is still a low risk of HBV reactivation in patients with low anti-HBs titers (<100 mIU/mL), which explains the monitoring of these patients. The loss of anti-HBs in patients with rheumatic diseases under treatment with b/tsDMARDs is greater in patients who associate chronic kidney disease (32).

We note a small percentage of patients vaccinated against B virus (9.90%). This revealed deficiencies in the management of these patients. An explanation can be the increased average age of the studied group, taking into account that in Romania mandatory B vaccination at birth only started in 1996. In Romania, a country with a high prevalence of viral hepatitis B, ideally all patients with RA and AS should be screened for markers of HBV infection, right from diagnosis. If they are negative, the complete vaccination schedule is indicated.

Regarding the C virus, there are no special recommendations related to immunosuppressive therapy, the risk of reactivation of the viral infection being reduced. In Patel's study (33), RA and HCV patients had higher rheumatic disease activity scores and were more likely to be treated with cortisone or biologics. Considering the hepatotoxicity potential of b/tsDMARDs, treatment with direct-acting antivirals is ideally recommended before starting immuno-suppressive treatment. Some recent data in the literature suggest improvement in RA activity scores after achieving a sustained viral response in patients with C virus treated with direct-acting antivirals (34). Italian group for the Study and Management of the Infections in patients with Rheumatic diseases (36) recommends screening for HCV infection and treatment with direct-acting antivirals for all patients, ideally before starting treatment with DMARDs. Anti-TNF agents can be administered concurrently with DAA therapy. In the case of patients in whom viral eradication cannot be done, DMARD therapy is not contraindicated, but liver function must be carefully monitored. In our studied group there were only 5 cases of viral C infection, all 5 having sustained viral response after oral antiviral treatment.

The limitations of our study are the short follow-up period (one year), the retrospective nature and the lack of evaluation of all parameters related to the viral infection (viremia level, hepatic fibrosis staging). However, it is a study conducted in a tertiary rheumatology center, which included a large number of patients, brought new epidemiological data (unknown in Romania), and analyzed patients with various biological and JAK inhibitors.

CONCLUSIONS

Viral B infection has a high prevalence in patients with RA and AS, most patients being negative HBsAg, positive anti-HBc, positive anti-HBs. Screening for chronic viral B and C infections should be done in all patients with autoimmune rheumatic diseases, right from the moment of diagnosis. Treatment with nucleotide / nucleoside

analogs is effective in preventing HBV flare regardless of the type of b/tsDMARDs. All patients with viral C infection should be treated with direct-acting antivirals. Additional efforts are needed so that all patients with autoimmune rheumatic diseases, likely to receive immunosuppressive treatment throughout their lives, should be vaccinated against the B virus.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest, and no funding regarding this scientific research.

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FIRST-LINE MEROPENEM ANTIBIOTIC THERAPY IN ACUTE PERITONITIS SECONDARY TO COLIC PERFORATION. CLINICAL AND HISTOPATHOLOGICAL STUDY ON THE LABORATORY ANIMAL

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FIRST-LINE MEROPENEM ANTIBIOTIC THERAPY IN ACUTE PERITONITIS SECONDARY TO COLIC PERFORATION. CLINICAL AND HISTOPATHOLOGICAL STUDY ON THE LABORATORY ANIMAL (Abstract): Acute peritonitis accounts for 1% of all on-call visits and is the second leading cause of sepsis worldwide. Annually, due to secondary peritonitis, it is estimated that 20 million years of life are lost, 25 million years are adjusted for disabilities and 896,000 deaths. Fecal peritonitis is one of the most common causes of intensive care admissions with a mortality rate between 19.1 and 31.6% in some centers exceeding 50%. **Material and methods:** Taking into account the above, we imagined a study, using laboratory animals, in which we produced sigmoid perforations, then followed their evolution without antibiotic treatment and under antibiotic therapy with Cefuroxime 25 mg/kg/24 hours intravenously or Meropenem 40 mg/kg intravenously, following the tissue changes both macroscopically and microscopically. **Results:** Our study revealed a mortality rate of 3.33%, the death occurring within 72 hours from the time of perforation. By observing the general condition (clinical point of view), no major differences between batches were found, neither from a macroscopic point of view (inspection of the peritoneal cavity). After microscopic analysis of the peritoneum and sigmoid colon meropenem treatment showed remarkably better results, especially at T4. **Conclusions:** Meropenem is a first-line antibiotic in acute peritonitis secondary to colonic perforation, which together with surgery can reduce the morbidity and mortality of this pathology. **Keywords:** PERITONITIS, DIGESTIVE PERFORATION, MEROPENEM, CEFUROXIME, MICROSCOPY.

Acute peritonitis accounts for 1% of all on-call visits and is the second leading cause of sepsis worldwide (1). Annually, due to secondary peritonitis, it is estimated that 20 million years of life are lost, 25 million years are adjusted for disabilities and 896,000 deaths (2).

Fecal peritonitis is one of the most common causes of intensive care admission for with a mortality rate between 19.1 and 31.6% (3) in some centers exceeding 50% (4), the average time of hospitalization in intensive care being about 5 - 12 days (5, 6). The most important predictors of mortality in fecal peritonitis are acute renal failure, acute heart dysfunction, elevated APACHE II scores, hypothermia, advanced age, and elevated lactate levels (3, 5, 7). The main causes of fecal peritonitis are colonic perforations (8), especially perforated diverticulitis and anastomosis dehiscence (3), with surgical treatment in the foreground, respecting three goals: removal of the septic source, excision of necrotic tissue and drainage of purulent collections (9), accompanied by appropriate intensive care measures and good pre- and postoperative antibiotic therapy. Good detection of the microbial factor involved, and the administration of effective antibiotic therapy can reduce the average number of days of hospitalization in intensive care from 12 to 6.6 days (6), thus reducing the costs by over 65,000,000 USD (6).

At present, inadequate antibiotic therapy is administered in approximately 20% of cases of septic shock, leading to increased mortality and morbidity (10).

In view of the above, we imagined a study using laboratory animals, in which we produced perforations of the sigmoid colon, then we followed their evolution without antibiotic treatment and under antibiotic therapy with Cefuroxime 25 mg/kg/24 hrs. intravenously (11) or Meropenem 40 mg/kg intravenously (12), following at certain times the tissue changes of the affected organ and of the peritoneum both macroscopically and microscopically.

MATERIAL AND METHODS

The study was done at the National Research Institute for Medical-Military Development "Cantacuzino", Băneasa Department (Romania), Preclinical Testing Unit, according to the rules of the Organization for Economic Cooperation and Development (OECD) in good laboratory practice(GLP), the Romanian laws and the internal protocols, with the authorization of the Veterinary Authority and the Ethics Committee of the "Cantacuzino" Institute. We used the Wistar strain rats, aged between 14-16 weeks and with body weights between 150-300 g at the beginning of the study. We used 35 animals, both female and male, healthy young adults, who had not been involved in previous tests. The rats were bred by the Băneasa Animal Breeding Department of the "Cantacuzino" Institute, and the identification of the laboratory animals was done individually. The acclimatization of the animals lasted eight days and was carried out under the same conditions as the study. The animals were housed in individual cages, identified by a label indicating the species, strain, age, sex, identity of the animals, group, cage number, project acronym, and experimental procedure, according to the protocols. The cages were made of polycarbonate and complied with the surface area established by the rules in force. The animals were kept at a temperature of 18-24°C, with a relative humidity of 30-70%, ensuring a light and dark cycle of 12 hours. The animals were fed standard granulated food and drinking water, ad libitum. The

feed and water did not contain any known contaminants to interfere with the results of the experiment. The test lab and pet rooms were also restricted areas.

The animals were divided into four groups (A, B, C, D). Group A, consisting of 5 rats, represented the control group, and the 10 rats in each group - B, C and D were perforated in the sigmoid colon, which was defined as T0. General anesthesia of the animals was performed with a mixture of Ketamine (0.35 mg/kg body weight) + Aceprosamine (0.3 mg/kg body weight) + O2 with Isoflurane. Postoperatively, the animals in groups B, C and D received treatment with Ketoprofen, and antibiotic therapy was administered as follows: group B did not receive antibiotic treatment, group C received antibiotic treatment with Cefuroxime, and group D received treatment with Meropenem.

Four points of time were defined: T0 - the time when the sigmoid perforation was produced (all subjects from the group A were sacrificed), T1 - 24 hours postoperatively, T2 - 48 hours postoperatively, when some of the subjects (5 from each group B, C, D) were sacrificed and histopathological samples were collected, T3 - 72 hours postoperatively and T4 - 96 hours postoperatively, at which time the subjects were sacrificed and histopathological samples were collected.

At time T0, in the case of all subjects in group A, a median incision was made through which it entered the peritoneal cavity, and then a sigmoid colon resection was performed (fig. 1a) and a sample from the parietal peritoneum. The samples were processed and stained with Hematoxylin-Eosin (HE), then analyzed using an optical microscope (OM). After resection of the sigmoid colon and the sample from the parietal peritoneum, subjects in group A were euthanized, according to internal euthanasia procedures, with an overdose of anesthetics. In the case of groups B, C and D, a median incision was made in the upper half of the abdomen through which it entered the peritoneal cavity, and intraperitoneally an incision was made in the sigmoid colon, about 0.5 cm (fig. 1b).

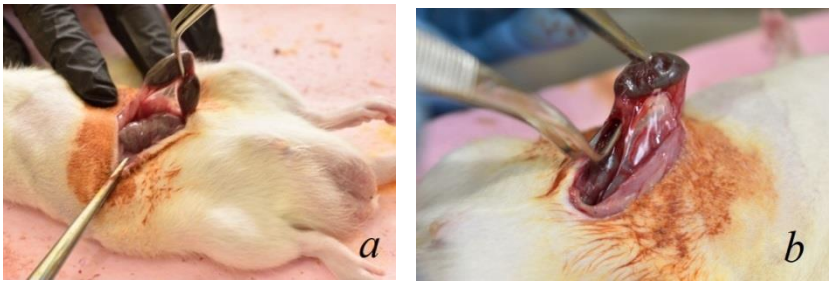

Fig. 1. a) Penetration into the peritoneal cavity and isolation of the sigmoid colon; b) Colon perforation at time T0

The sigmoid was carefully handled to prevent further intraperitoneal damage. After making the incision at the level of the sigmoid, a parietoraphy was performed in 2 planes (suturing the musculo-aponeurotic layers and suturing the skin) with a suture type with polypropylene 0 thread. The postoperative wound was cleaned, and the subjects were placed in their cages. Please note that no problems were encountered during the proceedings. Following the procedures from time T0 in all 3 groups, B, C

and D, each subject was monitored until returning from anesthesia. In the following days, each live animal received anti-inflammatory treatment (Ketoprofen 3 mg/ kg body weight, administered intramuscularly), the antibiotic treatment is administered as follows: in the case of subjects in group B no antibiotic was administered, in the case of subjects in group C it was administered Cefuroxime 25 mg / kg / 24 hrs. intravenously, and Meropenem 40 mg/kg intravenously was administered to subjects in group D. Throughout the study, the animals received food and water at their discretion.

At time T2, we intended to randomly select five subjects from groups B, C and D, which entered the peritoneal cavity by removing the sutures from the parietoraphy, in order to observe local macroscopic pathological changes, and after resection sigmoid colon and a sample from the parietal peritoneum, The samples were to be subjected to a histopathological examination to detect microscopic pathological changes.

At time T4, we intended that the remaining five subjects in each group (B, C, D) be subjected to procedures similar to those in T2, in order to observe the evolution of acute peritonitis in dynamics.

RESULTS

At time T0 we microscopically analyzed the sigmoid (fig. 2) and peritoneum resected from subjects in group A (control group), in which no perforation was produced in the digestive tract. No macroscopic and microscopic pathological changes were found.

From a clinical point of view, the subjects from all 3 groups showed favorable evolutions, being registered only one death at the level of group D at the time of T3. Subjects in all 3 groups showed good general condition and weight gain, except for subjects in group B who at time T2 showed a slight decrease in average weight. Thus, in the case of group B at time T0, the average weight was 219.52 grams, at time T2 218.84 grams, and at time T4 237.48 grams. In the case of group C at the time of T0, the average weight was 197.02 grams, at the time of T2 205.43 grams, and at the time of T4 214.98 grams, and in the case of group D the average weight was 247.94 grams, at time T2 256.46 grams, and at time T4 270.63 grams.

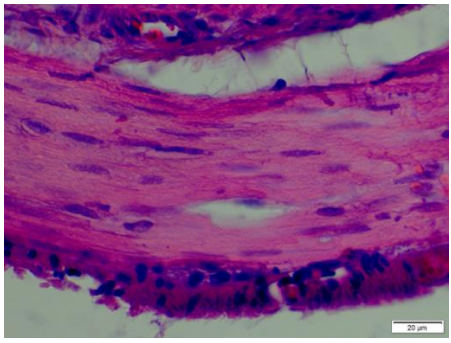

Fig. 2. Normal sigmoid colon (Group A)

At 48 hours after the production of the sigmoid perforation, at the time of T2, according to the planning, we randomly selected 5 subjects from groups B, C and D.

Macroscopically, when entering the peritoneal cavity, it was found in the case of group B the presence of a minimal amount of intraperitoneal fluid with serocitrin appearance, slightly relaxed colic, (fig. 3a) sigmoid perforation covered by intensely adherent intestinal loops (fig. 3b). By comparison, the subjects from group C showed an unfavorable evolution, intraperitoneally presenting both intestinal loops and a relaxed, edematous colic frame (fig. 3c) covering the perforation from which fecal matter is externalized (fig. 3d). Regarding group D, no significant changes were found compared to subjects from group B (figs. 3e, 3f).

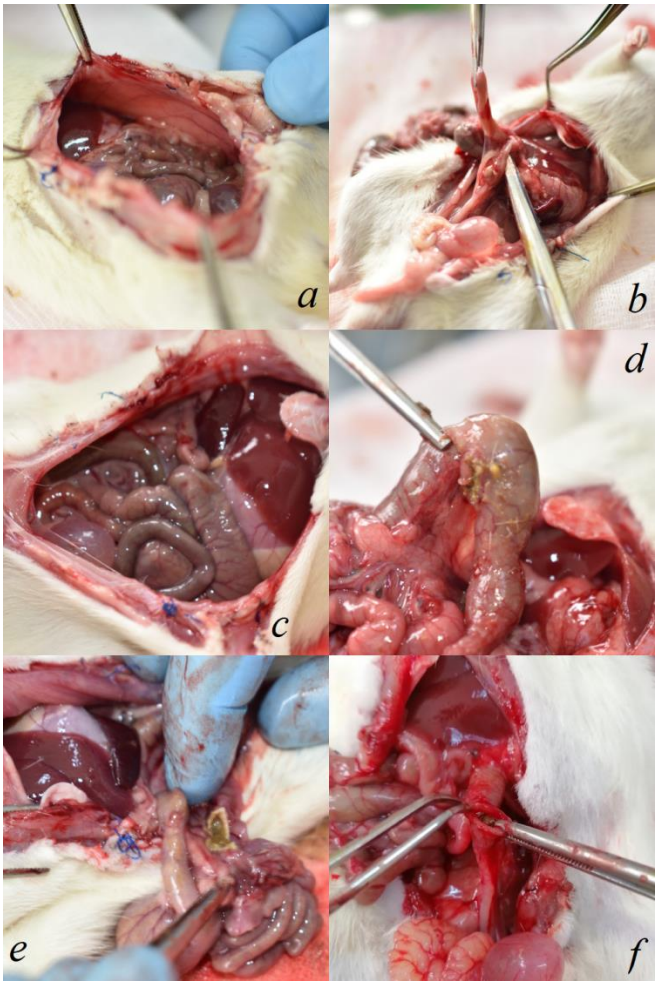

Fig. 3. a) Intraperitoneal appearance in a subject in group B at time T2; b) Intestinal loops adhering to the sigmoid perforation in a subject from group A at time T2; c) Intraperitoneal appearance of a subject belonging to group C at time T2; d) Sigmoid colon perforation with excretion of feces (Group C, T2); e) Intraperitoneal appearance group D at time T2; f) Intraperitoneal appearance group D at time T2.

At time T4, at 96 hours postoperatively, we sacrificed 5 subjects from groups B and C and 4 subjects from group D, one subject from group D died at time T3. Intraperitoneally, in subjects from group B, the minimum amount of serocitrin fluid, relaxed intestinal loops (fig. 4a), check and much relaxed colic frame, and edema are found (fig. 4b). In subjects from group C, distension of the intestinal loops with the presence of the minimum amount of intraperitoneal fluid was reported (fig. 4c), at the level of the perforation of the supple intestinal wall, without inflammatory signs (fig. 4d). The subjects in group D did not show free intraperitoneal fluid, instead, they showed relaxed intestinal loops, with the formation of plastrons at the level of perforation (fig. 4f).

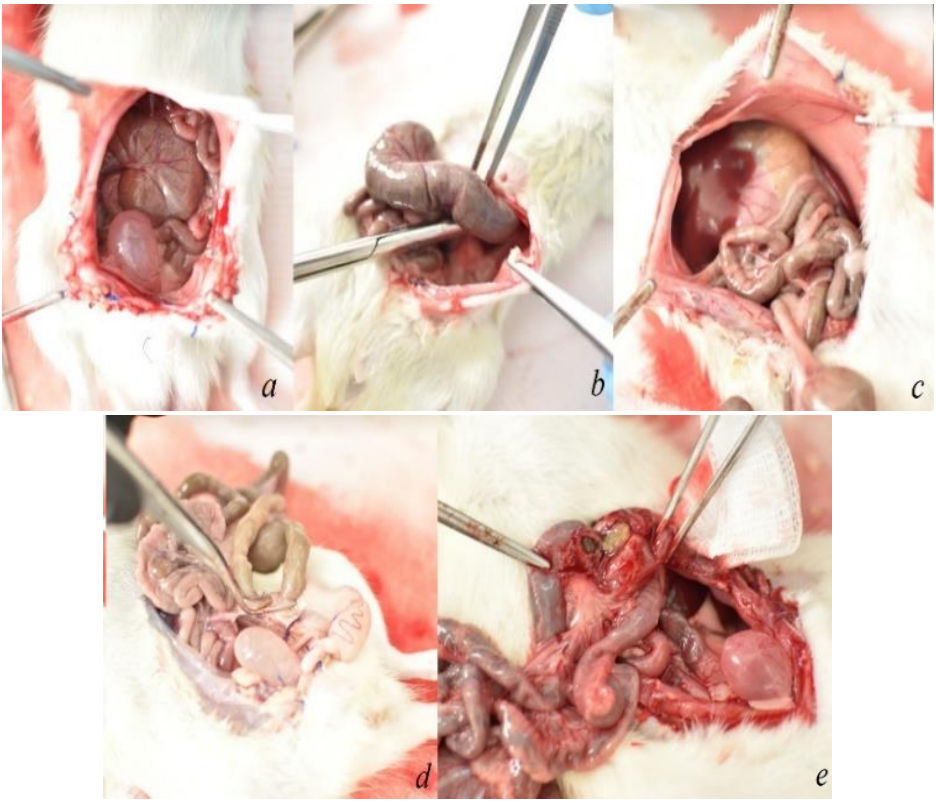

Fig. 4. a) Intraperitoneal appearance group B at time T4; b) Intraperitoneal appearance group B at time T4; c, d) Intraperitoneal appearance group C at time T4; e) Intraperitoneal appearance group D at time T4.

On microscopic examination, at time T2, a better evolution of groups C and D was observed, compared to group B, at the same time, group D showed the least inflammatory changes, at the level of the peritoneum being described as its discrete thickening and the presence of active mesothelial cells (fig. 5a) and at the level of the sigmoid being described thickened serous with vascular hyperemia at the level of the colic wall muscles and rare active mesothelial cells (fig. 5b). Subjects in group C showed serous peritoneum with hyper basophilic active mesothelial cells, hyperplastic lymph nodes, thickened mesenteric adipose tissue, edema with fibroblasts and active macrophages (fig. 5c), sigmoid colon with mesothelial cell activation, hyperemia moderate infiltration of mesenteric adipose tissue with lymphocytes, macrophages and neutrophils (fig. 5d). In group B, the peritoneum showed folding and detachment from adjacent muscles, infiltration rich in macrophages and neutrophils, fragmentation of connective tissue and basement membrane of the peritoneal sheet, mesothelial activity, sub peritoneal edema and fragmentary degeneration of adjacent muscle fibers, (fig. 5e) and at the level of the colon was observed serous bordered by mesothelial cells enlarged in volume, active, mucosa with abundant cellularity (fig. 5f).

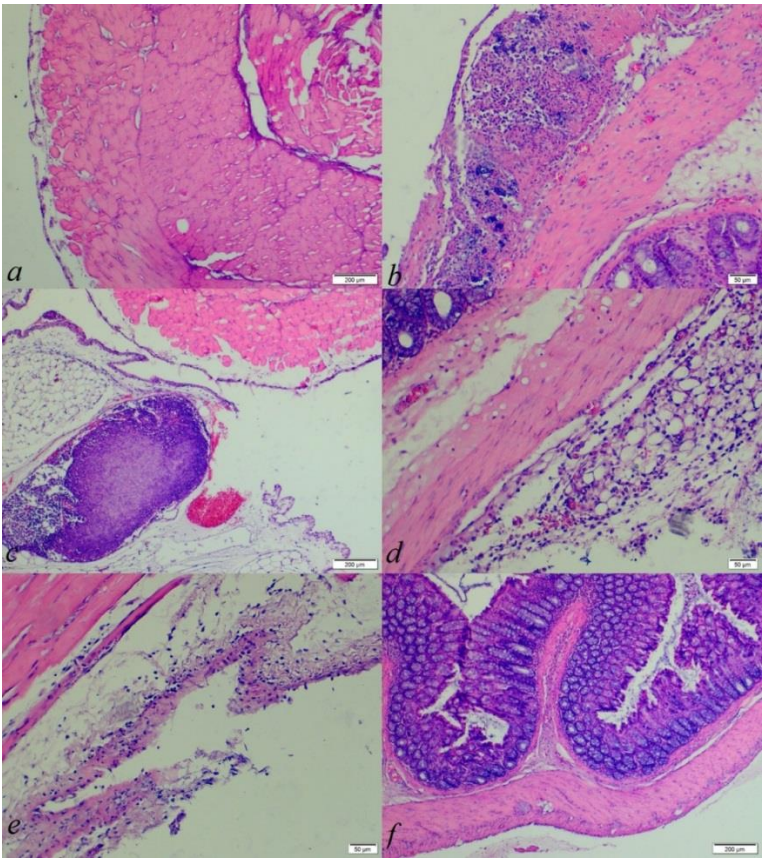

Fig. 5. a) Peritoneum with discrete thickening and the presence of active mesothelial cells, HE X4; b) Thickened serosa with vascular hyperemia in the colic wall muscles and rare active mesothelial cells, batch D, T2, HE, X10; c) Serous peritoneum with hyper basophilic active mesothelial cells, hyperplastic lymph nodes, thickened mesenteric adipose tissue, edematous fibroblasts and active macrophages, batch C, T2, HE X4; d) Sigmoid colon with activation of mesothelial cells, hyperemia of local vessels, with moderate infiltration of mesenteric adipose tissue with lymphocytes, macrophages and neutrophils, group C, T2, HE X10; e) Peritoneum with infiltrate rich in macrophages and neutrophils; fragmentation of connective tissue and basement membrane of the peritoneal sheet; mesothelial activity with sub peritoneal edema and degeneration of adjacent muscle fibers, group B, T2, HE X10; f) Serosa of colon bordered by mesothal cells enlarged in volume, active, mucosa with abundant cellularity, group B, T2, HE X4.

Microscopic analysis of the samples collected at T4 revealed a better evolution of the subjects in group D, so at the level of the parietal peritoneum there are rare active mesothelial cells, increased in volume (fig. 6a), and at the sigmoid without signs of inflammation (fig. 6b). Comparatively, group B showed an unfavorable evolution, so at the level of the peritoneum was detected edematous peritoneal serum edematous, interrupted, infiltrated with neutrophils and macrophages, with hyperemia of the vessels

(fig. 6c) and at the level of the sigmoid colon was detected peritoneal reaction that deforms the visceral peritoneum which is hyperplasia of hyperbasophilic cells, abundantly infiltrated with fibroblasts, neutrophils and macrophages, with edema, vascular hyperemia and capillary neoplasms (fig. 6d). Subjects in group C presented at the level of the peritoneum naked parietal sheet peritoneal, slightly thickened, with a tendency to detach, hyperemic skeletal muscle (fig.6e), and at the level of the sigmoid thickening of the visceral peritoneum with rare active mesothelial cells, adjacent tissue edema, invaded by groups of neutrophils and macrophages (fig. 6f).

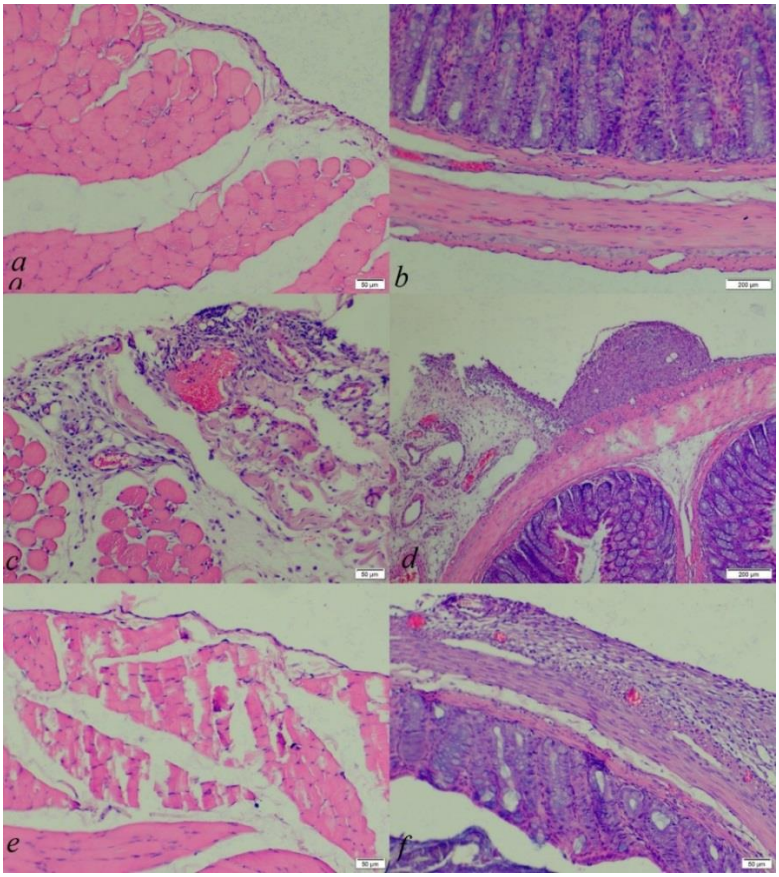

Fig. 6. a) Peritoneum with rare active mesothelial cells, increased in volume, batch D, T4, HE X1; b) Sigmoid colon without signs of inflammation, group D, T4, HE X10; c) Edematous peritoneal serum, interrupted, infiltrated with neutrophils and macrophages, with hyperemia of vessels, group B, T4, HE X10; d) Deformed visceral peritoneum, hyperplasia of hyperbasophilic cells, abundantly infiltrated with fibroblasts, neutrophils and macrophages, with edema, vascular and capillary hyperemia of neoformation, group B, T4, HE X4; e) Bare parietal leaflet, slightly thickened, with a tendency to detach, hyperemic skeletal muscles, group C, T4, HE X10; f) Visceral peritoneum with rare active mesothelial cells, adjacent edematous tissue with hyperemia, invaded by groups of neutrophils and macrophages, group C, T4, HE X1.

All subjects examined at T2 and T4 were euthanized according to internal procedures with an overdose of anesthetic after sampling.

DISCUSSION

In the case of perforation of the colon, mortality is between 11.1 and 31.6% (3, 13-15), studies in the laboratory animal found mortality of up to 80% within 72 hours of the perforation. (16), in our study a mortality of 3.33% was recorded, the death occurring within 72 hours from the time of perforation.

Studies in rats have shown that peritoneal lavage decreases mortality in acute peritonitis, (17, 18) in the absence of any treatment, which can reach up to 100% in 12- to 52-hour intervals. (17, 19) in our study, the mortality at 24 hours, in the case of subjects who did not receive treatment was 0% at 24-48 hours, similar to groups C and D, with the mention that at the time of T3, there was a death in group D, explainable in the context in which studies in rats showed mortality at 8 days of 10% (12).

With the formation of intraperitoneal abscesses, the effects of peritonitis begin to decrease, the clinical evolution being towards an apparent cure (20), in our study, all subjects showed intraperitoneal abscesses, formed by organs adjacent to the perforation, which had a role localization of the lesions, without noticing a significant decrease in macroscopic changes in the peritoneal cavity. At time T2 in all groups the presence of intraperitoneal fluid could be observed, at time T4 the amount of intraperitoneal fluid remained similar in the case of subjects in groups B and C, in the case of subjects in group D the absence of intraperitoneal fluid was found.

Paralytic ileus secondary to acute peritonitis has been explained, according to Stokes' law, as a result of serious inflammation (21) in the first phase, then bacterial toxicity and finally intraperitoneal adhesions (22) The main feature is the distension of intestinal loops (23-26).

In our study, ileus was detected in all subjects at both T2 and T4, with a slight improvement in subjects receiving antibiotic treatment at T4.

Intraperitoneal adhesions are classified by Adhesion grading scale: Score 0 - no adhesions, Score I - Filmy adhesions, Score II - Definitely localized adhesions, Score III - Dense multiple visceral adhesions, Score IV - Dense adhesions extending to the abdominal wall (27, 28). In our study in the case of each group we found in T2 moment score I to II adhesions and in T4 moment score II to III adhesions, with no notable differences between groups.

The inflammatory response of the peritoneum is produced by several steps, (29, 30) characterized by increased blood supply with the accumulation and attraction and infiltration of immune cells, with the accumulation of macrophages, which play a key role in the immune response of the peritoneum, which releases pro and anti-inflammatory factors (30, 31). Macrophages, resident in the peritoneal cavity and sub mesothelial stroma, play a key role in recognizing and digesting foreign matter and the introduction of inflammatory leukocytes into the blood, including monocytes, lymphocytes and neutrophils (30). In response to leukocyte infiltration and activation, mesothelial cells activate and begin to secrete proinflammatory factors (30, 32-35) at the level of the parietal peritoneum as well as at the level of the visceral peritoneum at the level of the affected organ. The exception was group D, in which, at the

time of T4, a minimal inflammatory reaction was found in the visceral peritoneum and the absence of inflammatory phenomena in the parietal peritoneum.

Exaggerated immune responses can lead to angiogenesis, fibrosis and later destruction of the peritoneum, (30, 36) such lesions were also found in our study.

Both meropenem and cefuroxime combined with or without metronidazole are used successfully in treating acute peritonitis with similar results (37, 38).

Our study showed better histopathological results in subjects treated with meropenem, especially at the time of T4, although no noticeable differences were observed macroscopically, and the only death occurred in a subject in group D. Subjects who did not receive antibiotic treatment showed the most important inflammatory changes on microscopic examination.

CONCLUSIONS

Our study proposed a model for simulating acute peritonitis by sigmoid perforation and tracking macroscopic and microscopic changes in both the parietal peritoneum and the visceral peritoneum. Both macroscopic and microscopic changes varied depending on the group, the treatment received, and the individual. From a microscopic point of view, the most important changes were of an inflammatory nature, the most important inflammatory reactions being in the parietal peritoneum. Although no major differences between batches were found in terms of general condition and macroscopic changes, in terms of microscopic changes, meropenem treatment showed remarkably better results, especially at T4. Meropenem is a first-line antibiotic in acute peritonitis secondary to colonic perforation, which together with surgery can reduce the morbidity and mortality of this pathology.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

RESEARCH ETHICS STATEMENT

The research was conducted according to the rules of the Organization for Economic Cooperation and Development (OECD) in good laboratory practice (GLP) and in accordance with the Romanian laws in force and the internal protocols, with the authorization of the Veterinary Authority and the Ethics Committee of the "Cantacuzino" Institute.

AUTHORS' CONTRIBUTION AND DISCLAIMER

The first and the second authors contributed equally to this paper's achievement.

The data presented in the article are part of a larger study, which is part of a Ph.D. thesis.

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A COMPARATIVE STUDY IN THE APPROACH OF INCISIONAL HERNIAS

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A COMPARATIVE STUDY IN THE APPROACH OF INCISIONAL HERNIAS (Abstract): Incisional hernias are one of the most frequent forms of complications derived from postoperative wound complications and must be addressed surgically. One of the best techniques is the Rives-Stoppa technique, which is also suitable for open surgery and minimally invasive procedures. **Material and methods:** We conducted a study to evaluate the results of open or laparoscopic surgery using the Rives-Stoppa method. **Results:** We completed the survey over three years and assessed the outcomes for 49 patients, of which 13 were treated with open surgery and 36 by using the minimally invasive technique, the average age of the patients being 52,02 ± 12,73 years old. **Conclusions:** The results show a significantly more extended hospital stay for the patients with open surgery combined with a considerably higher proportion of complications. **Keywords:** SECONDARY PARIETAL DEFECTS, RIVES-STOPPA TECHNIQUE, ABDOMINAL WALL, EVENTRATION.

Incisional hernias are one of the most frequent post-operatory complications of wounds, varying from 4% to 20% (1). Because the medial incision is the most frequent, most of the hernias are located at this level, some numbers suggesting 70-90% (2).

Initially described by Rives and Stoppa, this technique implies the incision of the skin and subcutaneous tissue, with the visualization of the sack and its release. After its opening, the viability of the content is verified, and intrasaccular and intraperitoneal adherences are dissected. Next, the reconstruction of the parietal wall follows the saccular resection. Next, the posterior fascia of the rectus abdominis is opened on the medial margin, and a retro rectal avascular plane is created through good resection. Here, the mesh is connected to the lateral margin of the muscle, where the vascular and nervous bundles are located.

The extraperitoneal method is done by repairing the hernial defect using extraperitoneal methods without opening the peritoneum (thus avoiding abdominal viscera and forming post-operatory adherences), is based on the procedures used in classic interventions and described by Rives and Stoppa. The techniques were first described in France by Dulucq J.L. (3-5), who used a surgical abutment of the dissection of the peritoneal space, followed by McKernan and Laws in 1993, which used the trocar with a dissecting balloon (6).

The advantage of using these techniques resides in intraabdominal pressure, which acts on the mesh's surface and fixates the

prosthetic material on the muscular plane. As a result, the laparoscopic anatomy of the region is seen differently from the classic anatomy. But, first, the surgeon must identify critical anatomical landmarks for incisional hernias. This study aims to evaluate the differences in recovery time and complications for patients with a hernia that are treated by using classic versus minimally invasive Rives-Stoppa techniques for eventrations.

MATERIAL AND METHODS

The prospective study was conducted on 49 patients treated for eventrations, over three years, between 2018 and 2021. Based on medical characteristics, patients were assigned to either classic or minimally invasive intervention (laparoscopic or endoscopic).

At the initial consult, we measured the socio-medical characteristics of the patients (age, sex, environment, weight, localization of the hernia, and comorbidities). Then, following the surgical procedures, we followed up with the patients and gathered data on their evolution, hospitalizations, and complications.

We used descriptive statistics, mean and standard deviation for continuous, numerical values, and counts (with percentages) for categorical variables. The tests used for determining the statistical significance of the results were adapted to the type of variable based on the existing general recommendations (7). For example, a "p" value less than 0.05 was considered statistically significant.

RESULTS

Characteristics of the sample

Table I summarizes the characteristics of the patients at admission. As can be observed, there are no statistically significant differences between the two groups of patients that underwent Rives-Stoppa intervention compared to the ones with minimally invasive interventions.

TABLE I.
Characteristics of the patients

	Total (49)	Minimally invasive (36)	Classic(13)	p-value
Age				
Mean ± SD	52.02 ± 12.73	50.33 ± 13.71	56.69 ± 8.24	0.12
Sex				
Male	25 (51%)	19 (52.8%)	6 (46.2%)	0.68
Female	24 (49%)	17 (47.2%)	7 (53.8%)	
Environment				
Urban	37 (75.5%)	28 (77.8%)	9 (69.2%)	0.54
Rural	12 (24.5%)	8 (22.2%)	4 (30.8%)	
Weight				
Mean ± SD	88.35 ± 15.55	90.39 ± 14.89	82.69 ± 16.51	0.13
Localization				
Supraumbilical	23 (46.9%)	18 (50%)	5 (38.5%)	0.58
Umbilical	10 (20.4%)	6 (16.7%)	4 (30.8%)	
Subumbilical	14 (28.6	10 (27.8%)	4 (30.8%)	
Inguinal	2 (4.1%)	2 (5.6%)	0 (0%)	
Size of the defect (cm)				
Mean ± SD	12.22 ± 6.4	10.03 ± 4.8	18.31 ± 6.5	<0.01

The average age of the patients was 52.02 years, with a standard deviation of 12.73 years. We observed that the average for the patients with minimally invasive interventions was more than six years smaller than those for whom the Rives-Stoppa intervention was used.

Even though there was a difference in the proportion of males and females, with a higher percentage of males being operated on using minimally invasive techniques, the results were not statistically significant.

At first sight, the proportion of patients according to the environment might give the impression that there is a significantly larger of patients living in urban areas. However, for the whole sample of patients, 69.2% were from urban areas. Considering where the patients come from, an acceptable percentage is in South-East Romania. According to official data, about 70% of the people live in urban areas, thus making these numbers normal. Regarding the localization of the hernia, again, there are no statistically significant differences (p=0.58) between the two groups. For minimally invasive interventions, half of them were supra-umbilical. When the hernia was in the umbilical area, the percentage of Classic-type interventions was almost double in frequency (30.8%) compared to the frequency of umbilical hernia for patients treated using minimally invasive techniques (16.7%). Also, for larger hernias, the classic intervention was preferred. The average size of the abdominal wall defect is almost double for patients treated using traditional interventions. When comparing the frequency of comorbidities at admissions, we observe essential differences in proportions regarding high blood pressure and obesity, cases in which minimally invasive techniques were preferred (tab. II). However, differences were not statistically significant (p=0.1).

TABLE II.
Comorbidities

	Classic	Minimally Invasive	Total
Atrial Fibrillation	1 (7.7%)	0 (0%)	1 (2%)
Cardiac Insufficiency	1 (7.7%)	2 (5.6%)	3 (6.1%)
Diabetes Mellitus Type 1	1 (7.7%)	0 (0%)	1 (2%)
Diabetes Mellitus Type 2	1 (7.7%)	0 (0%)	1 (2%)
High Blood Pressure	2 (15.4%)	9 (25%)	11 (22.4%)
Obesity	2 (15.4%)	13 (36.1%)	15 (30.6%)
Pulmonary Tumor	1 (7.7%)	0 (0%)	1 (2%)
Thyroiditis	0 (0%)	1 (2.8%)	1 (2%)
No comorbidity	4 (30.8%)	11 (30.6%)	15 (30.6%)

Post-surgery evolution

We analyzed the way patients evolved following the surgical intervention. We observed an almost four times higher percentage of complications following the Classic techniques than minimally invasive

procedures. The difference was statistically significant (p=0.01), suggesting an increased association between the Classic method and the appearance of complications (fig. 1).

When analyzing the days of hospitalization, the mean value for the number of hospitalization was 2.72 ± 0.944 days for the minimally invasive techniques and significantly higher, with an average of 4.69 ± 1.18 days for the patients for which Classic styles was used (tab. III). The difference was statistically significant, with p<0.001.

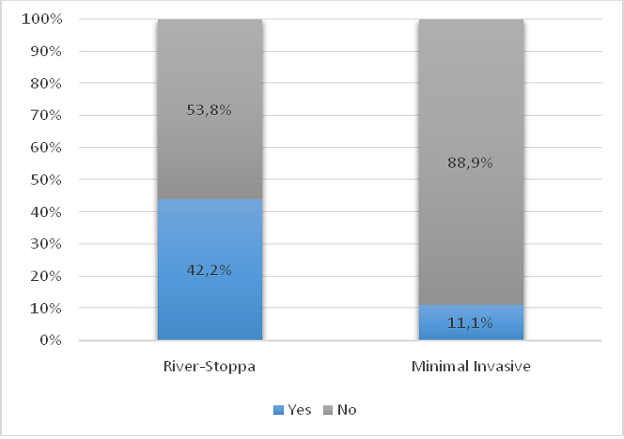

Fig. 1. Proportion of complications after the surgical intervention

TABLE III.

Descriptive analysis – days of hospitalization

Days of hospitalization	Type of Surgical Intervention		
	Classic	Minimally Invasive	Total
N	13	36	49
Mean	4.69	2.72	3.24
Std. Deviation	1.182	.944	1.331
Median	4.00	2.50	3.00
Minimum	3	2	2
Maximum	7	5	7
Skewness	1.065	1.465	1.075

DISCUSSION

Our study focused on identifying differences in the evolution of patients with an incisional hernia, depending on the surgical interventions used (Classic or Minimal Invasive). The Rives-Stoppa technique offered excellent long-term results in patients with large hernias (primary of incisional) (8).

Our study's sample population consisted of patients with different degrees of incisional hernias. We observed no statistically significant differences in patients within the arms of the study. Even though there is a very high percentage of patients within the urban area, this is considered normal for where the study took place, so we cannot say that there are issues related to health

inequalities, as observed in other studies or on different types of pathologies. (9, 10).

The primary outcome of the study was the presence of post-surgery complications.

Complications were observed in a relatively large number of patients treated with Classic intervention (42.2%) compared to Minimally Invasive methods (11.1%). The Classic intervention represented the complications caused by suppurations, hematomas, or seromas. In the case of Minimally Invasive interventions, the complications were described by local pain and thread granuloma.

Considering that the patients presented with incisional hernias, with one-quarter previously treated for hernia, we believe the observed results are within typical values. Other studies reported similar rates of complications, with significant variability (11, 12). Differences in the percentage of complications may also appear because of the difference in the size of the treated eventration, open surgery being preferred in the case of more significant abdominal wall defects.

The economic aspect is also essential when analyzing surgery outcomes. For example, using Minimally Invasive techniques, not only did the patients have significantly fewer complications, but the length of hospital stays was significantly reduced, from a median of 4 days (open surgery) to a median of 2.5 in the case of Minimally Invasive techniques, this represents one of the significant advantages of Minimally Invasive techniques (13, 14).

The present study adds to the existing literature by comparing surgical techniques for incisional hernias. Compared to open surgery, we demonstrated that minimally invasive procedures have better results in terms of recovery time and lack of complications.

We would have compared patients with eventrations of similar sizes for a better comparison. Also, the study focuses on immediate complications without considering possible recurrent hernias that can appear in time.

CONCLUSIONS

The minimally invasive approach offers shorter recovery time, thus decreasing the length of the hospitalization, with all the associated benefits and lower percentages and seriousness of the complications.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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EXCELLENT LONG-TERM NEGATIVE PREDICTIVE VALUE OF CAPSULE ENDOSCOPY IN SUSPECTED ISOLATED SMALL BOWEL CROHN'S DISEASE

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EXCELLENT LONG-TERM NEGATIVE PREDICTIVE VALUE OF CAPSULE ENDOSCOPY IN SUSPECTED ISOLATED SMALL BOWEL CROHN'S DISEASE (Abstract): There is no unique diagnostic test for Crohn's disease (CD), and the diagnosis of isolated small bowel Crohn's disease (SBCD) is even more challenging, due to non-pathognomonic clinical picture and low availability of accurate exploration methods. Small bowel capsule endoscopy (SBCE) revolutionized the approach of middle gastrointestinal tract, becoming the first investigation tool when small bowel pathology is suspected. The **aim** of our study was to assess the value and safety of SBCE in diagnosing isolated SBCD. **Materials and methods:** We retrospectively studied the patients undergoing SBCE for suspected isolated SBCD, in a six-year period, including in our analysis only the patients with a minimum of 18-month follow-up period. We assessed the global diagnostic yield (DY) of SBCE, as well as its yearly variation in time over the six-year period, and the complication rate. Subsequently, analyzing follow-up data, positive and negative predictive values were assessed. **Results:** 78 patients were investigated by SBCE for suspected isolated SBCD, mostly men (60.3%), with age ranging from 20 to 69 years old. SBCE showed lesions compatible with the diagnosis of SBCD in 49 patients, corresponding to a specific diagnostic yield of 62.8%, while for the remaining 29 patients (37.2%), no suggestive lesions were found. Following additional investigations, three of the 49 patients initially thought as having isolated SBCD were reclassified as intestinal lymphoma, eosinophilic gastroenteritis and intestinal tuberculosis, respectively, corresponding to a positive predictive value of 93.8%. During follow-up, none of the 29 patients with negative SBCE was diagnosed with CD, corresponding to a negative predictive value of 100%. We found a progressively increase of the diagnostic yield over time (from 42.6% in the first year to 70.2% in the last year of the analyzed period), corresponding the most probably to the refinement of the indication. **Conclusions:** SBCE has good DY and a high positive predictive value for isolated SBCD. However, it remains a purely visual technique with no capability of taking biopsies, so final diagnosis needs confirmation by additional investigations. Nevertheless, SBCE has a 100% long-term negative predictive value, being an excellent tool for ruling out isolated SBCD. **Keywords:** CAPSULE ENDOSCOPY, SMALL BOWEL, CROHN'S DISEASE.

Crohn's disease is a chronic inflammatory bowel disease, with various phenotypes and different particularities in each individual's clinical picture. Crohn's disease may affect any part of the gastrointestinal tract, the most frequent being the ileocecal localization - when both terminal ileum and part of colon are involved, while in approximately one third of cases, patients have only small bowel involvement (1). As there is no unique gold standard diagnostic test for Crohn's disease, the positive diagnosis is based on a combination of clinical evaluation, together with biological, imaging, endoscopic and histologic data (2). Moreover, due to the fact that clinical manifestations are not always pathognomonic, and that accurate exploration methods for the small bowel are not universally available, the diagnostic of isolated SBCD is even more challenging.

However, the introduction into the clinical practice of the capsule endoscopy, which has become the first investigation tool when small bowel pathology is suspected, has revolutionized the approach of the middle gastrointestinal tract (3). SBCE is nowadays consensually indicated in several IBD situations, as suspected Crohn's disease, evaluation of extension and activity of known CD, assessing the response to therapy, identifying the postoperative CD recurrence, and evaluation of unclassified colitis (4). The use of SBCE is justified by its advantages compared to other investigation methods; firstly, the safety and the non-invasiveness versus enteroscopy, and the superior diagnostic yield compared to imaging methods as enteroclysis, computed tomography-enterography, and magnetic resonance-enterography for incipient lesions (5-7). Nevertheless, SBCE has its limits, the main one being the inability of taking biopsies. Thus, SBCE remains a visual technique, and in some cases, for arriving at the final diagnosis, supplementary investigations might be necessary. Consequently, the discriminative power of SBCE as a single technique for exploring the small bowel is a question of debate. In this context, we aimed to assess the value of SBCE in diagnosing isolated SBCD. In the same time, considering that CD is one of the conditions predisposing patients to the retention risk, we also aimed to evaluate the safety of SBCE in investigating patients with suspected isolated SBCD.

MATERIAL AND METHODS

Patients

We retrospectively studied the patients undergoing SBCE for suspected isolated SBCD, in a six-year period (January 1st2016 – December 31st2021), in the Institute of Gastroenterology and Hepatology. We included in our analysis patients with clinical, biologic and/or imaging suspicion of CD, with no diagnostic findings at conventional ileocolonoscopy, who were followed after SBCE exploration for at least 18 months period.

Methods

We assessed the performance parameters of SBCE, as well as the complication rate.

The performance parameters were the diagnostic yield (DY), defined as number of capsule endoscopy exams with positive findings from the total number of investigations, the positive prediction value (PPV), defined as ratio of patients truly diagnosed as positive to all those who had positive results, and the negative predictive value (NPV), defined as the ratio of subjects without disease to all those who had

negative test results. Both PPV and NPV were retrospectively assessed, by analyzing follow-up data.

All patients were investigated after contraindications were excluded, and every patient signed the informed consent for SBCE prior to the investigation. The diagnostic work-up was conducted following the actual guidelines for the use of SBCE. Before SBCE, all patients underwent colonoscopy, which did not CD lesions.

The preparation was made with two liters of polyethylene-glycol the evening before the procedure, and the patients fastened overnight. The third-generation endoscopic capsule for small bowel examination (PillCam SB3) manufactured by Given Imaging, Yoqneam, Israel, was used. Interpretation of data was made with RAPID™ reader software.

The diagnostic of Crohn's disease was sustained according to Mow's criteria – more than three ulcerations, in the absence of nonsteroidal anti-inflammatory drugs (NSAIDs) administration (8). Anyway, patients with recent (four weeks or less) history of NSAIDs use were excluded from the analysis. For all the patients with Mow's criteria fulfilled, the Lewis score (LS) were afterwards assessed. The Lewis score is an incorporated software algorithm which sums up the points assigned to three characteristic findings – strictures, ulcers, fistula in the three segments of the small bowel, and classify the severity of inflammation into three categories: insignificant, mild or moderate-to-severe (9).

Complications of CE were noted. Incomplete examinations were not included in the study.

Statistical analysis was conducted using the *SPSS version 17.0 software* (SPSS Inc., Chicago, IL, USA).

RESULTS

Patients' characteristics

In the analyzed six-year period, 78 patients underwent SBCE for suspected isolated CD. Their characteristics are presented in the first table.

TABLE I.

Clinical characteristics of patients, findings at SBCE and follow-up data

	Total number of patients = 78
Age, years, mean ± SD	43 ± 12.5
Range, years	20-69
Gender ratio	31/47
Women/Men, n (%)	(39.7%/60.3%)
SBCE exam results	
Positive for CD, n (%)	49 (62.8%)
Negative for CD, n (%)	29 (37.2%)
Follow-up data	
CD confirmed	46/49 (93.8%)
CD ruled out	29/29 (100%)

Diagnostic yield

SBCE showed lesions compatible with the diagnosis of CD in 49 patients, corresponding to a diagnostic yield of 62.8%. For the remaining 29 patients (37.2%), no suggestive lesions were found, and consequently CD was ruled out.

We found a progressively increase of the diagnostic yield over time (from 42.6% in the first year to 70.2% in the last year of the analyzed period), corresponding the most probably to the refinement of the indication.

Lewis score

For the inflammation quantification, the Lewis score was used; 22 patients presented a score between 135 and 790, being classified as having mild inflammation, while the remaining 27 patients had a score > 790 points, corresponding to moderate-to-severe inflammation.

Positive predictive value

Analyzing follow-up data, we found that three of the 49 patients initially considered as having isolated SBCD were consequently further explored, and eventually a different diagnosis was made, corresponding to a positive predictive value of 93.8%. Out of the three patients, one patient was diagnosed with eosinophilic enteritis, one patient was confirmed as having intestinal T-cell lymphoma, and one patient had intestinal tuberculosis. All the three patients had a baseline moderate-to-severe inflammation, according to the LS.

Negative predictive value

During the follow-up period, none of the 29 patients with negative SBCE exam was diagnosed with CD, corresponding to a negative predictive value of 100%.

Complications

One case of capsule retention was noted (retention rate 1.28%), due to an ileal stricture in a patient with small bowel CD. Medical treatment was not successful, so the patient underwent surgery; stricturoplasty, as well as removal of retained capsule, were performed.

DISCUSSION

SBCE has emerged as a valuable tool for the diagnosis of CD. The ability to directly visualize the intestinal mucosa allows a comprehensive evaluation of the small bowel. Because CD may involve only small bowel segments, SBCE is a particularly valuable method in cases where other diagnostic methods, such as conventional endoscopy, are not contributive.

Studies have reported diagnostic yields of SBCE ranging from 50% to 80%, generally superior compared to other diagnostic modalities (5, 7, 10, 11). Ileocolonoscopy with biopsy remains the gold standard for the diagnosis of CD (4), but if it is normal, it must be followed by the SBCE in the absence of obstruction. A large meta-analysis showed superiority of SBCE compared to small bowel follow-through (52% *vs.* 16%), computed tomography-enterography (68% *vs.* 21%), and ileocolonoscopy (47% *vs.* 25%) (7). SBCE also proved its significantly higher sensitivity for incipient lesions, especially in the jejuni and ileum, compared to magnetic resonance-enterography, as a recent Spanish study showed (77% *vs.* 48%) (12).

Our study showed a global diagnostic yield for isolated CD of 62.8%, proving the value of SBCE in this particular type of CD patients, with exclusive small bowel involvement, where conventional endoscopy has limited role. The yearly analysis of the DY showed a higher DY over time (up to 70.2%), which we interpret as refinement of the indication.

Even if SBCE is a sensitive tool for mucosal abnormalities, one important limitation consists in the lack of definitive diagnostic criteria for CD. Mow's criteria are generally used for diagnosis, while other scoring systems, as Lewis score and, more recently, the Capsule Endoscopy Crohn's Disease Activity Index, are useful especially for disease monitoring and treatment guidance (13). Moreover, for suspected SBCD, it has as drawback a relative lack of specificity. In our analysis, three patients initially considered as having isolated SBCD were further explored and reclassified. They underwent SBCE for suspected CD based on their clinical picture; abdominal pain, chronic diarrhea, weight loss and anemia are overlapping symptoms for CD, eosinophilic enteritis, intestinal tuberculosis and intestinal lymphoma. SBCE findings consisted in oedema, hyperemia, erosions and superficial and deep ulcerations, which may appear

both in CD and in other inflammatory or non-inflammatory conditions. All the three patients had a baseline moderate-to-severe inflammation, according to the LS. Notably, all the three patients had a baseline moderate-to-severe inflammation, according to the Lewis score; however, the most appropriate role of the Lewis score is monitoring established CD. Additionally, imaging data may be similar; for both eosinophilic gastroenteritis and lymphoma, entero-CT showed similar SB wall modifications. Following the final diagnosis, made by device-assisted enteroscopy with biopsy, different therapeutic strategies were applied.

Indeed, a main limitation of SBCE is its inability of taking biopsies, while a definite diagnostic of CD would require, in order to be considered beyond any doubt, histologic confirmation. Small bowel ulcerations are not pathognomonic for CD; even they are a common finding in CD and the diagnostic criteria rely on their presence, they however may appear in a variety of other small intestine disorders, such as: infectious enteritis - like intestinal tuberculosis or cytomegalovirus infection, NSAID enteropathy, eosinophilic enteritis, ischemic enteritis, or intestinal lymphoma (14). Moreover, mucosal erosions may be found even in approximately 10% of healthy subjects (15). Precautions must be taken before SBCE, such as stopping NSAIDs at least four weeks before the investigation. Also, sometimes, further evaluation modalities must be considered. Enteroscopy with biopsy is a complementary investigation, which could ensure a final diagnosis. SBCE acts as a non-invasive evaluation tool, guiding the decision and the access way for a further enteroscopy. Current guidelines recommend upper or lower device-assisted enteroscopy after SBCE, allowing targeted biopsies.

In our study, follow-up data showed that none of the patients with negative SBCE exam was diagnosed afterwards with CD. Basically, normal SBCE can rule out CD with maximum confidence. As SBCE is recommended as a first line test for SB pathology suspicion, its capability to exclude CD makes it a very reliable tool in CD work-up.

SBCE is generally considered a safe technique; however, retention can occur, if narrowing, stricture, or obstruction of a certain intestinal segment exists. Other complications are extremely rare. The retention rate varies according to the indication, from 1%-2% in patients with suspected CD, up to 10-13% in patients with known CD (16). In our study, the retention rate was 1.28%, similar to other reports. Nevertheless, concerning capsule retention in suspected CD, several comments should be made. Apart known strictures, all other contraindications may be considered relative, and even in the event of retention with no response to conservative management, surgery will solve both the capsule impaction and the obstructive lesion, allowing also a definite histologic diagnosis. Patency capsule is indicated in order to confirm the permeability of gastrointestinal tract only when there is a clinical suspicion of small bowel obstruction, otherwise it is not routinely advised (4). Showing a retention rate close to that reported for obscure gastrointestinal bleeding, which remains the most common indication - pooled retention rate 1.2%, as reported by a systematic review by Liao *et al* (17), SBCE in suspected CD appears to be a safe technique.

CONCLUSIONS

SBCE has a good diagnostic yield and a good positive predictive value for isolated

SBCD. It is a safe first line tool in suspected CD, with low retention rate. However, it is a purely visual technique with no capability of taking biopsies. Hence, its results should be interpreted in conjunction with other relevant clinical data, and the final diagnosis needs confirmation by additional investigations. Definitive diagnostic criteria for CD are still lacking. Nevertheless, SBCE showed a very high clinical accuracy for excluding CD. With a 100% long-term negative predictive value, SBCE emerges as an excellent tool for ruling out isolated SBCD.

CONFLICT OF INTEREST AND FUNDING

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PREDICTING INTRAUTERINE GROWTH RESTRICTION: A PILOT STUDY WITH FEED-FORWARD BACK PROPAGATION NETWORK

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PREDICTING INTRAUTERINE GROWTH RESTRICTION: A PILOT STUDY WITH FEED-FORWARD BACK PROPAGATION NETWORK (Abstract): The prediction of intrauterine growth restriction (IUGR) represents a challenge for obstetricians throughout pregnancy, and the use of artificial intelligence could improve its screening. The **aim** of this pilot study was to prospectively design and test a Feed-Forward Backpropagation neural network (FFBPN) for the prediction of IUGR and its subtypes. **Materials and methods:** Between January and September 2023, we included 108 pregnant patients with singleton pregnancies who underwent conventional first trimester screening. Clinical and paraclinical data was used to construct a FFBPN, and its predictive performance was assessed using a sensitivity analysis. **Results:** Our results indicated that the FFBPN predicted the development of IUGR during pregnancy with a sensitivity (Se) of 94.7%, specificity (Sp) of 97.7%, and a false positive rate of 2%. The value of the area under the curve for this neural network was 0.978. On the other hand, when used for the prediction of IUGR subtypes, the FFBPN achieved lower predictive performance. Also, the sensitivity analysis revealed that the FFBPN could better predict early IUGR, with a Se of 71.4%, Sp of 94%, and accuracy of 90.7%. **Conclusions:** The FFBPN could be an important tool for the prediction and stratification of various disorders, including IUGR. **Keywords:** INTRAUTERINE GROWTH RESTRICTION PREDICTION, NEURAL NETWORK, FEED-FORWARD BACKPROPAGATION.

Intrauterine growth restriction (IUGR) is a condition described as a fetal growth rate that is less than normal based on race and gender (1). Although there are slight variations between the two, the words IUGR and small for gestational age (SGA) are frequently used interchangeably. SGA is diagnosed when the birth weight is less than the population norms on the growth charts (less than the 10th percentile for that gestational age) (2).

SGA refers to birth weight without considering in-utero growth. An IUGR newborn, on the other hand, may have an ac-

ceptable gestational weight but may have experienced any in-utero growth retardation as a result of a perinatal injury (3). It is important to note that a neonate with a birth weight less than the tenth percentile can be SGA but not an IUGR, whereas a neonate with a birth weight greater than the 10th percentile can be an IUGR (4).

Every year, an estimated 13.7 million newborns are born at term with low birth weight (LBW), accounting for 11% of all neonates delivered in developing nations (5). When compared to industrialized countries, this incidence is six times higher. IUGR accounts for 23.8% of all infants, or nearly 30 million every year. The prevalence of IUGR varies by country, population, and race, and it rises with decreasing gestational age. Asia bears the heaviest burden of these IUGR neonates, accounting for nearly three-fourths of all affected infants, followed by Africa and Latin America (5).

The current literature outlined multiple risk factors for IUGR such as race and maternal age (below 16 and above 35 years), low socioeconomic status, various maternal illnesses that impact blood circulation can decrease uteroplacental blood flow (i.e. hypertension, diabetes, chronic renal disease, thrombophilia, systemic lupus erythematosus, antiphospholipid syndrome, sickle cell disease, infections) (6-8).

Artificial reproductive technologies (ART) use increases the incidence of IUGR in both singleton and multiple gestation pregnancies (9). Low pre-pregnancy weight and pregnancy weight growth put women in the lowest quartile at the highest risk of having an IUGR fetus (10). Finally, smoking, drinking, drug use, and certain living conditions increase the risk of IUGR. Placental (abnormal placentation and inflammation) and fetal (genetic, metabolic or infectious disorders) risk factors should also be taken into consideration for the screening of IUGR (11-13).

Besides maternal-fetal characteristics, it is important to note that several ultrasound and chemical biomarkers have been included in the screening of IUGR. These include uterine artery Doppler pulsatility indexes, placental growth factor (PlGF), placental protein-13 (PP-13), soluble FM's-like tyrosine kinase-1 (sFlt-1) or vascular endothelial growth factor (VEGF) (14, 15).

IUGR can be defined and classified using the Delphi consensus (16), that is based on EFW and abnormal fetoplacental Doppler parameters: into early IUGR (< 32 weeks of gestation), and late IUGR (≥ 32 weeks of gestation).

Although current conventional antenatal screening of IUGR has a good predictive performance determined in numerous studies and meta-analyses, there are important nuances that can influence these results, and are represented by individual genetic potential, epigenetic influence, specific growth charts, or various definitions of IUGR. Thus, it is important to optimize the current screening strategies in a cost-effective manner in order to offer the best results with minimal efforts.

In the last decade, the artificial intelligence approaches such as machine learning algorithms and artificial neural networks have been successfully used for the prediction of a variety of medical disorders such as small for gestational age fetuses, preeclampsia, HELLP syndrome, seropositivity for hepatitis viruses, (7, 17, 18). Their results are encouraging, many of

them obtaining good predictive performances. In this study we aimed to design and test a Feed Forward Backpropagation neural network (FFBPN) for the prediction of IUGR and its subtypes.

MATERIALS AND METHODS

We performed a prospective observational study at "Cuza-Voda" Clinical Hospital of Obstetrics and Gynecology, Iasi, Romania, between 3rd of January 2023 and 10th of September 2023. The study included pregnant patients with singleton pregnancies who underwent conventional first trimester screening between 11 and 13+6 weeks of gestation.

The exclusion criteria included pregnancies with multiple fetuses, maternal age below 18 years, inaccurate dating of gestational age in the first trimester, abortions in the first and second trimesters, fetal death inside the womb, loss of follow-up, incomplete medical records, or the mother's inability to provide informed consent.

The study was carried out as part of the "Net4SCIENCE: Network for Applied Doctoral and Postdoctoral Research in the Fields of Smart Specialization - Health and Bioeconomy" project, with project code SMIS: 154722. Ethical approval for the study was obtained from the Institutional Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi (No. 151/13 February 2022).

All patients underwent ultrasound examination between 11 + 0 and 13 + 6 weeks of gestation by certified obstetricians using an E8/E10 (General Electric Healthcare, Zipf, Austria) scanner to measure crown–rump length (CRL) and nuchal translucency (NT) of the embryo as well as the maternal mean uterine artery pulsatility index (UtA-PI).

A 5 mL blood sample was collected from patients and was stored at at -20°C until we determined the serum levels of human chorionic gonadotropin (β-HCG), pregnancy associated plasma protein-A (PAPP-A), PlGF, and PP-13 using Brahms Kriptor analyzer (Thermo Fisher Scientific, Germany) and quantitative sandwich ELISA. Multiple of medians (MoM) values were calculated for all these markers, and were used for further data analysis. We also calculated the mean arterial pressure with a calibrated Omron device, and we transformed it in MoMs.

This study evaluated data from 108 pregnant patients that included demographic data, body mass index (BMI), smoking status, personal and family history of adverse pregnancy outcomes, maternal comorbidities, gestational age at IUGR detection, gestational age at birth, birthweight, and Apgar score.

The primary outcome of our study was represented by the detection of IUGR, while the secondary outcomes were represented by IUGR subtypes: early and late IUGR.

In the first step of our statistical analysis, we used descriptive statistics and comparisons of categorical variables (Pearson's χ^2 test) or continuous variables (student t test) for evaluated the differences between the main group: group 1 (with IUGR, n= 19 patients) and group 2 (control, n= 89 patients). *P* values under 0.05 were statistically significant. STATA SE (17, 2023, StataCorp LLC, College Station, TX, USA) was used for these analyses.

Our second phase involved creating of a FFBPN for the prediction of IUGR and its

subtypes that included maternal characteristics (age, BMI, smoking status, adverse pregnancy outcomes, comorbidities), MAP, and serum biomarkers (BHCG, PAPP-A, PlGF, and PP-13). Five-fold cross validation was performed on 80% testing and 20% training data.

A sensitivity analysis was done to characterize the predictive performance of the FFBPN for IUGR and its subtypes (early IUGR- 7 patients, and late IUGR- 12 patients). The sensitivity (Se), specificity (Sp), false positive rate (FPR), Matthews correlation coefficient, accuracy, precision, and F1 score were determined. The FFBPN was built and analyzed in Matlab (version R2023a, The MathWorks, Inc., Natick, MA, USA) (fig. 1).

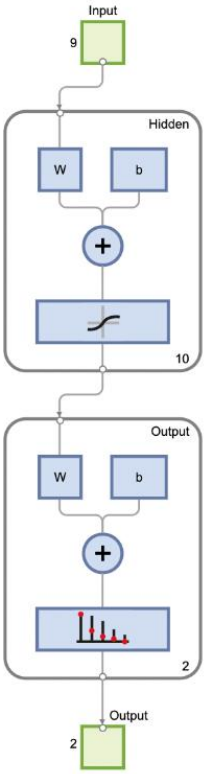

Fig. 1. Architecture of a FFBPN that comprises 10 neurons in the hidden layer. (FFBPN: feed-forward backpropagation neural network)

RESULTS

This study enrolled 108 pregnant patients that underwent first trimester screening. Their demographic and clinical characteristics are presented in table I. Both groups were relatively homogenous considering their clinical characteristics, and we could not find any statistically significant difference between both groups regarding their age (p= 0.32) or BMI (p= 0.76).

On the other hand, the patients who later developed IUGR presented significantly higher rates of smoking during pregnancy

(p= 0.007), autoimmune disorders (p= 0.02) or a personal history of adverse pregnancy outcomes such as intrauterine growth restriction, preeclampsia, abnormal placentation, preterm birth or premature rupture of membranes (p= 0.02).

TABLE I.

Demographic and clinical characteristics of patients included in the study

Clinical characteristics	IUGR group (n= 19 patients)	Control group (n= 89 patients)	*p* value
Age, years (mean ± SD)	25.16±4.32	27.13±3.22	0.32
BMI, kg/m^2, (mean and standard deviation)	23.25± 2.81	23.56± 3.16	0.76
Smoking habit (n/%)	Yes= 6 (40%)	Yes= 8 (9.4%)	0.007
Diabetes (n/%)	Yes= 1 (5.2%)	Yes= 2 (2.2%)	0.46
History of chronic hypertension (n/%)	Yes= 2 (13.3%)	Yes= 2 (2.7%)	0.08
History of autoimmune disorders (n/%)	Yes= 2 (13.3%)	Yes= 1 (1.1%)	0.02
Chronic kidney disease (n/%)	Yes= 0 (0%)	Yes= 2 (2.7%)	0.47
History of adverse pregnancy outcomes (n/%)	Yes= 4 (21%)	Yes= 5 (5.6%)	0.02

IUGR- intrauterine growth restriction, SD- standard deviation, n- number of patients, BMI- body mass index.

A comparison between the main groups regarding the MoM values of the biochemical markers and MAP is presented in table II and figure 2. Our results indicated a statistically significant difference between the two groups regarding the mean serum value of PlGF (p< 0.001) and PP-13 (p< 0.001). Other biomarkers and the MAP did not significantly differ between the two groups.

TABLE II.

Group comparisons of first trimester biochemical markers and mean arterial pressure.

Marker	IUGR group (n= 15 patients)	Control group (n= 180 patients)	*p* value
β-HCG, MoM (mean ± SD)	0.96± 0.15	1.14± 0.44	0.16
PAPP-A, MoM (mean ± SD)	0.81± 0.33	1.04± 0.57	0.06
PlGF, MoM (mean ± SD)	0.68± 0.14	1.26± 0.24	<0.001
PP-13, MoM (mean ± SD)	0.67± 0.16	1.08± 0.43	<0.001
MAP, MoM (mean ± SD)	1.05± 0.08	1.05± 0.06	0.53

IUGR- intrauterine growth restriction, SD- standard deviation, n- number of patients, MoM- multiples of median, β-HCG- beta-human chorionic gonadotropin, PAPP-A- pregnancy-associated plasma protein-A, PlGF- placental growth factor, PP-13- placental protein 13, MAP- mean arterial pressure.

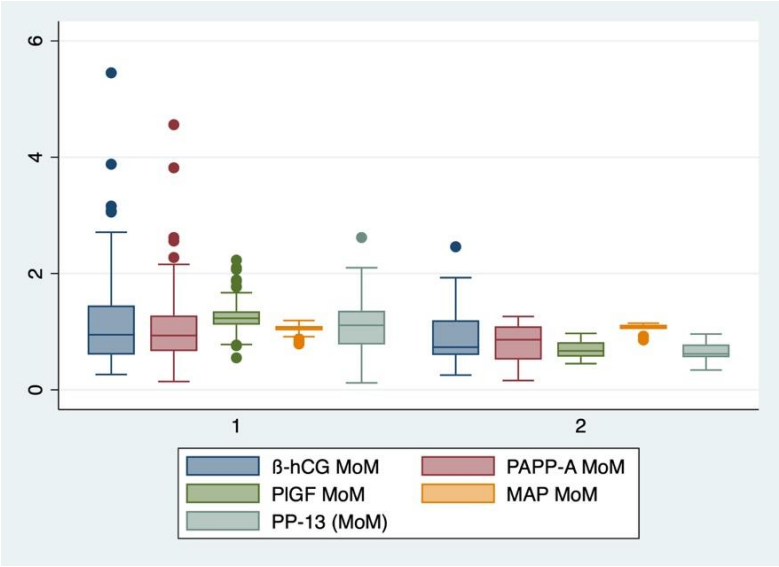

Fig. 2. Boxplot representing the comparisons of individual first trimester markers between groups. (1- control group, 2- IUGR group, 3- PE group, 4- PE and IUGR group).

In the second stage of our analysis, we determined the predictive performance of the FFBPN for the prediction of IUGR and its subtypes.

The results are presented in table III and figure 3. Our results indicated that the FFBPN predicted the development of IUGR during pregnancy with a Se of 94.7%, Sp of 97.7%, and an FPR of 2%. The value of the area under the curve for this neural network was 0.978 (figure 3).

On the other hand, when stratified for the prediction of IUGR subtypes, the FFBPN achieved lower predictive performance. Also, the sensitivity analysis revealed that the FFBPN could better predict early IUGR, with a Se of 71.4%, Sp of 94%, and accuracy of 90.7%.

TABLE III.

The predictive performance of individual and combined tests for the prediction of ptb at 14 days

AI model	Groups	Se (%)	SP (%)	FPR (%)	Matthews coefficient	Accuracy (%)	Precision	F1 score
FFBPN	IUGR (19 patients)	94.7	97.7	2	0.90	97.2	0.90	0.92
	Early IUGR (7 patients)	71.4	94	5	0.53	92.6	0.45	0.55
	Late IUGR (12 patients)	66.6	93.7	6	0.56	90.7	0.57	0.61

IUGR- intrauterine growth restriction, Se- sensibility; Sp- specificity,
FPR- False positive rate, FFBPN- feed-forward backpropagation neural network.

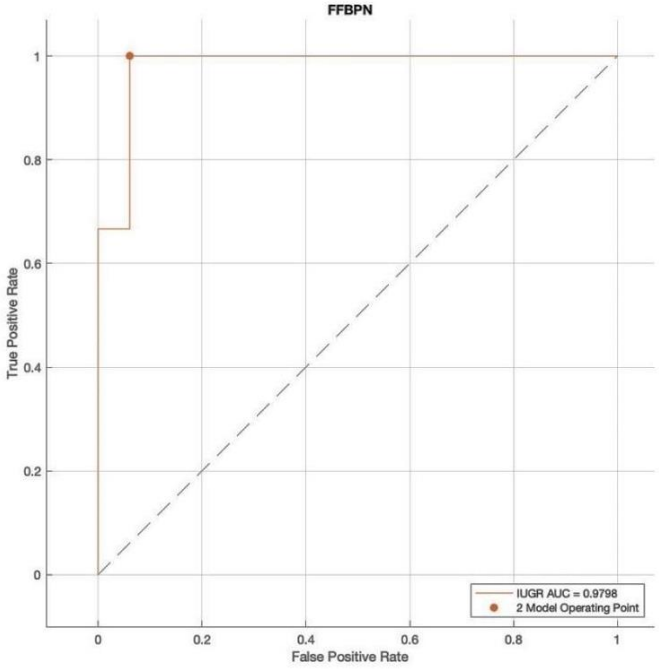

Fig. 3. AUC curve of the FFBPN for the prediction of IUGR.
(AUC- area under the receiver operating characteristic curve; IUGR- intrauterine growth restriction; FFBPN- feed-forward backpropagation neural network)

DISCUSSION

This pilot study evaluated the predictive performance of a FFBPN for the prediction of IUGR and its subtypes. Our results indicated that this type of neural network was good in discriminating those patients who would later develop intrauterine growth restriction. On the other hand, when faced with the prediction of IUGR subtypes, its discriminatory and predictive power were lower, but achieved overall good predictive performance for both early IUGR (accuracy of 92.6%), and late IUGR (accuracy of 90.7%).

Our results are comparable to those reported in previous literature. A recent retrospective study by Vicoveanu *et al.*, included 466 cases with thrombophilia, and used a FFPBN for the prediction of SGA newborns (18). Their results indicated a good predictive performance for the prediction of this disease, with an AUC value of 0.95, a true positive rate of 86.7%, and a false discovery rate of 10.5%. The overall accuracy of that model was 90%.

Moreover, Rescinito *et al.* performed a comprehensive systematic review and meta-analysis, encompassing a total of 20 studies, which investigated the utilization of artificial intelligence and machine learning models in the prediction of IUGR (19). The findings of this analysis revealed that the employed techniques exhibited a favorable diagnostic performance, with a Se of 0.84 (95% confidence interval [CI] 0.80-0.88), a Sp of 0.87 (95% CI 0.83-0.90), a positive predictive value of 0.78 (95% CI 0.68-0.86), and a negative predictive value of 0.91 (95% CI 0.86-0.94).

Although literature data is scarce regarding the use of this specific neural network for the prediction of IUGR, other studies have demonstrated overall good performance for other types of neural networks. For example, in a retrospective case-control study, Huang *et al.*, developed an artificial neural network, based on maternal peripheral blood parameters, that had an overall good predictive value for the occurrence of preeclampsia associated with IUGR (accuracy of 84.3%,Se- 97.7%, and Sp- 78%), as well as for the detection of severe IUGR (accuracy of 81.9%,Se- 97.2%, and Sp- 51%) (20).

Our study also confirmed the association between maternal risk factors such as smoking, autoimmune disorders and previous history of adverse obstetrical outcomes, and the occurrence of IUGR (21). Moreover, it outlined the significant differences regarding the serum levels of PlGF and PP-13 between the IUGR group and control, supporting the deficient angiogenesis theory in the development of this disorder (22).

This study has several limitations: small cohort of patients and the limited number of parameters included. On the other hand, this pilot study evaluated the predictive performance of a FFBPN in a prospective setting. Further studies, on larger cohorts of patients, could determine the cost-effectiveness of this type of FFBPN for the prediction of IUGR.

CONCLUSIONS

Neural networks are important tools for the prediction and stratification of various disorders, including IUGR.

It is important to provide a combined screening that included clinical, biochemical and ultrasound markers throughout pregnancy because later IUGR could be difficult to predict, especially from the first trimester.

Both PlGF and PP-13 could serve as important first trimester serum biomarkers for the prediction of ischemic placental disease, along with specific maternal and fetal risk factors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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THE AWARENESS OF MEDICAL WORKERS ON THE ARTIFICIAL INTELLIGENCE-BASED SOLUTIONS USED IN GASTROENTEROLOGY: AN EXPLORATORY SURVEY

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THE AWARENESS OF MEDICAL WORKERS ON THE ARTIFICIAL INTELLIGENCE-BASED SOLUTIONS USED IN GASTROENTEROLOGY: AN EXPLORATORY SURVEY (Abstract): Recently, significant changes regarding the approaches in several areas of daily life, healthcare, and research are emerging due to technological advance. The benefits of using artificial intelligence (AI) in medical care are suggestive. However, the opinion of the users could suggest several potential limitations. We **aimed** to evaluate the trends in understanding and using the AI-based solutions available in Medicine. **Materials and methods:** An online survey was designed and distributed to the members of the local academic and healthcare community regarding their perception on the AI-based applications used in their area of expertise. Furthermore, several items aimed to evaluate the perception of the potential users on the perceived advantages and weaknesses of AI-based applications in healthcare and medical research. **Results:** One hundred and fifteen medical workers were recruited for this study, of mean age 41 ± 10.07 years, and of whom 56.5 % were women. The majority of the responses demonstrated general understanding of AI-based applications in daily life and in medical act. As emphasized by several participants, some of the limitations of AI use in healthcare could arise based on AI-based solutions. Despite this, AI-based resources could offer high performance solutions to medical care and research consequent to technological progress. However, one of the most important drawbacks of AI-based solutions used in medical care could be related to the empathy involved in patient management. **Conclusions:** Further studies regarding the possible means to improve algorithm-based decision-making processes could be addressed to minimize AI-based solutions in medical care. **Keywords:** ARTIFICIAL INTELLIGENCE, MEDICAL CARE, AWARENESS, PERCEPTION, GASTRO-ENTEROLOGY.

Artificial intelligence (AI) is currently known as the most advanced technical field in data analysis and research, as the novel algorithms closely resemble human intelligence through high-performance computer simulations (1). The concept of AI dates back to the mid-90s when it was first proposed by Alan Turing (2) and technically applied by John McCarthy throughout the Dartmouth Summer Research Project (3). The latter forecast the integration of AI in medical care, as the Turing test results were groundbreaking (associating the complexity of AI with the human brain) (4, 5). However, despite the initial excitement, the practical applications only recently materialized and led to the third scientific and technological revolution prior to unlimited data storage and instantaneous long-distance communication (6-8). While there are studies that point to some limitations (for example, unwanted effects on human cognition, as discussed by Firth *et al.* (9), AI technology was quickly introduced into different domains in daily life as well as in different professions, such as healthcare and medical practice, with promising applicability (10). In this context, AI-based solutions provided significant improvements in medical services, as reflected by high-resolution for the interpretation of images, prompt data processing, workflow enhancement, and lessening errors (11). Moreover, some opinions acknowledge the utility of AI in improving the efficiency of several activities that are currently affected by limitations, such as information acquisition (12).

Besides the algorithms behind AI and its three core processes (Machine learning - ML, Deep learning - DL, and Artificial neural network - ANN) which transpose into novel solutions for the most complex challenges, controversies also arise. One pivotal part is the scarce transparency of the AI algorithms, notably the black box (13), and probable inaccuracies in disease diagnosis and treatment by eroding the patient-doctor relationship (14). AI could offer the achievement of personalized medicine and precision public health through its virtual (ML/DL) and the branches that offer user interfaces (carebots) (15,16), but not without raising, as anticipated, numerous ethical, professional, and technical questions. Considering the skepticism and divergent viewpoints on AI from medical students, physicians, and individuals, opinion surveys may help understand their overall perception.

Thus, we aimed to evaluate the trends in understanding and use of AI-based solutions available in medicine and healthcare. We also wanted to see whether if the lack of awareness, and mistrust of the users could be possible sources of controversy.

MATERIALS AND METHODS

Study design

A survey based on previously published studies from the UK (17, 18) and the US (19) via the Delphi modified method (17, 20, 21) was designed and disseminated among the medical workers from the Institute of Gastroenterology and Hepatology from the "St. Spiridon" County Emergency Clinical Hospital, Iasi, Romania, and the 42nd National Congress of Gastroenterology, Hepatology, and Digestive Endoscopy held June 7-10, 2023, in Iasi, Romania. Based on the previously reported data regarding similar outcomes, the survey was structured in four sections (17-19, 22, 23): (1) demographic and professional data; (2) the understanding of general and daily life artificial intelligence; (3) the understanding of medi-

cal care AI-based solutions and the willingness to use, if available; and (4) the human versus machine controversy. The inclusion criteria were: (1) Romanian medical doctors; (2) in Gastroenterology and Hepatology field of expertise; and (3) those who were admitted to the residency program or had already completed their training. The exclusion criteria consisted in: (1) medical workers that did not undergo residency programs; (2) medical studies; (3) currently not involved in medical care; or (4) medical workers whose expertise was not compatible with the Gastroenterology field.

Data analysis

All the data from the online surveying tool was collected in Excel format via the export option. *Version 26.0 of SPSS* (IBM SPSS Inc., Chicago, IL, USA) was used to statistically analyze the data. All the variables expressing categorical data were presented as percentages, while numerical variables were reported as mean and standard deviation (SD). Odds ratio, Wald test, and Fisher exact probability test were used to determine the influence of the demographic/professional variables on the binary answer items.

Ethical considerations

The survey was designed to be appropriate for online distribution via a free survey platform and was approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy (No. 301/4 May 2023). All the participants agreed with written informed consent and were recruited voluntarily, anonymously, and free of charge. Efforts were made not to collect personal data.

RESULTS

Demographic and professional characteristics

One hundred and fifteen medical care workers positively responded to the recruitment announcement via online notification. The description of the recruited participants in terms of demographic and professional characteristics is presented in tab. I. The sex distribution of the study group was 43.4 % males and 56.5 % females, while the mean age was 41 ± 10.07 years. The age interval of 30 - 39 years was predominant (47.82 %). All the participants came from the urban area. Finally, concerning the profession, about half of them (52.17 %) were primary care physicians.

TABLE I.

Demographic and professional characteristics of the population included in the study

Characteristics		Study group (N=115) n (%)
Gender	Male	55 (43.4)
	Female	65 (56.5)
Age	< 30	10 (8.69)
	30-39	55 (47.82)
	40-49	20 (17.39)
	> 49	20 (17.39)
	Total (years, mean ± SD)	41 ± 10.07
Medical experience	Resident doctor	15 (13.04)
	Medical specialist	40 (34.78)
	Primary care physician	60 (52.17)

Participants perceptions on artificial intelligence and everyday use

The second section of the survey focused on evaluating the general perceptions of the participants toward AI and their preferences to use basic and general AI-based applications, as they are available in daily life technology. The definitions offered by the participants for AI ranged between "algorithms" and "technology" (fig. 1). While 69.56 % of the participants use AI-based applications in their everyday lives, most of the participants generally enumerated chatbots (20.18 %), medical apps and AI-based scores (20.18 %), statistical tools (7.89 %), voice commands and recognition (11.40 %), and other multimedia apps and platforms (10.53 %) as the main AI-based solutions they encountered.

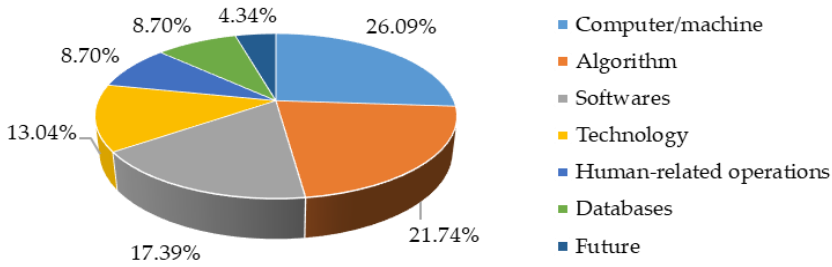

Fig. 1. Participants' preferences to use basic and general AI-based applications

Regarding the limitations of AI technology, 73.91 % of the respondents agreed that AI-based applications equally imply both advantages and disadvantages. While more than half of the respondents (52.17 %) do not consider that AI-based solutions could be the subject of serious security breach, emotional and social issues (26.09 %), addiction (17.39 %), and predisposition to errors (16.52 %) were also mentioned as limitations (fig. 2). Furthermore, almost 70% of the participants stated that AI could possibly replace humans in certain tasks at some point in the future.

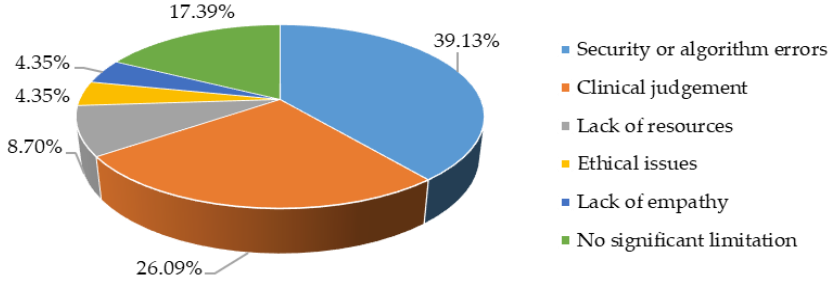

Fig. 2. Limitations of AI-based applications that are available for gastroenterology practice

Moreover, our results showed that neither age (OR=1.0085, 95% CI=(0.9661, 1.0527), p=0.7), nor the sex (OR=1.0370, 95% CI=(0.4650, 2.3127), p=0.92), did

significantly contribute to a segregation of responses regarding the idea of human being replaced by AI in certain tasks. On the other hand, all the medical residents and most of the primary care physicians (83.33 %) stated that AI could possibly replace humans in certain tasks at some point in the future. By contrast, only 37.5 % of the medical specialist doctors had the same opinion, this being a significant difference (χ^2=31.36, p < 0.001, in Fisher exact probability test).

The understanding and use of AI-based solutions in gastroenterology

The third section of the survey aimed to evaluate the awareness of the participants on the AI-based solutions available for clinical practice in Gastroenterology. Not necessarily connected to the current field of practice, the most of the respondents (86.95 %) stated that Telemedicine would be a valuable tool in primary care. When we applied data stratification tests, we found that age (OR=0.9427, 95% CI=(0.8951, 0.9928), p=0.025) was significantly correlated as contributor to this opinion. Moreover, we found that men were less likely to report a positive response, as compared to women (OR=0.33, 95% CI=(0.1060, 1.0482), p=0.05). The analysis of the data showed that the medical experience of the respondents did not pose a significant difference in responses on the value of Telemedicine, as we found that all the medical residents, 12.5 % of the medical specialist doctors, and 21.27 % of the primary care physicians gave a positive answer at this question (χ^2=2.95, p=0.22, in Fisher exact probability test).

More than half of the participants (52.17 %) considered that AI-based applications in Gastroenterology equally imply both advantages and disadvantages. However, most of the respondents pointed out that the limited resources (finances, time, and trainers) and AI-specialized personnel could be possible sources of reduced use of AI-based applications in their field of activity. Also, almost half of the respondents stated that they had not previously attended scientific events (43.47 %) or were not planning to register for training courses (56.52 %). In addition, the participants highlighted that the lack of clinical judgment and empathy, security or algorithm errors, the expense of resources, and ethical issues could raise some concerns regarding the use of various applications based on AI that are available for Gastroenterology practice (fig. 3).

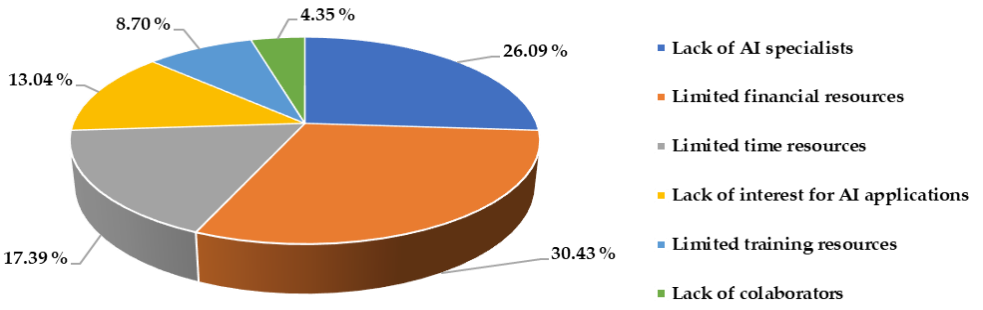

Fig. 3. Limitations of AI-based applications that are available for gastroenterology practice

Nevertheless, 86.95 % of the participants suggested that AI-based applications used in Gastroenterology could lead to some discordance between algorithmic response and medical decision. Despite this, 91.3 % of the medical doctors that were interviewed are not concerned about the possibility of being replaced by AI in their field. Our results showed that the age (OR=1.1798, 95% CI=(1.0393, 1.3394), p=0.01) of the participants significantly contributed to the stratification of the results in terms of the possibility of being replaced by AI in the field. Moreover, we observed that men are more likely to report this possibility, as compared to women, but not in a significant manner (OR=1.3333, 95% CI=(0.3639, 4.8848), p > 0.05). Also, we observed that neither medical residents, nor primary care physicians positively responded to this item (Fisher exact probability test not performed). In this context, most of the participants agreed that AI-based applications could be useful in diagnosis and treatment. However, the need for human validation was repeatedly mentioned in the situation of receiving AI-based suggestions on differential diagnosis, paraclinical investigations, and drug interactions. Moreover, some of the participants highlighted that these suggestions would not be as useful as predicted if a dichotomous response is present when the medical decision implies temporization or prioritization rather than a choice between action and non-action.

DISCUSSION

Healthcare is currently one of the most important branches in our society, and thus continuous development to improve diagnosis and treatment is needed. Together with the development of AI technology in the last decade, many solutions also address the medical care. In this way, as Gastroenterology also vastly benefited from AI-based solutions in paraclinical investigation, diagnosis, and treatment, we aimed to evaluate the perception of gastroenterologists regarding the extension of AI-based solutions used in their field. The results of our survey showed that, in contrast to previous similar studies, the participants recently gained more knowledge regarding the general understanding of AI (as seen in daily use) and in their area of expertise. However, this perception improvement did not significantly change the opinion of the participants on the advantages and disadvantages of daily life and professional AI-based solutions, findings that were consistent with previous similar studies (22, 24, 25).

The IMDRF SaMD Working Group (26) initially described medical devices as tools to be used in prevention, diagnosis, and disease management (ranging from treatment monitoring to symptoms alleviation or life support). The implications of AI-based software in these healthcare endeavors are meant to bring important improvements in the speed, precision, and accuracy of the medical act. However, the majority of the participants agreed that human validation is needed when AI-based applications are used in medical care and that the medical decisions should only be attributed to the human involved in the medical act, regardless of the assistance of the AI. In accordance with this, a recent study that evaluated the opinion of the American public on triage or discharge decisions showed a clear preference for human medical professionals over an AI-based algorithm (27). With regards to the possibility of being replaced by AI in their domain, almost all the participants in our study expressed no concerns about this aspect, as they clearly pointed out that the lack

of empathy of the AI-based applications could possibly prevent this. Goldhahn *et al.* (28) described the patient inputs during the medical act as a variety of emotions, values, personal preferences, and non-verbal communication that could hardly be substituted by technology. In this context, recent efforts were made to improve AI-based technology used in healthcare to recognize and respond to the facial emotions of the users, especially in carebots and online platforms/apps for mental therapy (29). However, the most difficult challenge for AI-based solutions in healthcare is their implementation in daily clinical practice, instead of determining whether the technologies will be capable enough to prove their effectiveness (30-32). In the present study, most of the respondents noted that AI-based technology could be helpful in diagnosis and treatment, leading to significant improvements in the patient's life. In addition, a recent review suggested that by adding AI technology to the healthcare system, the expertise of medical personnel could be complemented and enhanced, thus contributing to improved service quality, patient outcomes, and system performance (33).

Lately, due to the fast progress of technology, many AI-based resources have successfully been implemented in Gastroenterology. For an instance, high-performance recognition software was technologically customized for real-time endoscopy, along with high-quality graphical interfaces (34,35). Despite the fact that these tools proved their utility, some arguments are brought by the manifestation of obsolete behavior in clinicians when AI-based technology advances in performance and quality. In this context, the refusal of healthcare workers to use AI-based resources could be explained due to inflexibility or a lack of AI specialists, as seen in our study and also suggested by Quinn *et al.* (36) and Lambert *et al.* (37). Moreover, we identified that the lack of significant resources, such as financial support or time, could contribute to the limitation of AI-based solutions use during the medical act.

Regarding the possible errors that could occur during implementation and use, most of the participants in our study highlighted that AI-based solutions could be the source of some security issues. This limitation was documented by Pedro et al (38) and solutions such as permanent evaluation and update of the software by technical engineers, as well as standard, logistic, and ethical guidelines for AI-based solution implementation in healthcare were proposed.

Some of the main limitations of the present study address the reduced number of participants, the complexity of the survey, and the focus on just one medical specialty. However, we are currently considering to further extend the number and the medical field of the recruited participants. Concussive with this, we aim to improve the survey to include more aspects of AI-based solution awareness, as the current form of the survey was an initial and a less complex design. AI-based solutions are of great variety throughout the medical fields, thus the survey could also evaluate some field-specific aspects. Consequently, this preliminary data could only provide more information regarding the awareness of medical workers involved in Gastroenterology and Hepatology area of expertise.

CONCLUSIONS

This study reflected the awareness of the recruited medical workers regarding the potential of the use of AI-based solutions as helpful tools in the medical field, offer-

ing substantial advantages in both diagnosing patients' conditions and resolving their issues through the use of pre-established algorithms. However, AI-based solutions could need substantial resources, such as finances, time, and specialists, to provide the expected results. Nevertheless, AI-based solutions could be seen as valuable assets that could bring important improvements in the speed, precision, and accuracy of the medical act. However, AI-based solutions could require solid validation and thorough training of the users.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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CRIP-1 OVEREXPRESSION-PROGNOSTIC FACTOR IN CHILDREN'S OSTEOSARCOMA?

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CRIP-1 OVEREXPRESSION - PROGNOSTIC FACTOR IN CHILDREN'S OSTEOSARCOMA? (Abstract): Predicting the clinical evolution of patients with osteosarcoma is an essential condition for personalized treatment. In researching new and reliable biomarkers we identified CRIP-1 (Cysteine-Rich Intestinal Protein 1). Its *overexpression* has been documented to have a significant prognostic impact on gastric and colorectal cancer. Therefore, we aimed to investigate the *overexpression* of CRIP-1 in pediatric osteosarcoma and assess its potential as a prognostic biomarker. **Materials and methods:** We analyzed 65 samples from patients diagnosed with osteosarcoma at "Sf. Maria" Emergency Clinical Hospital for Children from Iasi, between 2017 and 2021. Two staining kits were used: Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC Kit from Abcam and Novolink Poly HRP Kit. **Results**: CRIP-1 was over expressed in 92.6% of the cases. The lower the degree of differentiation, the less CRIP-1 is over expressed and the more frequently it is found in the nucleus. **Conclusions:** Could this mean that CRIP-1 overexpression is associated with a better prognosis? The study highlights the clinical implications of CRIP-1 in osteosarcoma, emphasizing its importance for prognosis, and personalized treatment strategies. However, it calls for further investigation into CRIP-1 role in cancer biology (molecular pathways and interactions with other prognostic factors). **Keywords:** OSTEOSARCOMA, CRIP-1 OVEREXPESSION, CANCER, PROGNOSIS.

INTRODUCTION

Osteosarcoma is the most common malignant bone tumor in children and adolescents, characterized by aggressive growth (1, 2). One of the significant challenges in managing osteosarcoma is the high rate of metastasis observed at the time of diagnosis (3). This underscores the critical need for early detection and the identification of biomarkers that can predict therapeutic outcomes and disease progression.

Although advancements in treatment protocols have increased the 5-year survival rate to 50–70% (4, 5), a considerable number of patients still face recurrent or refractory metastatic disease with limited therapeutic options (6). To address this, there is an urgent need to identify reliable biomarkers that can forecast the prognosis of osteosarcoma patients, ultimately facili-

tating personalized therapy (7).

Identifying tumor markers and elucidating their interrelationships could pave the way for targeted and individualized therapies, thereby improving the survival rates and quality of life for cancer patients.

Studying the osteosarcoma microenvironment, both *in vitro* and *in vivo*, can enhance our understanding of the tumor's evolutionary behavior. Combining the expression profiles of specific biomarkers with responses to monotherapy or combined therapies in pediatric osteosarcoma could explain various clinical and evolutionary characteristics, leading to improved clinical and therapeutic management (8).

Cysteine-Rich Intestinal Protein 1 (CRIP-1) is a member of the LIM/ double zinc-finger protein family, involved in regulating cellular processes such as growth, differentiation, and apoptosis (9, 10, 11). CRIP was first identified as a developmentally regulated intestinal gene and has since been found in a variety of different tissues and cells (11). CRIP-1 has emerged as a potential biomarker of interest in various cancers. Studies have indicated that CRIP-1 expression is altered in several malignancies, including breast, colorectal, gastric, cervical, ovarian, thyroid, pancreatic, and prostatic cancers, suggesting its role in tumor genesis and cancer progression (9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20).

However, the role of CRIP-1 in osteosarcoma, particularly in the pediatric population, remains underexplored. Given its involvement in other cancer types, CRIP-1 may serve as a prognostic factor in osteosarcoma, potentially influencing tumor behavior and patient outcomes. Investigating CRIP-1 expression in osteosarcoma could provide new insights into the disease's molecular mechanisms and identify targets for novel therapeutic interventions.

This study aims to evaluate CRIP-1 overexpression as a prognostic biomarker in pediatric osteosarcoma. By analyzing CRIP-1 expression levels in osteosarcoma tissue samples and correlating these levels with clinical outcomes and pathological features, we seek to determine its potential utility in predicting disease progression and guiding treatment decisions. Our findings could contribute to the development of more effective, individualized treatment strategies for young osteosarcoma patients, ultimately improving survival rates and quality of life.

MATERIALS AND METHODS

A total of 65 samples from patients diagnosed with osteosarcoma at “Sf. Maria” Emergency Clinical Hospital for Children from Iasi, between 2017 and 2021, were analyzed. The tissue samples obtained from the cases in this study were processed using a standardized protocol. They were fixed in 10% buffered formalin, decalcified with EDTA, and embedded in paraffin. Sections of 5/4 µm were then prepared and transferred to electrostatically charged slides for subsequent immunohistochemistry analysis.

Two staining kits were used: Novolink Poly HRP and Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC Kit and CRIP-1 antibody from Abcam.

Initially, we used the Novolink Poly HRP kit, but it proved incompatible with the antibodies. Consequently, we switched to the Abcam Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC kit, which yielded positive results. Each staining process included both positive and negative controls to ensure that the staining

system functioned correctly, the positive and negative staining procedures were specific, and the specific protocol was properly followed.

CRIP-1 shows cytoplasmic and nuclear staining. The expression of CRIP-1 was assessed using the scores reported by Yu *et al* (21). The H-scores were calculated by determining the intensity of the staining and the percentage of the staining region with that intensity. Only stained cancerous cells were examined. The samples were divided into four different categories based on the intensity of nuclear or cytoplasmic staining: none = negative (0), weak (1), medium (2), and strong (3). The indexed total was calculated by multiplying the intensity grade by the percentage of stained region.

Specificity and sensitivity points can help maximize the positive rate. A score was assigned based on the number of stained cells and ranges from 0-4% to 80–100%. The score was judged poor from 1 to 5 (1 = 0-4%; 2 = 5-19%; 3 = 20-39%; 4 = 40-59%; 5 = 60-79%) and high from 6 (= 80-100%) to 18. We have multiplied 0 - 18.

The research was approved by the Ethics Committee of the "Sf. Maria" Emergency Clinical Hospital for Children from Iasi

RESULTS

In our research, CRIP-1 expression was assessed in 65 osteosarcoma tissue samples that met histopathological criteria. Patient observation sheets provided clinical and pathological information such as age, sex, tumor size, tumor grading, and the presence or absence of metastases. In the majority of cases, a diagnostic bone biopsy was the primary surgical procedure. By comparing the anatomopathological reports to the clinical data and immunohistochemistry findings, we discovered that the majority of patients were male, with ages ranging from 8 to 19 years; the highest incidence occurred at age 16. The most prevalent tumor location was the femur, namely the distal femur. The most common histological diagnosis was osteoblastic osteosarcoma (figs. 1, 2), followed by chondroblastic osteosarcoma, pleomorphic osteosarcoma, and clear-cell osteosarcoma.

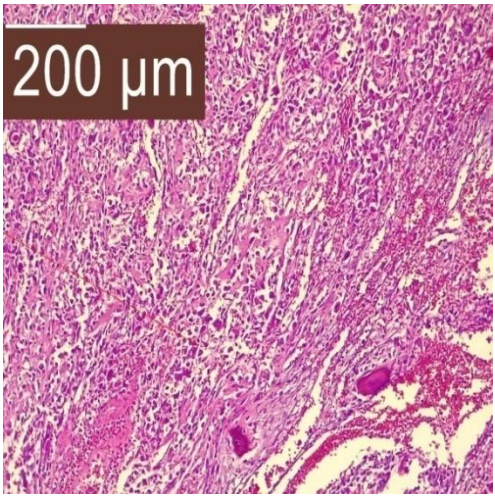

Fig. 1. Osteobalstic osteosarcoma, staining HEx10

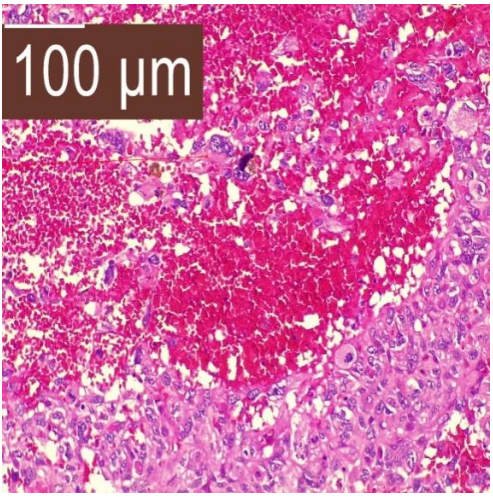

Fig. 2. Osteoblastic osteosarcoma, staining HEx2

From the perspective of tumor grading, our findings indicated that over 70% of the osteosarcoma cases were classified as high-grade, characterized by poorly differentiated and undifferentiated tumors (tab. I).

Metastasis was identified in two cases: one with lung metastasis and the other with skin metastasis.

TABLE I.
Anatomopathological characteristics of the cases

Variables	No. of cases	%
Tumor grade		
Grade 1 - well differentiated	8	12.3
Grade 2 - moderate differentiated	11	16.9
Grade 3 - poorly differentiated	38	58.5
Grade 4 - undifferentiated	8	12.3
H-score (CRIP-1% of nuclear/ cytoplasmic staining)		
3 = between 20-39	1	1.5
4 = between 40-59	2	3.1
5 = between 60-79	25	38.5
6 = between 80-100	37	56.9
CRIP-1 intensity		
negative	3	4.6
weak	19	29.2
medium	29	44.6
strong	14	21.6

Regarding CRIP-1 antibody staining, one case could not be assessed due to a technical defect. However, in the remaining cases, cytoplasmic and/or nuclear staining was observed, with over 60% of tumor cells showing positive staining (scoring 5 and 6) (tab. I).

Medium CRIP-1 staining was observed in 88.88% of osteoblastic osteosarcomas (figs. 3, 4, 5) and 85.71% of chondroblastic osteosarcomas (fig. 6). In pleomorphic osteosarcomas, 50% of cases exhibited strong CRIP-1 staining.

In the majority of examined cases, the staining intensity was medium and localized either cytoplasmic, nuclear, or both (Table I). Strong staining was noted in all cases of well-differentiated osteosarcoma. Among poorly differentiated osteosarcoma, 50% demonstrated intense staining. Also, high-score nuclear staining was identified in 50% of the undifferentiated osteosarcoma forms.

CRIP-1 was over expressed in 92.59% of cases. In the 2 metastatic blocks, staining was absent in the case with frontal skin metastasis and weak in the case with pulmonary metastasis, despite the overexpression (strong staining intensity) observed in the primary tumor. A correlation between histopathological type and staining intensity for CRIP-1 could not be established.

The lower the degree of differentiation, the less CRIP-1 is over expressed and the more frequently it is found in the nucleus.

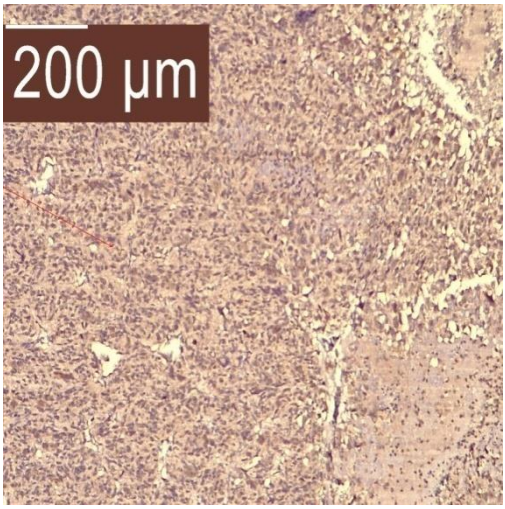

Fig. 3. Osteoblastic osteosarcoma, CRIP-1 weak to medium cytoplasmic staining x10

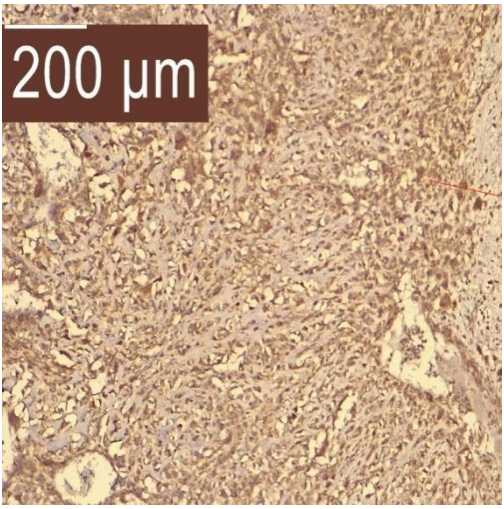

Fig. 4. Osteoblastic osteosarcoma, CRIP-1 medium to strong cytoplasmic staining x10

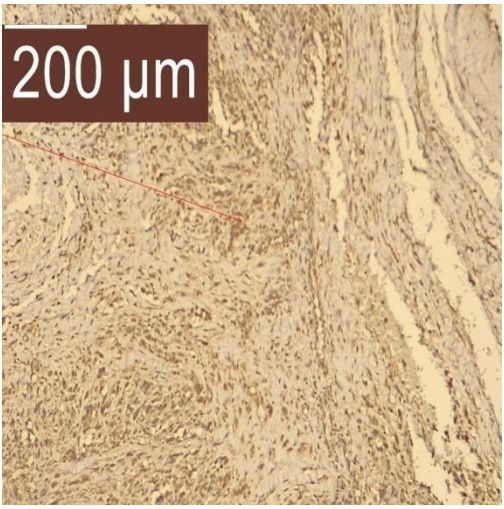

Fig. 5. Osteoblastic osteosarcoma, CRIP-1 medium to strong staining x10

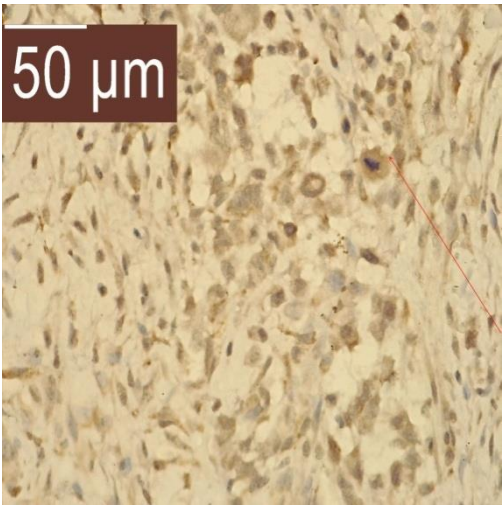

Fig.6. Chondroblastic osteosarcoma, CRIP-1 medium cytoplasmic staining x 40

DISCUSSION

Even though several studies have linked CRIP-1 expression to a poor prognosis in colorectal, breast, gastric, prostate, cervical, ovarian, thyroid, pancreatic, and endometrial cancer (10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25), there are very few published papers on the role of CRIP-1 in osteosarcoma (9, 22).

The data reported on the topic revealed contradictory results regarding the impact of CRIP-1 on osteosarcoma. A study published in 2011 demonstrating that CRIP-1 expression is strongly associated with a

favorable outcome and as significant negative predictors of systemic spread in osteosarcoma (9). A more recent work reported that overexpression of CRIP-1 was associated with a poor survival of osteosarcoma patients (22). Probably CRIP-1 has oncogenic and tumor suppressor properties specific to the tumor type, which require further clarification of the pathogenesis.

Our study aimed to investigate the expression of CRIP-1 in osteosarcoma tissue samples and its correlation with various clinical and pathological features, exploring the potential of CRIP-1 as a prognostic biomarker. The results revealed significant patterns in the demographic distribution, tumor characteristics, and CRIP-1 staining intensity, providing insights that contribute to the existing body of knowledge on osteosarcoma.

The demographic data showed that the majority of osteosarcoma patients were male, with ages ranging from 8 to 19 years, and the highest incidence occurring at age 16. These findings are consistent with previous research, which indicates a higher prevalence of osteosarcoma in males during adolescence-a period marked by rapid bone growth (22, 23). The predominant tumor location was the distal femur, aligning with literature that highlights the long bones around the knee as common sites for osteosarcoma (9, 22).

Osteoblastic osteosarcoma was the most frequent histological subtype, followed by chondroblastic, pleomorphic, and clear-cell osteosarcomas. This distribution mirrors established patterns, where osteoblastic osteosarcoma is commonly the most diagnosed subtype (22, 24). In the current research, over 70% of cases were classified as high-grade, characterized by poorly differentiated and undifferentiated tumors, which are known to be associated with aggressive disease and poorer prognosis (23). The presence of metastasis in two cases further underscores the aggressive nature of high-grade osteosarcomas.

Our findings on CRIP-1 expression revealed that over 60% of tumor cells exhibited positive staining for CRIP-1, with varying intensities and localization patterns, results that were in line with the literature (9). Baumhoer and collaborators reported 45% of cases with CRIP-1 expression (9). In the current research, CRIP-1 was overexpressed in 92.59% of cases, predominantly showing medium staining intensity. Notably, strong CRIP-1 staining was observed in all cases of well-differentiated osteosarcoma, and high-score nuclear staining was identified in 50% of undifferentiated osteosarcomas. This suggests that CRIP-1 expression may be associated with tumor differentiation status, where better-differentiated tumors show stronger CRIP-1 expression.

Interestingly, the correlation between CRIP-1 staining and metastatic potential revealed that metastatic tumors exhibited weaker CRIP-1 staining compared to their primary counterparts. Specifically, the case with pulmonary metastasis showed weak staining, and no staining was observed in the case with frontal skin metastasis. This disparity suggests that CRIP-1 expression might be reduced in metastatic lesions, indicating a possible role of CRIP-1 in the primary tumor environment but not in metastatic sites.

The study is limited by the small sample size and the retrospective nature of data collection, which may affect the general applicability of the findings. Additionally, technical issues in assessing one sample highlight the need for robust methodologies

in future studies. Prospective studies with larger cohorts are necessary to validate our findings and further elucidate the role of CRIP-1 in osteosarcoma pathogenesis. Functional studies exploring the molecular mechanisms of CRIP-1 could provide deeper insights into its role as a potential biomarker and therapeutic target.

CONCLUSIONS

Our study offers significant insights into the demographic and clinical characteristics of osteosarcoma, with a particular focus on the expression of CRIP-1. The data indicate that CRIP-1 is markedly overexpressed in osteosarcoma tissues and its expression appears to be correlated with the differentiation status of the tumor. Specifically, well-differentiated tumors tend to exhibit stronger CRIP-1 staining. Furthermore, the observed disparity in CRIP-1 expression between primary and metastatic tumors suggests that CRIP-1 may play a distinct role in the primary tumor environment compared to metastatic sites. These findings highlight the potential of CRIP-1 as a biomarker for osteosarcoma and suggest avenues for future research into its mechanistic roles and therapeutic potential. Prospective studies with larger patient cohorts and functional analyses of CRIP-1 are essential to validate these observations and to fully elucidate the implications of CRIP-1 expression in osteosarcoma. Understanding the role of CRIP-1 could lead to the development of novel diagnostic and therapeutic strategies, ultimately improving clinical outcomes for patients with osteosarcoma. We propose to expand this study by investigating the correlation between CRIP-1 overexpression and survival rate in patients with osteosarcoma.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest in their scientific research.

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TESTICULAR TUMORS IN PEDIATRIC POPULATION: SINGLE CENTER EXPERIENCE

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TESTICULAR TUMORS IN PEDIATRIC POPULATION: SINGLE CENTER EXPERIENCE (Abstract): Our study **aims** to provide valuable contributions to the understanding and management of this rare condition, with a particular focus on our single-center experience and analysis of cases in light of limited worldwide reports. **Materials and methods:** This study analyzed 18 patients between 2003 and 2023. We categorized patients into two age groups: prepubertal (ages 0-11 yrs.) and postpubertal (ages 12- 18 yrs.), based on the typical onset of puberty in males, in order to compare distinct tumor characteristics. We assessed clinical data, staging imaging, pre-operative serum tumor marker values, surgical approaches, chemotherapy protocols, and clinical outcomes. **Results:** There was no significant difference in the prevalence of the different types of clinical presentations between groups. The overall survival (OS) rate was 94.4%, while the progression free survival (PFS) was 77.8%. The postpubertal age group had a significantly lower PFS period compared to patients in the prepubertal age group. **Conclusions:** This research highlights the importance of precisely performing physical, imaging, and biological tests for the early detection of testicular cancer. It emphasizes the significance of ongoing research and collaborative efforts to enhance testicular cancer management. **Keywords:** TESTICULAR, TUMORS, PREPUBERTAL, POSTPUBERTAL, CHILDREN, ADOLESCENTS.

Testicular tumors (TT) are uncommon across the pediatric population, with an annual incidence of between 1.6 and 2 cases per 100,000 children (1, 2). Based on age, TT in boys exhibits a bimodal distribution, occurring between 2 and 4 years or during infancy (prepubertal group) and after 12 years (postpubertal group) (3). Clinical considerations during these age periods are essential for choosing appropriate and distinct treatment strategies. Given the scarcity of cases and the limited literature available globally on this topic, our goals are to underline the clinical, pathological, and outcome data from single-center cases to enhance understanding and inform future clinical practice.

MATERIALS AND METHODS

This study analyzed only the children with TT who were admitted to our institution between January 2003 and January 2023. We retrospectively selected patients using the ICD-10 coding system and re-

viewed medical chart records for epidemiological and clinical data, staging imaging, pre-operative serum tumor marker levels (STM), surgical procedures, medical oncology therapy(s), clinical outcomes, and the histological subtype. We categorized patients into two age groups: prepubertal (0- 11 years) and postpubertal (12- 18 years), based on the typical onset of puberty in males, approximately at 12 years of age.

Data from the study were analyzed using IBM *SPSS Statistics versions 20 & 25* and illustrated using Microsoft Office Excel/Word 2013. Quantitative variables were tested for normal distribution using the Shapiro-Wilk Test and expressed as averages with standard deviations or medians with interquartile ranges. Kaplan-Meier curves were used to illustrate and calculate overall survival time (OS) and progression-free survival time (PFS) in the analyzed groups. Tarone-Ware tests were used to compare OS and PFS between age groups.

RESULTS

We have identified 18 children with TT, 10 cases in the prepubertal group and 8 cases in the postpubertal group (tab. I). Detailed family histories were limited in the study. One 7-year-old patient had a history of multicystic renal dysplasia. Incidental palpable testicular mass was most common in the 12-18 age group (8/8), but there was no significant difference compared to the 0-11 age group (9/10) (p-value = 0.387). In our study, right-side tumors were more common (10/18), and no patients had bilateral tumors. Testicular pain was more prevalent in postpubertal patients (5/8) compared with prepubertal patients (1/10). Only one child from the postpubertal cohort presented with respiratory distress syndrome because of pulmonary metastasis.

TABLE I.

Comparison of diagnostic age according to age groups

Age	Average ± SD	Median (IQR)	Mean rank
Prepubertal	1.46 ± 2.01	0.91 (0.64-1.25)	5.50
Postpubertal	15.87 ± 1.8	17 (15-17)	14.50

Ultrasound was performed on all symptomatic and diagnosed patients, and only 7 required additional imaging. Computed tomography (CT) was used in 5 out of 18 patients, while magnetic resonance imaging (MRI) of the abdomen and pelvis was used in 2 out of 18 cases. The decision to conduct additional imaging studies was made based on clinical judgment and specific indications. These indications primarily included symptoms suggestive of advanced disease or findings on initial ultrasound examinations warranting further evaluation for accurate staging and treatment planning. In our study cohort, only a subset of patients met these criteria and thus required additional imaging studies. CT scans were exclusively performed on post-pubertal patients (n = 5), and only two results revealed significant findings related to disease spread (p-value = 0.016).

Positron emission tomography (PET-CT) was performed on three postpubertal patients. In the first case, the patient follows the chemotherapy protocol with favorable clinical progress. We conducted imaging one year

after diagnosis, which revealed certain lung abnormalities on CT scans; subsequently, a PET-CT scan was performed, which identified a probability of secondary pulmonary dissemination and hypermetabolism at the mediastino-pulmonary and bilateral axillary levels. While complete exclusion of dissemination was not possible, suspicion of pulmonary and nodal metastasis was high, leading to the initiation of a second-line chemotherapy protocol. In the second case, the patient presented with metastases upon admission and underwent initial chemotherapy for six months; subsequently, a PET-CT was deemed necessary, revealing metabolically active tumor remnants. In the last case, it was performed two months after finishing chemotherapy regimens, prompted by the parents' insistence on obtaining this exam and no detectable malignant FDG-hyper captured lesions were observed.

Histologic subtypes included 7 (38.88%) mixed germ cell tumors (mixed GCTs), 4 (22.21%) immature teratomas, 3 (16.66%) Yolk Sac Tumors (YSTs), 2 (11.1%) PT-RMS, 1 (5.55%) embryonal carcinoma (EC), and 1 (5.55%) cystic dysplasia of the testis (tab. II). Even though PT-RMS is not a TC, we included these 2 patients in our study due to the paratesticular mass. Immunohistochemistry diagnostic procedures were performed on a limited sample, comprising three patients (16.67% of the cohort). These evaluations were specifically focused on 2 PT-RMS (11.1%) and, regarding non-GCNIS derived tumors, were exclusively performed on an 8-month-old patient diagnosed with mixed GCT.

TABLE II.

Patient distributions according to age and histologic subtypes

Tumor subtype	0 - 11 yrs.	12 - 18 yrs.	p-value
Germ cell tumors derived from germ cell neoplasia in situ			0.002
-Mixed Germ Cell Tumors	1	6	
-Nonseminomatous germ cell tumors: Embryonal carcinoma	-	1	
Germ cell tumors unrelated to germ cell neoplasia in situ			
-Teratoma, prepubertal type (immature)	4	-	
-Yolk sac tumor, prepubertal type	3		
Paratesticular tumors (PT-RMS)	1	1	
-Cystic dysplasia of testis (benign)	1	-	

STM values were available for all cases, and measurements were performed preoperatively (tab. III). Alpha-fetoprotein (AFP) levels (normal value <34.8 ng/mL) ranged from 39 ng/mL to 5321 ng/mL (median 2255 ng/mL) in malignant cases. Patients from the postpubertal group had human chorionic gonadotropin (hCG) levels (normal value <1.2 mIU/mL) ranging from 150 mIU/mL to 45654 mIU/mL in malignant cases. The highest hCG value was observed in the patient with mixed GCTTs who had metastases at the time of the presentation. hCG and lactate dehydrogenase (LDH) levels were within the normal range in the prepubertal group. Elevated levels of both AFP and hCG were observed in six postpubertal patients. Among them, five patients were diagnosed with mixed GCTs, while one patient had EC.

TABLE III.
STM distribution according to age groups and histological subtype

Age group (No)/ STM	5 - 11 yrs.	12 - 18 yrs.	p-value
AFP elevated	3	7	0.038
- histological subtype	YST- 2 IT- 1	Mixed GCTTs- 5 EC- 1 PT-RMS- 1	
- highest value (ng/mL)	1800	5321	
hCG elevated	-	6	0.036
- histological subtype	-	Mixed GCTTs- 5 EC- 1	
- highest value (mIU/mL)		45654	
LDH elevated	-	6	0.001
- histological type	-	Mixed GCTTs- 6	
- highest value (U/L)		1200	

All patients within the age range of 12 to 18 years underwent radical inguinal orchidectomy (RO). Testicular sparing surgery (TSS) was deemed appropriate for three patients in the prepubertal age group; Initially, in two patients, the strategy involved performing a total excision of the tumor while preserving the seemingly unaffected testicular parenchyma. However, after a few days, a RO was decided upon following the pathological examination, which revealed a mixed GCT/ YST. In the last case of TSS, a surgical procedure was performed through a trans scrotal approach. Additionally, the right testicle showed enlargement attributed to the presence of a multicystic, septated tumor that was composed of serous citrine fluid. A biopsy was performed on the cystic mass and the testicle, with an extemporaneous evaluation ruling out malignancy. A 1-year-old patient, previously diagnosed with YST and who underwent trans scrotal orchidectomy at another medical facility, presented to our department with a local recurrence in the left hemiscrotum. As a result, a second surgery was performed to remove the recurrent area.

In the prepubertal group, seven patients received platinum-based chemotherapy (CHT) protocol and the SIOP MMT 95.3 protocol was used for patient with PT-RMS. In the postpubertal group, six children underwent platinum base CHT protocol, and patient diagnosed with PT-RMS was initially treated with vincristine, dactinomycin, and cyclophosphamide (VAC Protocol), but due to metastasis spreading to the lungs and lymph nodes, a second regimen with temozolamide and irinotecan was required.

Among the study participants, metastatic spread was observed in two postpubertal cases at initial presentation in our institute. One patient with mixed GCT and another case involved a patient with EC. After initial diagnosis, subsequent metastasis developed in one prepubertal patient after 14 months, and in three patients from the postpubertal group at 8, 12, and 18 months, respectively. The most frequently affected sites were the lung and retroperitoneal lymph nodes, followed by the brain and bones.

To obtain details concerning survival and mortality, an official request was sent

to the National Register of Persons (No. 19315-2022) for the public disclosure of relevant data. Due to the low frequencies of deaths (1 case- 5.6%) and disease events (4 cases- 22.2%), medians with interquartile ranges could not be computed for the OS and PFS periods. As such, the overall survival rate was 94.4%, while the PFS was 77.8%. According to the Tarone-Ware tests, the PFS period was significantly different (**p= 0.047)**. Patients in the postpubertal age group had a significantly lower PFS period (mean = 14, 95% C.I.: 8.15- 19.84 months) in comparison to patients in the prepubertal age group (mean = 187.33, 95% C.I.: 147.29- 227.37 months) (fig. 1).

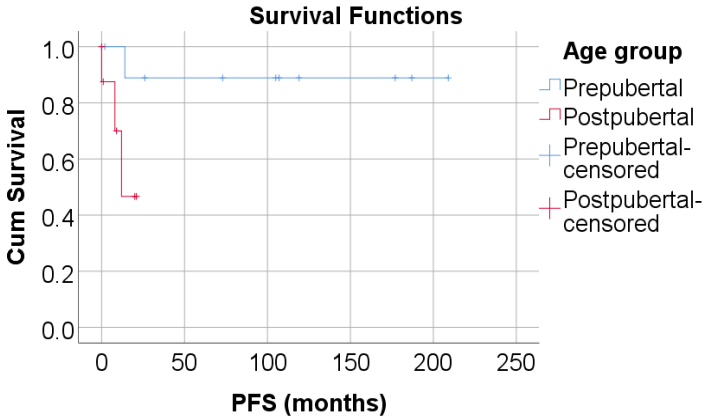

Fig. 1. Comparison of PFS period according to the age groups

DISCUSSION

In this article, we present our experience from a single center focusing on the pediatric population up to 18 years of age. Our hospital serves as a primary referral center for pediatric oncology in Eastern Romania, covering a significant region where the majority of pediatric tumors are treated. The risk of TC is 2-5 times higher in individuals with a history of cryptorchidism (the normal incidence is 1 in 400 men) (4). In contrast to documented risk factors, data on the risk associated with testicular microlithiasis (TM) are contradictory (5). In our study, none of the patients had a known history of cryptorchidism, and only one patient with cystic dysplasia of the testis had a personal history of multicystic renal dysplasia. The majority of TGCTs (85%-90%) manifest as asymptomatic testicular masses, which can be discovered by the patient, parent, or physician (6,7). Scrotal pain, with or without a mass, has been attributed to intratumorally hemorrhage or infarction in up to 50% of TC cases. In our study, beyond a testicular mass, pain was reported only in 5 postpubertal patients, and only 3 of them had the presence of tumoral necrosis or infarction on the histological report.

The connection between age at diagnosis and histologic characteristics, etiology, treatment strategies, and prognosis has been determined to be substantial (8). The age of 12 is commonly used as a proxy for the onset of puberty. Our cohort precisely matched the described bimodal distribution of disease, with peaks of incidence in both

the first year of life and the seventeenth year. Notably, among prepubertal malignant tumors, only a small proportion (5%) of prepubertal YSTs exhibited dissemination at the time of diagnosis, contrasting with postpubertal GCTs, where the percentage of cases with metastasis at presentation ranges from 20% to 30%. Additionally, nearly 90% of prepubertal children are stage 1 at the first presentation, compared to only 35% of postpubertal cases with testicular-limited cancer. This further demonstrates the highly aggressive and distinct nature of TGCTs in adolescents (9).

Increased AFP values were identified in more than half of the patients included in the study, which contrasts with findings by Kashyap, who observed that among 66 children with abnormally high AFP levels (>20 ng/mL), only 18 (27.3%) showed signs of malignancy (10). Preoperative serum AFP levels ranged from 39 ng/mL to 5321 ng/mL (median 2255 ng/mL) in malignant cases. In prepubertal patients, the AFP value was increased in two YSTs and in one case with an immature teratoma. Based on a meta-analysis, AFP was found to be very useful in distinguishing YSTs in prepubertal age cohorts (median 90%). These values were typically >100 ng/mL, which proved very useful for distinguishing teratomas, as they do not always produce elevated AFP values (11). Elevated hCG values were identified in 6 postpubertal patients included in the study. Regarding the type of tumor, we observed the highest value of hCG (45654 mIU/mL) in the patient with mixed GCT who had metastases at the time of presentation. In the literature, choriocarcinomas had the highest incidence of elevated levels, followed by seminomas (15 to 20%) and EC. It has been shown to be considerably higher in just two boys with mixed GCT in a study involving 34 male patients, leading some published research to conclude that testing for it in prepubertal disease age cohorts is minimally useful (11). Significant increases in LDH, have been linked to high tumor burden and may indicate recurrence (12). Elevated LDH values were identified in six postpubertal patients included in the study.

Imaging plays a significant role in the clinical staging and subsequent restaging of patients with GCTs (13,14). US has been used for all diagnosed patients, with only 7 requiring additional imaging studies. Recently, 18F-FDG PET has been used alongside CT to monitor patients for metastasis or recurrence (15). In our series, PET-CT was performed on 3 postpubertal patients. Considering the findings of some researchers, who reported a high incidence of false-negative PET scans within two weeks of chemotherapy, it is imperative that the examination be performed prior to chemotherapy (16). In our series, we provided a rational use for elucidating its diagnostic significance beyond seminoma.

Pohl *et al*. reported that 74% of the testicular masses identified in the prepubertal group in their research were determined to be benign (17). However, in our study, only one prepubertal patient was diagnosed with a benign lesion, which does not align with findings in existing literature. Previously, it was believed that YSTs were the predominant pathology in prepubescent males. Other research has acknowledged that there may have been selection bias in their report due to the absence of benign mature teratoma lesions among participants (18). In our sample population, we had 4 testicular teratoma with immature tissue and only 3 cases with YST.

A meta-analysis combining studies from IGCCCG and COG revealed excellent 6-year event-free survival (EFS) and overall survival (OS), with EFS of 78.5% and OS of 100% for stage 1, 100% for stage 2, 94.1% for stage 3, and 90.6% for stage 4 (19). In our study, the average OS rate was 94.4%, while the overall progression-free survival rate was 77.8%. Patients in the postpubertal age group had a significantly lower PFS period compared to those in the prepubertal age group. Several investigations in the literature have examined the genetic foundation of male infertility following cancer. These studies shed light on the genetic vulnerability associated with different cancer treatments and explore the possibility of men recovering reproductive function at the end of cancer treatment (20, 21).

Our study has a few limitations. These include the relatively small number of patients, the long-time span (20 years), the retrospective nature of the study, and possible selective bias because our institution is not used as a national referral medical center for TT.

CONCLUSIONS

Testicular tumors form a heterogeneous group that varies considerably in clinical presentation, location, histology, and biology. There is a great necessity to include patients in international partnerships, data exchange, and clinical trials at all stages and risk categories for understanding these uncommon tumors and, most critically, to improve outcomes. This study represents the largest pediatric testicular case series in eastern Romania, emphasizing the significance of ongoing research and collaborative efforts to enhance TC management. Achieving this necessitates further investigation through targeted studies and involvement in clinical trials, particularly given the limited number of worldwide reports.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of "Sf. Maria" Emergency Children Hospital's (No. 29289/12.10.2022) and the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi Ethics Committee (No. 247/15.12.2022) both approved the publication of this report.

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ANALYSIS OF THE IMPACT OF TELEREHABILITATION ON CARDIOVASCULAR RECOVERY IN PATIENTS DIAGNOSED WITH PERIPHERAL ARTERIAL DISEASE OF THE LOWER LIMBS

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ANALYSIS OF THE IMPACT OF TELEREHABILITATION ON CARDIOVASCULAR RECOVERY IN PATIENTS DIAGNOSED WITH PERIPHERAL ARTERIAL DISEASE OF THE LOWER LIMBS (Abstract): Peripheral arterial disease (PAD) is a significant public health concern, given its debilitating consequences, diminished quality of life (QoL), and the associated increased cardiovascular risk. Cardiac rehabilitation (CR) based on supervised exercise therapy (SET) is recommended as first-line treatment, but the patient adherence to these programs is low. Home-Based Exercise Therapy (HBET) may be an effective alternative, and can be achieved by telerehabilitation (TR), where patients receive exercise recommendations and are monitored remotely using current technological means. **Objectives:** The aim of this study was to analyze the impact of a TR program on the improvement of general health status in patients with PAD. **Materials and methods:** 20 people diagnosed with PAD followed a 12-week TR program based on walking at home with a progressive increase in the number of steps. Physical activity monitoring was performed using electronic devices, and participants' progress was assessed weekly by individual telephone calls with the investigator. Patients were reassessed at the end of the TR intervention. **Results:** Statistically significant improvements were observed in the majority of clinical and paraclinical parameters, alongside functional status, cardiovascular risk, and quality of life of the patients, from baseline to the end of the 12-week training period. **Conclusions:** Home-based exercise therapy monitored with digital technology may be an effective strategy for CR of patients with PAD. **Keywords:** PERIPHERAL ARTERIAL DISEASE, TELEREHABILITATION.

INTRODUCTION

Cardiovascular diseases (CVDs), particularly those of atherosclerotic etiology, are the leading cause of death worldwide (1). Mortality associated with these pathologies has risen by approximately 7.4 million over the past four decades (2), with a balanced distribution between genders (1).

The most common CVDs are coronary artery disease and stroke, followed by peripheral arterial disease (PAD) (3). PAD represents a significant public health issue globally, affecting around 5.6% of adults, with a 72% increase in prevalence observed from 1990 to 2019 (4). The main clinical manifestation of PAD is intermittent claudication (IC), defined by pain or discomfort in the legs, onset on exertion and improving at rest (6). This symptom leads to limitation of functional capacity, thus reducing patients' social participation, quality of life (QoL) and life expectancy (6,7).

In terms of rehabilitative treatment of PAD, supervised exercise therapy (SET) is the first-line recommendation according to the ***European Society of Cardiology Guidelines*** (8), but recent studies have demonstrated low patient adherence to these cardiac rehabilitation (CR) programs (9,10). Consequently, the guidelines offer an alternative approach of home-based exercise therapy (HBET) monitored remotely by electronic devices, telephone communication and logbooks (8). Thus, telerehabilitation (TR) could be a potential way to effectively implement HBET, implying that patients receive recommendations for their exercise program according to their PAD stage, their progress being followed with current technological means by their doctors (11-14).

We conducted a study based on a 12-week home-based exercise program, aiming to analyze the impact of TR on CR in patients diagnosed with PAD.

MATERIALS AND METHODS

Out of a total of 824 patients admitted to a CR clinic between January 1st and March 1st, 2024, 27 were diagnosed with PAD, of whom 20 were enrolled in our study after applying inclusion and exclusion criteria (fig. 1).

The first stage of the study aimed to establish the clinical-paraclinical profile of the patients by performing anamnesis, clinical examination, laboratory tests, 6-minute walk test, cardiopulmonary exercise test, ankle-brachial index measurement, carotid Doppler ultrasound, cardiovascular risk calculation and QoL assessment by specific questionnaire (PADQOL - Peripheral artery disease quality of life, QOLPAD - Quality of life of patients with peripheral artery disease, VASQOL-6 - Vascular quality of life).

We prescribed to each patient a personalized TR program based on walking at home for 12 weeks, with the frequency, intensity and duration of training adapted according to the individual clinical and paraclinical profile. Each participant received an electronic device (smart-watch) for monitoring physical activity and was trained in its appropriate use. An investigator conducted weekly individual telephone calls lasting approximately 10 minutes to assess general health, physical activity, and adherence to treatment, adjusting the exercise program by progressively increasing the number of steps performed daily by approximately 250 steps/day for the following week. Patients were re-evaluated at the end of the 12-week TR program, using the same methods applied at the time of enrollment in the study.

All participants provided written informed consent prior to enrollment in the study. The study protocol was approved by the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy Iași (approval certificate 10.01.2024) and by the Ethics Committee of Iași Clinical Rehabilitation Hospital (approval certificate dated 16.11.2022).

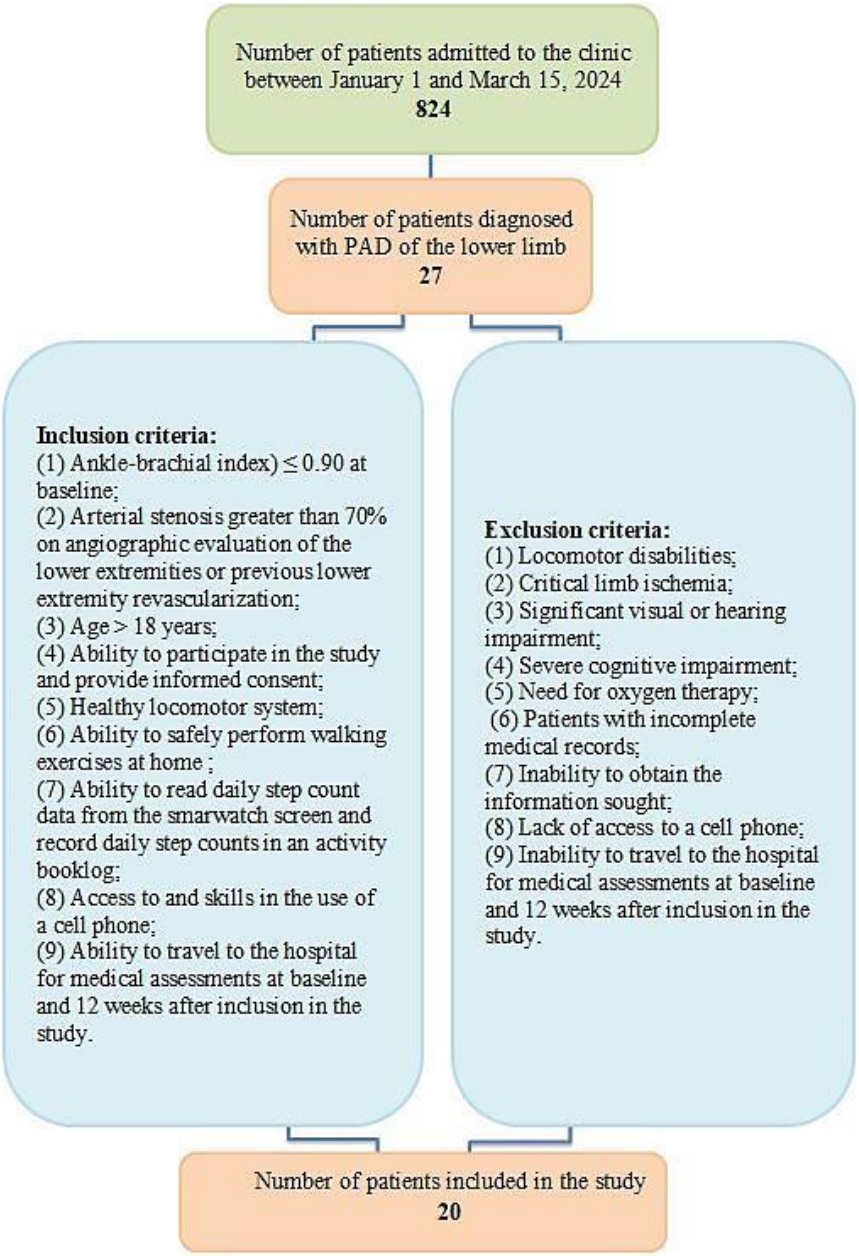

Fig. 1. Criteria for inclusion and exclusion of patients from the study

RESULTS

Of the 20 patients, 15 were male and 5 were female, with a mean age of about 67 years, mostly from rural areas. 40% of the studied population had a PAD course between 1 and 5 years. In terms of behavior, we found a preponderance of smoker or ex-smoker status. Regarding the clinical char-

acteristics of PAD, 80% of patients exhibited symptomatic (effort related) peripheral artery disease and 20% had chronic limb-threatening ischemia.

Concerning associated comorbidities, 80% of the individuals had diabetes mellitus or impaired fasting glucose, 85% hypertension, 15% elevated blood pressure, 40% chronic kidney disease, and 80% obesity or overweight. Additionally, 80% of population had dyslipidemia. A significant proportion of the cohort was diagnosed with heart failure (35%), chronic coronary syndrome (15%), chronic obstructive pulmonary disease (15%), and 10% had a history of stroke. A small proportion of studied population had documented lower limb arterial stenosis (20%) or other arterial stenosis (10%). Carotid atheromatosis was detected at Doppler ultrasonography in 90% of the participants.

Regarding medication, 65% of the patients had oral Cilostazol or Pentoxifylline as treatment and 55% of the patients had previously undergone intravenous treatment in hospital with Alprostadil or Pentoxify-lline. 25% of participants received a medical prescription for oral home treatment with oral Cilostazol or Pentoxifylline.

All 20 patients had completed the TR program and attended the follow-up medical visit at the end of the 12 weeks of training. 5 of these patients had difficulties using the smartwatch, encountering technical problems such as device failure or discharge and thus not fully recording physical activity. These problems were solved by replacing the device or providing additional instructions for its correct use.

After the 12 weeks of walking at home, we found statistically significant decrease in diastolic blood pressure in the study population (tab. I). This reduction can be attributed to improved endothelial function and increased nitric oxide production, leading to vasodilation and reduced peripheral vascular resistance. In contrast, systolic blood pressure remained stable, likely due to its stronger correlation with cardiac output. Walking, as a moderate-intensity aerobic exercise, also improves peripheral vessel elasticity and reduces diastolic pressure more effectively than systolic pressure.

TABLE I.

Changes in the blood pressure values between baseline and 12-week assessment

Parameter	The baseline evaluation	The final evaluation	p
SBP (mmHg)	147.00(131.00;153.00)	137.5(125.00;147.5)	0.841
DBP (mmHg)	78.25 ± 9.34	78.00 ± 8.55	0.003

Diastolic blood pressure – DBP; Systolic blood pressure – SBP.

In addition, we found a statistically significant decrease in body mass index (BMI) (tab. II) and glycemia (tab. III) between baseline and final assessment. This reduction in BMI is linked to increased energy expenditure from physical activity, promoting fat loss. Sustained physical activity improves insulin sensitivity and accelerates metabolism, contributing to better weight management and glycemic control, particularly in individuals with diabetes or impaired fasting glucose.

TABLE II.

Changes in the body mass index values between baseline and 12-week assessment

Parameter	The baseline evaluation	The final evaluation	p
BMI (kg/m²)	29.02 ± 4.87	28.62 ± 4.48	0.000

Body mass index – BMI.

TABLE III.

Changes in the glucose profile between baseline and 12-week assessment

Parameter	The baseline evaluation	The final evaluation	p
BG (mg/dL)	117.90(106.85;137.90)	118.55(96.00;131.15)	0.022
HbA1c (%)	6.23(5.89;7.33)	6.18(5.74;7.23)	0.003

Blood glucose – BG; Glycated hemoglobin – HbA1C.

At the end of the TR program, improvements in the lipid profile were observed (tab. IV), attributed to a combination of a hygienic-dietary regimen and medication. The diet reduced intake of saturated fats and cholesterol, while hypolipidemic treatment inhibited cholesterol synthesis in the liver.

Additionally, physical activity stimulates the enzymes responsible for the catabolism of low-density lipoproteins (LDL) and increases the levels of high-density lipoproteins (HDL), which contributes to a decrease in total cholesterol (TC) and LDL.

TABLE IV.

Changes in the lipid profile between baseline and 12-week assessment

Parameter	The baseline evaluation	The final evaluation	p
TC (mg/dL)	181.00 ± 40.70	171.86 ± 43.83	0.024
HDL-C (mg/dL)	51.70(48.50;62.15)	48.65(44.85;67.85)	0.001
LDL-C (mg/dL)	108.13 ± 36.07	100.82 ± 37.29	0.039
TG (mg/dL)	119.15(95;142.10)	111.10(91.25;148.16)	0.000

HDL-cholesterol - HDL-C**;** LDL cholesterol – LDL-C; Total cholesterol – TC; Triglyceride – TG.

Inflammatory markers such as erythrocyte sedimentation rate (ESR) and white blood cell count (WBC) significantly decreased (tab. V). This can be explained by the anti-inflammatory effects of moderate exercise like walking, which lowers pro-inflammatory cytokines as TNF-alpha and IL-6, and increases the production of adiponectin, an anti-inflammatory protein. A balanced diet further aids in reducing systemic inflammation.

The improvement in serum iron values (tab. VI) after completing the TR program may be the result of reduced inflammation, which improves iron bioavailability. In the presence of chronic inflammation, hepcidin is increased, which limits iron absorption and release from stores. As inflammation is reduced by exercise and diet, hepcidin levels decrease, allowing more efficient utilization of iron from stores and improved intestinal absorption.

Kidney function also improved, as seen by reduced urea and creatinine levels and enhanced creatinine clearance (tab. VII). This improvement is likely due to better blood flow, reduced oxidative stress, and improved glycemic control, which support kidney health over time.

Carotid Doppler ultrasound showed a stationary state of carotid atheromatosis post-TR intervention, likely due to the short duration being insufficient for visible plaque changes. The TR program improved inflammation and lipid profiles, stabilizing plaques without altering their size, while advanced plaques resist rapid changes and need more time to regress.

TABLE V.

Changes in the inflammatory status between baseline and 12-week assessment

Parameter	The baseline evaluation	The final evaluation	p
CRP (mg/dL)	0.82 ± 0.58	0.69 ± 0.39	0.338
ESR (mm/h)	12.5 (9.5;28.5)	11.5(9;24.5)	0.000
WBC (x10³/mm³)	6.94 ± 2.09	7.25 ± 2.06	0.000

C-reactive protein – CRP; Erythrocyte sedimentation rate – ESR; WBC – white blood cell.

TABLE VI.

Investigation of anemia syndrome at baseline and 12-week assessment

Parameter	The baseline evaluation	The final evaluation	p
Hb (g/dL)	12.93 ± 1.70	13.43 ± 1.32	0.000
Fe (µg/dL)	74.98 ± 29.59	75.12 ± 25.54	0.022

Hemoglobin – Hb; Serum iron – Fe.

TABLE VII.

Changes in the renal function between baseline and 12-week assessment

Parameter	The baseline evaluation	The final evaluation	p
Urea (mg/dL)	43.70(38.90;51.60)	39.91(34.15;48.60)	0.000
Cr (mg/dL)	1.07(0.92;1.27)	1.06(0.98;1.29)	0.001
Cr clearance (ml/min)	66.09 ± 12.62	63.96 ± 13.60	0.001

Creatinine – Cr; Creatinine clearance – Cr clearance.

In terms of functional status, there was an improvement in the onset threshold of IC (tab. VIII), an increase in the walking distance on the 6-minute walk test (6MWT) (tab. IX), and in exercise capacity assessed by cardiopulmonary exercise testing (CPET) (tab. X). QoL as assessed by PADQOL, QOL PAD, VASQOL-6 (tab. XI), and 10-year CV risk (tab. XII) were statistically significantly improved between the two assessments. The improvements can be attributed to regular physical activity, which enhances blood flow and perfusion to peripheral tissues, thereby alleviating symptoms of IC. Continuous exercise promotes cardiovascular adaptations, leading to increased aerobic capacity and endurance. Furthermore, physical activity reduces systemic inflammation and improves lipid profiles, positively influencing overall QoL. These physiological changes collectively contribute to a significant reduction in long-term cardiovascular risk, supporting better health outcomes for participants.

TABLE VIII.

Changes in the onset threshold of intermittent claudication and in the ankle-brachial index value between baseline and 12-weeks assessment

Parameter	Baseline evaluation	Final evaluation	p
The onset of IC (m)	192.50 ± 163.92	484.00 ± 687.93	0.000
Right ABI	0.76 ± 0.17	0.76 ± 0.19	1.000
Left ABI	0.75 ± 0.19	0,75 ± 0.17	1.000

ABI - ankle-brachial index; IC - intermittent claudication.

TABLE IX.

Changes in the in the walking distance and symptom-free walking time on the 6-minute walk test

Parameter	Baseline evaluation	Final evaluation	p
Distance (m)	175.00(110.00;275.00)	250.00(135.00;450.00)	0.000
Duration (min)	6.00(3.50;6.00)	6.00(6.00;6.00)	0.541

TABLE X.

Changes in the exercise capacity assessed by cardiopulmonary exercise testing

Parameter	Baseline evaluation	Final evaluation	p
Maximum LOE (Watt)	73.50(65.50;105.00)	78.00(71.00;107.50)	0.000
VE/VCO2	39.14 ± 11.14	42.07 ± 12.83	0.000
Target HR (bpm)	88.00(78.00;96.00)	89.00(83.00;100.00)	0.004

HR – heart rate; Level of effort – LOE; VE/VCO2 -ventilation/carbon dioxide production slope.

TABLE XI.

Changes in the quality of life between baseline and 12-week assessment

Parameter	Baseline evaluation	Final evaluation	p
VASQOL6 (points)	13.65 ± 3.23	18.50 ± 2.50	0.006
QOLPAD (points)	38.35 ± 6.96	46.05 ± 3.99	0.003
PADQOL (points)	120.80 ± 25.34	149.90 ± 18.60	0.002

PADQOL – Peripheral artery disease quality of life questionnaire;
QOLPAD - Quality of life of patients with peripheral artery disease questionnaire,
VASQQOL6- Vascular quality of life questionnaire.

TABLE XII.

Changes in the cardiovascular risk at 10 years

Parameter	Baseline evaluation	Final evaluation	p
10-years CR (%)	27.43 ± 17.95	25.19 ± 15.82	0.000

CR – cardiovascular risk.

Evaluation of adherence to the TR program showed that during the 12 weeks, 55% of the time patients achieved the target number of daily steps set for the following week, 59% of the time they increased the average number of steps / days compared to the previous week, 55% of the time they averaged more than 5,000 steps/day, and 11% of the time they averaged more than 10,000 steps/day (tab. XIII).

TABLE XIII.

Adherence to the telerehabilitation program

Target	% of time
Achievement of average number of target steps/day set for the following week	55.49 ± 35.65
Increase in average steps/day compared to last week	58.74 ± 9.55
Average more than 5 000 steps/day	55.46 ± 42.36
Average more than 10 000 steps/day	11.29 ± 26.37

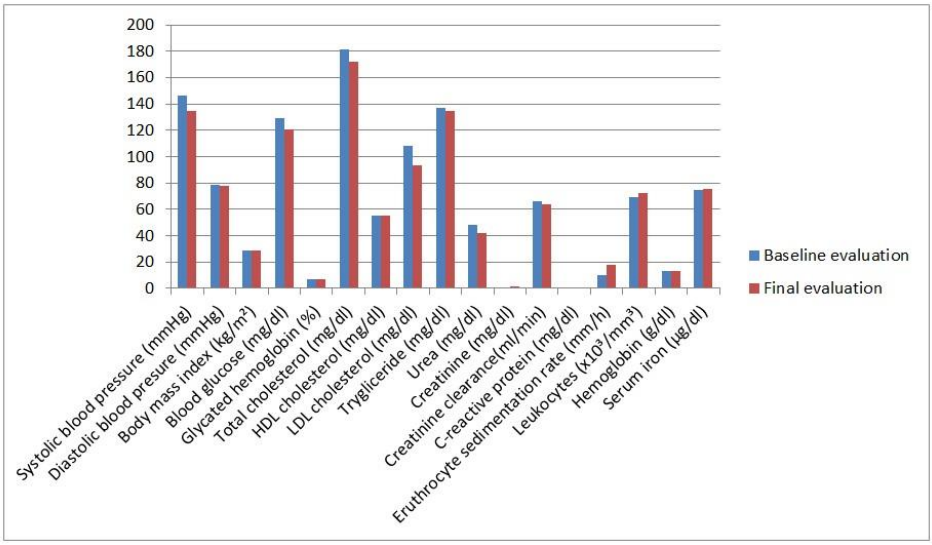

Fig. 2. Changes in the clinical-paraclinical profile between baseline and final evaluation

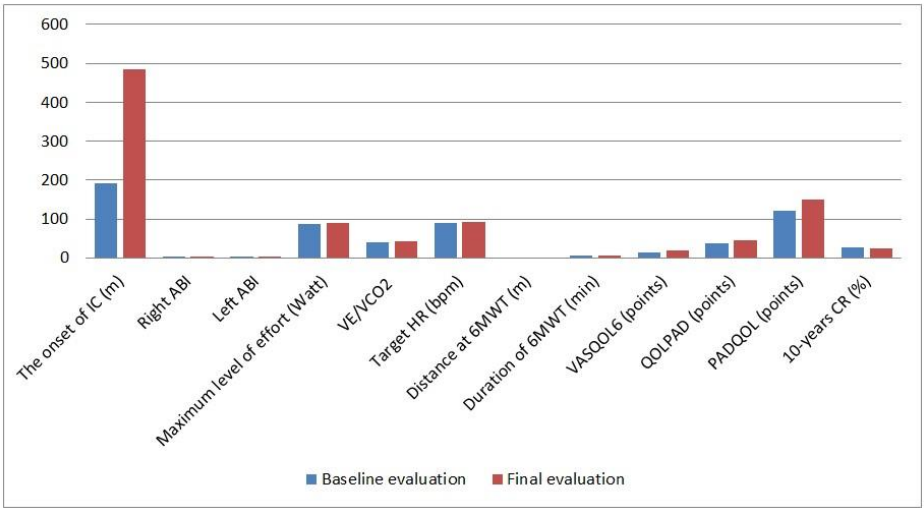

Fig. 3. Changes in functional capacity, quality of life and cardiovascular risk at 10 years between baseline and final evaluation

DISCUSSION

Telerehabilitation, an innovative method of delivering rehabilitation services through digital technologies, has demonstrated multiple benefits in CR of patients with PAD of the lower limbs.

Faster social and family reintegration

A key aspect of TR is that patients are not limited to regular visits to CR centers, as it is the case in SET, allowing them to spend more time at home with their families. This is important because early reintegration into the community and family supports patients' emotional and psychological health. Varnfield M *et al*. (2014) conducted a study in which patients engaged in a home-based rehabilitation program following acute myocardial infarction, in which smartphones were used. The authors demonstrated that the patients had a rapid reintegration into social and family life. This has profound implications for the patient's emotional support and motivation to continue the rehabilitation program in the long term (15).

Reduction of hospitalization-dependent depression and anxiety

TR reduces the need for frequent hospitalization and stressful interactions with the medical field, which can aggravate anxiety and depression associated with chronic diseases. A study by Frederix *et al*. highlighted the positive effect of TR in reducing levels of anxiety and depression among patients with CVDs, including those with PAD. Lack of dependence on hospitalization and continuous monitoring through TR helped to decrease patients' psychological distress, thus improving not only physical but also emotional recovery (16).

Increased functional and self-care capacity

Another significant benefit is the increased functional capacity of patients. TR gives them the possibility to manage their exercise program under remote supervision of specialists. Research conducted by Duscha *et al*. (17) and Schopfer (18) has shown a notable improvement in exercise tolerance and walking ability among patients with cardiovascular diseases who underwent TR interventions. These findings were similar to the ones observed in our study. Also, in two studies on the effects of TR in patients with acute myocardial infarction and coronary artery disease, respectively, an increase in self-care capacity was observed by developing skills necessary for long-term disease management (19, 20).

Increasing quality of life

QoL is a key aspect in evaluating the success of a rehabilitation program. TR allows a personalized and flexible recovery, reducing the discomfort and effort associated with frequent travels to rehabilitation centers. Otsuka at al observed a significant improvement in QoL assessment scores in PAD patients who underwent a HBET program after endovascular treatment (22). In our study we obtained similar results regarding the improvement of QoL assessed by specific questionnaires for patients with PAD, after the home rehabilitation program. The increase in patient satisfaction could be due to the fact that they are able to participate more actively in their social and family life and are able to maintain their daily routine with fewer interruptions.

Decreased cardiovascular risk and adverse events

Another important aspect of TR is its impact on cardiovascular risk reduction. In our research we that estimated cardiovascular risk decreased in 10 years. Our results are similar to the findings of the recent study by Kunutsor and Laukkanen, which demonstrated the role of physical activity in reducing the risk of major cardiovascular events in patients with pre-existing CVDs, including PAD (22). In addition, TR allows constant monitoring of clinical parameters and adaptation of the exercise program according to patients' responses, which offers the advantage of earlier intervention in case of cardiovascular complications.

Case stratification and hospitalization only of patients who need it

Another important benefit of TR for people with PAD is the ability to continuously monitor patients' health status and stratify cases according to severity. By using telemonitoring technologies, specialists can obtain real-time data on vital parameters and functional evolution of patients, which allows a more accurate assessment of the need for hospitalization or treatment adjustment. In this context, telerehabilitation can significantly reduce the number of hospitalizations by focusing medical resources on patients who really need intensive care in hospital. According to a study by Scherrenberg *et al*, remote monitoring allows early identification of signs of worsening disease and rapid adjustment of therapy without the need for hospitalization. In the same study, it was highlighted that patients with PAD monitored by TR had significantly fewer hospitalizations compared to those who followed conventional CR (23).

Reduced costs and optimization of human resource requirements

One of the major advantages of TR in CR of patients with PAD is the significant decrease in costs associated with treatment. The implementation of TR significantly reduces direct costs, including patient transportation to rehabilitation centers, hospital room and board expenses, and the intensive use of hospital equipment and infrastructure. In a study conducted by Cottrell *et al.*, TR was shown to reduce costs per patient by approximately 30-50% compared to conventional CR (24). This cost reduction can make rehabilitation accessible to a larger number of patients, especially in countries or regions with limited medical resources.

In addition, TR reduces the need for human resources involved in the rehabilitation process. In traditional CR, each patient requires frequent and direct interactions with a team of physicians, physiotherapists, nurses and other specialists. By remotely monitoring and guiding patients through automated programs or mobile applications, telerehabilitation allows medical teams to manage multiple patients simultaneously with the same resources. A study by Kraal *et al.* showed that medical staff were able to monitor and coordinate twice as many patients through telerehabilitation compared to traditional treatments without compromising quality of care (25).

Also, reducing the need to be physically present in medical facilities for constant monitoring, frees the hospital staff to focus on critical cases, which contributes to the overall efficiency of the healthcare system. In the context of increasing demand for healthcare services and staff shortages, telerehabilitation is becoming a practical solution to optimize available resources

and improve access to treatment for the largest possible number of patients.

CONCLUSIONS

HBET performed by using digital technology is an effective approach for CR in patients with PAD. TR programs require adaptation according to the clinical and paraclinical profile and psycho-emotional status of the patients to maximize benefits. Such individualized therapeutic interventions could provide significant benefits, including faster social and family reintegration, reduced levels of anxiety and depression associated with hospitalization, improved functional capacity and self-care skills, decreased cardiovascular risk and thus increased life expectancy, contributing to improved QoL of patients with this pathology. In terms of the public health system, TR could reduce the need for hospitalizations and the human resources involved in the treatment of patients with BAP, providing an efficient and sustainable approach for remote care and stratification of case severity, with identification of those requiring hospitalization.

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EFFICACY OF COMPUTER-ASSISTED COGNITIVE STIMULATION IN PSYCHIATRIC DISORDERS: CORRELATIONS BETWEEN DIAGNOSIS, TREATMENT AND EVOLUTION OF COGNITIVE SCORES

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EFFICACY OF COMPUTER-ASSISTED COGNITIVE STIMULATION IN PSYCHIATRIC DISORDERS: CORRELATIONS BETWEEN DIAGNOSIS, TREATMENT AND EVOLUTION OF COGNITIVE SCORES (Abstract): This study **aims** to investigate the effectiveness of computer-assisted cognitive stimulation (CACS) in improving cognitive deficits associated with psychiatric disorders. **Materials and methods:** The study evaluated the impact of the CACS intervention on cognitive functions using standardized instruments such as the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA). In addition, the influence of diagnosis and demographic variables on the results obtained was analyzed, highlighting significant variations depending on the typology of the psychiatric disorder treated. **Results:** The data indicate considerable improvements in the cognitive domain, with differences between diagnostic groups highlighting the importance of a differentiated approach. **Conclusions:** The results support the need for personalization of interventions and highlight the relevance of assessing qualitative factors in the application of CACS, opening new research directions for optimizing the treatment of psychiatric disorders through cognitive technology.
Keywords: COMPUTER-ASSISTED COGNITIVE STIMULATION (CACS), PSYCHIATRIC DISORDERS, COGNITIVE ASSESSMENT, MMSE, MOCA.

INTRODUCTION

Psychiatric disorders, such as depression and schizophrenia are often associated with profound cognitive deficits, which significantly impact the quality of life of patients. These cognitive impairments not only occur frequently but also vary depending on the type of disorder, the patient's age and the severity of symptoms (1). In particular, the importance of cognitive assessment has been emphasized in the context of neurodegenerative diseases, for example, (2) highlight the

essential role of standardized instruments, such as Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA), in identifying cognitive deficits which are sometimes not detectable by conventional clinical assessments.

Non-pharmacological interventions, such as computer assisted cognitive stimulation (CACS) have shown considerable potential in improving these deficits. Recent studies suggest that the effects of CACS may be influenced by factors such as the specific diagnosis, age and intensity of the intervention. (3) and (4) showed that the response to CACS varies depending on the clinical characteristics of the patients and (5) reported moderate improvements in cognitive function, although with notable differences between different psychiatric disorders (6).

These findings highlight the need for personalized interventions, adapting them to the particularities of each patient to maximize cognitive benefits.

Another aspect of interest is the impact of age on the effectiveness of CACS (7) found that age is not always a robust predictor of intervention outcomes, suggesting that variables such as the number of sessions and the severity of initial cognitive deficits may play a more determining role. In this regard, (8) demonstrated that an increased number of CACS sessions is associated with significant improvements in MMSE and MoCA scores, highlighting a positive relationship between the intervention and cognitive performance.

Therefore, analyzing the efficacy of CACS, assessed changes in MMSE and MoCA scores, as well as investigating the influence of diagnosis and other factors (age, number of sessions) on cognitive outcomes, are crucial for the development of personalized and evidence-based therapeutic strategies in the treatment of cognitive deficits associated with psychiatric disorders.

MATERIALS AND METHODS

This study included 92 patients with psychiatric disorders. Inclusion criteria for selecting patients with a confirmed diagnosis of psychiatric disorders who had participated in a minimum of 10 sessions of computerized cognitive stimulation. Exclusion criteria included the presence of major comorbidities that could influence the assessment of cognitive function, the inability to provide informed consent and the existence of severe medical conditions requiring urgent interventions. These patients completed at least 10 sessions of CACS. Diagnoses were numerically coded to facilitate statistical analysis (e.g., 1 - emotionally unstable personality disorder, 2 - bipolar affective disorder - depressive episode, 3 - severe depressive episode - generalized anxiety disorder). In the first table is presented the full list of psychiatric conditions included in the study.

Patients were selected according to the inclusion and exclusion criteria established in the study protocol.

Cognitive function was assessed using the MMSE and MoCA, both before and after CACS intervention. The number of CACS sessions ranged from 3 to 233, depending on the protocol individualized for each patient.

The procedure included an initial assessment of patients using MMSE and MoCA, followed by CACS, with a frequency of 1-2 sessions per week, each session lasting 60 minutes.

After completing the CACS session, patients were re-assessed using the same cognitive scales.

TABLE I.

Psychiatric diagnoses included in the study and numerical coding

Numeric code	Diagnostic
1	Emotionally unstable personality disorder
2	Bipolar affective disorder - depressive episode
3	Severe depressive episode, Generalized anxiety disorder
4	Depressive episode with anxiety elements
5	Severe depressive episode
6	Organic mood disorder
7	Mixed dementia, Recurrent depressive disorder
8	Acute psychotic disorder with schizophrenia symptoms
9	Recurrent depressive disorder, severe current episode
10	Mixed dementia, Recurrent depressive disorder
11	Generalized anxiety disorder, Obsessive-compulsive disorder
12	Severe depressive episode with atypical features
13	Mild cognitive disorder
14	Atypical autism
15	Mixed dementia - mild form
16	Paranoid schizophrenia
17	Attention and activity disorder
18	Dependent personality disorder
19	Recurrent depressive disorder - severe current episode
20	Recurrent depressive disorder - current episode moderate
21	Dementia in early-onset Alzheimer's disease
22	Emotionally unstable personality disorder, Mild depressive episode
23	Generalized anxiety disorder, Depressive episode with atypical features
24	Panic disorder, Mild depressive episode, Mixed dementia
25	Recurrent depressive disorder - current moderate episode with psych cognitive features
26	Severe depressive episode, generalized anxiety disorder, Sleep disorder
27	Recurrent depressive disorder - current moderate episode, Nonorganic insomnia
28	Mild depressive episode, Adjustment disorder
29	Mild cognitive disorder, Anxiety disorder
30	Recurrent depressive disorder
31	Emotionally unstable personality disorder
32	Adjustment disorder, Nonorganic insomnia
33	Mild cognitive disorder, Moderate depressive episode, Paroxysmal anxiety disorder
34	Posttraumatic stress disorder

Numeric code	Diagnostic
35	Recurrent depressive disorder - mild current episode
36	Anxiety disorder, Recurrent depressive disorder - moderate depressive episode
37	Mixed anxiety and depressive disorder
38	Mixed dementia, Organic personality disorder, Severe depressive episode
39	Cognitive disorder, Recurrent depressive disorder, Generalized anxiety disorder
40	Recurrent depressive disorder - severe current episode without psychotic symptoms
41	Recurrent depressive disorder - severe current episode without psychotic symptoms, Generalized anxiety disorder
42	Mild depressive episode, Panic disorder
43	Moderate depressive episode
44	Panic disorder
45	Cognitive disorder, Severe current depressive episode with psychotic symptoms
46	Cyclothymia
47	Bipolar affective disorder - depressive episode, Cognitive disorder
48	Anxiety disorder, Mild depressive episode
49	Sleep disorder
50	Schizophrenia
51	Emotionally unstable personality disorder, Anxiety disorder
52	Cognitive disorder, Chronic organic cerebral disorder
53	Moderated mixed dementia, Moderate depressive episode
54	Moderate depressive episode with anxious - interpretive elements
55	Moderated mixed dementia, Severe depressive episode
56	Late-onset *alz*heimer's disease dementia
57	Sexual identity disorder, Disturbance of attention and activity
58	Mixed dementia, Recurrent depressive disorder - severe current episode
59	Cognitive disorder
60	Mixed dementia

Statistical analyses included the paired-samples t-test used to compare MMSE and MoCA scores before and after the intervention, to determine whether there was a significant improvement in cognitive function. One-way ANOVA was used to compare differences between diagnostic groups in terms of MMSE and MoCA scores. Pearson correlations were calculated to assess the relationship between age, number of CACS sessions and differences in cognitive scores (ΔMMSE and ΔMoCA).

All personal data of patients were stored and processed in accordance with GDPR regulations. Patients signed an informed consent agreement regarding the processing of personal data. The data were stored electronically in digital form using the *Mi-*

crosoft Excel program, ensuring confidentiality and security of the information.

RESULTS

The study aimed to evaluate the effects of the CACS intervention on cognitive function, using two internationally recognized instruments: MMSE and MoCA. The results indicate a clear improvement in cognitive performance, illustrated both by the increase in the mean scores and by the statistical significance of the observed changes.

Specifically, the mean score on the MMSE test improved from 25.17, recorded before the intervention, to 26.33 after its application. This increase is supported by a t-statistic of -3.686 and a p-value of 0.000386, which means that the probability that the observed difference is due to chance is practically negligible. In addition, a correlation coefficient of 0.798 between the pre- and post-intervention scores confirms the existence of a strong linear relationship, indicating that the initial performance is a good predictor of subsequent cognitive improvement. Similarly, for the MoCA test, there was an increase in mean scores from 24.29 to 26.40. This difference is marked by a t-statistic of -10.737 and extremely small p-values (p one-tail = 3.72453x10^(-18) and p two-tail = 7.44906x10^(-18)), which emphasizes that the improvement is not attributable to chance. A correlation coefficient of 0.909 between pre- and post-intervention measurements highlights an almost perfect association, demonstrating the consistency and robustness of the cognitive improvements.

Statistically, an exceedingly small p-value indicates that the differences between measurements are significant and unlikely to arise from chance, which validates the effectiveness of the CACS intervention. Also, the remarkably high correlation coefficients (0.798 for MMSE and 0.909 for MoCA) reflect a close and predictive relationship between cognitive performance before and after the intervention, thus confirming the positive impact of applied method.

ANOVA analysis revealed that the specific diagnosis of the patients significantly influences cognitive performance, with an F of 142.86 ($p \approx 1.12x10^{(-24)}$) for MMSE scores and 133.58 ($p \approx 1.59x10^{(-23)}$) for MoCA scores. In addition, the correlation analyses between age and score variations (ΔMMSE and ΔMoCA) generated coefficients close to zero (approximately -0.00739 and -0.03891), indicating that age does not play a determining role in the evolution of cognitive performance post-intervention. Similarly, the weak relationship between the number of CACS sessions and changes in scores ($r = 0.01645$ for ΔMMSE and $r = -0.03203$ for ΔMoCA) suggests that the intensity of the intervention, measured by the number of sessions, is not a significant predictor of cognitive improvement.

For a visual understanding of the relationship between the studies variables, the following graphs were developed.

This graph shows the distribution of MMSE score variations according to age, highlighting a very low correlation coefficient ($r \approx -0.00739$). Thus, it can be concluded that the age factor does not have a significant impact on cognitive improvement, suggesting that the benefits of CACS apply uniformly across the age range of patients.

Similarly, this graph indicates a correlation coefficient of approximately -0.03891, confirming that age differences are not

associated with significant variations in MoCA scores. This finding supports the idea that the intervention produces positive results regardless of the age group.

Visual analysis of the data shows a weak correlation (r = 0.01645) between the number of sessions and MMSE score improvement. This observation suggests that although the number of sessions varies significantly between patients, the intensity of the interventions is not a direct predictor of cognitive improvement measured by MMSE.

Similarly, the analysis of the relationship between the number of sessions and the variation of MoCA scores indicates a non-significant correlation coefficient (r = -0.03202). This confirms that intensity (in terms of number of sessions) does not directly influence cognitive outcomes and other variables, such as the specificity of the diagnosis or individual characteristics of the patients, may play a more determining role.

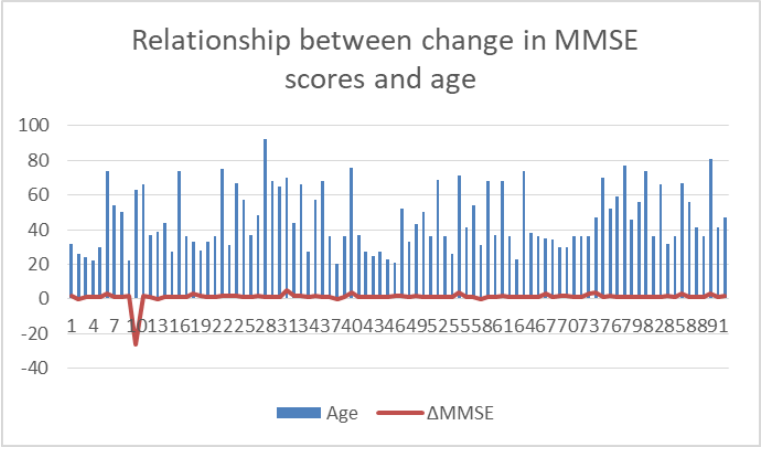

Fig. 1. Relationship between change in MMSE scores and age

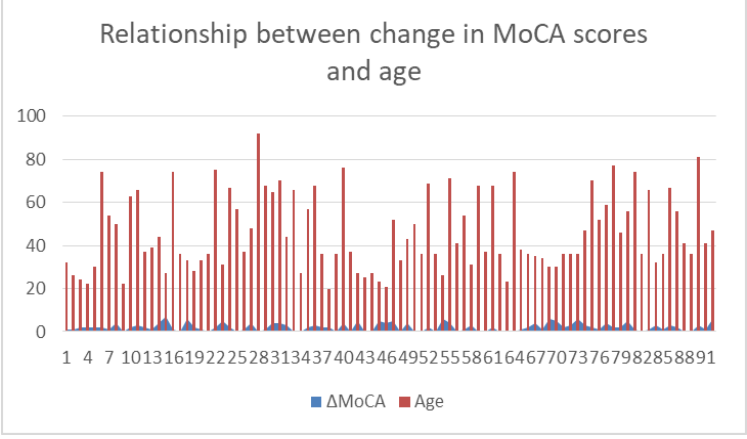

Fig. 2. Relationship between change in MoCA scores and age

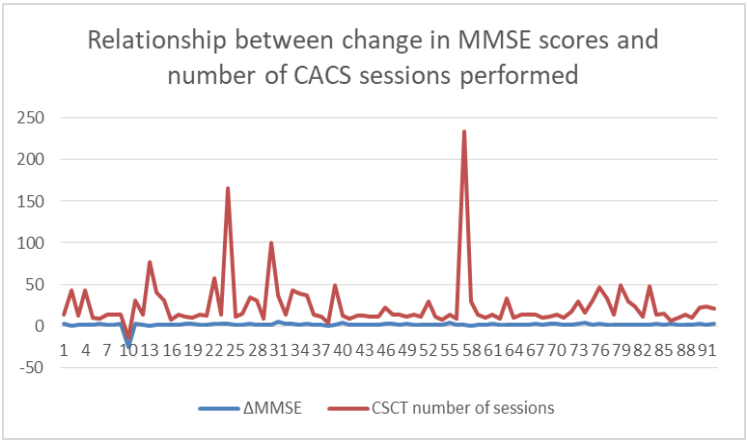

Fig. 3. Relationship between change in MMSE scores and number of CACS sessions performed.

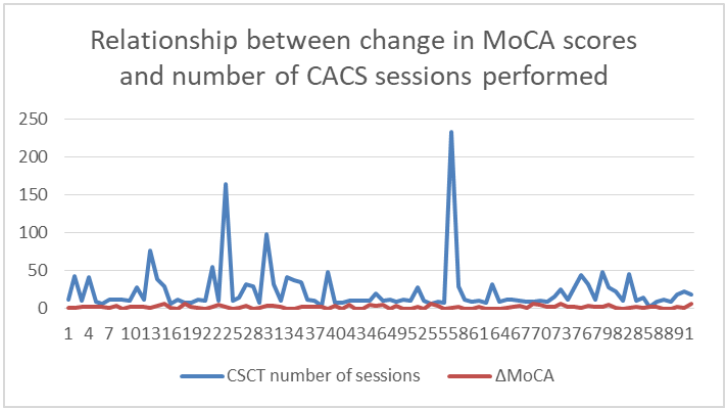

Fig. 4. Relationship between change in MoCA scores and number of CACS sessions performed.

Thus, the CACS intervention generated significant increases in cognitive function, and the statistical values obtained confirm both the real significance of these changes and the consistency of the improvements, demonstrating the effectiveness of the method within the study.

DISCUSSION

The study clearly highlights that CACS intervention leads to significant improvements in cognitive function, as reflected in the notable increase in MMSE and MoCA scores post-intervention. Paired-samples t-tests showed a significant mean difference: MMSE scores increased from 25.17 to 26.33 ($t = -3.686$; $p = 0.000386$; $r = 0.798$), and MoCA scores improved from 24.29 to 26.40 ($t = -10.737$; p one-tail $\approx 3.72 \times 10^{-18}$, p two-tail $\approx 7.45 \times 10^{-18}$; $r = 0.909$). ANOVA analysis also demonstrated that specific diagnosis has a significant impact on cognitive performance, with very high F-values ($F = 142.86$ for MMSE and $F =$

133.58 for MoCA, with $p < 0.001$ in both cases). These findings are in line with the literature, which supports that CACS can bring relevant benefits, but the response to treatment varies depending on the patient's diagnostic profile (3, 4).

The graphs completed the statistical analysis and provided additional visual interpretation. Figure 1 illustrates the relationship between age and ΔMMSE, and Figure 2. shows the relationship between age and ΔMoCA; both graphs highlight the correlation coefficients close to 0 ($r \approx -0.00739$ and $r \approx -0.03891$, respectively), confirming that age is not a significant predictor of improvement in cognitive function. Similarly, Figure 3 and Figure 4 show the relationship between the number of CACS sessions and the changes in scores (ΔMMSE and ΔMoCA) and the results show a very weak correlation ($r = 0.01654$ for ΔMMSE and $r = -0.03203$ for ΔMoCA). These observations suggest that, although it can be assumed that a higher intensity of the intervention could lead to superior outcomes, the number of sessions per se is not a significant predictor of cognitive improvements in this study.

Therefore, in the current context, the results demonstrate that the beneficial effects of CACS are determined by the specificity of the diagnosis rather than by demographic factors or the intensity of intervention, thus opening the way for future investigations to explore other moderating factors, such as the quality of the sessions or the initial stage of cognitive deficits. These aspects, highlighted both by statistical analysis and graphical representations, will be subject to detailed analysis in future discussions, thus providing a solid basis for optimizing non-pharmacological interventions in the management of cognitive deficits in patients with psychiatric disorders.

Despite the encouraging findings related to CACS, several limitations of the current study must be acknowledged. First, the inherent heterogeneity of psychiatric disorders and the variability in cognitive impairments among patients complicate the evaluation of CACS efficacy (9, 10. Many of the existing studies have been conducted with relatively small sample size and without rigorous control groups, which limits the generalizability of their findings (11, 12. Additionally, the reliance on self-reported measures of cognitive functioning may introduce bias, as patients' insight into their own cognitive deficits can vary considerably (13).

Furthermore, the current literature often does not adequately account for confounding variables such as medication effects, comorbid conditions and socio-demographic factors, all of which can significantly influence cognitive outcomes (14, 15). Future studies should strive to address these limitations by recruiting larger, more diverse samples and incorporating objective measures of cognitive functioning alongside self-report instruments (16, 17).

The promising efficacy of computer-assisted cognitive stimulation (CACS) in psychiatric disorders opens several exciting avenues for future research. As psychiatric treatment continues to advance, integrating CACS with conventional therapeutic modalities may further enhance cognitive outcomes in patients with diverse psychiatric conditions. Future investigations should prioritize longitudinal studies that examine the long-term effects of CACS on both cognitive functioning and psychiatric Symptoms, especially in populations with comorbid conditions (18, 19. In addition, employing advanced neuroimaging techniques to explore the neurobiological mechanisms underlying CACS may shed light on its influence on brain function and

structure (20).

Moreover, the potential constructive interaction of combining CACS with non-invasive brain stimulation methods, such as transcranial magnetic stimulation (TMS) or deep brain stimulation (DBS), merits further exploration. Early evidence suggests that these combined approaches can amplify cognitive improvements and alleviate psychiatric symptoms (21, 22, 23).

This study has some limitations that need to be considered. First, the retrospective design may introduce biases related to the selection and quality of the available data. Second, the sample size and diversity of the study population may limit the generalizability of the results. Also, the lack of a control group reduces the ability to attribute direct causality to the intervention. In addition, variables such as treatment adherence and socio-economic factors were not controlled for, which could influence the results obtained.

CONCLUSIONS

The present study provides compelling evidence that CACS exerts a significant positive impact on cognitive function among patients with psychiatric disorders. Our statistical analyses revealed that both MMSE and MoCA scores improved markedly following the intervention, thereby confirming the clinical efficacy of CACS. Notably, the data indicates that the specific psychiatric diagnosis plays a pivotal role in determining cognitive outcomes, underscoring the importance of tailoring interventions to the individual's clinical profile. In contrast, neither age nor the number of cognitive stimulation sessions exhibited a significant correlation with the magnitude of cognitive improvements, suggesting that the therapeutic benefits of CACS remain consistent across different demographic groups and intervention intensities.

These findings advocate for the advancement of personalized CACS protocols, wherein interventions are adapted to the unique cognitive profile and diagnostic characteristics of patients. Future research should focus on refining these personalized approaches, as well as on exploring additional factors that might modulate treatment efficacy to ultimate optimize cognitive outcomes in psychiatric populations.

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THE INFLUENCE OF BODY MASS INDEX ON THE PROGNOSIS OF PATIENTS WITH COPD: THE EFFECT OF METABOLIC SYNDROME AND THE VALIDITY OF THE OBESITY PARADOX

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BODY MASS INDEX- MORE THAN JUST A NUMBER IN THE PROGNOSIS OF PATIENTS WITH COPD AND METABOLIC SYNDROME (Abstract): Chronic obstructive pulmonary disease (COPD) is increasingly recognized as a complex systemic disease rather than a condition limited to the lungs. Malnutrition, cachexia, and obesity contribute to decreased exercise capacity, higher rates of exacerbations and increased mortality. The **aim** of the study was to investigate the influence of the body mass index (BMI) on the prognosis of patients diagnosed with COPD, in the presence or absence of MetS. **Materials and methods**: A total of 100 patients with a confirmed diagnosis of COPD were enrolled in the study, of whom 65 patients met the diagnostic criteria for MetS. Patients were evaluated based on clinical, anthropometric, and laboratory parameters. BMI was calculated for each patient, and its association with disease prognosis was subsequently analyses, considering the presence or absence of MetS. **Results:** The patients were divided into two groups based on the presence or absence of metabolic syndrome. Both BMI and waist circumference showed statistically significant differences between groups ($p<0.001$). Plasma glucose levels were significantly higher in COPD patients with MetS and BMI$\geq$ 25 kg/m^2 compared to those without MetS ($p= 0.020$). Plasma triglyceride levels were also significantly higher in the same group ($p= 0.034$). No statistically significant differences were observed for systolic and diastolic blood pressure across the groups ($p> 0.05$). **Conclusions:** Continuous monitoring of BMI and the prevention of cachexia are essential strategies for reducing mortality rates, whereas the prevention of obesity contributes to the reduction of cardiovascular complications. **Keywords**: COPD, METABOLIC SYNDROME, BMI, PROGNOSIS.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major global health problem, characterized not only by persistent respiratory symptoms and airflow limitation, but also by significant systemic effects (1, 2).

Among these systemic manifestations, nutritional disturbances such as malnutrition, sarcopenia, and obesity have been increasingly recognized as important factors influencing disease progression and prognosis (3-5).

In particular, body composition abnormalities and the presence of metabolic syndrome (MetS) are highly prevalent among COPD patients and are associated with increases morbidity and mortality (6, 7). Therefore, assessing and addressing nutritional status represents a crucial component of comprehensive COPD management (8). Moreover, the coexistence of MetS adds a substantial burden of cardiovascular morbidity. As pulmonary function worsens, COPD patients often reduce their physical activity levels, creating conditions that facilitate weight gain (9, 10). In COPD patients, there exists a paradox where individuals with higher body mass index (BMI) tend to have better survival rates than those with normal or low BMI, especially among those with advanced disease (11). One possible reason is that the severity of COPD may be overestimated in obese patients due to the physiological reduction in lung volumes (12).

However, when factors such as carbon dioxide levels, muscle mass, and exercise capacity are accounted for, this "obesity paradox" often disappears (13). Both physical inactivity and co-existing conditions negatively affect survival outcomes. Additionally, the presence of heart failure, diabetes, and hypertension in COPD patients is linked to heightened systemic inflammation, suggesting metabolic abnormalities may contribute to disease mechanisms (14-16).

Patients with COPD who have an elevated BMI are more likely to develop MetS, a cluster of conditions that includes abdominal obesity, insulin resistance, dyslipidemia, and hypertension (17). These metabolic disturbances contribute to a heightened risk of cardiovascular disease, which is already a major cause of morbidity and mortality in COPD populations (18). The presence of excess adipose tissue, particularly visceral fat, may exacerbate systemic inflammation and oxidative stress, further promoting insulin resistance and abnormal lipid profiles (19, 20). Consequently, the combination of COPD and MetS poses a significant challenge in clinical management and underscores the need for early screening and targeted interventions to reduce cardiovascular risk in this subgroup of patients (21, 22).

MATERIALS AND METHODS

This prospective study was conducted at the Clinical Rehabilitation Hospital in Iași and included 100 patients diagnosed with COPD. The study was carried out between January 2022 and June 2023. The diagnosis of COPD was established by spirometry, based on a post-bronchodilator forced expiratory volume in one second (FEV1)/ forced vital capacity (FVC) < 0.70. Patients aged over 35 years who provided written informed consent were enrolled. Exclusion criteria included advanced renal or hepatic failure, neurological or psychiatric disorders, malignancies, and refusal to sign the informed consent form. All participants underwent comprehensive clinical and laboratory evaluations, with particular emphasis on analyzing the impact of BMI on pulmonary function. This study aimed to assess the relationship between BMI, MetS, and clinical outcomes in patients with COPD, emphasizing the importance of early nutritional monitoring as part of comprehensive disease management. Demographic data, anthropometric measure-

ments, cardiovascular risk indicators, and lung function were assessed at the baseline and again after 6 months.

Statistical Analysis. Descriptive statistics were performed using *SPSS software (version 27.0, SPSS Inc., Chicago, IL, USA)* for Windows. Data were reported as mean ± standard deviation (SD) for continuous variables or as percentages for categorical ones. The Kolmogorov-Smirnov test was applied to verify the normality of data distribution. For comparisons of continuous variables, a parametric t-test was used, while categorical variables were analyzed using Fisher`s exact test. To evaluate the strength and reliability of correlations, Pearson`s and Spearman`s (r) coefficients were employed.

Ethics. This study received ethical approval from the Research Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi, Romania (Approval No. 122, dated 11th of November 2021), as well as from the Ethics Committee of the Iasi Clinical Rehabilitation Hospital on 10th of December 2021.

RESULTS

The study included 100 patients, of whom 68% were male (fig. 1). We focused on investigating BMI within the study group, highlighting an average BMI of 30.63 ± 6.842 kg/m^2 (mean ± standard deviation), with a higher value observed in subjects with COPD and MetS (32.57 ± 6.635 kg/m^2 vs. 26.68 ± 5.488 kg/m^2, p< 0.001). Moreover, 79% of the subjects had a BMI≥ 25 kg/m^2, with the majority of them being diagnosed with MetS (92.5% vs. 51.5%, p< 0.001). We also investigated abdominal circumference, and statistically significant differences were recorded between the two study groups (110.93± 13.120 vs. 99.52± 14.871, p< 0.001).

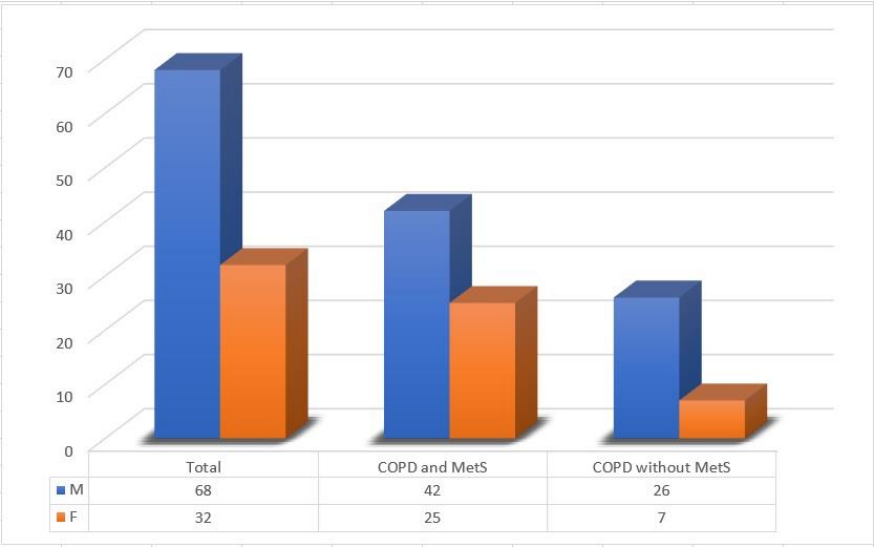

Fig. 1. Gender based distribution of COPD with and without MetS

The diagnostic criteria for MetS were analyzed by further subdividing the patients into two additional groups: those with a BMI< 25kg/m^2 and those with a

BMI≥ 25kg/m^2. The mean plasma glucose level was significantly elevated in patients with a BMI≥ 25kg/m^2 (p= 0.020). Moreover, statistically significant values were observed in the mean triglyceride levels among subjects with a BMI≥ 25kg/m^2 (p= 0.034). No statistically significant results were obtained regarding BMI, abdominal circumference, or systolic and diastolic blood pressure values (tab. I).

The patients underwent spirometric reevaluation at 6 months. At both baseline and 6- month follow-up, BMI showed a statistically significant positive correlation with FEV1 (%) and the FEV1/ FVC ratio in the total study population. These associations remained significant in the subgroup of patients with MetS, particularly for the FEV1/ FVC ratio, which demonstrated the strongest correlation (r= 0.379 at baseline and r= 0.349 at follow-up; p< 0.001). No statistically significant correlations were observed in the subgroup without MetS. Additionally, a weaker but significant correlation between BMI and FVC (%) was found at follow-up in the overall group (r= 0.223; p= 0.026) (tab. II).

The graphs illustrate the changes in BMI (fig. 2) and abdominal circumference by gender (fig. 3), between baseline and 6-month follow-up. At the initial evaluation, patients were educated on the importance of pulmonary rehabilitation programs. These programs were initiated during hospitalization and continued at home following discharge. Upon reevaluation at 6 months, both BMI and abdominal circumference showed a slight decrease in both male and female patients, reflecting a positive trend likely associated with adherence to the rehabilitation protocol.

TABLE I.

Criteria of MetS of the study population by BMI

	BMI < 25kg/m^2		**BMI ≥ 25kg/m^2**	
	COPD with MetS (n = 5)	COPD without MetS (n = 16)	COPD with MetS (n = 62)	COPD without MetS (n = 17)
BMI (kg/m^2)	21.76 ± 2.964	22.39 ± 1.815	33.44 ± 6.057	30.73 ± 4.619
	p = 0.905		p = 0.120	
Abdominal circumference (cm)	92.00 ± 11.225	90.44 ± 7.737	112.45 ± 12.093	108.06 ± 15.039
	p = 0.842		p = 0.397	
Plasma glucose level (mg/dL)	101.40 ± 7.807	100.29 ± 13.226	116.47 ± 29.446	106.31 ± 30.828
	p = 0.660		p = 0.020*	
Plasma TG level (mg/dL)	90.38 ± 12.912	98.90 ± 49.892	148.83 ± 67.618	114.71 ± 38.749
	p = 0.780		p = 0.034*	
SBP (mmHg)	136.00 ± 20.433	134.50 ± 19.135	137.10 ± 19.133	134.47 ± 12.319
	p = 0.603		p = 0.729	
DBP (mmHg)	82.00 ± 4.472	79.81 ± 11.173	81.81 ± 10.884	81.53 ± 5.970
	p = 0.548		p = 0.872	

Data are presented as mean±standard deviation (SD). Mann-Whitney test p<0.05* statistically significant
Abbreviations: BMI (body mass index), TG (triglyceride), SBP (systolic blood pressure), DBP (diastolic blood pressure)

TABLE II.

Correlations between BMI and spirometry results

Spirometry parameters	Total				COPD with MetS				COPD without MetS			
			95% C.I.				95% C.I.				95% C.I.	
	r	p	Lower bound	Upper bound	r	p	Lower bound	Upper bound	r	p	Lower bound	Upper bound
Baseline												
FEV1 (%)	0.342	<0.001*	0.157	0.505	0.389	0.001**	0.164	0.575	0.198	0.270	0.156	0.507
FVC (%)	0.171	0.089	0.026	0.355	0.071	0.570	0.173	0.306	0.114	0.526	0.238	0.440
FEV1/ FVC ratio	0.379	<0.001 *	0.198	0.536	0.508	<0.00 **	0.305	0.667	0.149	0.408	0.205	0.468
Follow-up												
FEV1 (%)	0.333	<0.001	0.146	0.497	0.361	0.003	0.132	0.553	0.149	0.409	0.205	0.468
FVC (%)	0.223	0.0260	0.028	0.402	0.127	0.304	0.116	0.357	0.202	0.259	0.151	0.510
FEV1/ FVC ratio	0.349	<0.001	0.164	0.510	0.484	<0.001	0.276	0.649	0.062	0.734	0.288	0.396

Abbreviations: FEV1, forced expiratory volume in the first second; FVC, forced vital capacity

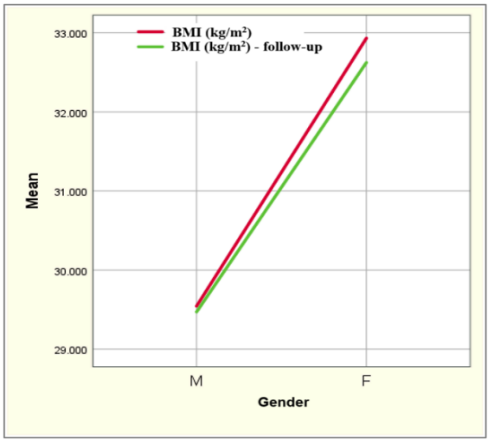

Fig. 2. Comparison of mean BMI (kg/m^2) at baseline and follow-up by gender

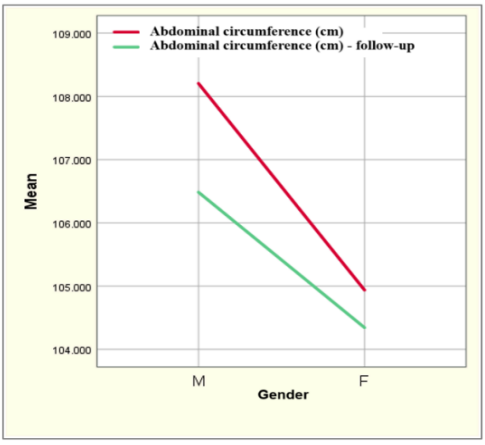

Fig. 3. Comparison of mean abdominal circumference (cm) at baseline and follow-up by gender

DISCUSSION

Earlier research has suggested a link between low BMI and decreased survival in individuals with COPD (23). However, more recent data indicate that specific adverse shifts in body composition may serve as more accurate predictors of mortality than BMI alone. In people with COPD, these changes are associated with reduced exercise capacity, poorer quality of life, and a higher risk of death (4). While some studies have explored the link between body composition and disease severity, few have investigated the role of sarcopenia in relation to COPD prognosis or addressed the importance of considering sarcopenia when evaluating BMI in overweight patients to prevent diagnostic errors (24, 25).

A retrospective cohort study involving 116,463 patients with COPD showed that individuals with both low and high BMI experienced a greater number of exacerbations over the course of one year. Moreover, a low BMI was associated with the presence of pulmonary comorbidities such as interstitial lung diseases, tuberculosis, and metabolic disorders (23, 26).

A decrease in BMI has been linked to an increased risk of overall mortality, even among overweight or obese individuals with COPD (27). Epidemiological studies in cancer patients have also found that being slightly overweight or in the early stages of obesity may lead to better survival outcomes. This trend has been consistently noted in COPD populations (28). One meta-analysis of five randomized clinical trials demonstrated that a higher BMI may help preserve lung function, with the slowest decline observed in those with a BMI of 30 kg/m² or higher (29). Another meta-analysis involving over 21,000 COPD patients found that individuals who were overweight or obese had lower mortality rates than those with a normal BMI (30). These findings support the concept of the "obesity paradox," where excess weight appears to be associated with reduced risk of death (23).

Our study highlights that in COPD patients with a BMI $\geq$ 25kg/m^2, the presence of MetS is associated with significantly higher plasma glucose and triglyceride levels, compared to those without MetS. These findings suggest that elevated BMI may exacerbate metabolic disturbances in patients with COPD, reinforcing the role of excess body weight as a contributing factor to the development and progression of MetS in this population. Consequently, they emphasize the importance of routine metabolic screening and individualized therapeutic strategies in COPD patients with increased BMI. Early detection and appropriate management of MetS may improve long-term clinical outcomes, reduce cardiovascular risk, and mitigate systemic complications in this vulnerable group.

Additionally, patients with lower BMI tended to have reduced spirometric values (FEV1 and FEV1/ FVC), both at baseline and follow-up, particularly in those without MetS. This pattern may reflect the detrimental impact of low body mass in respiratory muscle strength, nutritional status, and systemic inflammation. As supported by previous literature, a lower BMI in COPD patients is often associated with a poorer prognosis, increased risk of exacerbations, hospitalization, and overall mortality. These findings underscore the importance of early nutritional assessment and interventions aimed at preventing weight loss and muscle wasting in underweight or cachectic COPD patients.

In our patients, a positive correlation was observed between BMI and spirometric parameters (FEV1, FEV1/ FVC), both at baseline and at the 6-month follow-up, particularly in those with MetS. This suggests that a higher BMI is associated with better pulmonary function in this subgroup, supporting the concept of the "obesity paradox" in COPD.

Although the obesity paradox in COPD suggests that a higher BMI may be associated with lower mortality, this phenomenon should be interpreted with caution. Factors such as body composition, the presence of malnutrition or sarcopenia, and coexisting comorbidities must be considered. Consequently, weight management in COPD patients should be individualized, avoiding

both excessive weight gain and significant weight loss in order to optimize prognosis and enhance quality of life (31-33).

Recent studies have highlighted that the protective effect of obesity in COPD may be partially attributed to the greater nutritional reserves and muscle mass that overweight patients possess, which could contribute to better tolerance of disease exacerbations and improved survival (34). Moreover, adipose tissue may exert anti-inflammatory effects through the secretion of specific adipokines, potentially modulating the chronic systemic inflammation characteristic of COPD. However, this apparent advantage should not lead to the underestimation of obesity-related risks, such as cardiovascular disease, obstructive sleep apnea, and reduced physical performance. A nuanced understanding of the obesity paradox is necessary to tailor clinical interventions and to balance metabolic and respiratory risks in COPD management (30, 32).

CONCLUSIONS

BMI plays a significant role in the clinical profile of COPD patients, being associated with altered spirometric parameters and increased metabolic risk. Higher BMI values, particularly in patients with MetS, were significantly correlated with impaired pulmonary function, as demonstrated by reduced FEV1 and FEV1/ FVC ratios. Monitoring and managing BMI in COPD patients is essential, as excess weight contributes to increased systemic inflammation, cardiovascular risk, and reduced respiratory efficiency. Incorporating weight control strategies and structured pulmonary rehabilitation programs may improve both respiratory function and metabolic outcomes in COPD patients with elevated BMI.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that they have no conflict of interest.

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THE ROLE OF ROUTINE CARDIAC AND HEMATOLOGIC BIOMARKERS IN FORECASTING POSTOPERATIVE RECOVERY AND LENGTH OF STAY

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THE ROLE OF ROUTINE CARDIAC AND HEMATOLOGIC BIOMARKERS IN FORECASTING POSTOPERATIVE RECOVERY AND LENGTH OF STAY (Abstract): Perioperative morbidity remains a challenge in elderly orthopedic patients, particularly in the presence of cardiovascular comorbidities. Easily available biomarkers may provide prognostic value for postoperative recovery and length of hospitalization. **Aim**: To investigate the prognostic significance of cardiac and inflammatory biomarkers, including CK-MB, fibrinogen, red blood cell distribution width (RDW), and hemoglobin, on postoperative outcomes, with a focus on length of hospital stay. **Materials and methods:** We retrospectively analyzed 85 patients undergoing orthopedic surgery. Demographic, clinical, and laboratory data were collected pre- and postoperatively. Primary outcome was length of hospitalization. Associations between biomarkers and hospitalization were evaluated using Spearman correlations, group comparisons (median and quartile splits), and multivariable regression with robust standard errors. **Results:** The cohort had a mean age of 72.2 years, with equal sex distribution. Significant positive correlations were found between hospitalization days and preoperative CK-MB ($\rho = 0.286$, $p = 0.008$), postoperative CK-MB ($\rho = 0.277$, $p = 0.010$), preoperative fibrinogen ($\rho = 0.334$, $p = 0.002$), and preoperative RDW ($\rho = 0.257$, $p = 0.018$). In multivariable regression, postoperative CK-MB ($\beta = 0.190$, $p = 0.079$) and postoperative fibrinogen ($\beta = 0.115$, $p = 0.242$) emerged as the strongest predictors, though statistical significance was not reached. Variance inflation factors revealed substantial collinearity, particularly for RDW. **Conclusions:** Routine biomarkers such as CK-MB, fibrinogen, and RDW demonstrate prognostic associations with hospitalization duration in orthopedic surgery. These markers reflect myocardial stress, systemic inflammation, and hematologic resilience, respectively. Incorporating them into perioperative assessment may improve risk stratification and guide targeted management. Larger prospective studies with high-sensitivity troponin and composite indices are warranted. **Keywords:** CARDIAC BIOMARKERS, CK-MB, FIBRINOGEN, RED BLOOD CELL DISTRIBUTION WIDTH (RDW), POSTOPERATIVE OUTCOMES, HOSPITALIZATION LENGTH, RISK STRATIFICATION.

INTRODUCTION

Perioperative morbidity and mortality remain major concerns in surgical populations, particularly among elderly patients

with cardiovascular comorbidities. Accurate risk stratification and early identification of patients at higher risk for complications are critical to optimize surgical planning, anticipate adverse events, and guide perioperative management strategies. In recent years, biochemical and hematological biomarkers have gained considerable interest as potential tools for predicting short- and long-term outcomes (1).

Among these, creatine kinase MB isoenzyme (CK-MB) has been traditionally used as a marker of myocardial injury. While cardiac troponins have largely replaced CK-MB in the diagnosis of acute myocardial infarction, evidence suggests that postoperative CK-MB elevation, even within modest ranges, independently predicts mortality and adverse cardiac events following major non-cardiac surgery (1). This highlights its potential role as a valuable prognostic indicator in the perioperative context, particularly in elderly patients undergoing orthopedic procedures who frequently harbor silent or subclinical cardiovascular disease.

Red blood cell distribution width (RDW), a routinely available parameter reflecting anisocytosis, has emerged as a powerful prognostic biomarker across various clinical settings. Elevated RDW levels have been linked to increased mortality, arrhythmias, and renal dysfunction in patients undergoing cardiac surgery (2). Furthermore, in non-cardiac surgical cohorts, higher preoperative RDW has been associated with more than a twofold increase in 30-day postoperative mortality, suggesting it may reflect underlying chronic inflammation, nutritional deficiencies, and impaired erythropoiesis that collectively worsen recovery potential (3).

In parallel, fibrinogen, a key coagulation factor and acute phase reactant, has demonstrated prognostic utility in surgical patients. Hypofibrinogenemia following cardiac surgery independently predicts excessive postoperative bleeding and the need for transfusion (4). A meta-analysis confirmed that both pre- and postoperative fibrinogen concentrations inversely correlate with blood loss, reinforcing its role as a determinant of perioperative hemostasis and recovery (5). In orthopedic surgery, where perioperative bleeding and transfusion requirements significantly affect morbidity, fibrinogen dynamics may hold particular prognostic value.

Collectively, these biomarkers represent distinct yet complementary aspects of postoperative physiology: CK-MB as an indicator of myocardial stress or injury, RDW as a marker of systemic resilience and chronic disease burden, and fibrinogen as a determinant of coagulation and inflammatory status. Their combined evaluation may provide a multidimensional assessment of perioperative risk, enabling better prediction of outcomes such as prolonged hospitalization, postoperative complications, and recovery trajectories.

The present study **aims** to evaluate the prognostic value of CK-MB, fibrinogen, RDW, and related biomarkers in patients undergoing orthopedic surgery. By integrating these routinely measured parameters into perioperative risk assessment, clinicians may be able to refine patient stratification and optimize perioperative care (3).

MATERIALS AND METHODS

Study Design and Population. This was a retrospective observational study conducted on patients admitted for orthopedic surgery, primarily hip fracture repair

and prosthetic joint replacement, between (insert years, e.g., January 2018 and December 2023). The study cohort consisted of consecutive patients with complete clinical, laboratory, and surgical records available for analysis. Patients with incomplete datasets or missing postoperative biomarker measurements were excluded.

Data Collection. Demographic and clinical variables were extracted from medical records, including sex, age, primary diagnosis, and relevant comorbidities such as arterial hypertension, pulmonary hypertension, ischemic heart disease, chronic kidney disease, diabetes mellitus, and chronic liver disease.

Laboratory biomarkers were collected both preoperatively and postoperatively. The primary biomarkers of interest were:

- Creatine kinase MB isoenzyme (CK-MB), measured in U/L.
- Fibrinogen, measured in mg/dL.
- Red blood cell distribution width (RDW), expressed as a percentage.
- Hemoglobin, measured in g/dL.

Additional markers, including INR, APTT, creatinine, and electrolytes, were also recorded.

Surgical data included day of intervention (relative to admission), type of prosthesis, and perioperative transfusion requirements.

Outcomes. The primary outcome was length of hospitalization (days). Secondary outcomes included:

- Postoperative complications, defined as cardiac (arrhythmia, myocardial injury, heart failure) or non-cardiac (infection, bleeding requiring transfusion).
- Biomarker variation (preoperative vs. postoperative changes) and their association with outcomes.

Statistical Analysis. All statistical analyses were performed using Python (version 3.11) within the Visual Studio Code environment. The following Python libraries were employed: pandas (v.2.1.0) for data management and preprocessing, numpy (v.1.26.0) for numerical operations, scipy.stats (v.1.11.0) for correlation analyses and hypothesis testing (Shapiro-Wilk normality test, Spearman and Pearson correlation coefficients, Mann-Whitney U test, independent-samples t-test, Kruskal-Wallis test, and ANOVA), stats models (v.0.14.0) for regression modeling, including ordinary least squares (OLS) regression with robust (HC3) standard errors, multivariable linear regression, and logistic regression, matplotlib (v.3.8.0) and seaborn (v.0.13.0) for the creation of plots and data visualizations, pingouin (v.0.5.3) for effect size estimation and variance inflation factor (VIF) analysis to evaluate multicollinearity.

Continuous variables were assessed for distributional normality using the Shapiro-Wilk test and reported as mean ± standard deviation (SD) when normally distributed or as median with interquartile range (IQR) otherwise. Categorical variables were summarized as frequencies and percentages.

Associations between biomarkers and hospitalization duration were evaluated using Pearson's correlation for normally distributed variables and Spearman's rank correlation otherwise. Group comparisons were conducted using independent-samples t-tests or Mann-Whitney U tests, depending on data distribution. For comparisons across multiple groups, ANOVA or Kruskal-Wallis tests were employed.

Multivariable regression analyses were performed to identify independent predictors of hospitalization duration. Variables with $p < 0.10$ in univariate analyses were considered for entry into the multivariable

models. Variance inflation factors were computed to assess multicollinearity. Logistic regression models were additionally used to explore associations between biomarker levels and the occurrence of postoperative complications.

Ethical Considerations. The study was conducted in accordance with the Declaration of Helsinki. Approval was obtained from the institutional ethics committee. As this was a retrospective analysis of anonymized data, informed consent was waived.

RESULTS

A total of 85 patients were included in the final analysis. The mean age of the cohort was 72.2 years, with an equal sex distribution (50% female, 50% male). The majority were admitted for hip fracture repair with prosthesis implantation. Baseline biomarker levels and hospitalization duration are summarized in first table. The median hospitalization length was 10 days (IQR: 7-14).

Spearman correlation analysis revealed that several biomarkers were significantly associated with hospitalization duration. Higher preoperative CK-MB ($\rho = 0.286$, $p = 0.008$), postoperative CK-MB ($\rho = 0.277$, $p = 0.010$), preoperative fibrinogen ($\rho = 0.334$, $p = 0.002$), and preoperative RDW ($\rho = 0.257$, $p = 0.018$) were each correlated with prolonged hospitalization. In contrast, postoperative fibrinogen and postoperative RDW showed weaker, non-significant associations. Complete correlation coefficients are reported in Table II.

TABLE I.

Descriptive statistics of pre- and postoperative biomarkers and hospitalization days.

	Count	Mean	Std. dev.	Min.	25%	50%	Median	75%	Max.
CK-MB (0-25)	85	15.54	13.29	0.9	10	13	13	16	83
CK-MB post-op	85	23.7	48.4	1.5	7.3	12	12	20	379
Fibrinogen (238-498)	85	367	135.71	6.2	296	362	362	423	976
Fibrinogen post-op	85	403.62	219.11	5.3	331	423	423	544	769
RDW pre-op (12.1-16.2)	85	14.41	2.4	7.2	13.3	13.9	13.9	15	22.2
RDW post-op (12.1-16.2)	85	14.45	2.56	7.7	13	13.9	13.9	15.2	23.7
Hb (n: 12.5-16.3)	85	12.58	1.86	7.8	11.2	12.8	12.8	13.9	16.7
Hb post-op	85	11.14	1.64	6.7	10.3	11.2	11.2	12.3	17.6
Hospitalization days	85	9.75	3.56	5	7	9	9	11	28

TABLE II.

Spearman correlations between biomarkers and hospitalization days.

Biomarker	No. pairs	Spearman rho	p-value
CK-MB (0-25)	85	0.286	0.0079
CK-MB post-op	85	0.277	0.0103
Fibrinogen (238-498)	85	0.334	0.0018
Fibrinogen post-op	85	0.148	0.1752
RDW pre-op (12.1-16.2)	85	0.257	0.0176
RDW post-op (12.1-16.2)	85	0.273	0.0114
Hb (N: 12.5-16.3)	85	-0.234	0.031
Hb post-op	85	0.028	0.7965

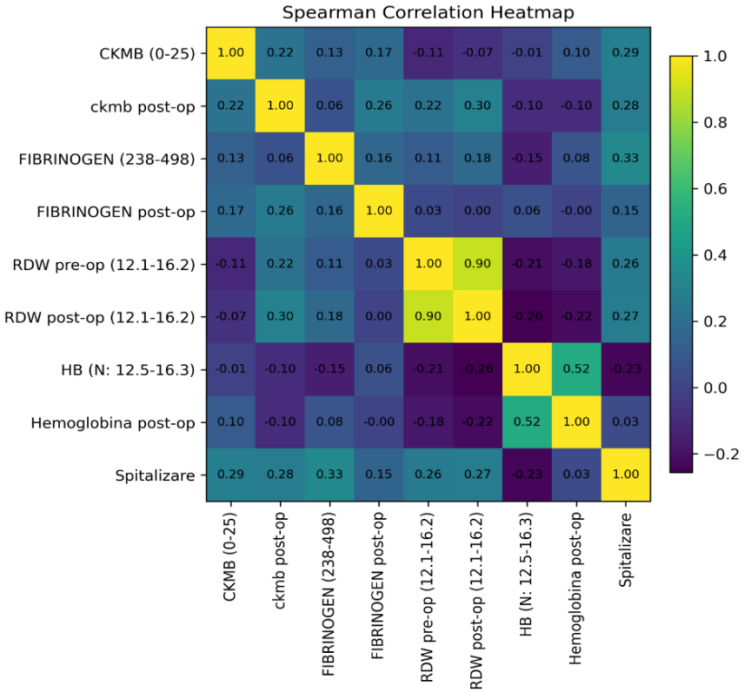

Fig. 1. Heatmap of Spearman correlations between biomarkers and hospitalization days.

The overall correlation structure between biomarkers and hospitalization is illustrated in first figure, where positive associations are evident for CK-MB, fibrinogen, and RDW.

Group Comparisons

When stratified by median values, patients with higher biomarker levels (≥ median) consistently demonstrated longer hospital stays compared to those below the median. This difference was most pronounced for preoperative fibrinogen and RDW (fig. 2).

Quartile-based analyses further highlighted a stepwise increase in hospitalization with rising biomarker levels. Patients in the highest quartile (Q4) of fibrinogen and RDW had the longest hospital stays, compared with those in the lowest quartile (Q1) (fig. 3).

Multivariate Regression Analysis

In the multivariable regression model including all biomarkers simultaneously, postoperative CK-MB (standardized $\beta = 0.190$, $p = 0.079$) and postoperative fibrinogen ($\beta = 0.115$, $p = 0.242$) emerged as the strongest predictors of hospitalization length, although statistical significance was not achieved. Preoperative CK-MB and RDW lost significance after adjustment, suggesting overlapping predictive domains. Full regression results are provided in Table III and standardized coefficients in Table IV.

A graphical summary of standardized coefficients with 95% confidence intervals is provided in fig. 4, highlighting the relative contribution of CK-MB and fibrinogen to hospitalization outcomes.

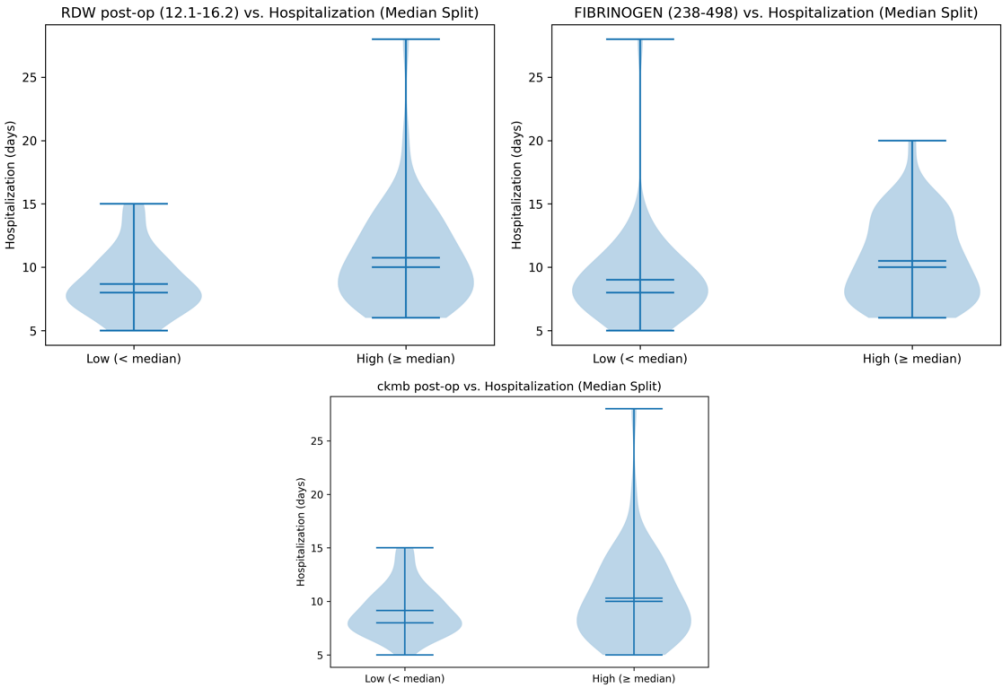

Fig. 2. Violin plots of hospitalization days by median split of biomarkers.

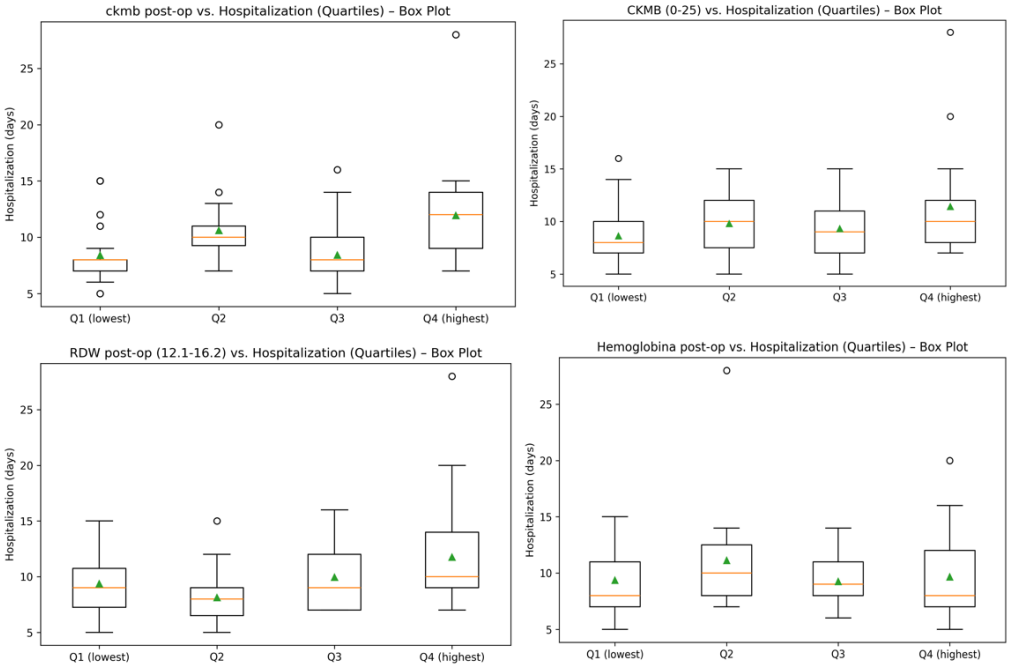

Fig. 3. Box and violin plots of hospitalization days across biomarker quartiles.

TABLE III.
Multivariate OLS regression coefficients for predictors of hospitalization.

Term	Coefficient	Std. err (hc3)	t	p_value	CI_low	CI_high
Const	3.8858	3.5293	1.101	0.2709	-3.0316	10.8031
CK-MB (0-25)	0.01	0.0272	0.3664	0.7141	-0.0433	0.0633
CK-MB post-op	0.0139	0.0079	1.7577	0.0788	-0.0016	0.0295
Fibrinogen (238-498)	-0.0004	0.0046	-0.0846	0.9326	-0.0094	0.0086
Fibrinogen post-op	0.0019	0.0016	1.1693	0.2423	-0.0013	0.005
RDW pre-op (12.1-16.2)	0.0886	0.1872	0.4735	0.6359	-0.2782	0.4554
RDW post-op (12.1-16.2)	0.2203	0.1753	1.257	0.2088	-0.1232	0.5639
Hb (N: 12.5-16.3)	-0.3248	0.213	-1.525	0.1273	-0.7421	0.0926
Hb post-op	0.3944	0.274	1.4393	0.1501	-0.1427	0.9315

TABLE IV.
Standardized β coefficients for predictors of hospitalization.

Term	Beta coefficient	Std._error_ beta (hc3)	t_beta	p_value_ beta	Beta_ CI_low	Beta_ CI_high
Const	0	0.1086	0	1	-0.2129	0.2129
CK-MB (0-25)	0.0373	0.1017	0.3664	0.7141	-0.162	0.2365
CK-MB post-op	0.1897	0.1079	1.7577	0.0788	-0.0218	0.4011
Fibrinogen (238-498)	-0.0149	0.1761	-0.0846	0.9326	-0.36	0.3302
Fibrinogen post-op	0.1153	0.0986	1.1693	0.2423	-0.078	0.3086
RDW pre-op (12.1-16.2)	0.0597	0.1261	0.4735	0.6359	-0.1875	0.3069
RDW post-op (12.1-16.2)	0.1586	0.1262	1.257	0.2088	-0.0887	0.4059
Hb (N: 12.5-16.3)	-0.17	0.1115	-1.525	0.1273	-0.3885	0.0485
Hb post-op	0.1823	0.1266	1.4393	0.1501	-0.0659	0.4305

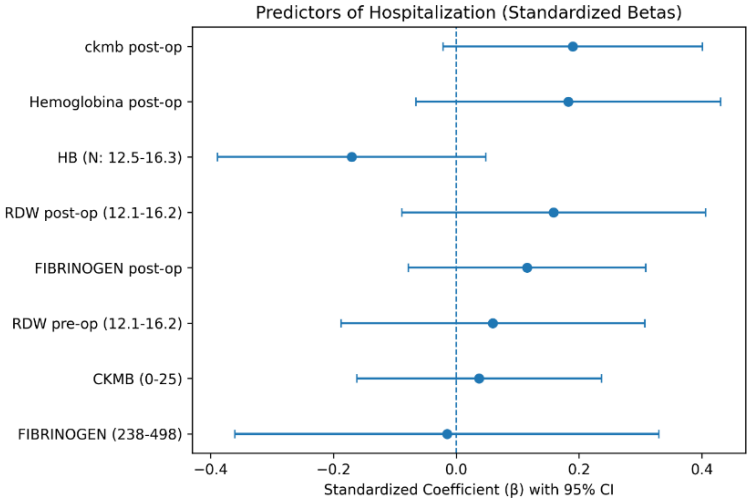

Fig. 4. Coefficient plot of standardized β values for predictors of hospitalization.

Multicollinearity Diagnostics

Variance inflation factor (VIF) analysis revealed substantial collinearity among some predictors, particularly RDW (VIF ≈ 102) and fibrinogen (VIF ≈ 9.3), indicating that results involving these variables should be interpreted with caution (tab. V).

TABLE V.

Variance inflation factors for included predictors.

Predictor	VIF
CK-MB (0-25)	3.11
CK-MB post-op	1.523
Fibrinogen (238-498)	9.342
Fibrinogen post-op	4.994
RDW pre-op (12.1-16.2)	101.956
RDW post-op (12.1-16.2)	94.245
Hb (n: 12.5-16.3)	60.146
Hb post-op	60.586

DISCUSSION

In this single-center cohort of older adults undergoing orthopedic surgery, we found that higher preoperative CK-MB, postoperative CK-MB, preoperative fibrinogen, and preoperative RDW correlated with longer hospital stay, whereas in multivariable analysis the postoperative CK-MB and postoperative fibrinogen showed the largest (albeit non-significant) adjusted effects. These findings align with the broader perioperative evidence that myocardial injury after non-cardiac surgery (MINS) and systemic inflammatory/coagulation signals track with worse short-term recovery and resource use, including length of stay (LOS). Our data, centered on routinely measured biomarkers, support the concept of a multidomain biological signal, myocardial stress (CK-MB), inflammatory/hemostatic tone (fibrinogen), and hematologic reserve/inflammation (RDW), that together shape postoperative trajectories (6-8).

Extensive literature shows that postoperative cardiac biomarker elevations (classically CK-MB and now predominantly troponin) are associated with adverse outcomes after non-cardiac surgery. In the landmark work by Landesberg *et al.*, even low-level postoperative CK-MB and troponin elevations independently predicted long-term mortality after major vascular surgery, highlighting their complementary prognostic value (1). Contemporary analyses of MINS consistently link postoperative troponin rises to higher short-term mortality and complications, and several cohorts also report longer hospital stays among patients with myocardial injury (7, 9, 10). Our observation that postoperative CK-MB exerted the strongest adjusted effect on LOS is therefore directionally concordant with the troponin-centric literature, even if CK-MB is a legacy assay; the mechanistic plausibility is that subclinical myocardial injury drives hemodynamic instability, arrhythmias, or non-cardiac organ dysfunction that prolongs recovery (1, 7, 9, 10).

Preoperative RDW has emerged as a robust, low-cost prognostic marker across surgical populations. Large studies demonstrate associations between higher RDW

and postoperative mortality in non-cardiac surgery (3, 11, 12), and in hip-fracture cohorts RDW predicts mortality as well (13). Importantly for our endpoint, RDW has also been linked to prolonged ICU and hospital LOS among critically ill fracture patients, dovetailing with our univariable correlations (14). RDW likely integrates chronic inflammation, iron/folate deficiency, renal function, and marrow stress, all of which impair physiologic reserve. In our multivariable model, the RDW signal attenuated, possibly reflecting multicollinearity with other inflammatory and hematologic variables (consistent with our high VIFs) and/or residual confounding by anemia or frailty (11, 12, 14, 15).

Fibrinogen is pivotal for clot firmness and is an acute-phase reactant. In orthopedic and spine surgery, lower preoperative fibrinogen has been associated with higher bleeding/transfusion needs (spine) (16), though findings are mixed in arthroplasty, where a 2023 study reported no association between preoperative fibrinogen and transfusion in THA (17). Interventional evidence suggests fibrinogen concentrate can reduce perioperative blood loss and transfusion in THA (18), reinforcing the biological relevance of fibrinogen to operative hemostasis. Beyond bleeding, higher preoperative inflammatory tone reflected by fibrinogen (or composite indices such as the fibrinogen/albumin ratio, FAR) has been linked to longer hospital and ICU LOS and greater morbidity in vascular disease and hip-fracture cohorts, mirroring our positive correlation between preoperative fibrinogen and LOS (13, 19). Taken together, our results likely capture two overlapping phenomena: (i) hemostatic sufficiency that reduces bleeding-related delays and (ii) systemic inflammation that prolongs convalescence (13, 16, 17, 19).

First, while CK-MB highlighted risk in our data, postoperative troponin monitoring is better validated and should be preferred where available; routine surveillance in higher-risk patients identifies those at risk for complications and longer LOS, enabling targeted monitoring and cardioprotective strategies (8, 9). Second, RDW can be integrated into preoperative assessment as a readily available marker of physiologic reserve; when elevated, it should prompt evaluation of reversible contributors (iron deficiency, malnutrition, renal dysfunction). Third, fibrinogen merits dual consideration, as a modifiable hemostatic variable (with goal-directed therapy when low) and as an inflammatory proxy when high; however, its relationship to LOS in arthroplasty appears context-dependent, warranting individualized interpretation and, where appropriate, adjunct measures (e.g., TXA protocols, blood management pathways) (7, 9, 10, 13, 14, 17).

Our study is retrospective, single-center, and modest in size, which limits power, particularly for multivariable inference where collinearity was substantial (e.g., RDW). We focused on LOS rather than adjudicated complications; while LOS is clinically meaningful, it is influenced by non-medical factors (bed management, rehabilitation logistics). Biomarker timing was limited to available clinical draws; serial measurements, especially of high-sensitivity troponin, could refine prognostication. Future multicenter studies should test parsimonious models that combine postoperative troponin with RDW and fibrinogen (or composite indices like FAR), and evaluate whether targeted interventions (anemia correction, anti-inflammatory strategies, fibrinogen-guided hemostasis, cardioprotective

pathways) can shorten LOS and improve recovery (1, 6, 7, 13, 14, 18).

CONCLUSIONS

In this retrospective study of patients undergoing orthopedic surgery, we found that preoperative CK-MB, fibrinogen, and RDW, as well as postoperative CK-MB, were significantly associated with prolonged hospitalization. Although the strength of association diminished after multivariable adjustment, postoperative CK-MB and fibrinogen remained the most consistent predictors of length of stay, underscoring the prognostic value of cardiac and inflammatory biomarkers in the perioperative setting.

These findings support the integration of routine laboratory parameters, including CK-MB, RDW, and fibrinogen, into perioperative risk stratification frameworks. They highlight the interplay between myocardial stress, hematologic resilience, and inflammatory/hemostatic balance in shaping recovery trajectories after major orthopedic procedures.

Future research should validate these results in larger, prospective, multicenter cohorts, ideally incorporating high-sensitivity troponins and composite indices such as the fibrinogen/albumin ratio. Interventional trials exploring the correction of modifiable factors, such as anemia, nutritional deficits, or hypofibrinogenemia, may further determine whether biomarker-guided perioperative strategies can shorten hospital stay and improve outcomes in this vulnerable population.

CONFLICT OF INTERESTS AND FUNDING

The authors declare no conflict of interests. The research did not receive external funding.

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DISTINCT METABOLIC AND STONE COMPOSITION PATTERNS IN FIRST EPISODE AND RECURRENT UROLITHIASIS PATIENTS

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DISTINCT METABOLIC AND STONE COMPOSITION PATTERNS IN FIRST EPISODE AND RECURRENT UROLITHIASIS PATIENTS (Abstract): The objective of this study was to identify clinical, metabolic, and compositional factors associated with stone recurrence, using morpho-spectroscopic stone analysis combined with biochemical and dietary assessment. **Materials and methods:** We retrospectively analyzed 1,380 patients with urinary lithiasis evaluated at the Michel Daudon Morphological and Spectroscopic Analysis Center, Iași, Romania, between 2018 and 2022. Patients were divided into first-episode (n=842) and recurrent (n=538) groups. Demographic data, metabolic profile, dietary habits, and stone composition were assessed. Multivariable logistic regression was used to identify independent predictors of recurrence. **Results:** Recurrent patients accounted for 39.1% of the cohort and were significantly older. Calcium oxalate monohydrate and dihydrate stones predominated in first episodes, whereas uric acid, urate-mixed, and struvite calculi were more frequent in recurrent cases. Obesity, hyperuricemia, hypertension, type 2 diabetes, and chronic kidney disease were significantly more prevalent among recurrent stone formers. Dietary assessment showed that low fruit and vegetable intake and high sodium consumption were strongly associated with recurrence. Logistic regression identified hyperuricemia (OR=2.3), obesity (OR=1.9), hypertension (OR=1.5), and chronic kidney disease (OR=1.6) as independent predictors. **Conclusions**: Stone recurrence is characterized by a distinct clinical and metabolic profile, compositional shifts toward uric acid and struvite calculi, and unfavorable dietary habits. These findings confirm the systemic nature of lithiasis and support the integration of morpho-spectroscopic, metabolic, and nutritional evaluation into standard urological practice. **Keywords:** URINARY STONE DISEASE, RECURRENCE, MORPHO-SPECTROSCOPIC ANALYSIS, METABOLIC SYNDROME, URIC ACID, PREVENTION.

INTRODUCTION

Urinary stone disease represents a major and growing public health challenge worldwide. Its lifetime prevalence is estimated between 10-12% in Europe and North America, with increasing incidence also reported in developing countries (1). The disease is highly recurrent: more than 50% of patients are expected to develop a new episode within 10 years after the first event. Beyond the acute burden of renal colic and the need for surgical or endourological interventions, recurrence is associated with progressive renal impair-

ment, reduced quality of life, and considerable healthcare costs (1, 2).

The etiology of urinary stone disease is multifactorial. Established risk factors include metabolic abnormalities such as obesity, hypertension, diabetes mellitus, dyslipidemia, and hyperuricemia, which are frequently clustered in the metabolic syndrome (3, 4). Dietary habits strongly modulate lithogenic risk: high sodium intake and an elevated sodium-to-potassium ratio significantly increase stone risk, whereas diets rich in potassium, fruits, and vegetables are protective (5). High intake of animal protein and oxalate-rich foods contributes to uric acid and calcium oxalate stones, respectively (6, 7). Lifestyle patterns also play a role: sedentary behavior increases risk, while vigorous physical activity has been shown to mitigate it (1, 8).

Epidemiological data reveal changing demographic trends. Traditionally, stone disease was more common in men; however, recent reports suggest a narrowing gender gap, with increasing incidence among women and even among adolescents. Moreover, disparities have been reported across ethnic and socioeconomic groups, with disproportionate increases among Black and Hispanic populations in the United States and with variable access to metabolic evaluation and surgical care (4). Such observations underline the systemic and societal dimensions of stone disease beyond its local urological manifestations.

Pathophysiological mechanisms of stone formation are closely linked to morphological and compositional features. Randall's plaques, intratubular crystallization, and papillary erosions represent distinct initiating pathways, each associated with specific stone types. Comprehensive morpho-constitutional analysis, as proposed by Daudon and colleagues, combines macroscopic morphology with infrared spectroscopy to provide insights into the mechanisms of stone genesis and recurrence. Stone composition also predicts clinical behavior: calcium oxalate monohydrate and dihydrate stones are usually small and more likely to pass spontaneously, whereas struvite, brushite, and cystine stones tend to be larger and associated with higher recurrence rates. Furthermore, metabolic conditions influence stone type: uric acid stones are strongly associated with obesity, diabetes, and insulin resistance, while calcium phosphate and brushite stones are linked to hypercalciuria and hyperparathyroidism (1, 3, 9–11).

In Romania, recent studies have highlighted the interplay between stone composition, metabolic comorbidities, and dietary risk factors, with hypertension associated with calcium oxalate monohydrate, diabetes with uric acid stones, obesity with calcium oxalate dihydrate, and specific dietary habits predisposing to urate and oxalate lithiasis. Such findings support the need for an integrated clinical, metabolic, and morpho-spectroscopic approach in the evaluation of stone formers(11-14).

Despite these advances, few studies have directly compared the metabolic and morpho-spectroscopic profiles of patients at their first stone episode versus those with documented recurrence. Given the clinical and economic impact of recurrent stones, identifying compositional shifts and associated metabolic abnormalities is crucial for guiding preventive strategies.

The aim of our study was to compare the clinic-biological metabolic profile and morpho-spectroscopic characteristics of urinary stones between patients presenting with a first episode and those with recurrence. By integrating morphological classification, infrared spectroscopic analysis,

and metabolic assessment, we sought to elucidate the factors driving recurrence and to highlight potential targets for prevention.

MATERIALS AND METHODS

Study design and setting. We conducted a retrospective, observational study at the Urology Clinic of "Dr. C. I. Parhon" Clinical Hospital, Iași, Romania, in collaboration with the Michel Daudon Morphological and Spectroscopic Analysis Center. The study covered the period from January 2018 to June 2025. All patients underwent standard evaluation and treatment for urinary stone disease according to current urological guidelines.

Study population. During the study period, 1,520 patients diagnosed with urinary lithiasis were evaluated. For each patient, only a single stone specimen was analyzed, either collected spontaneously or retrieved during surgical/endourological procedures. After exclusion of incomplete or fragmented samples, a total of 1,380 stones were available for analysis. Patients were categorized into two groups.

Clinical and anthropometric evaluation. All patients underwent standardized clinical examination including measurement of body mass index (BMI), waist circumference, and blood pressure. Relevant comorbidities such as type 2 diabetes mellitus, hypertension, dyslipidemia, hyperuricemia, and chronic kidney disease (CKD) were recorded based on clinical history and laboratory data.

Stone analysis. Macroscopic morphological assessment of calculi was performed according to the Daudon classification, documenting external surface, section, and crystalline organization. Infrared spectroscopy (FTIR) was used for compositional analysis, enabling precise identification of crystalline phases (e.g., calcium oxalate monohydrate, calcium oxalate dihydrate, uric acid, urates, carbapatite, struvite, cystine, protein matrix). Each stone was assigned a morpho-constitutional type based on combined macroscopic and spectroscopic findings.

Dietary evaluation. Patients completed a standardized dietary questionnaire developed in our department, focusing on the frequency and quantity of consumption of salt, animal proteins, oxalate-rich foods, dairy products, alcohol, fruits, and vegetables. Reported intake patterns were categorized as favorable or unfavorable based on established nutritional recommendations for stone prevention.

Statistical analysis. Continuous variables were expressed as mean ± standard deviation (SD). Categorical variables were presented as absolute numbers and percentages. Comparisons between groups (first episode *vs.* recurrence) were performed using Student's t-test or Mann–Whitney U test for continuous variables and χ^2 or Fisher's exact test for categorical variables. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using *SPSS v. 26* (IBM Corp., Armonk, NY, USA).

All patients signed informed consent forms prior to participation. The study protocol was reviewed and approved by the Research Ethics Board of the "Dr. C. I. Parhon" Clinical Hospital, Iași, Romania (approval no. 18/08.03.2022).

RESULTS

A total of 1,380 patients were included in the analysis. Of these, 842 (60.9%) presented with a first episode of urolithiasis, while 538 (39.1%) had a history of recurrence.

The overall mean age was 52.8 ± 13.4 years (range 18-79), with a male-to-female ratio of 1.4: 1. Patients with recurrence were older on average than first-episode patients (55.1 ± 12.9 *vs.* 51.2 ± 13.7 years, p=0.01).

TABLE I.

Demographic characteristics of the study population

Variable	First episode (n=842)	Recurrence (n=538)	Total (n=1,380)
Age, years (mean ± SD)	51.2 ± 13.7	55.1 ± 12.9	52.8 ± 13.4
Age range (years)	18–77	20–79	18–79
Male sex, n (%)	498 (59.1%)	333 (61.9%)	831 (60.2%)
Female sex, n (%)	344 (40.9%)	205 (38.1%)	549 (39.8%)
Urban residence	505 (60.0%)	334 (62.1%)	839 (60.8%)
Rural residence	337 (40.0%)	204 (37.9%)	541 (39.2%)
County of residence			
Iași	547 (65.0%)	350 (65.1%)	897 (65.0%)
Suceava	84 (10.0%)	55 (10.2%)	139 (10.1%)
Botoșani	76 (9.0%)	46 (8.6%)	122 (8.8%)
Neamț	63 (7.5%)	39 (7.2%)	102 (7.4%)
Vaslui	46 (5.5%)	33 (6.1%)	79 (5.7%)
Other counties	26 (3.0%)	15 (2.8%)	41 (3.0%)

Table II summarizes the clinical and metabolic characteristics of the study population. Patients with recurrent stones were significantly older and had higher BMI compared to those with a first episode. The prevalence of abdominal obesity, hyper-uricemia, hypertriglyceridemia, hypertension, type 2 diabetes mellitus, and chronic kidney disease was markedly higher among recurrent stone formers, highlighting their more unfavorable metabolic profile.

TABLE II.

Clinical and metabolic characteristics of first-episode *vs.* recurrent patients

Parameter	First episode (n=842)	Recurrence (n=538)	p-value
Age, years (mean ± SD)	51.2 ± 13.7	55.1 ± 12.9	0.01
Male sex, n (%)	498 (59.1%)	333 (61.9%)	0.37
Female sex, n (%)	344 (40.9%)	205 (38.1%)	—
BMI, kg/m² (mean ± SD)	27.1 ± 3.9	29.3 ± 4.4	<0.001
Abdominal obesity, n (%)	176 (20.9%)	168 (31.2%)	0.003
Hyperuricemia, n (%)	159 (18.9%)	187 (34.8%)	<0.001
Hypertriglyceridemia, n (%)	126 (15.0%)	141 (26.2%)	0.002
Hypertension, n (%)	178 (21.1%)	174 (32.4%)	0.004
Type 2 diabetes, n (%)	83 (9.8%)	93 (17.3%)	0.01
CKD (eGFR <60), n (%)	27 (3.2%)	37 (6.9%)	0.03

Clinical and metabolic characteristics. Patients with recurrence displayed a more adverse metabolic profile. They had higher mean BMI and significantly higher prevalence of abdominal obesity, hyperuricemia, hypertriglyceridemia, hypertension, diabetes, and CKD compared to first-episode patients. Recurrent stone formers were older, more frequently obese, and exhibited a significantly higher burden of metabolic comorbidities than first-episode patients.

Stone composition

Calcium oxalate was the predominant stone type (59.8%), with whewellite (calcium oxalate monohydrate, COM) at 37.1% and weddellite (calcium oxalate dihydrate, COD) at 22.7%. Uric acid stones accounted for 13.9%, urate-predominant mixed forms for 13.5%, carbapatite for 8.4%, struvite for 4.1%, and rare forms (cystine/protein) for 0.3%.

Marked compositional differences were observed between groups. First-episode patients predominantly formed calcium oxalate stones (COM 43.0%, COD 24.7%), while recurrent patients more frequently had uric acid (20.4% *vs.* 9.7%, p<0.001) and urate-containing mixed calculi (17.7% *vs.* 10.8%, p=0.002). Struvite stones were also more common in recurrent cases (6.6% *vs.* 2.4%, p=0.01). Recurrent stone formers showed a significant compositional shift toward uric acid–containing, mixed, and infection-related stones compared to first-episode patients.

TABLE III.

Stone composition in first episode *vs.* recurrence

Stone type	Overall (n, %)	First episode (n=842)	Recurrence (n=538)	p-value
COM	512 (37.1%)	362 (43.0%)	150 (28.4%)	<0.001
COD	314 (22.7%)	208 (24.7%)	104 (19.3%)	0.04
Uric acid	192 (13.9%)	82 (9.7%)	110 (20.4%)	<0.001
Urates (mixed)	186 (13.5%)	91 (10.8%)	95 (17.7%)	0.002
Carbapatite	116 (8.4%)	76 (9.0%)	41 (7.6%)	0.47
Struvite	57 (4.1%)	20 (2.4%)	36 (6.6%)	0.01
Rare (Cys / protein)	4 (0.3%)	3 (0.4%)	1 (0.2%)	0.68
Total	**1,380 (100%)**	**842 (100%)**	**538 (100%)**	—

Multivariable logistic regression analysis (tab. IV) identified several independent predictors of stone recurrence. Increasing age was significantly associated with higher odds of recurrence, with an adjusted OR of 1.18 per decade (95% CI 1.06–1.31, p=0.002). Among metabolic factors, obesity (BMI ≥30 kg/m²) nearly doubled the risk (OR=1.90, p<0.001), while hyperuricemia emerged as the strongest predictor, conferring more than a two-fold increased risk (OR=2.30, p<0.001). Hypertension (OR=1.50, p=0.012) and chronic kidney disease (OR=1.62, p=0.041) also remained significant after adjustment. From a dietary perspective, low fruit and vegetable consumption (OR=1.80, p=0.001) and high sodium intake (OR=1.42, p=0.023) were associated with recurrence, underlining the role of lifestyle factors. In contrast, sex, urban/rural residence, and alcohol intake were not independent predictors in the adjusted model. Overall, the model showed good discrimination (AUC=0.77), explaining approximately 22% of the variance in recurrence risk.

TABLE IV.

Multivariable logistic regression identifying independent predictors of recurrence

Predictor (reference)	Adjusted OR	95% CI	p-value
Age (per 10 years)	1.18	1.06–1.31	0.002
Male sex (female)	1.12	0.90–1.38	0.31
BMI ≥30 kg/m² (<30)	1.90	1.40–2.60	<0.001
Hyperuricemia (no)	2.30	1.70–3.10	<0.001
Hypertension (no)	1.50	1.10–2.00	0.012
Type 2 diabetes (no)	1.35	0.98–1.86	0.065
Hypertriglyceridemia (no)	1.28	0.96–1.70	0.089
CKD, eGFR <60 (no)	1.62	1.02–2.57	0.041
Low fruit/vegetable intake (no)	1.80	1.30–2.50	0.001
High sodium intake (no)	1.42	1.05–1.92	0.023
Frequent alcohol intake (no)	1.36	0.98–1.90	0.064
Urban residence (rural)	1.10	0.86–1.40	0.44

DISCUSSION

This study, performed in a large cohort of patients managed at the Michel Daudon Morphological and Spectroscopic Analysis Center, Iași, analyzed in detail the clinical, metabolic, and compositional differences between first episode and recurrent stone formers. The results demonstrate that recurrence is associated with older age, greater metabolic burden, specific dietary patterns, and a compositional shift toward uric acid-containing and struvite stones.

Recurrent cases represented nearly 40% of the cohort, a proportion comparable to the recurrence rates reported internationally, where up to half of patients develop a new episode within 10 years. Age was a significant determinant, with recurrent patients being older than those with a first episode. Similar observations were made in large European cohorts, where increasing age correlated with larger stones and lower likelihood of spontaneous passage. The male predominance persisted in our series (60.2%), which is in line with the traditional epidemiology of lithiasis, though other reports indicate a narrowing of the gender gap in Western countries(15, 16). The geographic distribution, with two-thirds of patients originating from Iași county, reflects the tertiary referral profile of our center and is consistent with regional clustering of lithiasis observed in Romanian populations (3, 17).

Morpho-spectroscopic analysis identified significant compositional differences between groups. COM and COD were more frequent in first-episode patients, whereas uric acid, urate-mixed, and struvite calculi were characteristic of recurrence. Such patterns are in line with the morpho-constitutional classification proposed by Daudon, which links uric acid and infection-related stones to systemic

and recurrent disease(4, 16).

Regional studies also documented correlations between comorbidities and stone type: hypertension was associated with COM, diabetes with uric acid, and obesity with COD (3,18). These data support the concept that recurrent lithiasis reflects not only anatomical predisposition but also persistent metabolic or infectious drivers.

Recurrent patients exhibited a significantly higher prevalence of obesity, hyperuricemia, hypertension, diabetes, and chronic kidney disease. These findings are concordant with large-scale studies that identified the metabolic syndrome as a major contributor to stone disease (16). In our cohort, hyperuricemia conferred the highest independent risk for recurrence, followed by obesity, hypertension, and CKD. These results are consistent with analyses of metabolically active lithiasis in Romanian populations, where clustering of biochemical disturbances was shown to increase recurrence risk. The association of metabolic abnormalities with recurrence strengthens the interpretation of urinary stone disease as a systemic disorder with renal, vascular, and metabolic implications (19, 20).

Dietary imbalance emerged as an additional determinant of recurrence. Patients with low intake of fruits and vegetables and high sodium consumption were significantly more likely to develop recurrent stones. This finding is supported by nutritional studies indicating that the sodium-to-potassium ratio is a reliable predictor of stone risk, with potassium-rich diets exerting a protective effect (21). Local observations also showed that obese stone formers often fail to adhere to dietary recommendations, which may explain the persistence of lithiasis despite medical advice (3, 22). Alcohol intake was more common in recurrent patients but did not remain significant in adjusted models, a result consistent with studies reporting no clear dose–response relationship between alcohol and stone risk. Recent evidence also suggests that beyond sodium and potassium balance, other dietary components exert significant influence on stone recurrence. High animal protein intake increases acid load, enhances urinary calcium and uric acid excretion, and reduces urinary citrate, thereby favoring both uric acid and calcium oxalate lithiasis (5). Conversely, plant-based proteins and diets rich in alkali-producing foods may offer protective effects. Fructose-containing sweetened beverages have been associated with increased uric acid production and recurrent lithiasis in several cohorts, while inadequate fluid intake remains a major risk factor, particularly in rural populations with limited access to structured dietary counselling. Emerging studies also highlight the role of gut microbiota, showing that reduced colonization with *Oxalobacter formigenes* diminishes intestinal oxalate degradation, thereby contributing to calcium oxalate stone formation (1, 7, 23). These aspects underline that recurrence is not only the result of isolated dietary imbalances but of broader lifestyle and environmental patterns that warrant multifaceted preventive interventions. Sedentary lifestyle, although not directly assessed in this cohort, has been linked elsewhere to increased risk of lithiasis (20, 21).

Stone composition has direct clinical

relevance for urological management. Clinical experience in Romanian series demonstrated that COM calculi are more resistant to ESWL compared to other types. Comparative evaluations of electrohydraulic and electromagnetic lithotripters confirmed that stone composition remains a critical determinant of fragmentation success, regardless of technology (24-26). These results provide context for the present findings, where recurrent patients more frequently presented with uric acid and struvite stones-calculi known for therapeutic difficulty and for requiring adjuvant medical or anti-infective strategies.

Several large-scale analyses have demonstrated that calcium oxalate monohydrate calculi, while frequent in first-episode cases, are often linked to lower recurrence rates compared with uric acid or struvite stones (27). The latter are frequently associated with systemic conditions such as metabolic syndrome, diabetes, or chronic infection, which perpetuate the lithogenic milieu and favor multiple episodes (16, 21, 28). Uric acid stones have been correlated with obesity, insulin resistance, and acidic urinary pH, supporting their role as markers of metabolic imbalance rather than isolated events (29-31).

The cumulative evidence demonstrates that stone recurrence is not a random phenomenon but the clinical expression of systemic derangements combining metabolic, dietary, and infectious factors. International data have also linked stone disease to accelerated chronic kidney disease progression and to disparities in access to metabolic evaluation (26, 32). Within this framework, the systematic use of morpho-spectroscopic analysis, as applied in our center, enables the identification of high-risk phenotypes and the implementation of tailored preventive interventions.

The strengths of this study include the large patient cohort, the systematic application of morpho-spectroscopic analysis in a specialized reference center, and the integration of dietary, metabolic, and clinical data. Limitations stem from its retrospective design and single-center setting, although the referral nature of our institution provides regional representativeness. Prospective, multicenter investigations are warranted to confirm these findings and to assess the effectiveness of targeted preventive strategies.

CONCLUSIONS

Stone recurrence reflects a specific pathophysiological profile characterized by adverse metabolic conditions, unfavorable dietary patterns, and a compositional shift toward uric acid and struvite calculi. Recognition of these determinants emphasizes the systemic nature of lithiasis and establishes morpho-spectroscopic analysis, combined with metabolic and nutritional evaluation, as essential tools for accurate risk stratification and the design of effective preventive strategies.

CONFLICT OF INTEREST AND FUNDING

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HEMOGLOBIN A1C AND BODY MASS INDEX AS PREDICTORS OF DISCREPANCIES BETWEEN CONTINUOUS GLUCOSE MONITORING AND GLUCOMETER READINGS IN PREGNANT WOMEN WITH GESTATIONAL DIABETES

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HEMOGLOBIN A1C AND BODY MASS INDEX AS PREDICTORS OF DISCREPANCIES BETWEEN CONTINUOUS GLUCOSE MONITORING AND GLUCOMETER READINGS IN PREGNANT WOMEN WITH GESTATIONAL DIABETES (Abstract): This study investigated the relationship between differences in continuous glucose monitoring (CGM) and glucometer measurements and the clinical factors hemoglobin A1c (HbA1c) and body mass index (BMI) in women with gestational diabetes. We also evaluated if HbA1c and BMI could serve as predictors of CGM-glucometer differences. **Materials and methods**: HbA1c was measured both continuously and categorically (≥5.9% vs <5.9%), and BMI was calculated in kg/m². An independent-samples t-test compared CGM differences between the two HbA1c categories. Pearson correlations examined relationships between CGM differences and HbA1c and BMI. A multiple linear regression assessed whether HbA1c and BMI predicted CGM differences. **Results**: Patients with elevated HbA1c (≥5.9%) had significantly higher CGM differences ($M = 28.71$, $SD = 0.73$) compared to those with lower HbA1c ($M = 23.17$, $SD = 0.91$), $t(42) = -19.94$, $p < 0.001$. CGM differences were very strongly correlated with HbA1c ($r = .996$, $p < 0.001$) and BMI ($r = .984$, $p < 0.001$). In the multiple regression model, only HbA1c significantly predicted CGM differences ($B = 26.52$, $SE = 2.18$, $p < 0.001$), whereas BMI was not statistically significant. The model explained 99.3% of the variance in CGM differences ($R^2 = 0.993$). **Conclusions.** The findings of our study suggest that HbA1c is strongly associated with CGM-glucometer differences, while BMI, despite a strong correlation, does not independently predict discrepancies when HbA1c is considered. These results underscore the importance of HbA1c in assessing CGM accuracy. **Keywords:** HEMO-GLOBIN, BODY MASS INDEX, PREDICTORS, CONTINUOUS GLUCOSE MONITORING, PREGNANT WOMEN, GESTATIONAL DIABETES.

INTRODUCTION

Continuous glucose monitoring (CGM) has become an increasingly important tool for measuring glycemic control, particularly in populations at risk for glucose abnormalities such as women with gestational diabetes (1). CGM offers frequent glucose readings over time, which can reveal fluctuations that are not captured by traditional glucometer measurements (2). However, discrepancies between CGM and glucometer readings, particularly in the context of gestational diabetes, have been understudied, and investigating the factors that contribute to these differences may be critical for clinical interpretation.

Hemoglobin A1c (HbA1c) is a form of hemoglobin with glucose bound to it (3). Since red blood cells have a lifespan of approximately three months, HbA1c can provide an estimate of average blood glucose levels over that period. Therefore, HbA1 is commonly used to evaluate long-term glycemic control (3). Hence, HbA1c reflects long-term glycemic control and its relation to the extent of CGM-glucometer differences should be examined.

Body Mass Index (BMI) is calculated by dividing a person's weight in kilograms by the square of their height in meters (kg/m^2) (4). BMI is a simple, widely used screening measure to estimate whether an adult's body weight is healthy relative to their height. While BMI does not directly measure body fat, it is utilized to categorize individuals as underweight, normal weight, overweight, or obese, and to examine potential health risks associated with obesity (4). Similarly, BMI has been suggested to influence glucose measurement accuracy, although evidence remains mixed (5). Therefore, the present study aimed to investigate the relationship between CGM-glucometer differences, HbA1c, and BMI, and to evaluate whether HbA1c and BMI can predict the observed differences.

MATERIALS AND METHODS

Participants. The study included 44 pregnant women. Demographic and clinical characteristics were recorded, including body mass index (BMI), and hemoglobin A1c (HbA1c). All participants underwent both continuous glucose monitoring (CGM) and glucometer measurements.

Measures. The primary variable of interest for our experimental design was the difference between CGM readings and standard glucometer measurements, recorded in mg/dL (CGM-diff). HbA1c was measured as a percentage using standard laboratory assays. For some statistical analyses, HbA1c was categorized based on a threshold of 5.9% into lower risk/normal (<5.9%) and higher risk/elevated (≥5.9%) (6). BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2) (4).

Procedures. Participants underwent simultaneous CGM and glucometer glucose measurements. Data for CGM-diff, HbA1c, and BMI were collected and entered into the SPSS data set prior to the statistical analysis.

Statistical Analysis. All statistical analyses were conducted using SPSS. An independent samples t-test was performed to compare CGM differences between patients with HbA1c <5.9% and ≥5.9%. Pearson correlations were also calculated to examine the relationships between CGM-glucometer differences and the continuous variables HbA1c and BMI. Finally, a multiple linear regression was conducted to assess whether HbA1c and BMI predicted CGM-glucometer differences. Model assumptions were evaluated, and statistical significance was set at $p = 0.05$.

RESULTS

Independent Samples t-Test. Our statistical analysis showed that patients with HbA1c ≥ 5.9% had a higher mean CGM-glucometer difference (M = 28.71, SD = 0.73, n = 14) compared to patients with HbA1c < 5.9% (M = 23.17, SD = 0.91, n = 30).

The independent-samples t-test indicated that this difference was statistically significant, t(42) = -19.94, p <0.001, with a mean difference of -5.55 (95% CI (-6.11, -4.99)). In addition, Levene's test confirmed that the assumption of equal variances was met, F = 0.77, p = 0.39. The effect size was very large, Cohen's d = 6.45 (fig. 1).

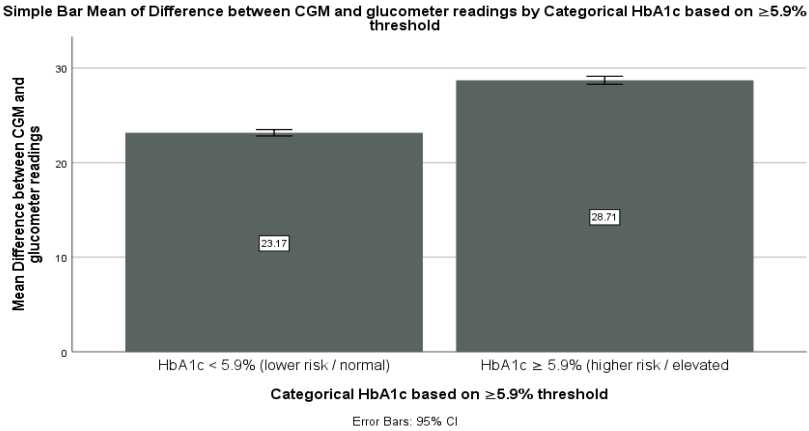

Fig. 1. Mean differences between continuous glucose monitoring (CGM) and glucometer readings for patients with HbA1c <5.9% (lower risk/normal) and ≥5.9% (higher risk/elevated). Error bars represent standard deviations. Patients with higher HbA1c levels exhibited larger CGM-glucometer differences.

Pearson Correlations

Furthermore, a Pearson correlation was conducted to examine the relationship between CGM-glucometer differences and HbA1c. There was a strong, positive correlation, r = 0.996, n = 44, p < 0.001 (fig. 2).

In addition, a second Pearson correlation was used to examine the relationship between CGM-glucometer differences and BMI. The results showed that there was a strong, positive correlation, r = 0.984, n = 44, p < 0.001 (fig. 3).

Multiple Regression

Lastly, a multiple linear regression was conducted to examine whether HbA1c and BMI can predict CGM-glucometer differences. The results showed that the overall model was statistically significant, F(2, 41) = 2910, p < 0.001, and explained 99.3% of the variance in CGM-glucometer differences (Adjusted R^2 = 0.993).

Examination of the coefficients indicated that HbA1c was a significant predictor of CGM-glucometer differences, B = 26.52, SE = 2.18, β = 1.03, t(41) = 12.17, p < 0.001. However, BMI was not a significant predictor, B = -0.05, SE = 0.13, β = -0.03, t(41) = -0.37, p = 0.717. The intercept was B = -134.51, SE = 9.75, t(41) = -13.80, p <0.001 (fig. 4).

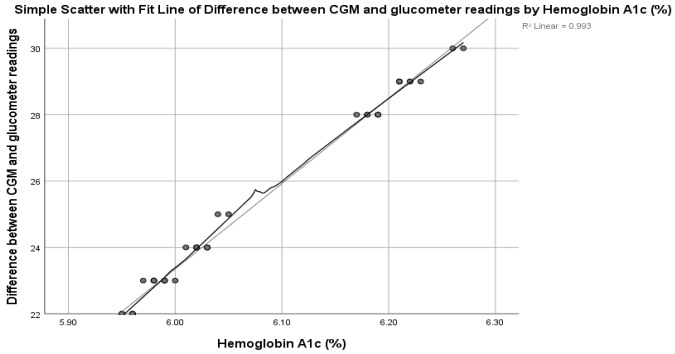

Fig. 2. Scatterplot showing the relationship between CGM-glucometer differences and hemoglobin A1c (HbA1c). The regression line illustrates the strong positive correlation ($r = 0.996$, $p < 0.001$), indicating that higher HbA1c levels are associated with larger CGM-glucometer differences.

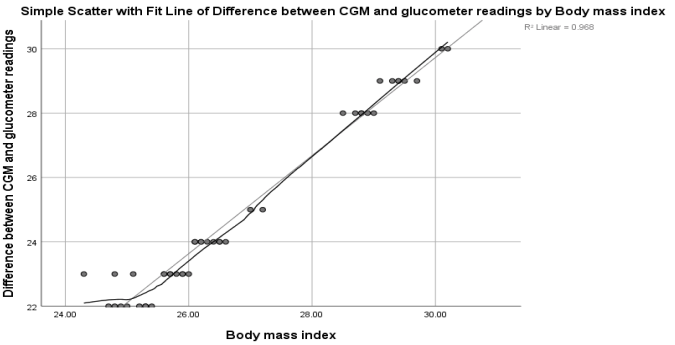

Fig. 3. Scatterplot showing the relationship between continuous CGM-glucometer differences and body mass index (BMI). The regression line illustrates the strong positive correlation ($r = 0.984$, $p < 0.001$), indicating that higher BMI values are associated with larger CGM-glucometer differences.

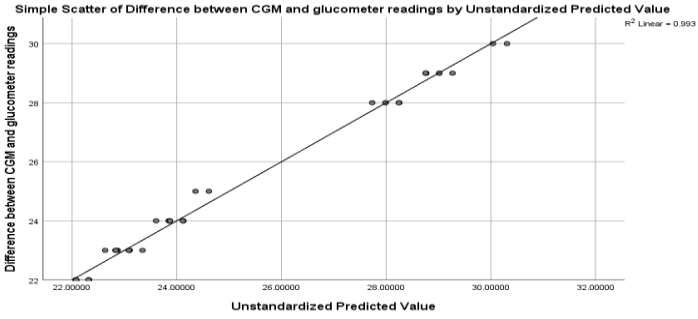

Fig. 4. Scatterplot of observed versus predicted continuous CGM-glucometer differences from the multiple regression model including hemoglobin A1c (HbA1c) and body mass index (BMI) as predictors. The X-axis shows unstandardized predicted values of CGM-glucometer differences, and the Y-axis shows the observed CGM-glucometer differences. Points close to the diagonal line indicate that the model predicts CGM-glucometer differences accurately.

DISCUSSION

The present study examined differences between continuous glucose monitoring (CGM) and glucometer readings in relation to HbA1c and BMI in a sample of 44 pregnant women. The independent-samples t-test indicated that patients with higher HbA1c levels (≥5.9%) exhibited significantly larger differences between CGM and glucometer readings compared to those with lower HbA1c levels. This suggests that HbA1c may be associated with the magnitude of discrepancy between these glucose measurement methods. Our results are in concordance with those found in the literature (7). For example, in a recent study, Lai *et al*. (2023) reported that in gestational diabetes patients with HbA1c < 6%, CGM reduced the mean glucose difference to around 23 mg/dL, closely matching our subgroup with HbA1c < 5.9%, which had a mean difference of 23.17 mg/dL (8).

In addition, our Pearson correlations revealed very strong positive relationships between CGM-glucometer differences and both HbA1c ($r = 0.996$) and BMI ($r = 0.984$). These findings indicate that as HbA1c or BMI increases, the difference between CGM and glucometer readings also increases. In another recent study on this subject, Zhu *et al*. demonstrated that CGM use in gestational diabetes resulted in a mean HbA1c of 6.0% and improved glucose detection, aligning with our results and the resulted high correlation of HbA1c with CGM-glucometer differences (9).

Ultimately, our multiple regression analysis further clarified the predictive role of these variables. While the overall model explained a very high proportion of the variance in CGM differences ($R^2 = 0.993$), only HbA1c emerged as a significant predictor. BMI did not significantly contribute to the model, suggesting that, in this dataset, HbA1c is the primary driver of CGM-glucometer discrepancies. Although previous work has demonstrated robust associations between HbA1c and CGM-derived metrics such as mean glucose (10), few studies have directly assessed whether HbA1c predicts the magnitude of discrepancy between CGM and glucometer readings, underscoring the novelty of our experimental design. Consistent with Guo *et al*, who reported a weak association between higher BMI and larger CGM-glucometer differences (11), our regression analysis also indicated that BMI was not a significant predictor, suggesting a minimal effect of BMI on glucose measurement discrepancies.

Taken together, these results highlight the potential importance of HbA1c in understanding differences between CGM and glucometer readings. Although BMI was strongly correlated with CGM-glucometer differences, it did not remain a significant predictor when considered alongside HbA1c, indicating potential collinearity between the two variables.

CONCLUSIONS

Our findings align with previous studies demonstrating that continuous glucose monitoring can detect fluctuations missed by home glucose monitoring (12) and that higher HbA1c is associated with larger CGM-glucometer differences (13, 14).

These results provide a foundation for further investigation into the factors influencing CGM accuracy, particularly in populations with elevated HbA1c. Future studies should explore additional clinical and behavioral variables that may contribute to these observed measurement discrepancies.

CONFLICT OF INTERESTS AND FUNDING

The authors declare no conflict of interests.

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BDNF AND ALOX12 DURING ANTIDEPRESSANT TREATMENT IN MAJOR DEPRESSION: A LONGITUDINAL STUDY

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BDNF AND ALOX12 DURING ANTIDEPRESSANT TREATMENT IN MAJOR DEPRESSION: A LONGITUDINAL STUDY (Abstract): This study investigated the longitudinal dynamics of brain-derived neurotrophic factor (BDNF) and arachidonate 12-lipoxygenase (ALOX12) during antidepressant treatment in patients with major depression. **Materials and methods**: Twenty-six patients were assessed at baseline, 4 weeks, and 8 weeks. Depressive and anxiety symptoms were measured using HAM-D and HAM-A, and plasma BDNF and ALOX12 levels were quantified using ELISA. **Results**: HAM-D decreased from 31.0 to 23.0 and HAM-A from 28.0 to 18.0. BDNF increased from 0.87 to 5.33, while ALOX12 decreased from 1.39 to 0.22 ($p<0.001$), with the largest change between weeks 4 and 8; ALOX12 at 8 weeks correlated inversely with HAM-D ($r = -0.416$; adjusted $r = -0.515$). **Conclusions**: BDNF and ALOX12 showed distinct temporal patterns. BDNF increase and ALOX12 reduction occurred without significant associations between their changes. **Keywords**: MAJOR DEPRESSION; BDNF; ALOX12; BIOMARKERS.

INTRODUCTION

Major depression is a clinical condition defined by persistent low mood, anhedonia, cognitive impairment, and somatic symptoms that impair daily functioning (1). The clinical presentation varies across individuals, with differences in symptom profiles, illness trajectory, and comorbidity patterns. This variability also extends to treatment response. A substantial proportion of patients fail to achieve full remission despite adequate pharmacological intervention. Data from the STAR*D study indicate remission rates of approximately 28–33% following first-line treatment, highlighting the limited effectiveness of standard approaches in a large segment of patients (2).

The disorder lacks a single identifiable biological mechanism. Structural and functional alterations have been described in corticolimbic circuits, including the prefrontal cortex, hippocampus, amygdala, and striatum (3). Cellular findings include reduced synaptic density, dendritic retraction, and altered excitatory and inhibitory neurotransmission (4, 5). Inflammatory activation and oxidative stress have been consistently reported, with increased circulating

cytokines and elevated markers of oxidative damage (6-8). These findings indicate a disorder involving multiple interacting biological systems rather than a unitary pathophysiological process.

Numerous biomarkers have been investigated in psychiatric disorders, particularly in depression. These include inflammatory markers, oxidative stress indicators, neurotrophic factors, and metabolic parameters. Nonetheless, no biomarker has demonstrated sufficient sensitivity and specificity for routine clinical use. Findings remain inconsistent across studies, influenced by methodological variability, heterogeneity of patient populations, and differences in biological sampling. Current research has shifted toward examining dynamic biological changes and interactions between systems rather than relying on single-marker approaches (9-12).

Brain-derived neurotrophic factor is one of the most extensively studied molecules in depression. It is involved in neuronal survival, synaptic plasticity, and activity-dependent signaling. BDNF is widely expressed in corticolimbic regions implicated in mood regulation. It's signaling includes functionally distinct isoforms, with mature BDNF promoting synaptic strengthening through TrkB receptors and proBDNF associated with synaptic weakening via p75 receptors (13, 14). Experimental studies have shown reduced BDNF expression following stress exposure and restoration after antidepressant treatment (13-15). Clinical studies report lower peripheral BDNF levels in depression and increases following treatment, although effect sizes are moderate and variability is high (16-18).

Peripheral BDNF reflects both central and systemic sources. Transport across the blood–brain barrier has been demonstrated, although circulating levels are influenced by platelet release and peripheral production (19). Associations with symptom severity are inconsistent. Changes over time appear more informative than baseline levels, particularly in relation to treatment response. These characteristics position BDNF as a marker of neuroplastic processes rather than a diagnostic indicator.

Lipid-mediated pathways represent another component of cellular stress response. Polyunsaturated fatty acids, including arachidonic acid, are integral to neuronal membranes and serve as precursors for bioactive lipid mediators (20). Enzymatic pathways involving cyclooxygenases and lipoxygenases generate molecules that regulate inflammation, oxidative balance, and intracellular signaling (21). Lipoxygenases catalyze the formation of oxidized lipid derivatives that influence membrane integrity and cellular responses to stress (22).

Arachidonate 12-lipoxygenase, encoded by the ALOX12 gene, converts arachidonic acid into 12-hydroxyeicosatetraenoic acid and related metabolites (23). Experimental data show activation of this pathway under conditions of oxidative stress, leading to lipid peroxidation, calcium dysregulation, and neuronal injury (24). Increased expression has been observed in models of cerebral ischemia, with reduced damage following enzyme inhibition (25). This suggests involvement in redox-sensitive cellular processes.

Clinical evidence linking ALOX12 to psychiatric disorders remains limited. Genetic association studies report relationships with bipolar disorder (26). Neuroimaging and genetic analyses suggest associations with cortical structural changes in

stress-related conditions (27). Experimental models demonstrate modulation of lipoxygenase-derived metabolites under chronic stress, particularly in reward-related brain regions (28). Direct clinical data on major depression are scarce, which limits interpretation but highlights the need for longitudinal investigation of this pathway.

Inflammatory, oxidative, and neurotrophic systems interact at multiple levels. Cytokines influence neurotransmitter systems and reduce neurotrophic support, with documented effects on monoaminergic transmission and BDNF expression (29, 30). Oxidative stress affects membrane integrity, mitochondrial function, and intracellular signaling pathways, contributing to neuronal dysfunction (6, 8). These processes converge on mechanisms regulating synaptic plasticity and neuronal adaptation, with inflammation and oxidative stress both implicated in impaired neurogenesis and synaptic remodeling (31, 32). BDNF and lipid-mediated pathways may represent distinct components of these interactions, reflecting different aspects of the biological response to stress and cellular injury (20, 22).

The present study investigates the dynamics of BDNF and ALOX12 during antidepressant treatment in patients with major depression. Biological assessments are performed at baseline, before treatment initiation, and at subsequent time points during treatment. Clinical evaluation is conducted using standardized measures of depressive and anxiety symptom severity, allowing examination of associations between biomarker changes and symptom trajectories.

MATERIALS AND METHODS

This study was designed as a prospective, longitudinal observational investigation conducted between January and June 2025 at the Socola Institute of Psychiatry in Iasi, Romania. The study was approved by the Ethics Committee of Grigore T. Popa University of Medicine and Pharmacy, in Iasi, Romania and the Socola Institute of Psychiatry. All participants provided written informed consent before inclusion.

Participants were evaluated at three predefined time points: baseline (T0), before initiation of pharmacological treatment, 4 weeks after treatment initiation (T1), and 8 weeks after treatment initiation (T2). All assessments were performed under standardized conditions.

A total of 26 patients aged between 24 and 63 years were included in this study. Participants were recruited from inpatient admissions and subsequently monitored in an outpatient setting, with rehospitalization when clinically indicated. Inclusion criteria comprised age between 18 and 65 years, a diagnosis of severe depressive episode or recurrent depressive disorder, and absence of psychiatric medication in the two weeks preceding baseline evaluation. Exclusion criteria included bipolar disorder, psychotic disorders, significant cognitive impairment, chronic alcohol or substance use, pregnancy or breastfeeding, severe untreated medical comorbidities, positive serology for hepatitis B, hepatitis C or HIV, known hypersensitivity to prescribed medications, poor compliance, and the presence of active suicidal ideation or aggressive behavior at baseline.

Clinical evaluation was performed by the same clinician at all time points and included both a general medical examination and a structured psychiatric assessment. Depressive symptom severity was measured using the 17-item Hamilton De-

pression Rating Scale, while anxiety symptoms were assessed using the Hamilton Anxiety Rating Scale. Anthropometric measurements were obtained for the calculation of body mass index (BMI), and relevant demographic and clinical variables, including smoking status and alcohol consumption, were recorded due to their potential influence on inflammatory and metabolic processes.

Venous blood samples were collected at each time point between 08: 00 and 09: 00 following an overnight fast of at least 8 hours in order to minimize circadian and metabolic variability. Samples were drawn into EDTA tubes and processed within 30 minutes. Platelet-poor plasma was obtained using a two-step centrifugation protocol, aliquoted into polypropylene tubes, and stored at −80 °C until analysis. Samples from the same participant were analyzed within the same experimental run to reduce inter-assay variability, and repeated freeze–thaw cycles were avoided.

Plasma concentrations of brain-derived neurotrophic factor were determined using a commercially available sandwich ELISA kit (IBL International GmbH/Tecan, catalogue number RB59041), following the manufacturer's instructions. All samples and standards were analysed in duplicate. The same applies to plasma levels of arachidonate 12-lipoxygenase, which were measured in duplicate using a commercially available ELISA kit specific for human ALOX12 (FineTest, catalogue number EH1980) according to the manufacturer's protocol.

Pharmacological treatment was initiated after baseline assessment and was prescribed individually by the treating psychiatrist in accordance with current clinical guidelines. The study followed a naturalistic design, with no intervention from the investigators in treatment selection or dosing.

Statistical analyses were performed using IBM SPSS Statistics v28.0. Data distribution was assessed using the Shapiro–Wilk test. As the data were not normally distributed, non-parametric tests were applied. Changes across the three time points were analyzed using the Friedman test, with pairwise comparisons performed using the Wilcoxon signed-rank test. Associations between biomarker levels and clinical measures were evaluated using Spearman's rank correlation coefficient. Statistical significance was set at $p < 0.05$ (two-tailed).

RESULTS

Clinical and biomarker evolution

At baseline, patients presented with high levels of depressive and anxiety symptoms, with median HAM-D and HAM-A scores of 31.0 and 28.0, respectively, indicating moderate-to-severe symptomatology. After 8 weeks of treatment, both measures showed a clear reduction, with median values decreasing to 23.0 for HAM-D and 18.0 for HAM-A (tab. I).

In parallel, biological markers showed distinct temporal patterns. Serum BDNF levels increased markedly over the course of treatment, from a median of 0.87 at baseline to 5.33 at 8 weeks. In contrast, ALOX levels showed a progressive decrease, from a median of 1.39 at baseline to 0.22 at 8 weeks (tab. I).

These temporal changes are illustrated in figure 1, which shows a progressive reduction in ALOX values over time, with the most pronounced decrease observed at 8 weeks. The distribution also indicates greater variability at baseline, followed by a reduction in dispersion at subsequent time points.

TABLE I.

Descriptive statistics of clinical variables and biomarkers across time points

Variable	T0 Median (IQR)	T1 Median (IQR)	T2 Median (IQR)
ALOX	1.39 (1.04–3.41)	1.17 (0.94–1.41)	0.22 (0.21–0.23)
HAM-D	31.0 (29.75–32.0)	30.0 (28.0–31.25)	23.0 (17.5–24.25)
HAM-A	28.0 (26.0–30.0)	24.0 (22.0–26.0)	18.0 (18.0–20.0)
BDNF	0.87 (0.54–1.43)	5.11 (2.53–8.89)	5.33 (3.15–9.42)

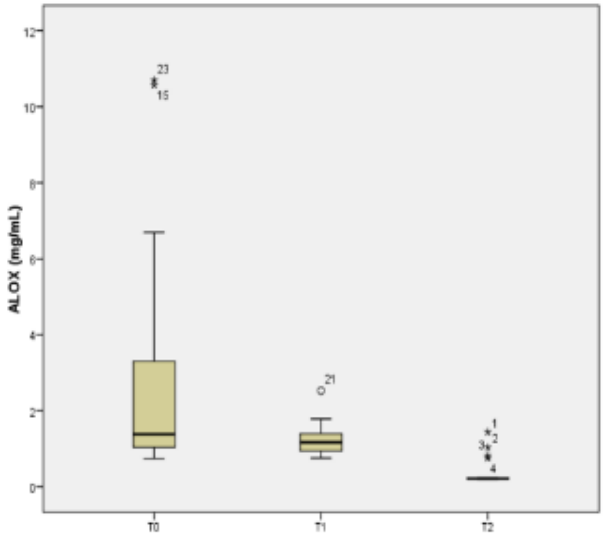

Fig. 1. Evolution of ALOX levels at T0, T1, and T2 during treatment

Given the non-normal distribution of most variables, non-parametric analyses were applied. The Friedman test revealed a significant overall effect of time on ALOX levels ($\chi^2(2) = 34.462$, $p < 0.001$). Mean ranks decreased progressively from T0 (2.62) to T1 (2.31) and T2 (1.08), indicating a consistent downward trajectory.

Post-hoc pairwise comparisons showed that the reduction between baseline and 4 weeks did not reach statistical significance after Bonferroni correction ($p = 0.027$). In contrast, differences between baseline and 8 weeks, as well as between 4 and 8 weeks, were statistically significant (both $p < 0.001$), indicating that the main change in ALOX occurred during the later phase of treatment. Stratified analyses across antidepressant classes showed similar temporal patterns of ALOX reduction across all groups (tab. II). Baseline values were numerically higher in the tricyclic antidepressant group, although interpretation is limited due to the small subgroup sizes. No statistically significant differences between classes were observed (Kruskal–Wallis χ^2 (3) = 6.769, $p = 0.080$), although the highest mean rank change was noted in the multimodal serotonergic group.

TABLE II.

Descriptive statistics stratified by antidepressant class

Variable	Time	SMS (n=4)	SNRI (n=9)	SSRI (n=10)	TCA (n=3)
HAM-D	T0	30.5 (30.0–31.5)	32.0 (31.0–32.0)	32.0 (30.0–32.0)	29.0 (28.5–29.0)
	T1	30.0 (29.0–30.0)	30.0 (30.0–32.0)	30.5 (28.0–32.0)	–
	T2	21.0 (16.5–24.0)	24.0 (20.0–25.0)	21.0 (16.0–24.0)	24.0 (20.0–25.0)
HAM-A	T0	28.0 (27.0–29.0)	28.0 (28.0–30.0)	26.0 (26.0–30.0)	24.0 (24.0–26.0)
	T1	25.0 (22.0–27.0)	26.0 (22.0–26.0)	23.5 (20.0–26.0)	22.0 (22.0–24.0)
	T2	19.0 (17.0–21.0)	20.0 (18.0–20.0)	18.0 (16.0–20.0)	20.0 (19.0–20.0)
BDNF	T0	0.50 (0.40–0.86)	0.86 (0.63–1.33)	0.98 (0.73–1.55)	0.72 (0.59–1.25)
	T1	9.09 (2.52–18.70)	5.39 (4.17–5.88)	3.67 (2.33–11.25)	5.46 (3.90–6.04)
	T2	9.81 (2.84–20.70)	5.65 (4.29–6.25)	4.15 (3.23–11.44)	5.41 (4.12–6.05)
ALOX	T0	1.10 (0.87–1.32)	1.21 (0.91–2.59)	1.60 (1.06–3.71)	3.31 (2.19–5.00)
	T1	1.37 (1.28–1.58)	1.07 (0.91–1.56)	1.15 (0.94–1.37)	1.15 (1.07–1.33)
	T2	0.52 (0.22–0.93)	0.22 (0.21–0.22)	0.22 (0.22–0.24)	0.23 (0.22–0.23)

*The symbol "–" indicates that the interquartile range (IQR) could not be calculated due to the small subgroup size (particularly TCA, n=3) and insufficient variability in the data at that time point.

Associations between biomarkers and clinical outcomes

Correlation analyses at 8 weeks revealed a significant negative association between ALOX levels and depressive symptom severity. Higher ALOX levels were associated with lower HAM-D scores in both zero-order analysis ($r = -0.416$, $p = 0.035$) and after adjustment for age and body mass index (partial $r = -0.515$, $p = 0.010$) (tab. III).

This relationship is illustrated in figure 2, which shows an inverse relationship between ALOX and HAM-D scores.

TABLE III.

Spearman and partial correlations at 8 weeks

Variable pair	r (zero-order)	p (zero-order)	r (partial)	p (partial)	Significant
HAM-D T2 vs ALOX T2	−0.416	0.035	−0.515	0.010	Yes
HAM-D T2 vs BDNF T2	−0.400	0.043	−0.402	0.052	Trend
HAM-D T2 vs Age	+0.501	0.009	—	—	Yes
HAM-D T2 vs BMI	+0.391	0.048	—	—	Yes
HAM-A T2 vs ALOX T2	−0.211	0.301	−0.240	0.259	No
HAM-A T2 vs BDNF T2	−0.250	0.218	−0.338	0.106	No
BDNF T2 vs ALOX T2	+0.282	0.163	+0.285	0.178	No
Age *vs.* BMI	+0.701	<0.001	—	—	Yes

In contrast, no significant associations were observed between ALOX levels and anxiety symptoms (HAM-A) at 8 weeks, either before or after adjustment (tab. III), suggesting the specificity of this relationship for depressive symptomatology.

BDNF levels at 8 weeks showed a negative association with HAM-D scores in zero-order analysis ($r = -0.400$, $p = 0.043$), which remained at a trend level after adjustment (partial $r = -0.402$, $p = 0.052$). The magnitude of this association remained stable after controlling for demographic variables.

No significant correlations were identified between changes in ALOX and changes in clinical scores or BDNF levels (all $p > 0.05$). A strong negative correlation was observed between baseline ALOX and its change over time ($r_s = -0.833$, $p < 0.001$), although this finding is influenced by mathematical coupling and should be interpreted cautiously.

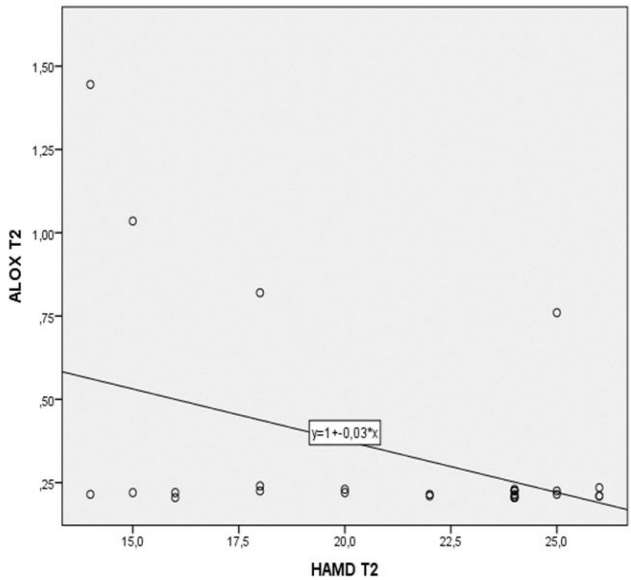

Fig. 2. Association between depressive symptom severity at 8 weeks and ALOX levels at T2

Age and body mass index were both positively associated with HAM-D scores at 8 weeks ($r = +0.501$, $p = 0.009$ and $r = +0.391$, $p = 0.048$, respectively). These variables were strongly correlated with each other ($r = +0.701$, $p < 0.001$), supporting their inclusion as covariates in adjusted analyses.

Exploratory subgroup and predictive analyses

No statistically significant differences in ALOX change were observed between responders and non-responders after 8 weeks of treatment (Mann–Whitney $U = 35.5$, $p = 0.269$), although responders showed higher mean ranks, suggesting a tendency toward greater reduction.

The distribution of ALOX values across these groups is shown in fig. 3 and fig. 4. Responders exhibited more homogeneous changes, whereas non-responders showed greater variability.

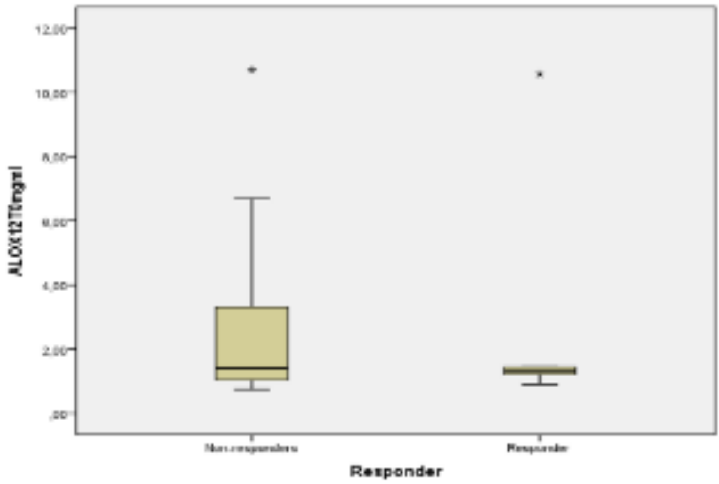

Fig. 3. Comparative boxplot of baseline serum ALOX levels in responders and non-responders

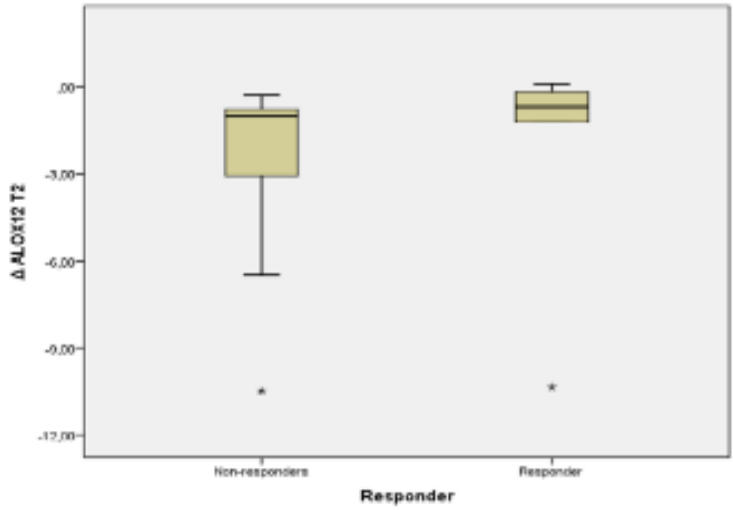

Fig. 4. Change in ALOX levels between T0 and T2 in responders and non-responders

Receiver operating characteristic analysis did not demonstrate predictive value of baseline ALOX levels for treatment response (AUC = 0.510, p = 0.948; 95% CI: 0.232–0.787).

Exploratory analyses of demographic and lifestyle factors did not identify significant associations with ALOX change (all p > 0.05) (tab. IV). The closest trend toward statistical significance was observed for smoking status (p = 0.143), with smokers showing higher mean ranks compared to non-smokers, which may indicate a more pronounced change in ALOX in this subgroup. In the absence of statistical significance, this observation remains exploratory.

TABLE IV.
Exploratory subgroup analyses of ALOX change

Variable	Group	N	Mean Rank	Mann-Whitney U	Z	p-value
Sex	Female	20	13.03	50.500	-0.578	0.563
	Male	6	15.08			
Residence	Rural	15	14.30	70.500	-0.623	0.533
	Urban	11	12.41			
Marital status	In a relationship	18	14.19	59.500	-0.695	0.487
	Not in a relationship	8	11.94			
Smoking	Smoker	12	15.88	55.500	-1.466	0.143
	Non-smoker	14	11.46			
Physical activity	Moderate	12	14.29	74.500	-0.489	0.625
	Low	14	12.82			
Alcohol use	Occasional use	15	15.00	60.000	-1.168	0.243
	No use	11	11.45			
Occupation status	Employed	12	13.33	82.000	-0.103	0.918
	Unemployed	14	13.64			

DISCUSSION

This study identified three principal observations: a reduction in depressive and anxiety symptom severity over 8 weeks, a marked increase in BDNF levels, and a progressive decrease in ALOX levels. The reduction in depressive severity was accompanied by an increase in BDNF and a decrease in ALOX, although these biological changes followed distinct temporal patterns and showed limited direct correlation with each other.

The absence of significant correlations between changes in ALOX and changes in clinical scores suggests that these markers capture partially independent biological processes. This dissociation is consistent with previous evidence indicating that biological markers in depression often reflect heterogeneous mechanisms rather than a unified pathophysiological pathway (33).

Depressive and anxiety symptoms showed a progressive reduction over the 8-week treatment period, with the most substantial improvement observed at the final time point. This temporal pattern aligns with clinical data indicating that antidepressant effects accumulate over several weeks rather than emerging immediately (34, 35). The magnitude of symptom reduction observed in this study is consistent with large clinical datasets showing partial response rather than full remission in most patients during early treatment phases (35). Previous studies have shown that early symptom reduction does not necessarily correspond to full biological normalization, supporting the interpretation of clinical improvement as a gradual and multidimensional process (36).

BDNF levels increased significantly over the course of treatment, consistent with the neurotrophic hypothesis of depression. This increase is in line with meta-

analytic findings reporting elevated peripheral BDNF following antidepressant treatment (16, 18). However, the lack of strong correlations between BDNF changes and symptom improvement in this study reflects the variability described in the literature. Several longitudinal analyses have reported weak or absent associations between peripheral BDNF changes and clinical outcomes, suggesting that BDNF does not function as a direct proxy for symptom severity (16, 17, 33).

The negative association between BDNF and depressive severity at endpoint, although modest, is consistent with evidence that reduced neurotrophic support is linked to impaired synaptic plasticity (4, 13). Some studies indicate that inflammatory processes can suppress BDNF expression, while BDNF itself may regulate neuroinflammatory activity, supporting a bidirectional relationship between these systems (30, 31).

ALOX levels showed a progressive and statistically significant decrease over time, with the most pronounced reduction occurring between weeks 4 and 8. This delayed dynamic suggests that ALOX-related pathways may reflect biological processes evolving later in treatment. The absence of significant correlations between ALOX changes and symptom improvement suggests that this marker does not directly reflect clinical response. However, the significant inverse association between ALOX levels and depressive severity at the endpoint indicates a more complex relationship. Higher ALOX levels at 8 weeks were associated with lower depressive severity, a finding that does not align with a simple pro-inflammatory or neurotoxic interpretation. This pattern may reflect compensatory or regulatory mechanisms within lipid signaling pathways. Lipid mediators derived from arachidonic acid metabolism participate in both pro-inflammatory and resolution processes, and their functional role may depend on context, timing, and cellular environment (20, 37).

The findings indicate that BDNF and ALOX reflect different aspects of biological response during antidepressant treatment. BDNF appears to capture processes related to neuroplastic adaptation, while ALOX may reflect changes in lipid-mediated oxidative pathways that evolve later in treatment. The absence of predictive value of baseline ALOX for treatment response suggests that this marker is more relevant for monitoring biological changes over time rather than predicting outcomes. The variability observed in non-responders further supports the presence of biological heterogeneity within depressive disorders (38, 39).

The relatively small sample size limits statistical power and may reduce the ability to detect subtle associations. The use of peripheral biomarkers introduces uncertainty regarding their relationship to central nervous system processes, particularly for BDNF, where peripheral levels may not directly reflect brain concentrations (17). The naturalistic design introduces variability related to treatment heterogeneity, although it enhances ecological validity. The absence of a control group limits causal interpretation of observed changes.

CONCLUSIONS

The study demonstrates that antidepressant treatment is associated with increased BDNF and decreased ALOX levels over time, alongside clinical improvement. These markers show distinct temporal patterns and limited direct association with each other.

BDNF appears to reflect neuroplastic processes associated with treatment, while ALOX may represent a separate biological

pathway related to oxidative and lipid-mediated mechanisms. Their combined assessment provides a broader perspective on biological changes occurring on treatment.

Longitudinal evaluation of multiple biomarkers may offer a more accurate representation of the biological processes underlying treatment response in major depression.

CONFLICT OF INTERESTS AND FUNDING

The authors declare no conflict of interest and they received no financial support was received for perfecting this article.

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INCREASING INCIDENCE OF RECURRENT *CLOSTRIDIUM DIFFICILE* INFECTION

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INCREASING INCIDENCE OF RECURRENT *CLOSTRIDIUM DIFFICILE* INFECTION (Abstract): *Clostridium difficile* infection continues to be a major cause of infectious enterocolitis, commonly associated with antibiotic use. Infection can lead to relapses, reason why it is considered by clinicians a true challenge. **Aim**: To determine the incidence of recurrent *Clostridium difficile* infection in patients admitted to the Iasi Infectious Diseases Hospital over a six-month period. **Material and methods**: Of the 195 admitted patients, 46 presented for the recurrence of colitis. Data from medical records had been used to identify the patients who met the inclusion criteria. **Results**: Most of the 46 relapses were recorded in individuals aged over 60 with multiple associated comorbidities. The test for *Clostridium difficile* toxin was positive in a significant percentage of cases and the treatment consisted in the administration of vancomycin and normix alone or in combination, along with the treatment of comorbidities. **Conclusions**: Once a relapse occurs the risk of a second relapse is rising, the elderly being most vulnerable. The use of antibiotics should be limited to situations where they are absolutely needed.
Keywords: RECURRENT CLOSTRIDIUM DIFFICILE INFECTION, VANCOMYCIN, COMORBIDITIES, MULTIDISCIPLINARY COLLABORATION.

Ubiquitous bacterium, *Clostridium difficile* (*C. difficile*) has been incriminated in the causation of infectious enterocolitis, the most common cause of nosocomial diarrhea. The bacterium is part of the normal intestinal microbiota in 3% of the adult population, 10-30% of hospitalized patients, and 60-70% of infants.

C. difficile enterocolitis can occur at any age after antibiotic administration and rarely spontaneously. Patients who receive antibiotic therapy are at 7-10 times higher risk for developing the disease (1).

Also, patients who are hospitalized for other diseases and those with gastrointestinal surgery in recent history are at higher risk, the incidence being higher among people over the age of 65 (2).

The disease can be cured, may recur or can be fatal. Recurrent *C. difficile* infections represent a major clinical challenge due to their growing incidence. According to the Center for Disease Prevention and Control each year *C. difficile* is involved in

over 500,000 illnesses and one in five people diagnosed with *C. difficile* colitis returns due to the recurrence of symptoms and positivity of stool cultures. Once a relapse occurs, the risk of a secondary recurrence is 45%. It is also estimated that this bacillus is the cause of more than 15,000 deaths in people over 65, about one in 11 patients diagnosed with the infection dying within 30 days of diagnosis (3).

The source of infection are people (with C. difficile infection or carriers) who spread the bacteria or contaminate surfaces through hand contact and the environment of patients with colitis induced by this bacterium. Human transmission can be prevented by isolating the *C. difficile* infection cases, the use of gloves and thorough handwashing, environmental disinfection, and by educating the patients/ families (1, 4).

Recurrences are caused by reinfection with the original C. difficile strain (due to the presence of resistant spores) and/or reinfection with a new strain from the environment, the exact cause being difficult to determine. What can be said for sure it is that resistant strains do not exist, and therefore, the treatment may be the same as for the primary infection (5, 7). Treatment options for patients with more than one recurrence are becoming more and more limited. Prolonged use of metronidazole is associated with a risk of neurotoxicity, which is why oral v vancomycin is preferred in these cases. Other drugs have been approved as an alternative treatment for relapses: fidaxomicin, rifaximin, probiotics, ion exchange resins (cholestyramine) or fecal microbiota transplantation (6, 7).

MATERIAL AND METHODS

This retrospective observational study was conducted at the "Sf. Parascheva" Infectious Diseases Hospital from Iasi. It included all patients diagnosed with *C. difficile* colitis admitted between July 1st and December 31st, 2016, the aim being to determine the incidence of recurrent infections. The presence of the causative agent was confirmed by the rapid test for the detection of *C. difficile* toxins A and B, this being the most important inclusion criterion.

A descriptive analysis based on the demographic, clinical, and laboratory data, disease course, therapeutic approach and underlying diseases was carried out using data from patient medical records.

RESULTS

Between July 1st and December 31st, 2016, 195 patients diagnosed with *Clostridium difficile* colitis were admitted. The data collected from medical records as well as from the accompanying documents revealed that 46 patients presented for relapse, the remaining patients being at their first admission for this disease.

The most affected age group was over 60 (71.73% of the cases), with relatively small gender differences (46% women, 54% men). Similar percentages were recorded for the place of residence (54% urban patients, 46% rural patients).

According to history and accompanying documents these 37 patients received antibiotic treatment prior to the first episode of C. *difficile* colitis, the most commonly being used the cephalosporins and fluoroquinolones.

Of the total of 46 patients, 63% were at their first relapse, 17% at the second, and 9% at their third relapse. In 58.69% of the cases, the patients presented themselves directly to our hospital (at the triage or the doctor who had previously treated him),

32.61% of cases were referred by other specialists and only 8.7% of the cases were referrals from family doctors.

The disease had a favorable course in most cases, with remission of diarrheal symptoms and negativizing of *C. difficile* toxin in stools. Three cases had an unfavorable course, resulting in death due to complications of the associated comorbidities. Because in most cases patients were retired (86.95%), they also had several underlying disorders, the most common being the digestive (including liver cirrhosis, hepatitis B, C, colorectal cancer, chronic cholecystitis, gastric/duodenal ulcers), cardiovascular and surgical ones, as shown in figure 1.

Analyzing the observation charts, it was found that all patients had the specific symptoms of the disease (moderate or severe diarrhea, accompanied by abdominal pain), to which symptoms of nausea and loss of appetite were added in 8 (17.4%) patients, vomiting and fever in 5 (10.9%) and 2(4.37%) patients experienced vertigo and abdominal bloating.

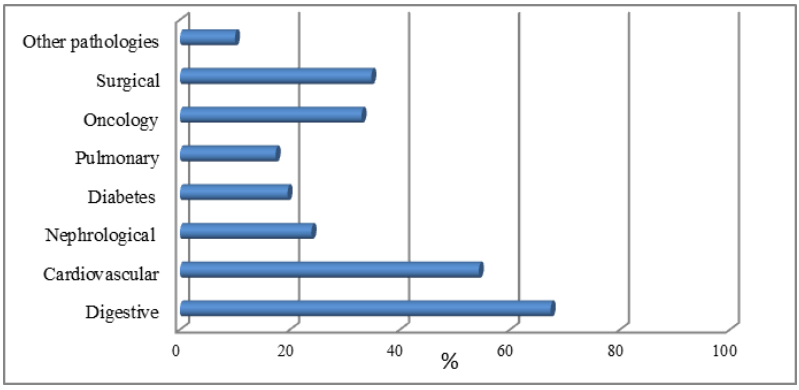

Fig. 1. Distribution of associated comorbidities

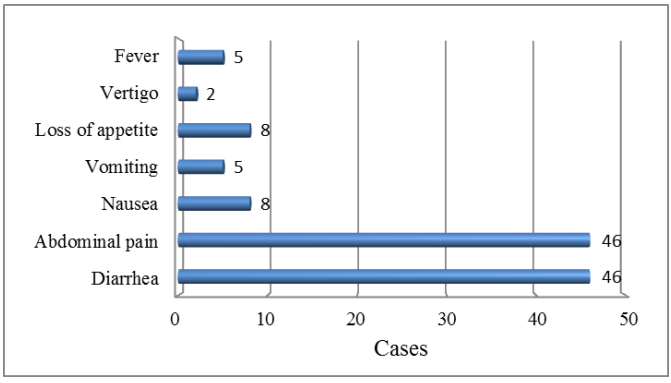

Fig. 2. Distribution of cases by associated symptoms

The positive diagnosis is based on the rapid detection of C. difficile toxins A and B in stool samples. In our study group both toxins (A and B) were detected in 80.43% of cases, toxin A alone in 10.9% cases, GDH antigen in 19.5% and 2.17% of the

results identified nontoxigenic strains.

A significant number of patients also presented clinical-biological changes associated with the disease: anemia (54.34% of cases), predominantly microcytic anemia, the most frequent cause being iron deficiency, or secondary, intrainfectious hypokalemia (39.13% of cases) and hypoproteinemia (39.95% of cases).

Most patients had been hospitalized for more than 8 days (range 3-26 days). All patients received antibiotic treatment during hospital stay. Therapy was initiated after the confirmation of relapse, and its average duration was 7-10 days. Vancomycin treatment was instituted in 80.43% of relapses, normix in 34.78%, and 15.21% of cases were treated with vancomycin and normix combination. In addition to the treatment for *C. difficile* colitis, 67.4% of the patients also received treatment for the underlying diseases.

In the case of patients with a poor general health status with or with associated comorbidities, intensive care physicians as well as physicians of various specialties were consulted to provide the best recovery therapy.

DISCUSSION

Unfounded use of antibiotics has led to a significant increase in the resistance of microorganisms to antibiotics. A major side effect of unfounded antibiotic use is the increased incidence of post-antibiotic diarrhea.

Any antimicrobial medication with intestinal penetration is thought to trigger the disease, even at very low doses. Therefore, those who received prior antibiotic treatment and the elderly are the most vulnerable groups of patients who can develop *C. difficile* colitis, an infectious disease with interhuman transmission, with increased risk of rebound and death.

The analysis of the data from our study showed that the people aged over 60 years and retired were most commonly affected, with a slightly higher proportion of male and urban population.

Clostridium difficile toxins A and B were positive in most cases, which is essential in the present study, with the occurrence of the specific symptoms of disease in 100% of cases. Patients had multiple associated comorbidities, especially surgical and digestive, but also disturbances in biological assays, which impeded the favorable outcome in three patients who died.

Due to the risk of neurotoxicity of prolonged use of metronidazole, antibiotic therapy with vancomycin (80.43%) and normix (34.78%) alone or in combination was chosen in parallel with the treatment of comorbidities.

CONCLUSIONS

The results of the present study show that recurrences of C. difficile infection are frequently diagnosed in patients with multiple comorbidities, especially surgical and gastrointestinal, and additional risk factors include advanced age and history of cancer that represent major problems in the clinical management of patients with *Clostridium difficile* infection.

Our recommendation for preventing the disease is the limitation of antibiotic use, as well as maintaining proper hygiene (of the skin, surfaces and the environment). Also, observance of the indications on food consumption leads to a favorable course of the disease and prevents relapses.

It can be noted that in case of complex treatments the multidisciplinary approach plays an important role in the evolution of the disease and recovery of patients.

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NEWS

HISTOPATHOLOGIC FEATURES OF PROGNOSTIC SIGNIFICANCE IN HIGH-GRADE OSTEOSARCOMA

Osteosarcoma is a malignant mesenchymal neoplasm characterized by bone production. Several types of primary high-grade osteosarcoma are recognized by the World Health Organization, including the "conventional" types encompassed by the chondroblastic, fibroblastic, and osteoblastic subtypes, among others. Despite heterogeneity in histomorphology, most high-grade osteosarcomas receive similar treatment, namely, neoadjuvant chemotheratypically consisting of doxorubicin, cisplatin, and high-dose methotrexate, followed by surgical resection and maintenance chemotherapy. Although many groups have assessed the contribution of clinical parameters—namely, patient age, sex, anatomic site of primary tumor, tumor size, and metastasis at presentation—in predicting survival outcomes for patients with osteosarcoma, there are limited data concerning histopathologic attributes. Histopathologic prognostic factors represent an essential component to the treatment stratification of many types of cancer. The authors suggest that detailed microscopic examination (tumor pleomorphism, mitotic activity, and morphologic subtype) yields useful prognostic information in high-grade osteosarcoma. In biopsy samples, necrosis and chondroblastic differentiation are adverse features. In resections, quantitation of the extent of residual viable tumor may be of greater clinical relevance than attempting to distinguish necrosis from other noncellular elements. In summary, the integration of clinical and microscopic features is a feasible and effective means for improving prognostication in patients with osteosarcoma. The effective stratification of patients using the proposed scoring system merits further investigation, ideally in prospective cohorts, to validate interobserver reproducibility and predictive ability (Chui MH, Kandel RA, Wong M, *et al*. Histopathologic Features of Prognostic Significance in High-Grade Osteosarcoma. *Arch Pathol Lab Med* 2016; 140(11): 1231-1242).

Doina Butcovan

BALANCING THE THROMBOTIC RISK AND THE HEMORRHAGIC RISK IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE AND DEEP VEIN THROMBOSIS. CASE REPORT

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BALANCING THE THROMBOTIC RISK AND THE HEMORRHAGIC RISK IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE AND DEEP VEIN THROMBOSIS - CASE REPORT (Abstract): The thrombotic events occurring in patients with inflammatory bowel disease are currently recognized as a form of extraintestinal manifestation of the disease. We present the case of a 35-year-old Caucasian male with inflammatory bowel disease and deep vein thrombosis, drawing attention to the particularities of the venous thrombotic events in this category of patients: high thrombotic risk, peak incidence at young age, high risk of recurrence. We state the correct anticoagulant treatment based on current evidence and guidelines recommendations, highlighting the importance of a careful assessment of both the thrombotic and hemorrhagic risk of the patient. Special attention has been paid to the new oral anticoagulants and to the latest recommendations of specialized guidelines.
Keywords: ANTICOAGULANTS, VENOUS THROMBOEMBOLISM, INFLAMMATORY BOWEL DISEASE.

Inflammatory bowel disease (IBD) is a chronic inflammatory condition characterized by local and systemic inflammation, predominantly affecting the gastrointestinal tract. The two major forms are Crohn's disease (CD) and ulcerative colitis (UC).

The association between IBD and venous thrombosis was first described in 1936 by Bargen and Barker (1). Further studies (2,3) have shown that IBD patients have a 2 to 3 fold increased risk of developing deep vein thrombosis (DVT) and pulmonary embolism (PE) compared to the general population and the thrombotic risk is almost three times higher in males than in females. No significant differences in the risk of venous thromboembolism (VTE) are found between UC and CD patients (4). The main sites of thrombosis are the deep veins of the lower limbs and the pulmonary system (5). Less frequently, the thrombosis may develop in the cerebrovascular system, hepatic, portal, retinal and mesenteric veins (4).

CASE REPORT

We present the case of a 35-year-old Caucasian male who presented with pain in

his right thigh with a 24-hour onset. The thigh was enlarged in volume, but without changes in color or local temperature. The patient denied any trauma in that area. At the age of 30 he was diagnosed with a limited form of ulcerative colitis (proctosigmoiditis) and at the time of the current presentation he was on treatment with mesalazine 1000 mg twice daily. He was a non-smoker. Venous ultrasound confirmed the deep vein thrombosis of the superficial femoral vein, the fresh thrombus occupying the lower third of the vein. Anemia was absent. The IBD was in remission. The patient's chronic treatment does not favor thrombosis; on the contrary, mesalazine has the advantage of reducing platelet activation.

The anticoagulant treatment was started with low molecular weight heparin (LMWH - enoxaparinum), with favorable outcome. Pain and swelling have rapidly diminished, being absent at discharge. Given the presence of UC, the main challenge was the management of the antithrombotic therapy, because patients with IBD are at high risk of both VTE and gastrointestinal bleeding.

DISCUSSION

We started by evaluating the substrate of the prothrombotic state to fit the current thrombotic event into one of the two categories: provoked or unprovoked DVT.

The genetic mutations which are causes of inherited thrombophilia have similar incidence in IBD patients and in the general population, suggesting that the acquired factors play a crucial role in the occurrence of thrombosis. The clinical studies have shown no association between IBD and Factor V Leiden, prothrombin G20210, MTHFR or Factor XIII val34leu, respectively. Therefore, the available guidelines do not recommend thrombophilia testing in patients with IBD and VTE, including the patients with unprovoked VTE while their IBD is in clinical remission (6). Accordingly, we did not do thrombophilia testing in our patient.

The role of inflammation in IBD pathogeny has been heavily debated over the years. Indeed, the inflammatory cytokines affect the coagulation cascade, fibrinolysis, and platelet functions. Because some of the coagulation factors are also acute-phase reactants, if the prothrombotic state is triggered by the inflammation or represent a feature of the intestinal disease per se is still a matter of debate. More recently, high levels of circulating microparticles have been found in IBD patients during active phases of the disease. Decreased natural anticoagulants (antithrombin III activity, proteins C and S, tissue factor pathway inhibitor) (7) and a disturbed fibrinolysis (reduction in fibrinolysis activators such as tPA and increase in inhibitors such as PAI-1 and TAFI) complete the complex anomalies of the coagulation system. Endothelial dysfunction and the quantitative, morphological and qualitative alterations in platelet characteristics also contribute to the prothrombotic state of this disease.

Despite the many above mentioned, inflammation does not seem to be the main player in this disease. Miehsler et al. (8) compared the thromboembolic risk of patients with IBD with that of patients with celiac disease and rheumatoid arthritis. While an association between the high frequency of thromboembolic events and IBD was established, no such association was found in patients with other systemic inflammatory conditions such as celiac disease and rheumatoid arthritis. A pro-

coagulant tendency unequivocally exists in IBD patients, but unfortunately no abnormality has been shown to be a marker for the thrombotic risk in IBD.

The incidence of VTE in the general population increases with age, but in IBD patients the highest risk for VTE is observed among those less than 40 years of age, with an incidence rate ratio for VTE of 4.5 when compared with the non-IBD patients (9, 10). Our patient was 35 years old at the time of the thrombotic event, so he fit the pattern. The younger the age at the first thrombotic episode is, the higher the risk of recurrence (3). The thromboembolic complications occur more frequently during the active phases of the disease and in patients with extended disease (11). Moderate and severe disease flares significantly increase the risk of VTE in IBD patients and should be considered as a provoking factor. But the thromboembolic complications are also seen in almost one third of the patients with partial or full remission (11). In our case, the patient had a limited form of disease and he was in remission when the thrombotic event occurred, so disease flares were excluded as provoking factor. No other provoking factors have been identified. IBD patients who experience their first episode of unprovoked VTE have a 33% risk of recurrence within 5 years. The risk of recurrence is 2.5-fold higher than that of non-IBD patients after an initial episode of unprovoked VTE (3).

All these data suggest that IBD is a continuing risk factor for recurrent VTE and, therefore, indefinite anticoagulant therapy should be recommended in patients who are diagnosed with their first episode of unprovoked proximal DVT when in clinical remission (12). Is seems that benefits of long-term anticoagulation in reducing recurrent VTE in IBD patients who have had unprovoked VTE outweigh the risks of the associated bleeding (2). The need for anticoagulation should be revised at least annually.

The first European evidence-based consensus on extraintestinal manifestations in inflammatory bowel disease (13) states that the treatment of VTE in IBD patients follows the same protocols as for non-IBD patients. So, we start the treatment with LMWH. Long-term anticoagulant treatment usually comprises a vitamin K antagonist (VKA) or a non-vitamin K antagonist oral anticoagulant (NOAC). Meta-analysis regarding efficacy and safety of NOACs as compared with VKA in the treatment of VTE have shown that NOACs have comparable efficacy to that of VKAs and are associated with a significantly lower risk of bleeding complications (odds ratio 0.63, 95% CI 0.44-0.90) (14). In terms of major gastrointestinal bleeding, a recent meta-analysis (15) reported low relative risk for rivaroxaban and apixaban and elevated relative risk for dabigatran when compared to warfarin. This might be explained by an individual drug effect or a difference between drug classes (FIIa inhibitors and FXa inhibitors). No separate data regarding bleeding risk were reported for edoxaban.

A meta-analysis of the clinical trials in the atrial fibrillation population confirmed a significantly increased risk of gastrointestinal hemorrhage with NOACs compared with VKA (16). Apixaban was not associated with this bleeding outcome in either the VTE or atrial fibrillation studies (17), so this medication may be more appropriate in patients who experienced a gastrointestinal hemorrhage or who are at risk for this complication. No significant difference was found between NOACs and comparators

for extended treatment of VTE.

All studies on NOACs for VTE treatment suggest that they have comparable efficacy to that of VKAs with a more favorable safety profile, but there is no direct evidence for their use in IBD patients yet, so our treatment respected the so far existing evidence. We were able to find just one case report (18) of NOAC use (rivaroxaban) in a patient with VTE and IBD.

In terms of surgical recommendations, VTE is not an indication for an invasive procedure and despite a very limited experience colectomy per se seems not to be able to protect from recurrent thromboembolic events.

CONCLUSIONS

We presented a case of IBD complicated with DVT as extraintestinal manifestation of the disease with the aim of raising awareness of the thrombotic risk in these patients. Considering that patients with IBD are at high risk of both VTE and gastrointestinal bleeding, we highlighted the importance of accurately assessing the duration of anticoagulant therapy. The latest guidelines mention that in the treatment and secondary prevention of VTE, NOACs have comparable efficacy to VKAs, with a more favorable safety profile, but it is also stated that there is no direct evidence for NOACs use in IBD patients yet.

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NEWS

ELECTROSPUN NANOFIBER ORIENTATION INFLUENCES WOUND CLOSURE

Nanofibers resemble the natural extracellular matrix of the human body. Furthermore, polymer nanofibers provide an ideal environment for wound healing. A recent study aimed to evaluate the pure topographical effects that an underlying scaffold material may have on fibroblast behaviour in a novel *in vitro* wound model, which included poly-ε-caprolactone scaffolds with different nanofiber alignement. A stencil based wound assay with L929 murine fibroblasts was used to study the closure of circular wounds over time. Triggering fibroblast migration into the wound accelerates the remodeling of the dermis. Cell viability, morphology and mobility – in fact a combination of proliferation and migration, since both occur during the natural wound healing process - were assessed. The study emphasizes the important effect of orientation on increasing cell mobility, polarization and elongating cell morphologies compared to random fiber scaffolds (Ottosson M, Jakobsson A, Johansson F. Accelerated Wound Closure - Differently Organized Nanofibers Affect Cell Migration and Hence the Closure of Artificial Wounds in a Cell Based *in vitro* Model. *PLoS ONE* 2017; 12(1): e0169419. doi:10.1371/journal.pone.0169419).

Oana Maria Ionescu

COMPARATIVE MORPHOLOGICAL AND MORPHOMETRICAL ANALYSIS OF TWO THYMOMA CASES

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COMPARATIVE MORPHOLOGICAL AND MORPHOMETRICAL ANALYSIS OF TWO THYMOMA CASES (Abstract): Thymoma is a primary tumor of the thymus epithelial cells and one of the most common neoplasms in the anterosuperior mediastinum, especially in middle-aged or older adults. There were some difficulties in thymoma diagnosis due to high variability of the morphological thymoma aspects. WHO 2004 morphologic classification reached a kind of consensus of the morphological thymoma types and established a correlation with prognostic factors. **Aim:** The aim of this study was to assess morphologically and morphometrically the two thymoma specimens for searching prognosis according with atypical features and variable invasive character. **Material and methods:** The study included 2 thymoma cases, one AB and the other B1 type, both in adults, operated for mediastinal tumoral masses. Surgery specimens were processed for histological examination. Qualitative and quantitative assessment of thymoma specimens was applied. **Results:** The study reveals various histological aspects and cytological measurement results explaining different invasiveness features in the two thymomas. The two thymoma types show a close relationship to blood vessels. **Conclusions:** Usually, the current state of the art in thymoma diagnostic defines invasion, both clinically and histologically. The morphometrical analysis, rarely used in clinical practice, could be a useful tool in prognosis assessment and offer an evolutive behavior of the tumor. **Keywords:** THYMUS, THYMOMA, MEDIASTINUM.

Thymic epithelial neoplasms are usually seen in the fourth and fifth decades of life (1, 2). Thymoma is a primary mediastinal neoplasm arising from or exhibiting differentiation toward thymic epithelial cells, typically with the presence of non-neoplastic lymphocytes (3, 4). Thymomas can be classified as benign or malignant (5). Cytologically benign thymomas are of three major types, such as lymphocyte rich, epithelial cell rich, and spindle cell type or according with their architecture as cortical, medullary, and mixed. Thymomas that are cytologically malignant are uncommon, having as a hallmark a significant cytological atypia. The local spreading feature characterizes the invasive tumors (6). We hereby present two thymoma cases with different morphological and morphometrical features. In this article, we describe pathologic features of thymomas and discuss these disease entities in terms of classification and staging evalua-

tion, in which radiology plays a crucial role. The prognosis assessment for thymic masses is necessary, as well. Morpho pathologically, atypia, mitotic activity and invasiveness are histological features used in tumor prognosis assessment.

MATERIAL AND METHODS

We present two patients, who underwent tumor excision to treat surgically the thymomas, one in 2008 and the other one in 2010, both having myasthenia gravis. We described and assessed the histological characteristics of the two thymomas, and between thymoma types and cell variables.

Morphology assessment was made with an optical microscope (Olympus CX41). The measurements were analyzed with a color image analysis system: Quick PHOTO MICRO 3.0. We studied the tumor histological structure and cytology by using usual and specific (hematoxilin eosin, elastic von Gieson, and Gordon Sweet for reticulin) stains.

Quantitative morphometry was performed by appreciation of the ratio between lymphocytes and epithelial cells, micro vessel density and nuclear area of the epithelial cells. The measurements were expressed in mean values and frequencies.

RESULTS

Two biopsies from mediastinal masses were performed. Histopathological examination revealed features of thymoma. The results of histological and cytological analysis varied in few points.

In AB thymoma, surgical biopsy showed a nodular encapsulated tumor having 2 points of capsular infiltration (fig.1, a, b). Cytologically, we found a mixture of variable proportions of lymphocyte-poor (fig.1, c), lymphocyte rich areas (fig.1, d) and discrete intermixed foci. Lymphocyte deprived areas were composed from spindle epithelial cells with few lymphocytes. Epithelial spindled to oval cells were essentially the same as those of type A, having bland nuclei, medium sized to large and round to oval.

Morphometrically, tumoral area was composed from a dual population of cells with a lymphocyte predominance of 71, 52%. Lymphocyte to epithelial cell ratio was of 1.8. Tumoral epithelial cells had an average nuclear area of 45.3 μm2. All these morphological features are common in AB thymoma. Angiogenesis was another morphological feature of the tumor. We found an average of 3 micro vessels on HPF (HE, x400).

In B1 thymoma (fig.2, a), morphological analysis showed a multinodular tumor with a thick capsule and an area of infiltration with involvement of the adjacent fat tissue. The septa had different thicknesses.

Morphologically, we emphasized zones resembling with cortical areas composed from lymphocytes with inconspicuous epithelial cells (fig.2, b). In lymphocyte, rich-areas we saw densely packed (fig.2, c), small lymphocytes. Epithelial cells had nuclei ranging from medium sized to large, with nucleoli varying from inconspicuous to prominent. Epithelial cells ranged from infrequent to occasional Hassall corpuscles. Medullary foci appeared as circumscribed round pale zones due to lose packing of lymphocytes (fig.2, d).

Morphometrically, we identified the same dual cell population, with two thirds (2/3) or more small lymphocytes from entire cell mass. The lymphocyte to epithelial cell ratio was of 2.53. The most epithelial cells were small with polygonal shape and an average nuclear area of 21μm2. Angiogenesis was greater than in AB thymoma, represented by 4 vessels on HPF (HE, x400).

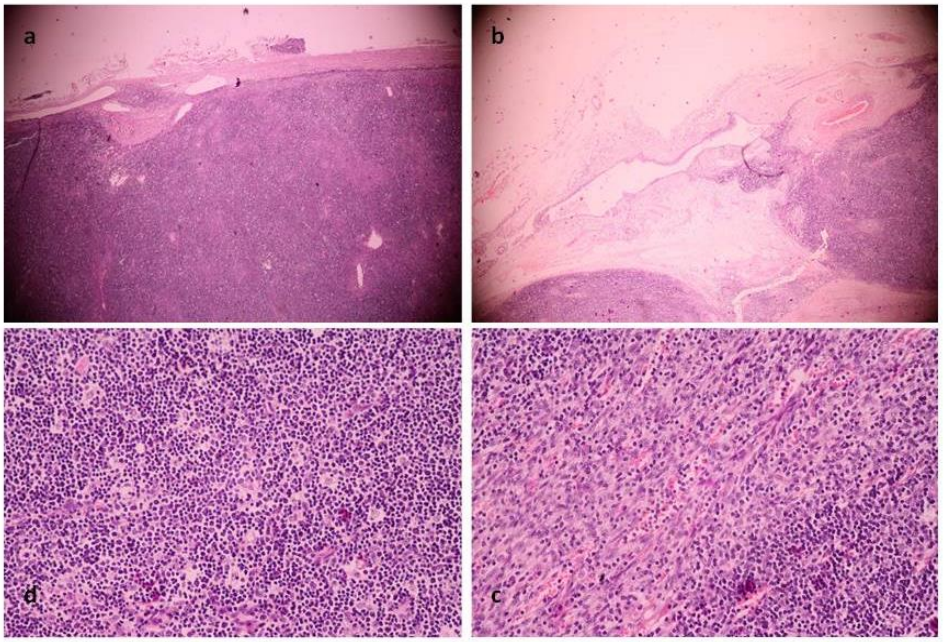

Fig. 1. Thymoma AB. a, b-foci of capsular infiltration (HE, x4); c-lymphocyte-poor area (HE, x40); d-lymphocyte-rich area (HE, x40).

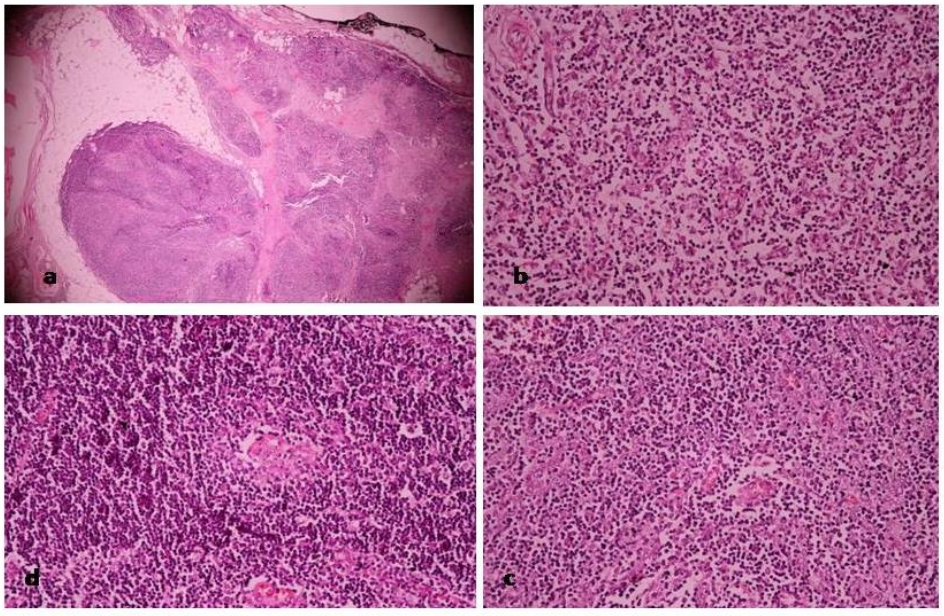

Fig. 2. Thymoma B1. a-area of fat tissue infiltration (HE, x4); b-lymphocyte rich-area (HE, x40); c-densely packed, small lymphocytes (HE, x40); d-loose packing of lymphocytes (HE, x40).

Morphometrically, we found different results between the two thymomas, according with epithelial cell sizes and shapes and their proportions reported to lymphocytes, all these distinctive features explaining differences in tumor invasiveness.

Despite angiogenesis study results are not too different; it may explain different degrees of malignancy among thymic epithelial tumors.

According with pathological stage (i.e., as defined after resection), both encapsulated tumors were in IInd stage of evolution, the first with microscopical invasion through the capsule (IIa stage for AB thymoma) and the second with invasion into surrounding fat (IIb stage for B1 thymoma).

DISCUSSION

Thymoma is a rare primary tumor of the epithelial cells of thymus and the most frequent tumor of the anterosuperior mediastinum. Its incidence increases with age (70% of cases are observed in patients past the age of 40) (7).

Distinctive features reminiscent of the normal thymus make the pathological diagnosis of thymoma easy in most cases. A fibrous capsule surrounds the tumor and sends thick, fibrous septa, dividing the tumor into well-demarcated lobules.

Microscopically, there is a distinctive dual cell population, including lymphocytes, small with dark nuclei, and epithelial cells, larger and lighter (1).

Quantitatively, the ratio between lymphocyte and epithelial cell varies widely. Thymomas are often classified according to the relative ratio of cells between predominantly lymphocytic, mixed epithelial–lymphocytic, and predominantly epithelial. In 40% of cases the tumors are predominantly lymphocytic, in 20% they are mixed and the rest are predominantly of epithelial type. The mean nuclear area increased significantly in the increasing order of noninvasive thymoma and invasive thymoma. The morphological and morphometric results demonstrated a significant difference in degree of malignancy between non- invasive and invasive thymomas. Cornea R shows a significant increase in the number of micro vessels in the temporal area, regardless the histological type of the tumor. So, in patients with thymic epithelial tumors, there appears to be a significant correlation between tumor angiogenesis and invasiveness (8).

The epithelial cells represent the neoplastic component of thymomas. They are typically round or oval, but sometimes have a spindle-shaped nucleus, which may represent the predominant cell in about 5 to 12% of thymomas, justifying the term “spindle cell thymoma,” which tends to grow more slowly. The lymphocytes are not considered neoplastic and, as in the normal thymus, are constituted mainly of T cells in various stages of maturation (1).

The clear majority of thymomas do not present considerable atypia or anaplasia. There are no reliable histologic features of “malignancy” for thymomas. The malignant behavior of a thymoma is indicated by microscopical or macroscopically invasion of the tumor capsule or of surrounding organs or by the presence of metastasis. In 90% of cases, they are well encapsulated and multilobulated, while in 10% of cases, the rupture of the capsule and invasion to the adjacent tissues are observed. About 30 to 40% of thymomas are invasive. On the other hand, a well-encapsulated thymoma may, on occasion, recur years after surgical resection (3).

It was recently postulated that thymomas may be further subdivided into those with cortical, medullary, and mixed differentiation, reflecting the anatomy of the normal thymus (2).

Cortical thymomas contain large epithelial cells with vesicular chromatin and prominent nucleoli, whereas medullary thymomas show oval to spindle-shaped epithelial cells with dispersed chromatin and inconspicuous nucleoli. Mixed thymomas contain both cortical and medullary components. Medullary and mixed thymomas appear to follow a more benign course than cortical ones. They show a lower incidence of invasiveness, lower recurrence rate, and better survival, even when capsular invasion is present. This classification has also been correlated with the size of epithelial cell nuclei using morphometrical analysis (3).

The thymomas behavior is unpredictable. The stage of the tumor at the time of diagnosis and the adequacy of the surgical excision are among the factors that influence the outcome of these tumors. The presence of clinical symptoms, large tumor size, local invasion or metastases at the time of the surgery, and predominant epithelial features are poor prognostic factors (9).

A multimodality approach that includes surgery, chemotherapy, and radiation therapy is suggested for treatment. Surgery is the mainstay of the treatment. However, complete resection is generally impossible or not feasible at diagnosis because of local invasion of important structures and metastasis (3).

The new WHO classification seems to correlate with invasiveness. The proportion of invasive thymomas was higher in B1 cases than in AB cases as in literature reports (Type B1, 69.1% comparing with Type AB, 47.3%) (10).

CONCLUSIONS

In our study, both thymoma types presented in a low stage of evolution. AB thymoma very rarely recurs while B1 thymoma may be sometimes aggressive. We noted both thymoma types show a close relationship to blood vessels. In our opinion, the micro vessel density is associated with thymoma invasiveness, and the higher radiotracer fixation may be due to increased angiogenesis. The histological prognosis assessment of these enlarged mediastinal masses is necessary, because thymomas could be sometimes aggressive tumors.

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NEWS

DIFFERENT VANCOMYCIN-INTERMEDIATE *STAPHYLOCOCCUS AUREUS* PHENOTYPES SELECTED FROM THE SAME ST100-HVISA PARENTAL STRAIN

The aim of this study is to characterize the factors related to peptidoglycan metabolism in isogenic hVISA/VISA ST100 strains. Recently, we reported the increase in IS256 transposition in invasive hVISA ST100 clinical strains isolated from the same patient (D1 and D2) before and after vancomycin treatment and two laboratory VISA mutants (D23C9 and D2P11) selected from D2 in independent experiments. High performance liquid chromatography-mass spectrometry (HPLC-MS) analysis of peptidoglycan muropeptides showed increased proportion of monomeric muropeptides and a concomitant decrease in the proportion of tetrameric muropeptide in D2 and derived mutants when compared to the original strain D1. In addition, strain D2 and its derived mutants showed an increase in cell wall thickness with increased pbp2 gene expression. The VISA phenotype was not stable in D2P11 and showed a reduced autolysis profile. On the other hand, the mutant D23C9 differentiates from D2 and D2P11 in the autolysis profile, and pbp4 transcription profile. D2-derived mutants exhibited differences in the susceptibility to other antimicrobials. Our results highlight the possibility of selection of different VISA phenotypes from a single hVISA-ST100 genetic background (Sabrina Di Gregorio, Silvina Fernandez, Arabela Cuirolo, Olivier Verlaine, Ana Amoroso, Dominique Mengin-Lecreulx, Angela Famiglietti, Bernard Joris and Marta Mollerach. Different Vancomycin-Intermediate *Staphylococcus aureus* Phenotypes Selected from the Same ST100-hVISA Parental Strain, Microbial Drug Resistance 2017; Vol. 23, Issue 1: 44-50).

Cristina Tuchiluş

WEIGHT STATUS AND QUALITY OF LIFE IN INFLAMMATORY BOWEL DISEASES

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WEIGHT STATUS AND QUALITY OF LIFE IN INFLAMMATORY BOWEL DISEASES (Abstract) The relationship between WEIGHT status and inflammatory bowel disease (IBD) is incompletely elucidated. Until recently, obesity among IBD patients was rarely found. However, in the past two decades the prevalence of obesity was rising in IBD patients. On the other hand, nutritive deficiencies and underweight status are part of the clinical manifestations of IBD and it is most commonly found in CD patients compared to UC patients. Both IBD and obesity can cause a negative impact on quality of life (QoL). **Aim:** to assess BMI in patients with IBD and quantify their QoL based on BMI. **Materials and methods:** We conducted a prospective study, over a period of 35 months (October 2011 - September 2014) that included 254 patients diagnosed with IBD (187 patients - UC and 67 CD). Disease activity was assessed by UCDAI/CDAI score. Weight status was quantified by BMI that classifies patients into four categories: underweight, normal weight, overweight and obesity. QoL was determined by the IBDQ-32 (Inflammatory Bowel Disease Questionnaire). **Results and discussion:** BMI ranged from 15.3 to 37.6 kg/m^2, with a mean significantly higher in the UC group compared with CD group (24.19 ± 3.60 *vs.* 22.04 ± 4 09 kg/m^2) (p = 0.001). The frequency of obesity was very low (4.13%). 25.96% of the patients were overweight, but frequency distributions show a significantly higher proportion with a high degree of overweight status in UC patients, while in CD patients we found a higher frequency of underweight status (p = 0.001). IBDQ-32 score values ranged from 66 to 207, with an average of 135.82. IBDQ-32 score was directly correlated with BMI, 13.8% of UC patients presented with higher scores of IBDQ-32 associated with higher BMI (p = 0.059) and in those with CD, 23, 6% were registered this association (p = 0.055). **Conclusions:** The results of our study cannot support that overweight status QoL of IBD patients, sooner we can say that the absence underweight status and obesity was associated with a better QoL. **Keywords:** INFLAMMATORY BOWEL DISEASES, QUALITY OF LIFE, WEIGHT STATUS.

The inflammatory bowel diseases (IBD) are chronic disorders whose etiology is yet to be clarified. This is the result of the interactions between genetic, immunologic and environmental factors (1). The IBD prevalence has increased over the last decades, especially in developing countries and in the areas characterized by high socio-economic development (2). This led to the hypothesis per which the emergence

and development of IBD is influenced by the changes underwent by changing food habits and the lifestyle associated with modern life (2).

The prevalence of obesity increased in parallel with the increase in IBD, suggesting the involvement of common environmental factors in the development of these disorders (3).

Until recently, the prevalence of obesity among the patients with IBD was low; nevertheless, the prevalence of obesity has increased in patients with IBD (4).

From another perspective, nutritional deficiencies and the underweight status have started to be part of the clinical picture of IBD, being more often met in Crohn's disease (CD) compared with ulcerative colitis (UC) (4). Starting from this data, we assessed the weight status in patients with IBD and we quantified the quality of life based on this index.

MATERIAL AND METHODS

1) We conducted a prospective study, carried out during 35 months (1 October 2011 - 30 September 2014). The sample consisted of 254 patients diagnosed with IBD (187 patients with UC and 67 patients with CD) who were attended at the Institute of Gastroenterology and Hepatology within the "Sf. Spiridon" County Clinical Emergency Hospital Iași.

2) The UC, respectively IBD diagnosis were supported by complete hematologic, biochemical tests, endoscopic and imagery examinations, as well as histopathological confirmation. The activity of the disease was quantified by means of the UCDAI/ CDAI score.

3) The weight status was quantified by the body mass index (BMI). This is a scientific official indicator for computing the ideal body weight for a given height. BMI is useful in establishing the weight group in which a person fits, classifying the weight status into four categories: underweight, normal weight, overweight and obesity,

4) The quality of life was assessed with the IBDQ-32 questionnaire (Inflammatory Bowel Disease Questionnaire). This is one of the most widely used questionnaires in QoL assessment. The questionnaire comprises 32 questions organised into four fields: gastrointestinal symptoms (diarrhoeal stoles, abdominal pain, rectorragia, rectal tenesmus), systemic symptoms (fatigue, sleep disorders), emotional functions (depression, irritability, anger, sexual activity) and social functions (absenteeism, affected social status). The responses in the questionnaire were graded from 1 (the worst) to 7 (the best). The total score is comprised in the range 32-224. Moreover, we computed the sub-scores for each distinct field (emotional functions: 12-84, gastrointestinal symptoms: 10-50, systemic symptoms: 5-35 and social functions: 5-35). The lower this score is, the more affected the HRQoL.

RESULTS

The study sample included 254 patients diagnosed with IBD and distributed into two groups:

- Group A - 187 patients with UC
- Group B - 67 patients with CD

The patients' distribution into the two samples is graphically presented in fig. 1.

BMI varied in the range 15.3-37.6 kg/m^2, registering an average value significantly higher in the group of patients with UC (24.19±3.60 *vs.* 22.04±4.09 kg/m^2) (p=0.001), classifying though the study groups as normal weight (fig. 2).

Of the total 254 subjects investigated, it can be noticed that the number of obese

patients was very low, with only 4% of the patients obese.

On the other hand, 31.9% of the cases were overweight, but the frequency distributions highlight a significantly higher ratio, with a high degree of the overweight status in the patients with UC, while in the patients with CD it can be noticed a higher frequency of the underweight status (p=0.001), (fig. 3).

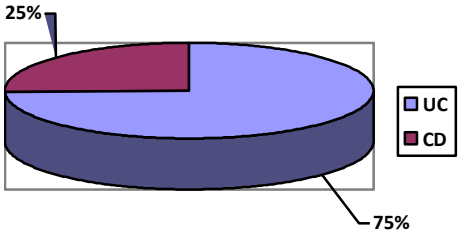

Fig. 1. - Structure of the studied sample

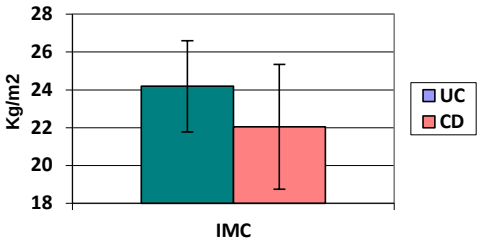

Fig. 2. The average values of BMI by study sample.

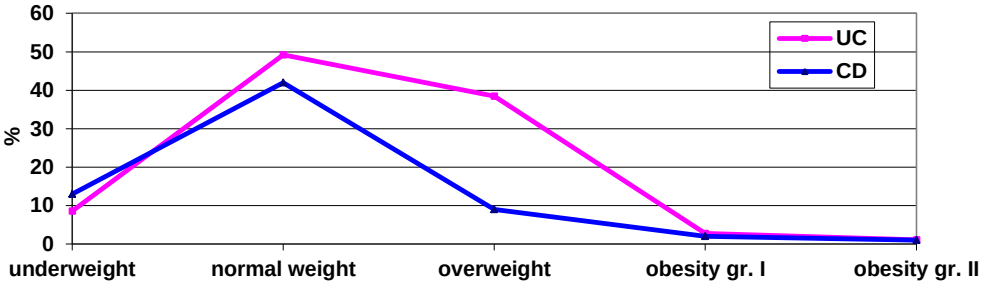

Fig. 3. Distribution of cases based on weight status.

The results of the present study could account for the pro-inflammatory role of the excessive fatty tissue in the patients with UC. Nevertheless, due to the low number of obese patients in the studied sample, we cannot support this hypothesis with certainty.

On the other hand, the higher frequency of the underweight status in CD confirms the fact that its mechanisms are more frequently met among patients with CD.

There are studies which demonstrated that malnutrition impacts the quality of life in patients with IBD. On the other hand, obesity also has a negative impact on the quality of life. Stating from this data, we assessed the quality of life in patients with IBD based on BMI.

In the studied cases, the values of the IBDQ-32 score varied from 66 to 207, with an average value of 135.82.

In the studied samples, the IBDQ-32 total score was directly correlated with BMI, 13.8% of the patients with UC presenting increased values of the total IBDQ-32 associated with increased BMI values (p=0.059); in the sample of patients with CD, 23.6% presented this association (p=0.055), (fig.4).

Since the overweight status was predominant in both studied samples, we can-

not support the hypothesis that this status contributed to improving the quality of life in the patients with IBD; we could rather infer that the absence of underweight and obese status was associated with improved quality of life.

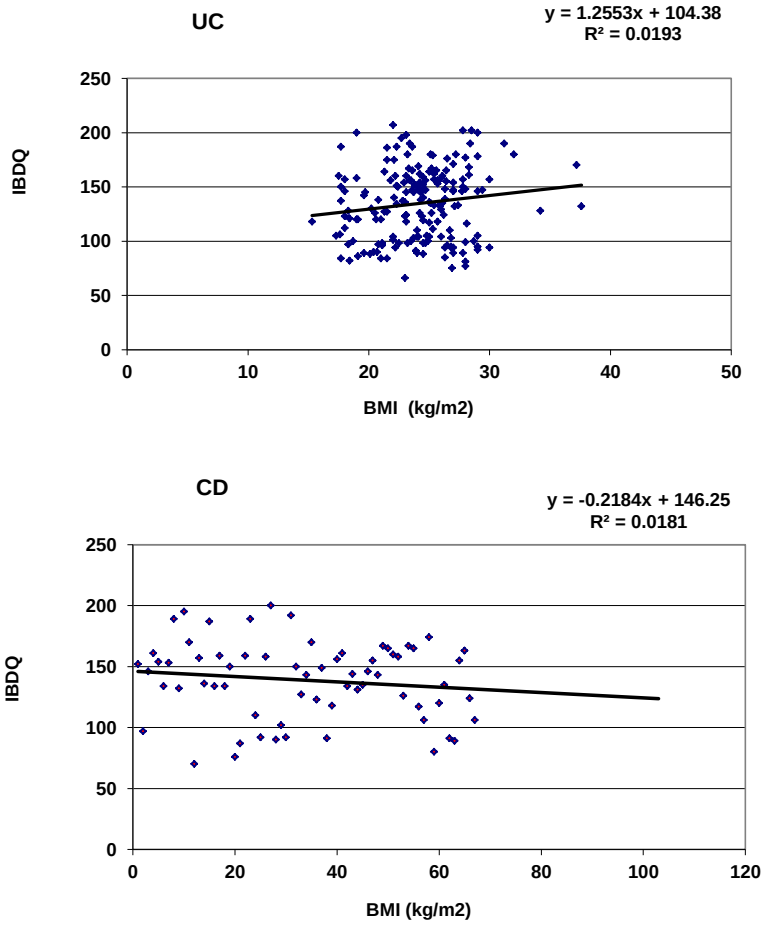

Fig. 4. Correlation between the IBDQ-32 score with BMI, by study samples.

We should notice the fact that among the patients with CD, the correlation of BMI with the IBDQ-32 score registered a higher percentage compared to the sample of patients with CD (23.6% *vs.* 13.8%). Since the frequency of the underweight status in the group of patients with CD was higher, this fact emphasizes the positive impact of an optimal nutritional status in the case of patients with CD.

The IBDQ-1 subscore quantified the impact of the gastrointestinal symptoms on the quality of life as being represented by the result of 10 of the 32 questions included in the IBDQ-32 questionnaire. Its values varied from 14 to 50, with limits comprised in the range 10-50. The patients with CD presented a significantly lower average

value compared to the patients with UC (29.78 *vs.* 33.64; p=0.001).

The analysis of the data assessing the sub score of the gastrointestinal symptoms (IBDQ-1), revealed a direct correlation of this subscore with BMI (fig. 5).

Only 12.7% of the patients with UC presented high values of the IBDQ-1 score associated with high BMI values (p=0.083), while in the CD sample, 27.1 of the patients registered this association (p=0.026). These results are like the total IBDQ-32 score. Again, it results that the gastrointestinal manifestations are reduced in patients with normal weight or overweight, in the absence of the underweight or obesity.

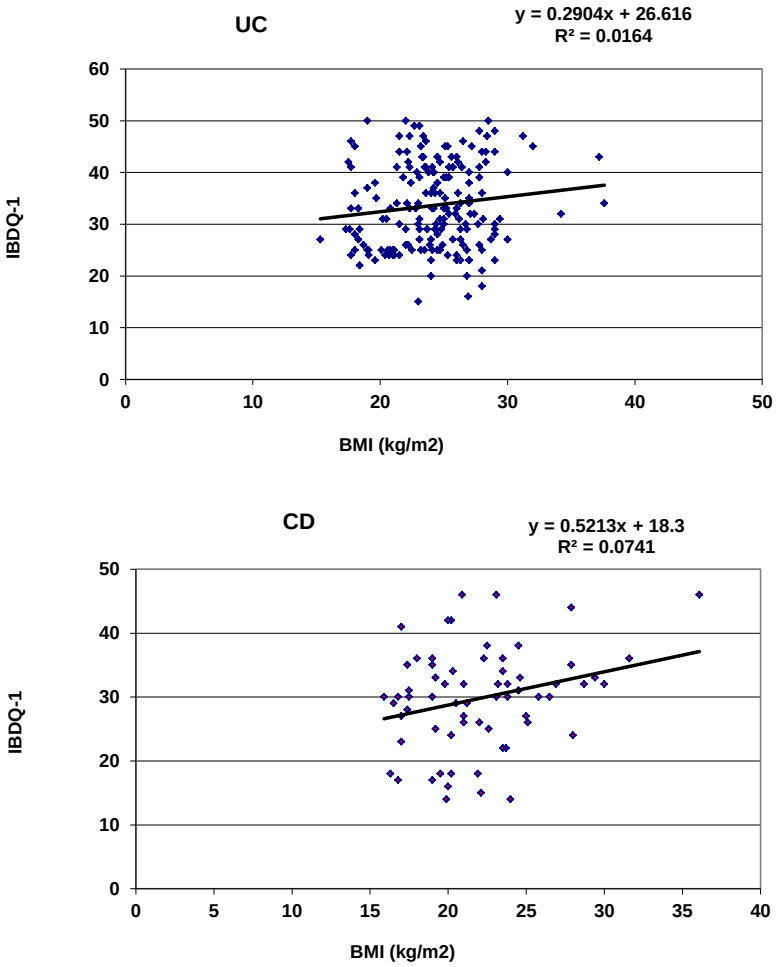

Fig. 5. Correlation between the IBDQ-1score with BMI, by study samples.

5) The IBDQ-2 subscore is the result of 5 of the 32 questions included in the IBDQ-32 questionnaire, with values in the range 5-35. This subscore quantified the impact of systemic symptoms - represented by chronic fatigue, energy level, general state of illness, quality of sleep and lack of energy - on the quality of life in patients with IBD.

In the study sample, the IBDQ-2 score varied from 5 to 35, without recording significant differences of the average values between the two disorders (22.50 *vs.* 21.24; p=0.256).

To assess the correlation between BMI and the systemic manifestations in IBD, we analyzed the average score of the systemic symptoms (IBDQ-2). The analysis of this data highlighted the presence of a significant correlation with BMI. Both in the patients with UC and in those with CD, high BMI was associated with lower systemic symptoms.

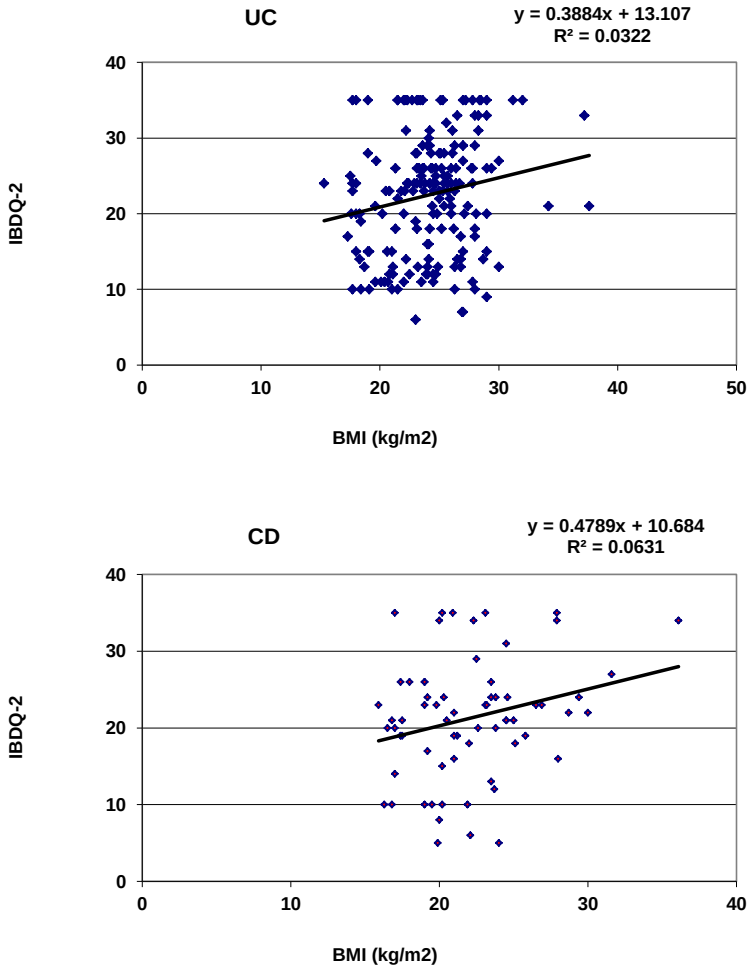

Fig. 6. Correlation between the IBDQ-32 score with BMI, by study samples.

We notice the fact that approximately 18% of the patients with UC (p=0.014) and 25% of the patients with CD (p=0.041) registered increased values in the IBDQ-2 score associated with increased BMI values (fig. 6). In both studied samples, the lowest values recorded for the score of the systemic symptoms were observed in underweight

patients, but were not significant from a statistic point of view. These results confirm once again the negative effect of underweight status on patients with IBD.

6) The IBDQ-3 subscore is the result of 12 of the 32 questions included in the IBDQ-32 questionnaire, with values in the range 12-84. This subscore quantified the impact of IBD on the patients' emotional functions (frustration, impatience, agitation, irritability, worries, depressive states, anger, (irrational) fear of being diagnosed with cancer, crying, sexual activity, anxiety, happiness).

In the studied sample, the IBDQ-3 values varied from 34 to 84, registering an average value significantly higher in the patients with CD.

About the emotional functions assessed through the IBDQ-3 subscore in both samples, they were not influenced by the weight status. It can be noticed, however, then only 10% of the patients with UC (p=0.153) and 18.6% of the patients with CD (p=0.133) presented high values of the IBDQ-3 score associated with increased BMI values (fig. 7). Thus, for both disorders, it appears that BMI did not influence the emotional functions.

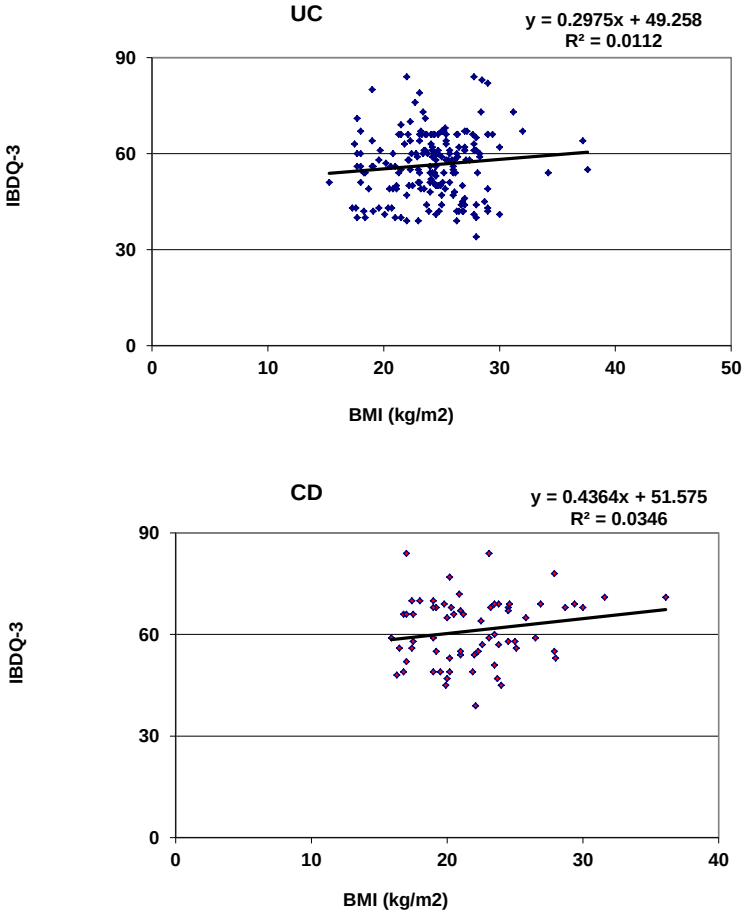

Fig. 7. Correlation between the IBDQ-3 score with BMI, by study samples.

Nevertheless, the patients with UC presented lower values of the IBDQ-3 subscore compared to the patients with CD, irrespective of the BMI. Because the emotional functions in the patients with UC were significantly more affected compared to the patients with CD, as previously mentioned, we consider this aspect to be a particularity of the study sample.

7) The IBDQ-4 subscore is the result of 5 of the 32 questions included in the IBDQ-32 questionnaire, with values in the range 5-35. This subscore quantified the impact of IBD on the social functions (absenteeism, social commitment, leisure and participation in certain social events).

In the studied sample, the values of the IBDQ-4 subscore varied from 5 to 35, registering a significantly lower average value in the patients with UC (22.27 *vs.* 26.21; p=0.001).

About the social functions, none of the samples revealed statistically significant differences based on BMI. In the case of the patients with UC, the score of the social functions (IBDQ-4) did not correlated with BMI (p=0.085). Additionally, in the case of the patients with CD, BMI was not associated with damage in the social functions either (p=0.090).

However, lower values of the IBDQ-4 subscore are noticed in the patients with UC compared with the patients with CD (fig. 8).

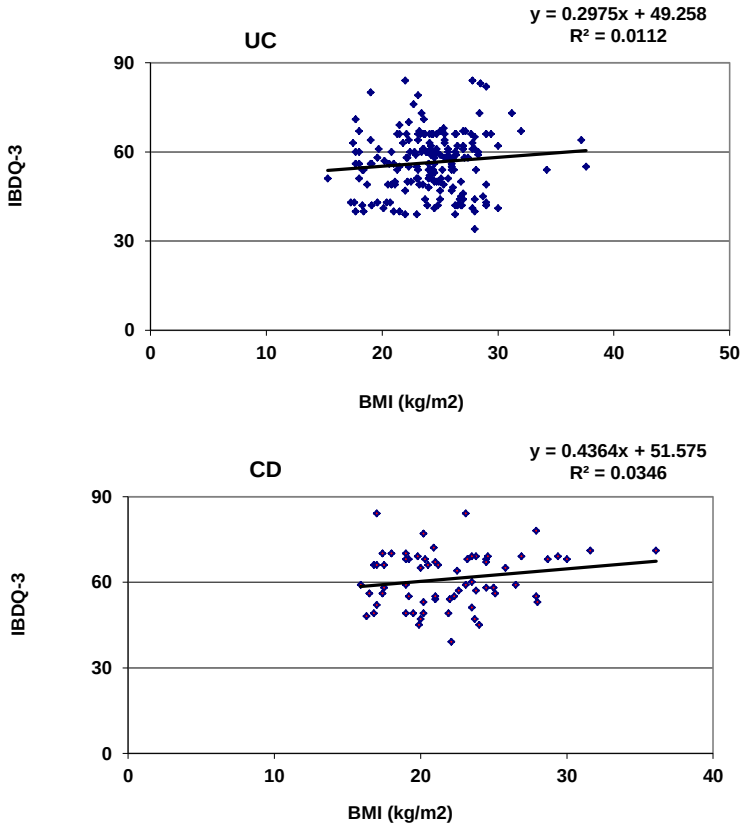

Fig. 8. Correlation of the IBDQ-4 score with BMI, by study samples.

To conclude, both in the patients with UC and in those with CD, high BMI was associated with higher quality of life, an aspect noticed in a higher ratio in the sample of patients with CD. The average IBDQ-32 total score and its 4 subscales were slightly higher in the patients with obesity in both study groups ($p>0.05$).

Based on these results, we cannot support the hypothesis that the overweight status improved the quality of life in patients with IBD; we could rather infer that the absence of underweight and obese status was associated with improved quality of life. Moreover, although the percentage of obese patients included in the study was lower, their quality of life was higher, indirectly refuting the presence of severe forms of IBD, respectively the pro-inflammatory role of the fatty tissue.

DISCUSSION

In the study presented above, most patients had normal weight. Of the total 254 subjects investigated, 4% were obese and 31.9% overweight. In the UC sample, we noticed a significantly higher ratio of overweight patients (38.5%), which can confirm the pro-inflammatory role of the excessive fatty tissue in patients with UC. On the other hand, although most patients with CD had normal weight (62.7%), as also shown in the specialized literature, the frequency of the underweight status was high compared to the sample of patients with UC ($p=0.001$). The results of the present study confirm the fact that for CD, the causes and mechanisms of the underweight status are more frequently met compared with the patients with UC.

The studies in the literature have shown that specific nutritional deficiencies and the underweight status are more frequent among patients with IBD (5).

In some studies, weight loss was met in 70-80% of the in-patients with IBD and in 20%-40% of the outpatients with CD. The prevalence of malnutrition was low among the patients with UC, but nutritional deficiencies can shortly occur in these patients as well, especially during the disease activity periods (4).

Malnutrition is often associated with acute or severe gastrointestinal disorders, having an impact both on their morbidity and mortality (5). Damaged nutritional status also impacts muscular strength, which eventually determines the decrease in the functional status. Moreover, malnutrition was associated with decreased functional state and damage of the immune system (5).

On the other hand, the prevalence of obesity increased over the last decades, being currently considered pandemic (4). While obesity is not frequently met in IBD, several studies focused on the increase in its prevalence among these patients (6, 7), being more frequent in patients with CD compared to patients with UC. A recent study carried out in Scotland showed that 18% of the patients with IBD are obese (BMI > 30 kg/m^2), compared to 23% of the general population in Scotland.

Although IBD are not considered "classic" comorbidities of obesity, these two entities have a cause-effect relation and are associated with increased inflammatory reactions (8). There are studies supporting the hypothesis that obesity accounts for the increase in inflammatory cytokines, leading thus to IBD development (9). Moreover, several studies have shown that morbidity increased among the obese patients with IBD, including the rate of hospitalization, perianal complications and the disease activ-

ity periods (10, 11). Other published data indicated the fact that the obese patients with IBD are more prone to complications in case of surgical intervention, and that these complications were determined especially by obesity comorbidities (12).

As far as the relationship between the nutritional status and the quality of life are concerned, research has sported the existence of a direct correlation between malnutrition and decreased quality of life, especially in older in-patients (13, 14), oncologic patients (15) and hemodialyzed patients (16, 17).

A study assessing the quality of life of patients with gastrointestinal disorders and malnutrition conducted in Germany demonstrated that malnourished patients with IBD presented lower QoL scores (18).

On the other hand, obesity generally has a negative impact on the quality of life (438).

CONCLUSIONS

In our study, BMI correlated significantly with the score for systemic symptoms (IBDQ-2). Higher BMI was associated with higher QoL scores. 25 % of the patients with CD (p=0.041) and 17.9% of the patients with UC (p=0.014) presented high values of the IBDQ-2 score associated with increased BMI values.

Based on these results, we cannot support the hypothesis that the overweight status improved the quality of life in patients with IBD; we could rather state that the absence of underweight and obese status was associated with improved QoL.

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NEWS

PREDICTION OF POSTOPERATIVE RENAL IMPAIRMENT USING A NEW URINE MARKER

Prognosis of cases in cardiac surgery is influenced by early detection of postoperative complications such as acute renal injury (ARI). Determination of creatinine serum levels is simple and inexpensive but can delay ARI diagnosis. The researchers of Department of Thoracic and Cardiovascular Surgery, University Medical Center Göttingen, Germany evaluated the importance of determining urinary neutrophil gelatinase-associated lipocalin (uNGAL) for predicting ARI occurrence after cardiopulmonary bypass (CPB). NGAL is a protein expressed by urothelial cells of the renal tubular system and it appears in the primary urine to 60–120 min after stress induction. The study included 81 adult patients with CBP without morphological or functional renal alterations. uNGAL level was determined in seven time points (before, during and after CBP). Also, were measured serum creatinine levels and glomerular filtration rate and was evaluated renal function per the Acute Kidney Injury Network criteria. The results showed that NGAL values increased after 120 minutes from the start of CPB and have remained elevated after CBP. Results of the study did not establish a correlation between uNGAL level and duration of CPB nor between uNGAL expression and occurrence and severity of ARI. The researchers suggested that "biomarker uNGAL failed to predict the onset and the severity of acute renal failure after cardiac operations" (Friedrich MG, Bougioukas I, Kolle J, Bireta C, Jebran FA, Placzek M, Tirilomis T. NGAL expression during cardiopulmonary bypass does not predict severity of postoperative acute kidney injury. *BMC Nephrol* 2017; 18(1): 73. doi: 10.1186/s12882-017-0479-8).

Cătălina Luncă

ETHNOBOTANICAL AND CANNABIS DRUG USE: A STEP BETWEEN DANGER AND PLEASURE

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ETHNOBOTANICAL AND CANNABIS DRUG USE: A STEP BETWEEN DANGER AND PLEASURE (Abstract): **Aim:** The study aimed to identify the risk factors and the particularities of ethnobotanicals and cannabis users who needed medical care in the Emergency Department of the „Sf. Spiridon" County Hospital. **Material and methods:** Retrospective study conducted over a period of 18 months (January 1, 2015- June 30, 2016). This study included all patients who presented to the E.D. after drug (cannabis and ethnobotanicals) consumption. The variables studied for creating the consumer profile were sex, age, social background, method of consumption, clinical and laboratory changes. **Results and discussion:** The study included 153 patients brought to hospital by ambulance (71.89%) for cannabis or ethnobotanical poisoning. Most patients were males (92.15%) from urban areas (75.97%), aged between 18 and 24 years (52.28%). At the time of admission to ED 96.07% of them needed medical care for consumption of ethnobotanicals through various methods and only 3.92% were brought to hospital for cannabis or marijuana use. For 61.70% of patients, curiosity was the reason behind their addiction. Toxicology testing (qualitative urine analysis) was useful in identifying the presence of tetrahydrocannabinol(THC) or a combination of THC and barbiturates, benzodiazepines, opioids in 26.53% of ethnobotanical and 66.67% of cannabis users. **Conclusions:** In Romania, the most commonly used drugs are cannabis followed by ethnobotanical products. For a while, ethnobotanical products were falsely considered as risk-free, but they contain dangerous substances with devastating effects, which, unless treatment is initiated immediately, can lead to death. **Keywords**: CANNABIS USE, ETHNOBOTHANICALS, POISONING.

Drugs have been known ever since antiquity, but their use has changed throughout history. In the beginning, they were used in medical and religious practice, being nowadays a source of expensive pleasure for teenagers and young adults (1).

In 2008 cannabis was the most widely used illicit drug in Romania. In 2008 Romania ranks fourth among the cannabis-consuming countries after England, Germany and Holland (2, 3, 4). Starting with 2009, new psychoactive substances have been increasingly used reaching a peak in 2010. The new legislation and the media

involvement led in 2013 to a slightly reduced usage of ethnobotanicals, which came second (18 per cent) immediately after heroine (42.7 percent) and before cannabis (15.6 percent). According to the 2014 National Antidrug Agency annual report the use of the new psychoactive substances has slight increased.

In 2014, the most commonly used drug in Romania was cannabis -10 percent in Bucharest and Ilfov, 5.9 percent in Southeast Romania, 2.9percent in the Western part of the country,2.5 percent in the Northwest, 1.6 percent in the South and Centre, 1.5 percent in the Northeast and 0.4 percent in Southwest (5, 6, 7, 8, 9, 10, 11).

Per "European Monitoring Centre for Drugs and Drug Addiction", Romania ranks eighth among the cannabis-consuming European countries, after France, Czech Republic, Italy, Denmark, Poland, Austria and Portugal (12, 13).

MATERIAL AND METHODS

This retrospective study covers 18 months and includes the patients cared at the Emergency Department of the Iasi "St. Spiridon" Hospital due to the use of the new psychoactive substances or cannabinoid substance.153 patients were included in this study, represented by 141 (92.15%) male and 12 (7.85%) female. The minimum age was 18.

Variables included were: age, sex, social environment, the main cause of addiction, drug type, method and age at onset of drug use, drug-related clinical and laboratory changes.

Vulnerable patients and patients having refused toxicological analysis were not included. Information was taken from patient medical records.

Data were analyzed and statistically processed using Microsoft Excel and *SPSS 18.0* statistics software. Patients characteristics were expressed as the mean standard deviation or as a number and percentage.

RESULTS

The study was conducted between January 1, 2015 and June 30, 2016 and included 153 patients who presented at the Emergency Department of the Iasi "Sf. Spiridon" Hospital for having used ethnobotanical and/or cannabinoid substances. Sex distribution of patients did not differ from that in the statistics of the Annual Reports of the National Antidrug Agency. Thus, in our study the clear majority of cases were male, 141 (92.15%) only 12 cases (7.85%) being female. Of the patients needing medical care following the use of ethnobotanical and/or cannabinoid substances 78.57% were brought to hospital by ambulance, 71.89% of them being hemodynamically stable. A significantly smaller percentage came by their own means (20.26%).

Educational, economic, and sociocultural factors contributed to an uneven distribution by place of residence –75.97% being urban patient. Age ranged from 18 to 54 years, with most cases aged 18-24 years (52.28%). 74.50% of the patients received medical care 1-3 hours after substance use. Per statistics, most patients had consumed ethnobotanical substances (96.07%) compared to 3.92% who used cannabis and marijuana. The most commonly used delivery method was via inhalation, declared by 98.69% of the users, the remaining 1.31% preferring the administration via ingestion or injection (fig. 1).

Of the ethnobotanical substance consumers (134 males and 13 females) 63.94% claimed to be occasional users, 30.61% chronic users (5 months-13 years) and 5.44% accidental user, smoking ethnobo-

tanical cigarettes offered by acquaintances or found on the street. Of the occasional users, 61.70% declared curiosity as main reason for substance use, the remaining 38.30% mentioning pleasure as main motivation (fig. 2).

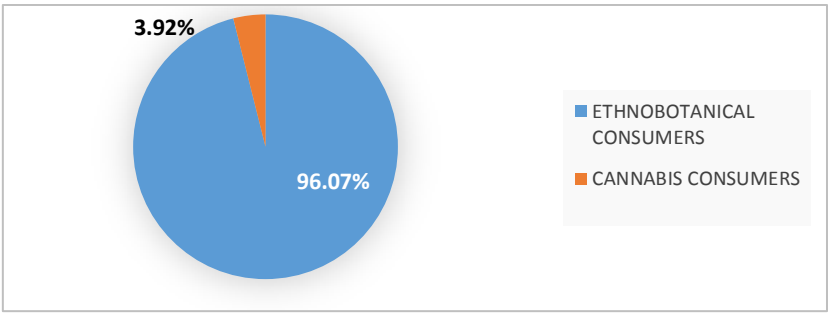

Fig. 1. Percentage distribution of used drugs

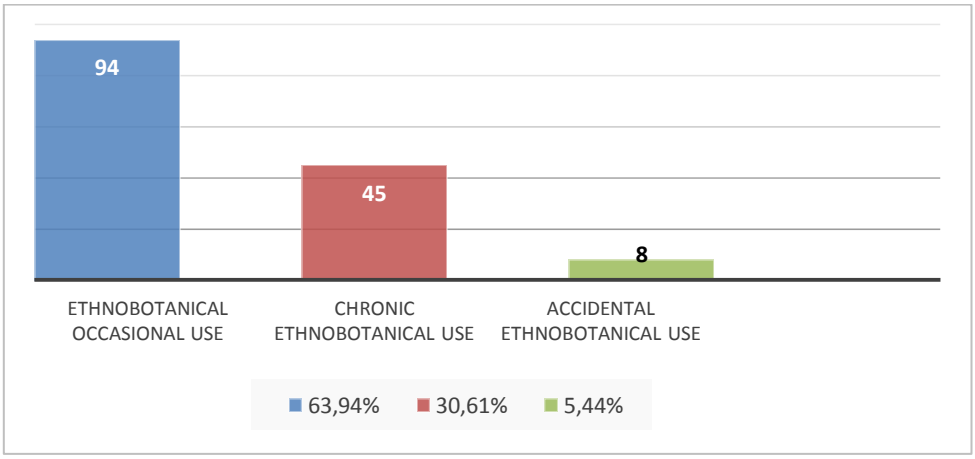

Fig.2. Patient distribution per substance use manner

Upon presentation to the Emergency Department, 11.56% of the chronic users had been admitted to substance abuse treatment and 2.72% had been treated for various psychiatric disorders at the Iasi Hospital of Psychiatry. Of the users of ethnobotanicals requiring medical care 70.06% were urban male patients (113/76.87%), aged 18-30 years (85.71%), brought by ambulance. 131 inhalation cases self-procured the ethnobotanical substance (89.11%) and 43 of them (29.25%) used ethanol as well.

Clinical symptoms included dizziness (19.04%), nausea and psychomotor agitation (18.36%), vomiting (14.28%) and palpitations (13.60%). ECG demonstrated sinus bradycardia, early repolarization, and minor right bundle branch block in 26.53% of ethnobotanical drug users. Urine toxicology testing done with the Triage Meter Pro using fluorescence for the immuno-

chemical tests performed at patient's bedside showed 26.53% patients positive for: BZO (10.88%), THC (10.20%), BAR (4.08%), APAP (2.72%), OPI (2.04%), TCA (1.36%). 7.47% of the toxicology tests could not identify the substance, although the patients declared and clinical evidence indicated ethnobotanical drug use.

The emergency physician has an extremely important role in case of overdose/intoxication where the substance, consumed amount, subsequent course and possible complications are unknown, in most such cases the doctor acting as interface between the patients in various stages of intoxication, and the hospital environment. Immediate emergency procedures are vital in such cases.

Early diagnosis and specific therapy reduce the risk of possible complications in ethnobotanical drug intoxication. History and clinical examination were thoroughly considered as they are key in diagnosing the patient with ethnobotanical drug intoxication.

Medical and scientific publications make little reference to the best treatment in cases of synthetic drug addiction. Early diagnosis and therapy are the best ways of reducing possible complications in ethnobotanical drug intoxication. There is no specific antidote or standard treatment because we have little information on the substance composition (substances being extremely varied), therefore vital signs (circulation and breathing) were stabilized first, and then the symptoms were treated.

Of the 147 patients consuming new psychoactive substances only 4.76% needed continuous follow-up at the Toxicology Clinic of the Iasi "Sf. Spiridon Hospital", the remaining 95.24% being referred to the Psychiatry Hospital (32.65%), to other hospitals (4.76%), back home (44.22%) and 13.61% left the hospital by themselves, being hemodynamically stable.

In the patients requiring medical care for cannabis and marijuana use (3.92%), aged 19-42 years, the sex ratio was like that recorded for the ethnobotanical drug users - 83.33% males. 66.67% arrived by ambulance, most patients residing in urban areas (66.67%). One patient reported cannabis cake ingestion (16.67%), 33.33% reported inhalation of cannabis and 50% inhalation of marijuana. 83.33% of the cases were occasional users. 50% of the cannabis and marijuana users reported pleasure as main reason whereas 33.33% reported curiosity as main motivation. The average time interval between inhalation and arrival at the Emergency Department was 1 to 2 hours, 83.33% of the patients being hemodynamically stable with palpitations (50%) and dizziness (33.33%).

Of the cannabis and marijuana users, 66.67% had positive urine toxicology tests in equal ratios for THC and BZO (33.33%).

The emergency department length of stay was approximately 5 hours, the vital signs being stabilized during this interval. 16.67% required hospital admission, the remaining patients being either discharged home or leaving the hospital after being stabilized.

DISCUSSION

The analysis and comparison of data provided by the National Antidrug Agency and the statistical data obtained from the present study show major differences between drug types consumed at a national level and drugs consumed in the North-East region, mainly in Iasi (5, 6).

Although during the past 8 years at national level cannabis has been the most

commonly used drug among teenagers and young adults, followed, starting with 2009, by the new psychoactive substances, most drug users in the Iasi area have been using ethnobotanical substances (96.07%), cannabis being consumed only by 3.92% (2, 3, 4, 5, 6).

The distribution by sex and area of residence is like that at the national level –the majority being male urban consumers.

Age group distribution of the study cases showed that most ethnobotanical drug users were young: 15-24 years (4.3%) with 52.38% in the 18-24 age group. Most cannabis consumers were aged 25 to 34 years (7.4%) at the general population level (4).

University students cover 29.41% of the studied population, percentage close to the one at the national level.

At the national level, 2 thirds of the ethnobotanical drug users (60% in 2014 compared to 61.2% in 2013) live in Bucharest, Iasi, Cluj, Galati.

Both cannabis and ethnobotanicals prevail in Bucharest-Ilfov (10% and 3.9%, respectively), whereas in the North-Eastern region the recorded percentages are only 1.5% and 0.8%, respectively (5, 6, 15).

Insufficient data concerning chemical composition, ingredients and possible dangerous substances used in the new psychoactive drug fabrication make more difficult the evaluation of health risks and toxicity levels. (1, 14, 15) Some drugs are combined with alcohol and other illegal drugs with dangerous side effects. Teenagers are tempted to diversify their drug consumption even if they know the risks involved by the association of illicit drugs with alcohol, tobacco, barbiturates and amphetamines (16).

Easy access and accessible prices make ethnobotanical drug intoxication an important issue. Alongside long term effects, ethnobotanical drugs are a social danger leading in time to the use of more dangerous drugs.

CONCLUSIONS

In Romania, the most commonly used drugs are cannabis followed by ethnobotanical products. For a while, ethnobotanical products were falsely considered as risk-free, but they contain dangerous substances with devastating effects, which, unless treatment is initiated immediately, can lead to death.

Data concerning chemical composition and ingredients are insufficient and these substances used among alcohol and other type of drugs can be dangerous and can lead to death of patient.

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NEWS

PHOTODYNAMIC THERAPY AS A PROMISING METHOD USED IN THE TREATMENT OF ORAL DISEASES

Photodynamic therapy is a innovative therapeutic method that has already been successfully adapted in many fields of medicine (e.g. gynecology, urology, dermatology) and cancer therapy. Dental medicine is also beginning to incorporate photodisinfection for treatment of the oral cavity. Photodynamic therapy consists of three elements: photosensitizer, light and oxygen. The photosensitizer has the property of selective accumulation in abnormal or infected tissues without causing any damage to the healthy cells. The antibacterial and fungicidal properties of the photosensitizer have been used to achieve better results in root canal treatment, periodontal therapy and the eradication of candidiasis in prosthodontics. Photodynamic therapy could be considered as an alternative treatment protocol in oncology, endodontics, periodontology and other fields of dentistry. (Prażmo EJ, Kwaśny M, Łapiński M, Mielczarek A. Photodynamic Therapy As a Promising Method Used in the Treatment of Oral Diseases. *Adv Clin Exp Med*. 2016 Jul-Aug; 25(4):799-807).

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OPTIMAL TEACHING OF CARDIOPULMONARY RESUSCITATION IN SCHOOLS: BETTER EDUCATION, MORE LIVES SAVED

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OPTIMAL TEACHING OF CARDIOPULMONARY RESUSCITATION IN SCHOOLS: BETTER EDUCATION, MORE LIVES SAVED (Abstract). Teaching cardiopulmonary resuscitation in Romanian schools is a joint initiative of the Acute Cardiac Care Working Group of the Romanian Society of Cardiology, the Romanian National Resuscitation Council and the Romanian Society of Emergency Medicine and Disaster. Teaching young generations to save people in cardiac arrest requires a structural approach, whose starting point is the launching of a large-scale campaign in favor of introducing the basics of cardiopulmonary resuscitation (CPR) in the school curriculum. The training stages can be set according to the principle of repetition (i.e. every two years). Teachers seem to be the best candidates for the instructor position, taking into account the knowledge and skills they acquired throughout the teacher training programs, as well as their continuous contact with the students; consequently, training teachers to become CPR instructors represents a long term investment. All acquired knowledge will implicitly spread to a secondary level (trainee's parents, relatives, etc.) and the number of individuals gaining access to CPR specific information will rise as a consequence. Entering the CPR training program should be promoted as a criterion for the accreditation and/or evaluation of primary schools, middle schools, and high schools. To begin with, this program must be implemented in Romania at first as part of the non-formal context of the special education week called *"Şcoala altfel"* ("School otherwise"). **Keywords**: RESUSCITATION, TRAINING, SCHOOL, CARDIAC ARREST.

Including basic cardiopulmonary resuscitation (CPR) in educational programs during schooling is a necessity of the modern world: with better education, more lives are saved. While in Norway, the country with the largest number of islands in Europe, CPR notions have been part of the school curriculum since 1961, an approach promoted only in 1992 as part of the European Resuscitation Council (ERC) policy (1, 2), this process has started in Romania only recently, as a result of the initiative of the Acute Cardiac Care Working Group of the Romanian Society of Cardiology,

which was also joined by the Romanian National Resuscitation Council and the Romanian Society of Emergency Medicine and Disaster ("You Can Also Save a Life!" Project) (3).

CPR in Romanian schools

Preparing a whole generation to be able to save people in cardiac arrest requires the initiation of a large-scale campaign in favor of introducing the basics of cardiopulmonary resuscitation in the school curriculum. The national impact of such an approach would be a considerable one; according to data provided by the National Institute of Statistics (2011/2012), the distribution analysis by level of education shows that of the total population of 3,823,515 individuals belonging to the school population, 673,641 are preschool children, 1,629,406 are part of the primary and secondary school cycles (1st–4th grades: 803,902 students; gymnasium 5th to 8th grades: 809,339 students, and special education classes 1st-8th grades: 16,165 students), 888,768 are in high school, 12,382 are enrolled in vocational education, 79,466 are in post-secondary education and apprenticeships, and 539,852 are in higher education (4). Therefore, the training of all these categories requires a considerable organizational effort, especially as such an approach needs to be repeated as a sequence (training–evaluation of training quality–re-training) at different stages of education. Moreover, as 55.1% of its inhabitants live in rural areas associated with poverty, Romania has the lowest level of urbanization in the European Union (by comparison, urbanization rates of our neighbor countries are: Bulgaria - 70.9%, Hungary - 67.8%) (4). These differences between urban and rural areas relate to infrastructure development, household equipment, access to public utilities, but also to a lower school instruction, limited access to forms of secondary education and a rural-urban gap in what concerns medical services (5).

A re-conceptualization of children as social actors is necessary; it is time for children to be regarded neither as "captive audience" when analyzed from the perspective of the information reception, nor as "adults in waiting" when assessing their effective participation, but as future citizens among whom altruism (the "Good Samaritan" attitude) should be encouraged (6, 7). Children are more easily motivated, they can easily learn various sequences of actions, but they obviously have a limited life experience, so they rely largely on the knowledge and guidance of teachers, who, in turn, should receive regular training in this area to avoid dilution of their knowledge (8). The young people of Romania's 2010-2020 decade are characterized by the presence of much more pragmatic values, which deserve to be brought to fruition.

In Romania, the "Good Samaritan Law" is currently in force, backed by the Law on Health Reform (Law 95/2006), which stipulates under Article 88 that "persons without medical training providing first aid on a voluntary basis, based on indications provided by a medical dispatcher or with some basic knowledge of first aid, acting in good faith and with the intention to save the life or health of a person, have no criminal or civil liability" (9). Thus, someone who wants to help can not be held liable even if something unpleasant happens to the victim.

Basic Life Support (BLS) training in schools

To meaningfully reduce the mortality in

out-hospital cardiac arrest (OHCA) cases, 15-20% of the population must know and perform BLS maneuvers (10). School is the place where such knowledge may be acquired. It is also a cost-effective and widely spread approach, as many local/ regional initiatives have proven: "ABC for life" (10), the Hampshire experience (11), the Washington experience (12), the Barcelona experience (13), or the Norwegian experience (14). However, the currently available data is still heterogeneous (15).

Optimal age for CPR training

There are studies on subjects at the kindergarten age (4-5 years) who were able to estimate the level of consciousness of a victim, whether the victim is breathing or not, to remember and dial the emergency alert service, to provide sufficient information to the dispatcher, to place the victim in the recovery position and to ensure the victim's airway permeability, skills that were maintained at a 2 months post-training examination (16). Two studies have found that children with a body mass index (17) over 15 or a minimum body weight (18) of 50 kg can correctly perform chest compressions.

The training of children in CPR must be differentiated according to age: young children involved in calling for aid, older children possibly involved in usual resuscitation maneuvers.

Optimal CPR training method

The manikin training is considered to be the most effective method (19), but video/DVD and interactive computer training can also help.

Theoretical training without practice turned out to be futile. In the case of participants without previous skills in resuscitation, training children to perform CPR using audio-visual animation available on mobile phones has proven to be more effective than that assisted by a dispatcher, in terms of completing the check list and complying to the time interval spent for performing each CPR maneuver (20).

A training program creating virtual avatars who participated in team (multiplayer online simulation) to the CPR efforts was recently tested and is well appreciated by high school students thus trained (21).

Optimal method for assessing the effectiveness of CPR training

In one study using a game-based assessment of children skills in performing CPR (the student's outcome was equal to the number of cards collected by answering questions correctly as deemed by peers) the results were comparable to those obtained by the classical test method of paper-and-pencil (22).

Drawings are considered a familiar activity for children and one of their preferred means of communication; therefore, we can use this as an effective strategy for engaging them in CPR research (23). For example, in "You Can Also Save a Life!" Project, the pre- and post-training evaluations were performed by analyzing questionnaires and drawings (as non-verbal means of expression and communication) (3).

Several studies that analyzed the dilution of CPR information showed significant improvement in knowledge immediately after training, which suffered a regression towards baseline levels over time (24). Thus, the sequence submitted by us (training–evaluation of training quality–retraining at different stages of development: 2nd grade, 5th grade, 7th grade, 9th and 11th grade) is highly pragmatic.

CPR optimal instructors

Teachers seem to be most effective as instructors: they have adequate teacher training background and continuous contact with students; thus, training them to teach CPR represents a long term investment. It also raises the problem of the available training time, considering the school curriculum, the availability of training space and necessary equipment, as well as the need for a quality training of the trainers. CPR training caravans can be a solution as far as rural areas are concerned.

A questionnaire addressed to the primary, secondary or higher school teachers from Belgium comprising four distinct components (demographic, CPR knowledge and skills, attitudes towards their possible involvement in CPR training and personal experience CPR-related) found that only 59% of the respondents (2539/4273) were involved in doing CPR, mainly primary school teachers (69%) belonging to the age group 21-30 years (68%); compulsory training the CPR at school is backed by only 41% of the respondents, 61% stated that they do not feel able and do not want to teach CPR concepts especially due to insufficient knowledge and competence in CPR, 73% expressing willingness to undergo further training in this field (250). Training teaching staff of any level is particularly important: while in Romania there are 52,541 doctors (2011 data), the body of school teaching staff is larger: 37,391 teachers in pre-primary education, 120,927 teachers in primary and secondary education, 59,380 teachers in upper secondary education, 64 teachers in vocational training, 1,360 teachers in post-secondary education and apprenticeship, and 28,365 teachers in higher education (4).

The optimal instructor-trainees' ratio was variable in different studies: from 1:10 to 1:25 (26).

Optimal duration of CPR training

A study was recently conducted in two secondary schools in Germany over a period of four years (26). It included pupils of different ages (10-13 years), with 3 hours training duration (one hour of theoretical training and two hours of individual training on the manikin). The training frequency was annual or biannual and there were two types of instructors (teachers of the school, and emergency physicians). Children who started training at the age of 13 had better results in terms of theoretical knowledge, but there was no difference in practical skills to those whose training started at the age of 10, even if the instruction was repeated biannually or not, if it was done by the teachers of the school or by emergency physicians (26). Moreover, the courses have doubled the number of children who said that they were confident that the newly gained knowledge would enable them to save lives.

The use of automatic/ semi-automatic external defibrillators (AED)

The current data on Public Access Defibrillators (PAD), although disparate, indicates that the incidence of the cardiac arrest is reduced in schools as compared to other locations, so placing AEDs in schools is currently recommended only in three cases: if there is a reasonable probability of a cardiac arrest occurring there in the next five years; if there is at least one school pupil with increased risk of cardiac arrest; if the response time (the "call-to-shock" time) of the emergency medical system is longer than 5 minutes (27).

In the case of 6-7-year-old children, the score obtained after training in the use of

an AED has made investigators note that "use of an AED is just as simple for them as using a TV remote control" (28). Training children in the use of AED is obviously much simpler if such a device is kept in school. Even if it is not used frequently, the school can be considered as a possible location for placing an AED in the PAD program. The obstacles posed by the costs of such a defibrillator can be surmounted by means of the collaboration between the Health Ministry and the National Resuscitation Council.

National CPR training program in schools

An updated version of the national CPR training program should include a chapter dedicated to CPR training in schools. Within this segment of human education, it is possible to easily pass knowledge-skills-attitudes related to the "Good Samaritan" intervention onto children belonging to all social classes and to all ethnic groups (29). Starting CPR maneuvers as early as possible after the cardiac arrest can thus become possible as a result of the altruism and empathy of these young resuscitators. Training should be repeated every two years; the level of knowledge provided can be increased gradually; also gradually, emphasis can be put on motivational dialogues, the amendment of incorrect skills and on increasing efficiency to carry out optimal maneuvers. Training stages will be tailored to children's age and will include specific goals adapted to each stage (Table I), respecting the principle of revisiting CPR notions every two years. Training time will be adapted to each stage: 30 minutes of theory plus 60 minutes of practice in the first two stages and 60 minutes of theory plus 60 minutes of practice for the last three stages. Emotional education, particularly important, will be conducted in each of the five stages and will have an affective component related to the victim (altruism, empathy, social responsibility), as well as a component relating to the resuscitator himself/herself (motivation, increased confidence in the need for the correct sequence and full compliance with the appropriate CPR maneuvers, together with the increased confidence in one's own forces during CPR maneuvers) (30).

In stages 4 and 5 trainees can participate as volunteers assisting authorized instructors during the training of children in the initial stages on the basis of the principle of pyramidal distribution of the theoretical and practical information. All acquired knowledge spreads also to a secondary level represented by the trainee's parents and relatives, increasing the number of people who have access to the information. Training of trainers is essential for an efficient transmission "in oil stain" (i.e. horizontal) of this educational approach dedicated to CPR. Knowledge-skills-attitudes must be thoroughly checked in a system that will provide candidates for the role of trainee, trainer and overseer of the entire educational process.

Costs can be minimized without compromising in terms of equipment and quality, according to AHA (31) at the level of the cost of teacher education (2-4 teachers from each school: travel cost, course cost, substitute teachers to fulfill classroom responsibilities cost), the cost of the necessary equipment (manikins for BLS training in each school, supplies for training in BLS, training AED, supplies for use the training AED); we must add the costs of all the training materials (instructors toolkits, students manuals and CPR cards).

TABLE I

CPR training tailored to children's grade/age

Stage 1 3rd grade (age 9-10 years)	Stage 2 5th grade (age 12-13 years)	Stage 3 7th grade (age 13-14 years)	Stage 4 9th grade (age 13-14 years)	Stage 5 11th grade (age 17-18 years)
• Basic notions on blood circulation and cardiac arrest; • Recognition of cardiac arrest; • Knowing the unique European emergency phone number, i.e. 112; • Chest compressions – demonstration; • Understanding of AED - demonstration	• Basic notions on blood circulation and cardiac arrest; • Recognition of cardiac arrest; • Getting on the spot / by phone help - knowing the unique European emergency phone number, i.e. 112; • Communication with EMS dispatcher; • Ensuring airway patency - practice; • Chest compressions - practice • Operating an AED - practice	• Deeper understanding of blood circulation and cardiac arrest; • Recognition of cardiac arrest; • Identifying the premonitory signs of cardiac arrest; • Getting on the spot / by phone help - knowing the unique European emergency phone number, i.e. 112; • Communication with EMS dispatcher; • Ensuring airway patency - practice; • Chest compressions – practice; • Operating an AED – practice.	• Pre-course questionnaire; • Deeper understanding of blood circulation and cardiac arrest; • Recognition of cardiac arrest; • Identifying the premonitory signs of cardiac arrest; • Getting on the spot / by phone help - knowing the unique European emergency phone number, i.e. 112; • Communication with EMS dispatcher; • Ensuring airway patency – practice; • Ensuring breathing – practice; • Chest compressions – practice; • Operating an AED - practice	• Pre-course questionnaire; • Deeper understanding of blood circulation and cardiac arrest; • Recognition of cardiac arrest; • Identifying the premonitory signs of cardiac arrest; • Getting on the spot / by phone help - knowing the unique European emergency phone number, i.e. 112; • Communication with EMS dispatcher; • Ensuring airway patency – practice; • Ensuring breathing – practice; • Chest compressions – practice; • Operating an AED – practice; • Recovery position – practice.

AED Automated External Defibrillator; EMS Emergency Medical System.

Training children in schools has proven to be more cost-effective than in a training center setting. In order to convince schools to join the CPR training program, such a program should be promoted as a criterion for the accreditation or evaluation of primary schools, middle schools and high schools. In Romania, this program can be preliminarily implemented within the framework of the special education week

called *"Scoala altfel",* which is dedicated to extracurricular activities carried out in a non-formal context. This became an important component of the 'applied cardiology' strategy of the Romanian Society of Cardiology (32).

The role of the Minister of Education and Scientific Research is particularly important in this field. The "European Cardiac Arrest Awareness Day" (16 October, each year), set up by the European Parliament and adopted by the European Resuscitation Council and Romanian National Resuscitation Council already had three editions and the main element of both was the CPR education of children.

CONCLUSIONS

The knowledge and implementation of CPR maneuvers constitute a social binder (an individual can be saved by any other individual) contributing to the culture of cohesion and to the respect of life itself. Upon graduation, each student is supposed to own a range of useful knowledge about day-to-day life. The basics of CPR are a vital part of this knowledge and should be valued accordingly.

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TREATMENT OF METABOLIC ALTERATIONS IN POLYCYSTIC OVARY SYNDROME

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TREATMENT OF METABOLIC ALTERATIONS IN POLYCYSTIC OVARY SYNDROME (Abstract). Polycystic ovary syndrome is a common endocrinopathy characterized by oligo ovulation or anovulation, signs of androgen excess and multiple small ovarian cysts. It includes various metabolic abnormalities: insulin resistance, hyperinsulinemia, impaired glucose tolerance, visceral obesity, inflammation and endothelial dysfunction, hypertension and dyslipidemia. All these metabolic abnormalities have long-term implications. Treatment should be individualized and must not address a single sign or symptom. Studies are still needed to determine the benefits and the associated risks of the medication now available to practitioners. **Keywords**: POLYCYSTIC OVARY SYNDROME, METABOLIC SYNDROME, COMBINED ORAL CONTRACEPTIVES.

Polycystic ovary syndrome (PCOS), a frequent endocrine dysfunction among women of reproductive age, has important short- and long-term metabolic consequences thus affecting the quality of life. According to the Rotterdam consensus, PCOS is defined by two of the following three criteria: irregular or absent ovulation, androgen excess (clinical or laboratory findings, following the exclusion of other pathologies, such as androgen-secreting tumors, Cushing's syndrome etc) and enlarged ovaries with at least 12 antral follicles each (1). This syndrome is characterized by various metabolic abnormalities: insulin resistance (IR), hyperinsulinemia, impaired glucose tolerance (IGT), visceral obesity, inflammation and endothelial dysfunction, hypertension and dyslipidemia. The management of these imbalances and their impact on health is still a challenge.

Etiology. The etiology remains unclear, but it involves interactions between endocrine (hypothalamic, ovarian, adrenal), genetic, environmental and behavioral factors (2). The sequence of the phenomena is unclear: it has not been established whether hyperandrogenism results from hyperinsulinemia secondary to insulin resistance (IR) or vice versa, but increasingly more studies show that hyperinsulinemia plays an important role in the pathogenesis of this syndrome.

Physiopathogenesis. Women with PCOS have certain metabolic and cardiovascular abnormalities. Obesity is common

among them and is associated with increased IR, IGT and dyslipidemia. IR is present in 60-80% of women with PCOS, and is more prevalent among the obese ones. Obesity seems to be the main factor for the development of IR, but defects in post-receptor intracellular signaling also seem to play a role. IR and the resulting hyperinsulinemia are found in both normal weight and overweight or obese women and significantly increase the prevalence of IGT and diabetes mellitus type 2 (DM2) in women with PCOS. Up to 35-40% of women with PCOS have IGT and 10% will develop DM2 in the third and fourth decade of life. IGT and DM2 are common among women with PCOS, and significant percentage of teenage women (mainly obese) are at risk of developing these diseases.

Dyslipidemia is another metabolic abnormality in women with PCOS and includes: increased levels of total cholesterol, LDL-C and triglycerides (TG) and low levels of HDL-C. Genetic and ethnic factors, hyperandrogenemia and obesity are linked to dyslipidemia in women with PCOS.

In women with PCOS, especially in the presence of obesity, elevated levels of the markers of cardiovascular disease have been reported (C-reactive protein (CRP), endothelin-1, adiponectin, homocysteine, plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor and markers of oxidative stress.

There is a substantial overlap between PCOS and the metabolic syndrome (MS), a group of metabolic disorders related to increased cardiovascular risk (3). The International Diabetes Federation consensus (2006) definition of MS is central obesity (waist circumference) and 2 of the following criteria: raised triglycerides: over 150 mg/dL (1.7 mmol/L), low HDL-C: below 40 mg/dL (1.03 mmol/L) in men and below 50 mg/dL (1.29 mmol/L) in women, raised systolic blood pressure > 130 mmHg or diastolic blood pressure > 85 mmHg, raised fasting blood glucose or DM2. The prevalence of MS in PCOS varies between 1.6% and 43% depending on the population studied (4, 5). In PCOS, the most common features are decreased HDL-C, obesity and high blood pressure (4). In the general population, MS leads to a 2-fold increase in cardiovascular risk. In women with PCOS there is limited evidence of cardiovascular events of increased heart disease mortality.

Given the multifactorial pathogenesis of PCOS, a multidirectional therapeutic strategy should be established. Currently there are many drugs that can be used, but which, although may be beneficial for certain metabolic aberrations, can aggravate other abnormalities. Some treatment options include: oral contraceptives and antiandrogens, oral antidiabetic agents (metformin, thiazolidinedione), statins, acupuncture, various dietary supplements (vitamin D, vitamin B1, folic acid) or hygienic-dietary regimes.

TREATMENT

1. CONTRACEPTIVES. Oral contraceptives have been the treatment of choice of PCOS for many years, especially combined oral contraceptives (COCs), but progestin only minipills have also been used. COCs contain an estrogen component (ethynyl-estradiol or estradiol) and a progesterone, which varies according to the type of pill. In most PCOS women, oral contraceptives resulted in amelioration of clinical manifestations, such as normalization of menstrual cycles, hirsutism and acne relief, and sometimes to regression of

male pattern alopecia. The effects are assigned to the decreased LH secretion, inhibition of ovarian and adrenal androgen production, and reduced free testosterone levels due to increased liver production of SHBG.

Contraceptives are not used specifically for the treatment of metabolic alterations, even though they inflict various influences on the metabolism. On the contrary, some studies suggest that oral contraception may have a negative effect on metabolic abnormalities in PCOS women.

There is an association between their use and development of IR, which suggested a potential adverse effect on the metabolism, but the results of the studies are yet inconclusive. Their influence on carbohydrate metabolism varies from DM to its improvement. Studies show that low-dose estrogen COCs (<50mcg) are associated with decreased risk for DM2, but investigations are still needed in order to determine the progestogen with the lowest impact on carbohydrate metabolism. Studies on the impact of COCs on lipid metabolism have limitations due to their heterogeneity, leading to inconsistent results. In general, it has been shown that treatment with oral contraceptives leads to an increase in LDL-C, total cholesterol, HDL-C and TG (6). Minipill administration seems not to interfere with lipid parameters (7). Increased TG seems to be the most common side effect of contraceptive treatment (6,7). The potential effect of this type of treatment appears to be the result of the action of estrogen on liver, by lowering TG clearance, so COCs with lower estrogen doses may lead to a lower increase of TG levels and transdermal systems might not affect the lipid profile (8). In addition, various COCs lead to increased HDH-C levels, the effect being attributable to the action of estrogen component on the apolipoprotein AI gene expression in liver cells. A Cochrane review (9) analyzed the results of four randomized trials comparing oral contraceptives with metformin and showed no differences between the 2 types of treatment in clinical metabolic effects (development of DM2 and cardiovascular diseases) and biological effects (insulin, glucose, total cholesterol), the only difference being the increase of TG in contraceptive therapy as compared to the metformin therapy.

Regarding hypertension, COCs can induce high blood pressure and are therefore contraindicated in hypertensive women with PCOS and should be stopped if hypertension develops during treatment.

The effect of oral contraceptives on weight is still controversial: some studies found an increase in body weight while others showed no differences in weight (10).

In conclusion, oral contraception may have a negative effect on metabolic abnormalities in PCOS women and the long term benefits are unclear, especially in patients with IGT, DM2 and dyslipidemia. The decision to administer this treatment must take into account the patient's phenotype and history.

2. ANTIANDROGENS. In women with PCOS, hyperandrogenemia was suggested as an initiating factor of metabolic abnormalities. Androgens exert their effects through the androgen receptor, which is expressed in the visceral adipose tissue, acting through competitive inhibition and by decreasing androgen production. In addition, the direct effect on insulin signaling pathway appears to contribute directly to IR in PCOS. Antiandrogens, such as cyproterone acetate, spironolactone and

flutamide, have been used in women with PCOS mainly for the treatment of hirsutism. They are generally used in combination with oral contraceptives, having synergistic effect, but also because a contraceptive method during the treatment with antiandogens is required because of the potential feminization of male fetuses.

Cyproterone acetate is a progestational antiandrogen which competitively inhibits the binding of testosterone and its more potent conversion product 5*a*-dihydrotestosterone to the androgen receptor.

Spironolactone is an aldosterone antagonist and a competitive inhibitor of the androgen receptors. Its use leads to the inhibition of androgen production and of the action of 5α reductase. In normal weight women it increases HDL-C and lowers TG (11).

Flutamide is a selective androgen receptor with progesterone-like activity. It reduces the conversion of testosterone to its more active metabolite, dihydrotestosterone, in the target tissues. In patients with PCOS it improves the androgenic manifestations. The effect on carbohydrate metabolism is insignificant, but it lowers total cholesterol, LDL-C and TG, independent of body mass. Used in conjunction with COC and metformin it causes a sharp decrease in LDL-C and increase in HDL-C, but does not prevent TG increase associated to COCs (12).

3. ORAL ANTIDIABETICS. The strong association between IR and PCOS led to the use of drugs that increase the sensitivity to insulin ("insulin sensitizers") in the treatment of this syndrome.

Metformin, a biguanide, acts mainly by suppression of hepatic gluconeogenesis, but also increases peripheral insulin action in skeletal muscle and decreases glucose uptake from the digestive tract, without having a direct effect on pancreatic insulin production. It improves the lipid profile through various mechanisms and directly inhibits thecal cell androgen production.

In women with PCOS, metformin appears to improve the cardio metabolic parameters by increasing insulin sensitivity, lowering blood glucose and androgenemia, but these effects are more obvious if there are associated with lifestyle changes. Metformin may normalize appetite in obese women with PCOS through normalization of neuropeptide Y, which is low in women with PCOS.

Some studies have shown that patients with PCOS and hyperinsulinemia treated with metformin benefited from a significant decrease in total cholesterol, LDL-C and TG and increased HDL-C, but these benefits have not been demonstrated in patients with severe obesity. Metformin appears to improve some atherogenic factors: endothelin-1, adhesion molecules, CRP, advanced glycation products (AGE).

Other drugs that increase insulin sensitivity are thiazolidinedione (glitazones), which act by binding to and activating the PPAR-gamma receptor (peroxisome proliferator-activated receptor gamma). Pioglitazone is the only one that can be used in Europe today. There is insufficient evidence of the metabolic benefits in women with PCOS, although pioglitazone monotherapy in obese women with PCOS improves the lipid profile (13) and decreases the serum PAI-1 and CRP levels.

4. OTHER THERAPEUTIC METHODS. Statins act by the selective inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which is involved in the biosynthesis of cholesterol. In

women with PCOS, statins have different effects, other than reducing lipids (especially LDL-C): they reduce the thecal production of androgens and steroidogenesis (14) and the effects of RI and associated hyperinsulinemia, have antioxidant and anti-proliferative effects, and appear to improve the cardiovascular morbidity and mortality associated with PCOS. However, they are teratogenic and cannot be used in women with PCOS who are planning a pregnancy.

As to the use of acupuncture in women with PCOS, although clinical trials are lacking, animal studies show that electro acupuncture could improve the cardio metabolic abnormalities in PCOS: it activates muscle fibers (which increase blood flow, peripheral insulin sensitivity and peripheral glucose utilization), stimulates the release of neuropeptides in the central nervous system (β-endorphins) and could reduce sympathetic activity, which results in lower levels of circulating testosterone (15).

In women with PCOS, low levels of vitamin D are associated with IR, obesity, IGT and MS, suggesting a possible role of this vitamin in the pathogenesis of PCOS (16). Administration of vitamin D in PCOS improves the lipid profile and IR, but further studies are needed.

It has been shown that the administration of vitamin B12 in patients with MS decreases IR. In women with PCOS, IR, obesity and elevated serum homocysteine levels are associated with low serum concentrations of vitamin B12 (17), suggesting a possible role of this vitamin in the treatment of PCOS.

CONCLUSIONS

Polycystic ovary syndrome is characterized by multiple metabolic abnormalities and has significant long-term implications. The therapeutic strategy encompasses numerous approaches and drugs, as the physiopathogenesis of the syndrome is being better understood.

In our opinion, the treatment should be individualized, must not address a single sign or symptom and should also take into account the possible side effects. We believe that further studies are still needed to determine the benefits and risks of medicines that are now available to the practitioners, as well as the long-term cardiovascular implications. Given the complexity of the syndrome, we advocate a multidisciplinary treatment approach (gynecologist, endocrinologist, cardiologist, nutritionist).

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NEWS

CLINICAL AND MICROBIOLOGICAL EVALUATION OF SCALING AND ROOT PLANING PER QUADRANT AND ONE-STAGE FULL MOUTH DISINFECTION ASSOCIATED WITH AZITHROMYCIN OR CHLORHEXIDINE

Conflicting data about the protocol of choice for non-surgical periodontal therapy with adjuvant use are still reported. The study realized by a group of brazilian researchers aimed to evaluate, through clinical and microbiological parameters, the systemic use of azithromycin as adjuvant to non-surgical periodontal treatment performed by one-stage full-mouth disinfection within 24 hours or conventional quadrant scaling in four weekly sections. In the randomized controlled trial, 85 patients diagnosed with chronic periodontitis underwent different treatment protocols, divided in 6 groups: 3 full-mouth disinfection groups (with no adjuvants, with chlorhexidine and with azithromycin) and 3 weekly sections groups (with no adjuvants, with chlorhexidine and with azithromycin). Clinical periodontal parameters, total and quantitative bacterial counts of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, and Streptococcus oralis were measured through real-time polymerase chain reaction at baseline, 90, and 180 days after treatment. In all groups, it was observed a significant reduction in the % of periodontal diseased sites, gingival index, plaque index, and clinical attachment level gain at 90 days of treatment, demonstrating effectiveness of the treatment, independently of the adjuvant. The full-mouth disinfection with chlorhexidine group showed higher reduction in probing depth and % periodontal diseased sites, as well as lower total bacterial count than all the other groups at 180 days. In conclusion, the adjuvant use of azithromycin did not provide any significant benefit, independently of the treatment protocol. The adjuvant use of the chlorhexidine showed a more expressive and significant improvement on clinical and microbiological parameters, especially in the full-mouth disinfection protocol, followed by weekly sections (Campideli Fonseca D, Cortelli JR, Cortelli SC, Miranda Cota LO, Machado Costa LC, Moreira Castro MV, Oliveira Azevedo AM, Costa FO. Clinical and Microbiological Evaluation of Scaling and Root Planing per Quadrant and One-Stage Full Mouth Disinfection Associated With Azithromycin or Chlorhexidine: A Clinical Randomized Controlled Trial. *J Periodontol.* 2015 ; 7: 1-16).

Irina Grădinaru

ASSESSMENT OF ADVERSE DRUG REACTIONS AS CARDIOVASCULAR RISK FACTORS

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ASSESSMENT OF ADVERSE DRUG REACTIONS AS CARDIOVASCULAR RISK FACTORS (Abstract): A wide range of pharmaceutical substances can induce side-effects expressed as cardiovascular changes or events, adding to other risk factors or worsening preexisting cardiovascular diseases. **Aim:** Continuous study focused on iatrogenic conditions representing cardiovascular risk factors. **Methods:** We developed a descriptive study of patients admitted to the Iasi Vth Internal Medicine and Geriatrics-Gerontology Clinic between 1998-2013, focusing on iatrogenic conditions representing cardiovascular risk factors. **Results:** We have diagnosed 81 cases of drug-induced hypertension, and 43 patients with hypertensive crises; 72 cases of iatrogenic hyperglycemia; 36 cases of drug-induced hyperuricemia; 50 cases of drug-induced dyslipidemias; and 17 cases of iatrogenic obesity. These iatrogenic diseases were more common in women and the elderly. Twenty-eight patients have developed simultaneous adverse drug reactions induced by the same drug and manifest as different cardiovascular risk factors. **Conclusions:** Cardiovascular risk factors can be induced to a significant extent by chronic drug administration. Some medications (e.g. NSAIDs, corticoids, β-blockers, diuretics, contraceptives) can act on the same patient by multiple pathogenic links. The adverse drug reactions can be cardiovascular risk factors that persist in time, or can be removed (by discontinuing the administration of the implicated drug). The highest importance of their acknowledgment relies on the possibility of their prevention through carefully balancing the benefits and the risk of each new medication. **Keywords:** DRUG-INDUCED SIDE-EFFECTS, CARDIOVASCULAR DISEASES, CARDIOVASCULAR RISK FACTORS, IATROGENESIS.

World Health Organization defines pharmacovigilance as "the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problem". The importance of pharmacovigilance lies in its key place in the public health care system (1).

A wide range of pharmaceutical substances can induce side-effects expressed as cardiovascular events, adding to other risk factors or worsening preexisting cardiovascular diseases. In countries with a very rigorous pharmacovigilance activity, such

as the Netherlands and UK, these cardiovascular iatrogenic effects reach up to 10% of all annual reported iatrogeneses (2). The most frequently found are heart rhythm disturbances, heart failure, high blood pressure, myocardial ischemia, thromboembolic disease, myocardial toxicity, valvular and pericardial heart disease (3,4,5).

Life-threatening adverse drug reactions (ADR) reports were submitted by patients in a range of 8.6% in Denmark up to 13% in Netherlands (2,6,7).

MATERIAL AND METHODS

We developed a continuous study aimed at identifying iatrogenic conditions representing cardiovascular risk factors in a population of hospitalized patients. Thus, we searched for iatrogenic hypertension, diabetes, dyslipidemia, obesity and hyperuricemia that might have occurred before admission or during hospital stay.

The observational study recorded the adverse reactions occurring in patients admitted between 1998-2013 to the Iasi Vth Internal Medicine and Geriatrics-Gerontology Clinic of the "Railway Hospital". The studied population consisted of 18,740 patients, of which 8,900 (47.49%) women and 9,840 (52.51%) men.

In order to establish the possibility of a drug side-effect the classical criteria of incrimination used in pharmacovigilance were used (8). Besides this intensive surveillance, some other clinical trials were designed in order to determine if the first assumptions are supported.

The study continued with a follow up of these patients during current and second admission for either an acute event or reassessment of their health status. One hundred ninety-nine patients only benefited from outpatient consultation.

RESULTS

1496 (7.98%) of the patients presented 1677 different types of adverse drug reactions (some of the patients experiencing more side effects) with different degrees of severity. Out of them 243 patients (16.24%) presented 299 adverse drug reactions that can be considered cardiovascular risk factors: hypertension, diabetes, dyslipidemia, obesity and hyperuricemia.

Age and sex distribution of the 299 cases with adverse reactions is shown in fig. 1 (some patients manifested multiple side effects) (fig. 1) and they were statistically analyzed (Data_cases of pharmacovigilance.xls).

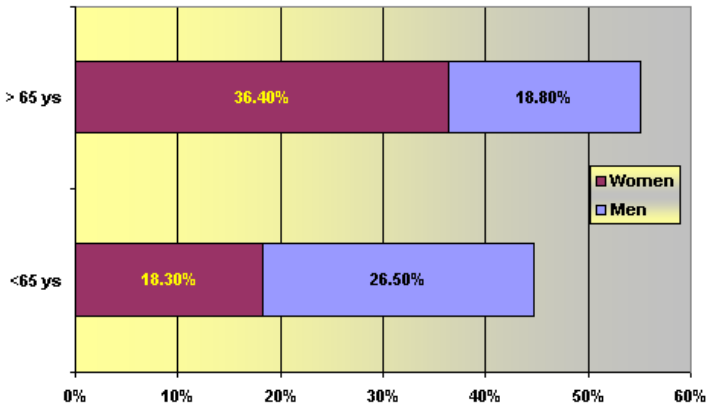

Fig. 1. The age and sex distribution of adverse drug reactions

The recorded iatrogenic effects were found in patients with cardiovascular diseases on long-term or short-term medication administered to treat different conditions or to reduce multiple risk factors (tab. I).

TABLE I

Adverse drug effects caused by the incriminated drugs

Adverse drug reaction	Incriminated drug	No of cases
Hypertension	Nonsteroidal anti-inflammatory drugs (NSAIDs)	25
	Oral contraceptives	17
	Corticosteroids	14
	Monoamine oxidase inhibitors (MAOIs)	7
	β-mimetics	5
	Thyroid hormones	5
	Anabolic steroids	5
	α-interferon	3
Hypertensive crises	Beta-blocker withdrawal syndrome	12
	NSAIDs	20
	Thyroid hormones	11
Diabetes mellitus/ impaired fasting glucose and/or impaired glucose tolerance	Diuretics (thiazide, furosemide, indapamide)	26
	Oral contraceptives	12
	Oral corticosteroids	9
	NSAIDs	5
	Atypical antipsychotic medications (olanzapine)	5
	β-blockers	4
	Calcium antagonists	3
	Nalidixic acid	3
	Intra-articular administration of corticosteroids	2
	Ciprofloxacin	2
	Propafenone	1
	Statin	1
Hyperuricemia	Diuretics (thiazide, indapamide)	25
	Salycilates	7
	Phenylbutazone	3
	Ranitidine	1
Dyslipidemias	Diuretics (thiazide)	17
	Oral contraceptives	11
	Corticosteroids	8
	Atypical antipsychotic medications (olanzapine)	8
	β-blockers	5
	Angiotensin -converting enzyme inhibitors	1
Obesity	Cortisone	6
	Atypical antipsychotic medications (olanzapine)	6
	Anabolic steroids	4
	Statin	1

Drug-induced hypertension was found as an adverse drug reaction in 81 cases (33.33%), more frequently involved being the nonsteroidal anti-inflammatory drugs (NSAIDs), oral contraceptives and corticosteroids. Monoamine oxidase inhibitors (MAOIs), β-mimetics, thyroid hormones, anabolic steroids or α-interferon were also incriminated in drug-induced hypertension, but with a lower frequency.

In agreement with other authors (9,10), we found neither specific clinical criteria for drug-induced hypertension (patients presenting with a wide range of clinical manifestations) nor temporal criteria (the onset as well as the time to the normalization of blood pressure in case of withdrawal being highly variable). In most of the cases with hypertension caused by NSAIDs and oral contraceptives (which were the leading cause), patients had taken the medication without medical prescription, and did not follow the instructions (low sodium diet).

The rise in blood pressure was usually mild, but 11 cases (4.53%) developed severe hypertension and discontinuing the incriminated drug led to the normalization of blood pressure within 2 weeks - 6 months.

The triggering of hypertensive crisis in 43 patients (17.70%) already known with high blood pressure was recorded in 3 situations: withdrawal syndrome following cessation of antihypertensive drug therapy (the abrupt discontinuation of β-blocker treatment) in 12 cases (4.94%), higher blood pressure levels associated with NSAIDs administration confirmed by 24-hours arterial blood pressure measurements in 20 patients ((8.23%), and hypertensive episodes in 11 patients (4.53%) on thyroid hormone replacement therapy.

Diabetes mellitus was the second most frequent cardiovascular adverse effect.

A correlation between the administration of a drug and the occurrence of hyperglycaemia was found in 72 patients (29.63%). In 58 cases (23.87%), the incrimination of the drug was very likely. The drugs most frequently involved were diuretics (thiazide, frusemide, indapamide), oral contraceptives and oral corticosteroids, followed by NSAIDs and atypical antipsychotic medications (olanzapine). Some patients had a history of chronic treatment for such diseases as tuberculosis (isoniazid and rifampicin), or persistent lumbar pain (NSAIDs). In a small number of patients we have noticed the involvement of amiodarone, statin, and atypical antipsychotic medication (olanzapine) in the onset of diabetes mellitus.

The relationship between drug use and hyperglycemia was weaker (possible or likely reaction) in 10 cases, and was related to β-blockers, calcium antagonists and nalidixic acid. Intra-articular corticosteroids, ciprofloxacin and propafenone produced this side effect with a lower frequency.

Of the drugs with hyperglycemic effect, only those well-known to induce this metabolic effect were most likely incriminated, but of the drugs with a smaller coefficient of incrimination (< 5), some less mentioned drugs like ciprofloxacin, or never mentioned – as propafenone – were responsible for this side-effect.

Hyperglycemic crisis, possibly secondary to drugs was found in 2 patients (0.82%) that were long-term treated with nifedipine and 1 patient receiving verapamil on long-term; if this side-effect of nifedipine is already known, the role of verapamil is controversial, some authors advocating

its hyperglycemic role or pleading for its hypoglycemic role, while others support its neutral role on the carbohydrate metabolism (11,12).

Drug-induced hyperuricemia (or iatrogenic hyperuricemia) (13) was found in 36 cases (14.81%) and was mainly caused by chronic diuretic use, as mentioned above, in 25 cases (10.29%). In other 7 cases (2.88%), secondary hyperuricemia was due to salycilates, while the frequency of this side-effect in patients treated with ranitidine and phenylbutazone was lower.

Diuretic-induced gout occurred in 11 cases (4.53%), and it was reversible following withdrawal of the drugs in 10 cases, while in 1 case it required treatment with allopurinol.

Drug-induced dyslipidemia (14) was diagnosed in 50 patients (20.58%). In the majority of cases, diuretics and oral contraceptives, together with corticosteroids and olanzapine caused different types of secondary iatrogenic dyslipidemias.

Obesity was recorded in 17 cases (~7%) on long-term medication use. Most drug-induced weight gain effects were the result of chronic treatment with cortisone and olanzapine. In few patients obesity was associated with an adverse drug reaction to anabolic steroids or statin regimen.

Our study proves that atypical antipsychotic medication increases the likelihood of many cardiovascular risk factors, confirming the conclusion that weight gain and metabolic disease in patients taking second-generation antipsychotic medications "represents an epidemic within an epidemic" (15).

The discontinuation of suspected drugs led to normalization of clinical and biological data in less than 3 months in the acute forms and in less than 6 months in the chronic ones. 17 patients (~7%) were lost tofollow up. No rechallenge was voluntarily administered.

DISCUSSION

Patients developing drug-induced diabetes mellitus or hyperglycemia were not previously known with diabetes mellitus or with abnormal glucose tolerance, although, theoretically, diabetics are more prone to develop this side-effect. On the other hand, the acknowledgement of the hyperglycemic effect of some drugs makes doctors to avoid their usage in diabetics.

We have found no causal relationship between the corticosteroid dose and the occurrence of hyperglycemia in non-diabetic patients; conversely, an increased likelihood of corticosteroids dose-related hyperglycemia was found in diabetics.

All patients with oral or intra-articular administration of corticosteroids were diabetics, and hyperglycemia occurred when, for various reasons, the patient has been given a higher dose than the initial one.

Statin-induced hyperglycemia was described in a 72-year-old woman, who also exhibited muscle weakness upon standing and walking, particularly in the quadriceps muscles, within 15 days of treatment for hypercholesterolemia. After statin administration was discontinued, blood sugar level returned to normal within 5 days, but, nevertheless, the patient was still complaining of muscle soreness and decreased exercise tolerance for another month. The cardiovascular risk might be augmented by the imposed sedentary behaviour. Symptoms had a lower intensity after one month, when she went to the ambulatory care clinic, and the caregiving team reassured and encouraged her to practice daily limb exercise training and walking.

A higher frequency of iatrogenic diseases was found in women, which general-

ly consume more frequently over-the-counter medication such as NSAIDs, diuretics, contraceptives. The long-term medication that led to the occurrence of adverse drug reactions was self-medication in 49 cases (20.16%), out of which almost 71% (35 cases) were women.

The higher frequency of iatrogenic disease in the elderly is caused by age-related alterations in pharmacokinetics and pharmacodynamics, polypragmasia (due to polypathology at this age), predisposition to many unwanted interactions and difficult cooperation with the old patient (16,17).

The importance of drugs as cardiovascular risk factors relies not only in their frequency (lower than other etiologies), but especially in the fact that they represent modifiable risk factors (therefore they are avoidable by ceasing treatment, if the administration is not absolutely necessary). It is also important to acknowledge the possibility that the same drug may simultaneously induce multiple risk factors (e.g.: corticosteroids, NSAIDs, β-blockers, diuretics, contraceptives) (18). The adverse drug reactions proved to be cardiovascular risk factors that disappeared within several days to several weeks after withdrawal of suspected drug and persisted for months only in three cases of obesity.

The more frequent occurrence of these iatrogenic effects in older women, as proved by this study, requires an increased focus on this segment of the population (considering that they additionally lose cardiovascular protection due to the drop in estrogen level associated with menopause).

Our study describes the following paradox: trying to treat cardiovascular risk factors may cause other cardiovascular risk factors. Moreover, a patient receiving multiple medication in order to avoid the occurrence of cardiovascular disease may become incompliant, ignoring the effects of diet and physical exercise. It is the physician's role to inform the patient on the possible negative effects of polypragmasia and the beneficial role of a healthy lifestyle (19,20).

CONCLUSIONS

1. The study proved that cardiovascular risk factors can be induced to a significant extent by chronic drug administration.

2. Some drugs (NSAIDs, β-blockers, diuretics, contraceptives) cause simultaneous cardiovascular adverse reactions in the same patient by multiple pathogenic links.

3. The adverse drug reactions disappeared within several days to several weeks after the discontinuation of the suspected drug and rarely persisted for months.

4. The highest importance of their acknowledgment relies on the possibility of their prevention through carefully balancing the benefits and risk of each new medication.

5. The adverse reactions can be counteracted by healthy lifestyle interventions, comprising balanced diet and regular exercise training, habits that can be enhanced through counselling and continuous medical education.

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EVALUATION OF PROGNOSTIC FACTORS IN DECOMPENSATED LIVER CIRRHOSIS WITH ASCITES AND SPONTANEOUS BACTERIAL PERITONITIS

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EVALUATION OF PROGNOSTIC FACTORS IN DECOMPENSATED LIVER CIRRHOSIS WITH ASCITES AND SPONTANEOUS BACTERIAL PERITONITIS **(Abstract)** Mortality in spontaneous bacterial peritonitis (SBP) decreased significantly from 90% in 1970 to 10-30% today, but SBP still remains a complication with a poor prognosis. Although there are new preventive measures, such as early diagnosis and treatment with albumin, the introduction of new antibiotics, the prognosis of patients with decompensated cirrhosis and SBP remains poor, with a mortality rate of 20-40%. The installation of an episode of spontaneous bacterial peritonitis reduces the survival rate at 1 year to 30% and to 20% at 2 years. **Purpose**: In this context, the identification of patients with increased risk of death is extremely important in order to improve prognosis. **Material and Methods**: The prospective study included 153 patients with cirrhosis admitted to the Institute of Gastroenterology and Hepatology Iaşi from 1 January to 31 December 2010, reevaluated during 2 years. Criteria for the diagnosis of SBP were the presence of a number> 250 PMN / mmc. The presence of ascites and / or upper gastrointestinal bleeding (UGB) marks the decompensated cirrhosis. To assess the severity of cirrhosis, there were used Child-Pugh and MELD scores. Diagnostic paracentesis and ascites fluid cultures were performed in all hospitalized patients with ascites and also in case of signs and symptoms of SBP, before and after antibiotic treatment. Lack of response to empirical therapy was considered in those cases with a decrease in the number of neutrophils <25% from baseline. **Results**: Identification of patients with increased risk of death is extremely important to improve prognosis. In peripheral leukocytosis and in the ascites fluid, low hemoglobin can be considered predictors of mortality in patients with PBS. Child-Pugh score, increased levels of bilirubin and creatinine and hyponatremia are independent risk factors of mortality in patients with SBP. Bacteremia and lack of therapeutic response are independent risk factors of mortality associated with SBP. Recent history of variceal bleeding, severity of infection and the degree of hepatic and renal impairment influence short-term prognosis of patients with SBP. **Conclusions**: Identification of patients with increased risk of death is extremely important to improve prognosis. Therefore, it is important to identify prognostic factors in patients with bacterial infection and cirrhosis, in order to identify high risk patients and to prevent complications and death. **Keywords**: SPONTANEOUS BACTERIAL PERITONITIS, RISK FACTORS, TREATMENT, PROGNOSIS.

Spontaneous bacterial peritonitis occurs in 24% of cirrhotic patients with ascites (1). Although preventive measures are taken, such as early diagnosis and treat-

ment with albumin, the introduction of new antibiotics, the prognosis of these patients remains poor, with a mortality rate of 20-40% (2, 3, 4). The installation of an episode of spontaneous bacterial peritonitis reduces the survival rate at 1 year to 30% and to 20% at 2 years. Almost one third of patients diagnosed with liver cirrhosis complicated with spontaneous bacterial peritonitis (SBP) develop hepato-renal syndrome, with a high mortality rate. Another complication that occurs in SBP is hepatic encephalopathy, ascites becomes refractory to treatment and recover rapidly after paracentesis (5, 6).

The appearance of multi-resistant (MR) bacteria has a major impact on the clinical course of patients with cirrhosis. In a prospective study on this topic, the response to empiric treatment in community-acquired infections was high (83%) but decreased in healthcare-associated infections (73%) and extremely low in nosocomial infections (40%) (7).

Bacterial infection is a leading cause of circulatory dysfunction. Infection in cirrhosis is associated with an inflammatory response more important than in non-cirrhotic population. Therefore, cirrhotic patients with SBP show very high levels of cytokines, which emphasizes the circulatory dysfunction (2). The infection in a patient with basic circulatory dysfunction can be devastating. Up to 30% of patients develop progressive circulatory dysfunction and acute renal failure, heart failure, hepatic encephalopathy and hepatorenal syndrome type-1 and death. Albumin increases the effective arterial volume, reduces renal failure and improves survival in SBP (8, 9).

MELD (Model for End-Stage Liver Disease) score predicts survival at three months in cirrhotic patients and in those enrolled in liver transplantation. As it proved to be insufficient, a new score iMELD was defined, with higher accuracy in predicting mortality. Besides bilirubin and creatinine levels, there were included age and serum sodium (Na lower 130mmol / l). This score shows that hyponatremia is an independent risk factor predicting mortality in cirrhotic patients with SBP (10). SBP mortality decreased substantially from 90% in 1970 to 10-30% today, but it still remains a complication with poor prognosis.

The purpose of the study is to evaluate patients with liver cirrhosis and SBP, assessing the survival rate of patients with ascites and SBP, and analyzing clinical, biological, echographic and endoscopic characteristics associated with worsening of the prognosis.

MATERIAL AND METHODS

The prospective study included patients with liver cirrhosis of different etiologies, hospitalized in the Institute of Gastroenterology and Hepatology Iasi in the period 1 January 2010 to 31 December 2010, which were clinically and laboratory investigated at the time of presentation and evaluated annually for 2 years. Socio-demographic data, personal history and symptoms were assessed and recorded in all patients. Complete clinical examination and history were achieved in all cases. The diagnosis of cirrhosis was established corroborating data obtained by objective clinical examination and laboratory investigations (hematology, biochemistry, abdominal ultrasound, upper gastrointestinal endoscopy).

A SBP essential diagnostic criterion was the presence of a number a PMN more than 250 PMN / mmc. Compensated cirrhosis was defined as cirrhosis without ascites. Ascites and / or variceal bleeding is a sign

of decompensated cirrhosis.

Child-Pugh score and MELD score were used to assess the severity of liver cirrhosis. Diagnose paracentesis and ascites fluid culture was performed in hospitalized patients at their admission and in case of signs and symptoms of SBP also during hospitalization period. Ascites fluid was investigated macroscopically, biochemically (albumin, protein), cytologically (cellular) and bacteriologically (smear and culture). Lack of response to empirical treatment was noticed in cases of general condition alteration and decrease PMN <25% of baseline.

Written informed consent for study participation was obtained after informing.

The processing of statistical data was performed with STATISTICA software var. 7.0, evaluating the annual rate of decompensation and analyzing the risk factors of decompensation with ascites and SBP.

RESULTS

The initial group in the study included 153 patients, 30.72% diagnosed with compensated cirrhosis and 69.28% in decompensated stage. At the end of the study, 9.87% of patients remained in the compensated stage of disease, and 91.45% were in decompensated stage. At the end of the study there were 14 deaths (tab. I).

The main causes of death were: spontaneous bacterial peritonitis (64.29%), hepato-renal syndrome (57.14%) and variceal bleeding (35.72%) (fig. 1).

TABLE I

Frequency of cases with compensated and decompensated cirrhosis

Patients	Stage I		Stage II		Stage III	
	n	%	N	%	N	%
Group with cirrhosis	153	100	153	100	152	99,35
Group with compensated cirrhosis	47	30,72	23	15,03	15	9,87
Group with decompensated cirrhosis	106	69,28	130	84,97	139	91,45
Deaths	0	0	1	0,65	13	8,55

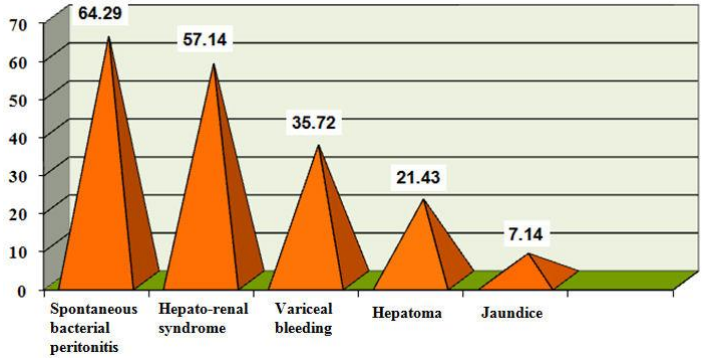

Fig. 1. Frequency of causes of death

To highlight potential risk factors and death associated with SBP, we compared different nonparametric variables in the subgroup of patients with SBP or the subgroup of patients who later died with similar values corresponding to the rest of the

group (patients without SBP), and we identified the possible risk factors of SBP and death.

The significant results were presented in the following tables and graphics.

In the patients who subsequently died, there were significantly higher mean values of blood leukocytes in both the onset phase of the study and also in the examinations at 1 and 2 years (fig.2). Mean values of leukocytes in ascites fluid at 1 and 2 years, and also the average values of PMN in ascites fluid at 1 and 2 years were higher (fig. 3).

Mean values of creatinine in surviving patients were lower compared to the dead ones (fig. 4).

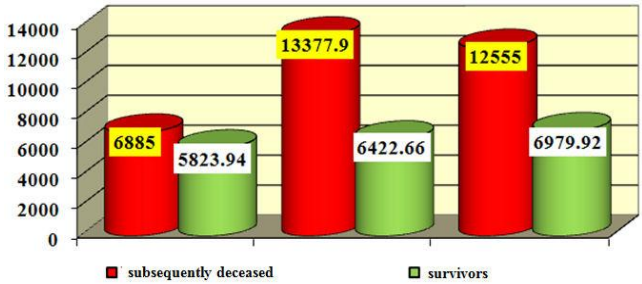

Fig. 2. Mean values of leucocytes (per mm^3)

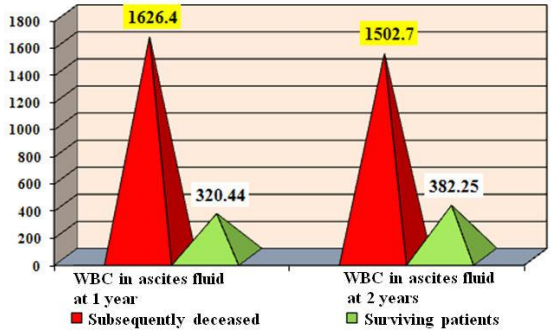

Fig. 3. Mean value of leucocytes in AF

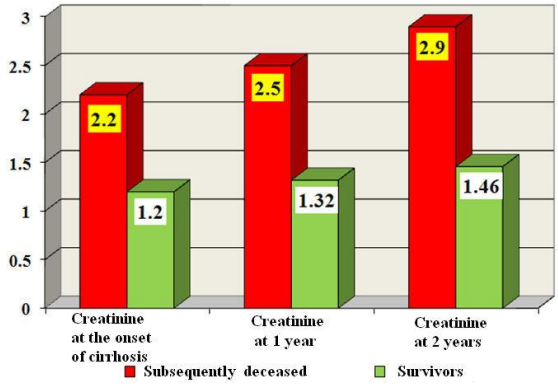

Fig. 4. Mean values of creatinine (mg/dL) in surviving patients compared to the dead ones

TABLE II

Statistical differences between the deceased and the survivors

Variables	Cases		Mean		Dev. std.		Minim		Maxim		p
	D	S	D	S	D	S	D	S	D	S	
Albumine	14	139	28.07	**32.05**	2.702	5.251	22	23	32	55	**0.0059**
Bilirubin	14	139	2.52	2.84	1.229	3.688	1.14	0.73	4.79	22	0.75
Creatinin	14	139	**2.94**	1.46	1.294	0.668	1.2	0.58	4.5	4	**<0,00001**
Natrium	14	139	127.79	130.84	2.694	10.360	120	65	131	154	0.28

The albumin levels are on average significantly higher in survivors, and creatinine is significantly higher in dead patients. So, maybe there is a significant relationship between death and these two variables. (tab. II, III, IV, fig. 5, 6)

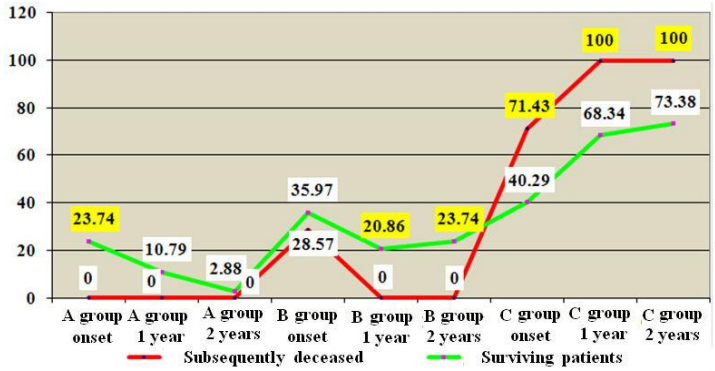

Fig. 5. Child classification in later deceased patients vs. Survivors

TABLE III

Frequency of hepato-renal syndrome in patients who subsequently died compared to survivors

Stages	Patients who subsequently died				Survivors				χ2, p
	With HRS		Without HRS		With HRS		Without HRS		
	n	%	n	%	N	%	n	%	
1 year	3	**21,43**	11	*78,57*↓	4	*2,88*↓	135	**97,12**	χ2=6,23, **p=0,01**
2 years	8	**57,14**	6	*42,86*↓	13	*9,35*↓	126	**90,65**	χ2=24,53 **p<0,00001**

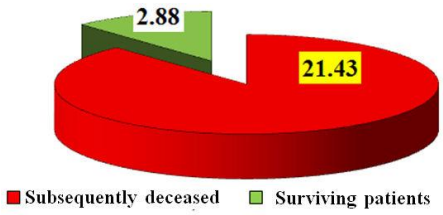

Fig. 6. Frequency of infectious complications in patients who subsequently died compared to survivors

TABLE IV

Frequency of variceal bleeding in patients who later subsequently compared to survivors

Stage	Patients who subsequently died				Survivors				χ2, p
	With VB		Without VB		With VB		Without VB		
	N	%	n	%	n	%	n	%	
1 year	4	**28,57**	10	*71,43*↓	11	*7,91*↓	128	**92,09**	χ2=6,14 **p=0,01**
2 years	3	**21,43**	11	*78,57*↓	6	*4,32*↓	133	**95,68**	χ2=3,99 **p=0,045**

DISCUSSION

Identification of patients with increased risk of death is extremely important in order to improve prognosis.

Terg and collaborators reported an increased mortality in patients with bilirubin over 4 mg / dL and serum creatinine over 1 mg / dl compared with those with low risk factors (23% vs. 6.5%) (10). Recent study by Luke et al. (11) showed that renal dysfunction, defined by a creatinine level higher than 1.5 mg / dl was the most important predictor of mortality in patients with SBP.

Almost one third of patients diagnosed with liver cirrhosis complicated with SBP develops hepato-renal syndrome, with a high mortality rate. Terg's recent study (10) showed that renal dysfunction, defined by a creatinine level higher than 1.5 mg / dl was the most important predictor of mortality in patients with SBP. They also demonstrate that hyponatremia is an independent risk factor of predicting mortality in cirrhotic patients with SBP (10, 12). MELD score predicts survival in cirrhotic patients at three months and in those enrolled in liver transplantation.

In those patients who subsequently died, the mean values of leukocytes in the blood, at both study onset and at examination 1 and 2 years, were significantly higher. Mean value of leukocytes and PMN in ascites fluid was significantly higher (about 4-5 times) in patients who subsequently died compared with the survivors.

Mean creatinine was significantly higher in patients who subsequently died, both at study onset and at examination 1 year and 2 years, assessing that renal dysfunction is a predictor of mortality in patients with decompensated cirrhosis.

Frequency of cases with hepato-renal syndrome at both examinations 1 year later and 2 years later was significantly higher in patients who later died.

Cases of infectious complications (other than SBP) were significantly more numerous (about 10 times) in patients who subsequently died (21.43% vs. 2.88%, p = 0.01). SBP frequency was significantly higher (3 to 7 times) in patients who later died, in all three stages of examination.

The frequency of variceal bleeding was significantly higher in patients who subsequently died, as also shown in the current literature (13).

Identification of new prognostic factors in patients with liver cirrhosis is important because recent research shows significant changes in the epidemiological profile, the number of multidrug-resistant bacteria is very high. Empirical therapy has been proved to be ineffective in these cases, antibiotics should be used according to the antibiogram results.

CONCLUSIONS

In our study the main conclusins are:

SBP mortality decreased substantially from 90% in 1970 to 10-30% today, but SBP still remains a complication with poor prognosis; Identification of patients with increased risk of death is extremely important to improve prognosis; Peripheral leukocytosis, and low hemoglobin in the ascites fluid, can be considered predictive of mortality in patients with SBP; Child-Pugh score, increased levels of bilirubin, creatinine, and hyponatremia are independent risk factors of mortality in patients with SBP; Bacteremia and the lack of therapeutic response are independent risk factors of mortality associated with SBP; Recent history of variceal gastrointestinal hemorrhage, severity of infection and the degree of hepatic and renal failure influence short-term prognosis of patients with SBP.

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VALIDATION AND EVALUATION OF MEMORIAL SYMPTOM ASSESSMENT SCALE SHORT-FORM (MSAS-SF) IN A ROMANIAN COHORT OF HEMODIALYSIS PATIENTS

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VALIDATION AND EVALUATION OF MEMORIAL SYMPTOM ASSESSMENT SCALE SHORT-FORM (MSAS-SF) IN A ROMANIAN COHORT OF HEMODIALYSIS PATIENTS (Abstract): **The aim** of this study was to validate the MSAS-SF in a Romanian hemodialysis (HD) population and to assess the prevalence and severity of physical and psychological symptoms of patients. **Materials and methods:** 102 stable patients (41 males, 61 females, mean age 52.51 ± 12.02 years), who were treated with hemodialysis (HD) three times weekly completed questionnaires on prevalence and severity of symptoms. To determine symptoms prevalence and severity for HD patients MSAS-SF was used. Cronbach`s alpha reliability test was performed to assess internal consistency of the MSAS-SF. **Results:** The Cronbach's alpha reliability score for the total number of items of the MSAS-SF was 0.838, while for the subscales of MSAS-SF the reliability scores ranged from acceptable to very good. The top three symptoms experienced by hemodialysis patients were lack of energy, followed by pain, and worrying. The most frequently reported physical symptoms included lack of energy (69.6%), pain (68.6%), and numbness /tingling in hands/feet (64.7%), difficulty sleeping (63.7%), dry mouth (61.8%), dizziness (58.8%), and problems with sexual interest or activity (52%). The most frequent psychological symptoms included worrying (65.7%), feeling sad (59.8%), feeling nervous (50%), and feeling irritable (40.2%). **Conclusions:** These results support the validity of the MSAS-SF in the assessment of Romanian patients undergoing hemodialysis. Hemodialysis patients have important psychological and physical symptoms burden. **Keywords:** END STAGE RENAL DISEASE, SYMPTOM BURDEN, HEMODIALYSIS, MSAS-SF.

The number of patients diagnosed with chronic kidney disease (CKD) is increasing, with recent studies suggesting that >10% of the population may have CKD (1). The total life expectancy of an individual with ESRD is only one-fourth to one-fifth that of the general population (2).

Physical and emotional symptoms are major concerns of chronic illnesses and represent a central role in patients` experience with life-threatening diseases. High symptom burden is associated with reduced health related quality of life (HRQoL) (3).

ESRD patients have a high burden of

disease (particularly cardiovascular co-morbidities) affecting their quality of life (QoL) and dramatically shortening life expectancy (4).

Stressors for individuals on hemodialysis can be treatment-related such as dietary and fluid restrictions and taking different medications, or psychosocial such as alterations in sexual function, changes in self-confidence, and panic of death (5). The symptoms that usually occur in treatment of dialysis are loss of appetite that may lead to a quick occurrence of metabolic disorders, mood reduction that also may result in willingness to give up treatment (6). Depressive symptoms and depression are the most frequent psychological problems reported among ESRD patients being treated by hemodialysis (*7*). Depression is significantly associated with mortality in dialysis patients (8).

Our objective was to validate, for the first time, the Memorial Symptom Assessment Scale (MSAS-SF) in a Romanian HD population and to assess the prevalence and severity of physical and psychological symptoms of HD patients using the MSAS-SF.

MATERIAL AND METHODS

We conducted an observational cross-sectional study, that comprised hemodynamic stable patients on hemodialysis treatment for at least six months from the Dialysis Center "Dr. C.I. Parhon" University Hospital, Iasi, Romania. The exclusion criteria were age under 18 years, psychiatric conditions, or any other medical condition that could severely interfere with completion of the scales. All included subjects gave informed consent before participating in the study. The study was approved by the Hospital's Ethical Committee and was performed in agreement with Helsinki's declaration of human rights.

Demographic characteristics, biochemical and dialysis parameters were obtained from the patients' medical records. All patients received HD a four hours/session, three times/week.. Kt/v represents a way of measuring dialysis adequacy. The current Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines on hemodialysis adequacy recommend that the minimally adequate dose for conventional, thrice-weekly treatment be a target dose of 1.4 (9). Interdialytic weight gain (IDWG) was calculated as the mean of IDWG of the last three dialysis sessions. Systolic and diastolic blood pressure values (SBP, DBP) were averaged, as well, from values before, during and after the finalization of the last three dialysis sessions.

The Memorial Symptom Assessment Scale - Short Form (MSAS-SF) (10) is a patient-rated instrument in which patient's rate symptom distress associated with 27 physical symptoms and the frequency of 4 psychological symptoms during the 7 past days. MSAS-SF subscales include: the global distress index (GDI) (4 psychological symptoms: feeling sad, worrying, feeling irritable, and feeling nervous, and 6 physical symptoms: lack of energy, pain, lack of appetite, feeling drowsy, constipation, dry mouth); the physical symptom distress score (PHYS) comprises 12 prevalent physical symptoms (lack of energy, pain, lack of appetite, feeling drowsy, constipation, dry mouth, nausea, vomiting, change in taste, weight loss, feeling bloated, and dizziness; the psychological symptom distress score (PSYCH) includes 6 prevalent psychological symptoms (worrying, feeling sad, feeling nervous, difficulty sleeping, feeling irritable, and difficulty

concentrating).

The data were analyzed using SPSS version 17.0 for Windows. Frequencies and descriptives statistics of the demographic and symptoms data of the study population were calculated. Cronbach alpha test was performed to assess internal consistency of the MSAS-SF. Independent Samples t test was used to compare means in male/female patients in MSAS-SF dimensions.

RESULTS

102 patients (41 males and 61 females) were enrolled in our study. The mean age was 52.51±12.02 years; patients` mean hemoglobin level 10.99±1.06 and mean kt/v 1.47±0.27. Patients had received renal replacement treatment (HD) for a mean duration of 10.79 ± 5.7 years (tab. I). The translated version into Romanian of the MSAS-SF proved to be comprehensible and easy to complete during the testing. All the participants considered that the items included in the test were relevant to patients with dialysis. Cronbach alpha reliability coefficients ranged from acceptable to very good, for all subscales of MSAS-SF. The Cronbach`s alpha coefficient for the total number of symptoms (MSAS-SF TNS) was 0.838. For GDI dimension (Global Distress Index) Cronbach`s alpha coefficient was 0.739, for PHYS dimension (Physical Symptom Subscale) was 0.648 and for PSYCH dimension (Psychological Symptom Subscale) the Cronbach`s alpha coefficient was 0.749.

The top three frequent symptoms experienced by hemodialysis patients were lack of energy, followed by pain, and worrying (tab. II).

The most frequently reported physical symptoms included lack of energy (69.6%), pain (68.6%), and numbness/tingling in hands/feet (64.7%), difficulty sleeping (63.7%), dry mouth (61.8%), dizziness (58.8%), and problems with sexual interest or activity (52%). The most frequent psychological symptoms that included worrying (65.7%), feeling sad (59.8%), feeling nervous (50%), feeling irritable (40.2%). The symptoms reported as most severe were difficulty sleeping (25.5%), numbness/tingling in hands/feet (11.8%), constipation (11.8%), itching (10.8%). HD patients seemed to ranked *difficulty sleeping* as the number one symptom that causes very much of distress. Also, we analysed the means scores of MSAS-SF dimensions in relation to gender of our included patients. There was no significant differences in symptoms frequencies between males and females for the all three MSAS-SF dimensions: GDI, PHYS and PSYCH ($p>0.050$) (tab. III).

TABLE I

Baseline characteristics of the included patients

	Males (N=39)	Females (N=63)	Total (N=102)
Age (years)	51.31±13.56	53.25±11.02	52.51±12.02
Hemoglobin (g/dl)	10.94±1.11	11.02±1.03	10.99±1.06
Kt/V	1.34±0.19	1.55±0.28	1.47±0.27
IDWG (kg)	1.85±0.61	1.76±0.59	1.80±0.60
Albumin (g/dl)	4.15±0.56	4.07±0.40	4.11±0.48
Dialysis vintage (years)	10.00±4.71	11.42±6.40	10.79±5.71

(Mean±Standard deviation) Kt/v- dialysis efficiency; IDWG (interdialytic weight gain)

TABLE II

Ranking and frequencies of symptoms MSAS-SF

Symptoms	Rank Ordering	Yes N (%)	No N (%)
Lack of energy	1	71 (69.6)	31 (30.4)
Pain	2	70 (68.6)	32 (31.4)
Worrying	3	67 (65.7)	35 (34.3)
Numbness/tingling in hands/feet	4	66 (64.7)	36 (35.3)
Difficulty sleeping	5	65 (63.7)	37(36.3)
Dry mouth	6	63 (61.8)	39 (38.2)
Feeling sad	7	61 (59.8)	41 (40.2)
Dizziness	8	60 (58.8)	42 (41.2)
Problems with sexual interest or activity	9	53 (52)	49 (48)
Feeling nervous	10	51 (50)	51 (50)
”I don t look like myself”	11	49 (48)	53 (52)
Difficulty concentrating	12	46 (45.1)	56 (54.9)
Sweats	13	45 (44.1)	57 (55.9)
Itching	14	43 (42.2)	59 (57.8)
Lack of appetite	15	42 (41.2)	60 (58.8)
Feeling irritable	16,5	41 (40.2)	61 (59.8)
Cough	16,5	41 (40.2)	61 (59.8)
Constipation	17	39 (38.2)	63 (61.8)
Hair loss	18	36 (35.3)	66 (64.7)
Swelling arms or legs	19	35 (34.3)	67 (65.7)
Feeling bloated	20	31 (30.4)	71 (69.6)
Shortness of breath	21	29 (28.4)	73 (71.6)
Feeling drowsy	22	26 (25.5)	76 (74.5)
Change in the way food tastes	23,5	25 (24.5)	77 (75.5)
Changes in skin	23,5	25 (24.5)	77 (75.5)
Nausea	24	24 (23.5)	78 (76.5)
Vomiting	25	22 (21.6)	80 (78.4)
Weight loss	26	19 (18.6)	83 (81.4)
Difficulty swallowing	27	12 (11.8)	90 (88.2)
Diarrhea	28	9 (8.8)	93 (91.2)
Mouth sores	29	7 (6.9)	95 (93.1)

TABLE III

Mean Scores of MSAS-SF Dimensions in Relation to Gender of Patients (N=102)

MSAS-SF Dimensions	Gender		p
	Male	Female	
Global Distress Index	11.30±7.04	10.74±6.64	0.688
Physical Dimension	10.56±6.63	10.23±6.24	0.801
Psychological Dimension	7.49±5.71	6.58±4.71	0.386

DISCUSSION

All three MSAS-SF subscales, namely GDI, PHYS and PSYCH had alpha Cronbach coefficients higher than 0.60, showing good reliability.

Symptom management became the focus of care for patients with advanced kidney disease including patients receiving renal replacement therapy. Lack of energy, pain and worrying were the most important physical symptoms, respectively psychological in our dialysis patients. In long-term dialysis patients, fatigue represents one of the common and troublesome problems that may influence the quality of life. Duration of dialysis is a factor that positively correlates with fatigue. Post-dialysis fatigue has been attributed to essential components of dialysis, i.e. ultrafiltration and diffusion, and the consequent rapid hydraulic and molecular flux (11).

Pain is common in patients who are in long-term hemodialysis (12). Headache is very common, usually restricted to the HD session. Typically, headache occurs in the last hour of the session (13). The incidence of headache was higher when patients had their treatment times increased from 4 to 5 h (14). Pain of muscoloskeletal origin was also found the most frequent form of chronic pain reported by patients who were on maintenance hemodialysis. Abdominal pain was the most gastrointestinal symptom, reported by 72% of HD patients (12).

Hemodialysis patients are subjected to multiple psychosocial and physiological stressors and may be threatened with many potential losses and lifestyle changes. In a recent research, limited vacantation, fatigue, uncertainty about future were the real causes for worry in HD patients (15).

It seems that the most distressful symptom reported by our hemodialysis patients was difficulty sleeping. From 65 patients who reported difficulty sleeping, a number of 26 mentioned this symptom as causing very much of distress. Sleep disorders are quite common in the hemodialysis patients, especially those who are anemic and hypoalbuminemic (16).

Our findings are consistent with those of other studies investigating the symptoms of chronic renal disease managed with dialysis. Weisbord et *al.* (17) found that HD patients reported a wide range of symptoms, including lack of energy (74%), feeling drowsy (63%), numbness/tingling in hands/feet (63%), dry mouth (58%), pain (53%), itching (53%).

In a study of assessing symptom prevalence in cancer patients, Tranner et al (18) found that lack of energy (83%), dry mouth (82%), pain (78%) were the most frequent physical symptoms. If findings from this study are compared with our study, then lack of energy and pain were equally common in both population, but dry mouth was much more prevalent for cancer population than for HD patients.

CONCLUSIONS

In our study the total number of symptoms experienced by our study patients was high and the most prevalent symptoms proving not to be the most distressing. Our results demonstrated that physical and emotional symptoms are prevalent and can be severe in patients who receive maintenance hemodialysis. MSAS-SF is a usefull tool in the assessment of physical and psychologial symptoms in Romanian patients undergoing hemodialysis.

ACKNOWLEDGEMENTS

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CONGENITAL HEART MALFORMATIONS IN NEWBORN BABIES WITH LOW BIRTH WEIGHT

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CONGENITAL HEART MALFORMATIONS IN NEWBORN BABIES WITH LOW BIRTH WEIGHT (Abstract): congenital heart malformations represent a public health problem, holding a significant percentage of the total of heart diseases. Beside the elevated frequency of the malformations, we also notice their occurrence in newborn babies with low birth weight, increasing, thus, the risk of complications and late therapeutic approach. **The goal** of the study was to highlight the general and particular aspects of cardiovascular malformations epidemiology in newborn babies with low weight at birth, the correlation of the malformations with implied genetic and environmental factors, assessing the complications and their procedures on the therapeutic management. **Material and methods**: Our study was performed on a group of 271 patients, hospitalized in the Department of Pediatric Cardiology of "Sf. Maria" Emergency Clinical Hospital for Children of Iasi, during January 2011 – December 2013. The patients were assessed based on anamnesis, clinical, biological and imagistic exam. **Results**: The study lot was divided according to the type of the structural defect: 95% of the patients were diagnosed with non-cyanogenic congenital heart malformations and 5% with cyanogenic congenital heart malformations. Regarding the patient's origin background, we notice an elevated frequency of the rural environment (71%). The incidence of the malformations was high in premature low birth weight (48%), followed by premature very low birth weight (22%). In evolution, congenital heart malformations often get more complicated heart failure, arterial hypertension and respiratory infections being most often met. Mortality was maximum in the first year of life, a third of the cases being associated with chromosomal malformations. **Conclusions**: Congenital heart malformations in newborn patients with low weight at birth represented an elevated percentage of 44.13% of the total of the cases hospitalized for cardiovascular diseases from the Department of Pediatric Cardiology of Iasi. Many cases were associated with other congenital malformations or different complications, being necessary an interdisciplinary collaboration to adequately monitor the anatom5ical and functional parameters and to ensure a somatic and mental development as normal as possible. **Keywords**: CONGENITAL HEART MALFORMATIONS, LOW WEIGHT AT BIRTH, COMPLICATIONS

Congenital heart malformations are the result of an abnormal embryogenesis, characterized by structure or function anomalies of the cardiovascular system (1). Of a big importance is the early diagnosis, before the settlement of the irreversible complications that create a vicious circle on infantile mortality and morbidity. Each

year in Romania over 20,000 prematurely born babies and new-born babies with low weight at birth are registered. Prematurity and its complications are accountable for more than a half of the neonatal deaths, representing one of the main causes of neonatal mortality in our country (3).

MATERIAL AND METHODS

The study was retrospectively performed on a group of 271 newborns with low weight at birth within the Department of Pediatric Cardiology of "Sf. Maria" Emergency Clinical Hospital for Children of Iasi, during January 2011 - December 2013. All patients were anamnestically, clinically, biologically and imagistically examined, being divided in 2 sub-groups: non-cyanogenic congenital heart malformations and cyanogenic congenital heart malformations.

RESULTS

In this study, the cases with congenital heart malformations in newborns with low weight at birth represented 44.13% of the total of cases hospitalized for cardiovascular diseases in the Department of Pediatric Cardiology of "Sf. Maria" Emergency Clinical Hospital for Children of Iasi, in the chosen period. From the background environment point of view, 192 (71%) patients came from rural environment and 79 (29%) patients from urban environment. Maternal anamnesis showed that 50% of these are young mothers (16-30 years of age) and only a percentage of 28% presents extreme ages (36-49 years of age). The incidence of malformations proportionally varied with age, being higher in the premature low birth weight (48%), very low birth weight (22%), extremely low birth weight (6%).

The analysis of maternal pathology with possible teratogenic implications demonstrated that chronic diseases were present in 21 (7.58%) in the mothers of the children diagnosed with congenital heart malformations: DZ (6 cases), hypothyroidism, (6 cases), epilepsy (2 cases), HTA pregnancy pre-existence (8 cases), TBC (1 case). Anti-epileptic and tuberculostatic chronic medication during pregnancy was present in 1% of the cases, alcohol chronic consumption – 0.8% and viral infections (rubella, varicella, toxoplamosis, TORCH infections) - 2%.

The study group was divided according to the type of structural defect (fig. 1): 95% non-cyanogenic congenital heart malformations and 5% cyanogenic congenital heart malformations.

In the non-cyanogenic congenital heart malformations subgroup, the highest frequency was met in atrial septal defect cases (with a percentage of 43.10), the majority being of OS type. As frequency, patent *ductus arteriosus* (23.28%) follows, than ventricular septal defect (13.36% - with localization on membranous septum level in most of the cases). The anomalies of the right ventricle exit tract (pulmonary valve stenosis) were presented in 7.54% of the cases and the anomalies of the left ventricle exit tract in 7.76% of the cases, of which 4.31 % presents aortic stenosis (of bicuspid valve type) and 3.45% presents coarctation of aorta (fig.2).

Upon sex repartition, we noticed a slight increase in favor of the males, (59% - the most frequent being diagnosed with ventricular septal defects, patent *ductus arteriosus*, pulmonary valve stenosis, coarctation of the aorta and atrio-ventricular canal defects; the females were noted in 41% of the cases being diagnosed in most of the cases with aortic stenosis (fig.3).

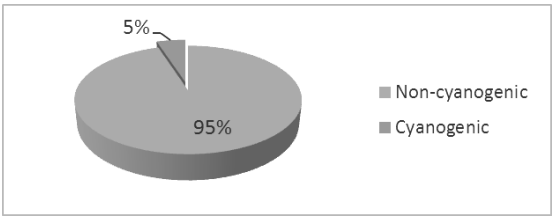

Fig. 1. Case distribution according to the type of the structural defect

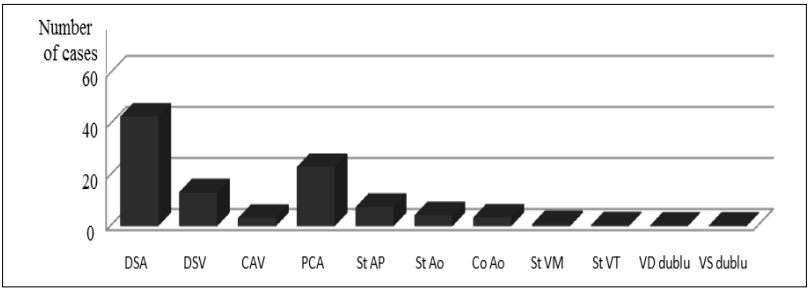

Fig. 2. Case distribution according to the different types of non-cyanogenic congenital heart malformations

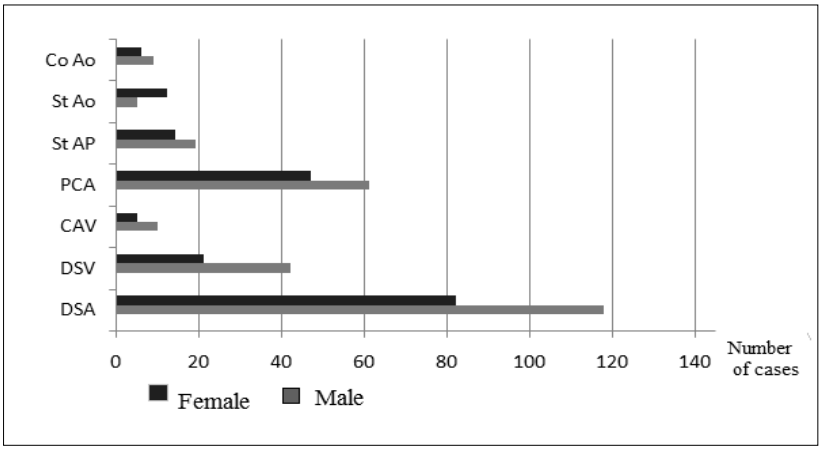

Fig. 3. Case repartition with non-cyanogenic congenital heart malformations according to the sex of the patients

Subsequent to the analysis of the anamnestic data regarding babies' gestational age and weight at birth, we notice that half of them had grade I prematurity, followed in equal proportions by cases with grade II prematurity and dismaturity.

The analysis of the electrocardiograms demonstrated that the majority of the patients were in sinus rhythm -97.37% and only 2.63% had irregular rhythm (atrial fibrillation). At the analysis of QRS complex morphology we noticed axis changes (16% - axis on the right 5% - axis on the left), atrial and ventricular hypertrophies and interventricular blocks (fig.4).

In evolution, non-cyanogenic CHM often complicated with IC, followed by pulmonary hypertension and respiratory condi-

tions (fig.5).

A number of 76 (17%) patients of the study subgroup were diagnosed with other congenital malformations. The analysis of associated genetic syndromes showed a predominance of chromosomal syndromes - 30 (6%) cases compared to monogenic ones -4 (0.89%) cases.

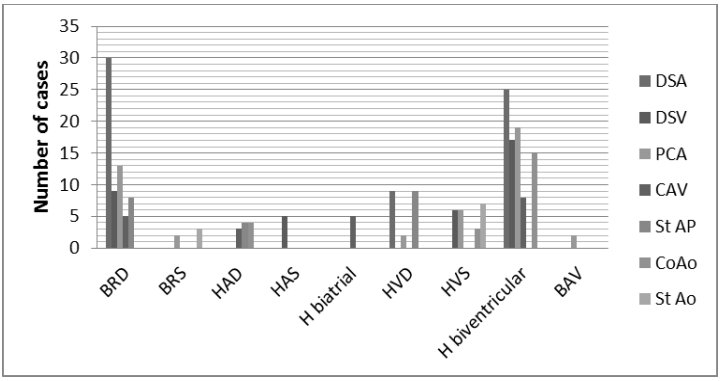

Fig. 4. Case repartition according to the electrocardiographic modifications

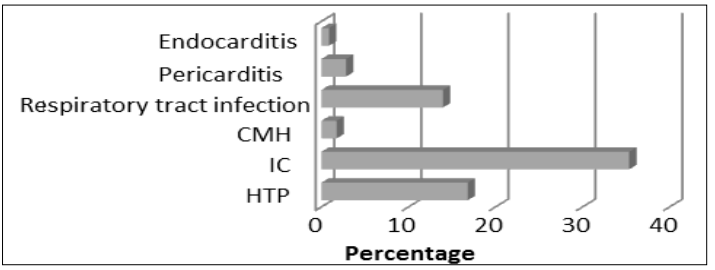

Fig. 5. Graphical repartition of the complications in patients diagnosed with non-cyanogenic congenital heart malformations

TABLE I

Genetic syndromes detected in children win non-cyanogenic congenital heart malformations

Chromosomal syndrome	Associated non-cyanogenic CHM	Number of total cases
Down	DSA OS	20
DiGeorge	DSA OS	4
Edwards	DSV	2
Turner	St AO	1
Cri du chat	PCA	1
Williams	St AP	1
Waardenburg	Co AO	1
Monogenic syndromes		
Marfan	DSA OS	1
Ehler Danlos	DSA OS	1
ADPKD	CAV	1
Dandy Walker	DSA OS	1

In the study subgroup of the cases with cyanogenic congenital heart disease, we noticed an increased frequency of cases with tetralogy Fallot (45%), followed by big vessel transposition (24%), Fallot pentalogy (19%). 0.2% of the total congenital heart disease represented the percentage in common for the cases that were diagnosed with common arterial trunk and sole ventricle (1 case each) (fig.6).

Repartition on sexes of cyanogenic congenital heart disease showed a slight difference in favor of the females (57%); males (43%) are especially met in cases diagnosed with transposition of the great arteries and sole ventricle (fig.7).

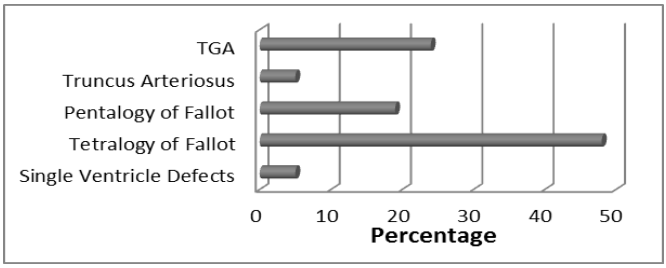

Fig. 6. Cyanogenic congenital heart disease case distribution

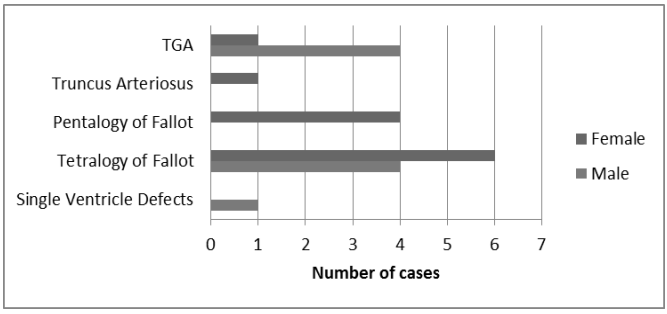

Fig. 7. Repartition to the sex of cases with cyanogenic congenital heart malformations

The X-ray exam brought a major contribution in Fallot tetralogy diagnosis (cord en sabôt-73% accompanied by pulmonary hyper-transparent - 55%) and transposition of the great arteries (narrowed superior mediastine 67%, ovoid heart 83% and pulmonary stasis 50%). In cases with sole ventricle and common arterial trunk, only an enlarged diameter of the heart and stasis aspect were described, requiring, thus, additional investigations.

At the analysis of the electrocardiograms, in 67% of the cases, we notice the deviation of the axis to the right, and in only 16% the intermediate axis. Right ventricular hypertrophy is presented in 7 cases of Fallot tetralogy of the 10 (64%) diagnosed, in 4 cases of transposition of the great arteries of the 6 (30.77%) non-diagnosed (30.77%) and in 1 case of Fallot Pentalogy of the 4 (25%) diagnosed. Biventricular hypertrophy was met in the case of the sole ventricle and common arterial trunk.

Three cases of syndromes were associated with other genetic syndromes: 2 cases with Di George syndrome and 1 case with fetal alcohol syndrome. Between 2011-2013, 18 children of non-cyanogenic CHM

group were operated (the most frequent defects being: DSV - 6 cases, PCA - 4 cases, and some 2 cases of CAV, Co AO, DSA, St AO), and 4 children were operated form the cyanogenic CHM group (3 cases with Fallot tetralogy and a case of TVM). An increased frequency of the surgery procedure was noticed in 2012 (fig.8).

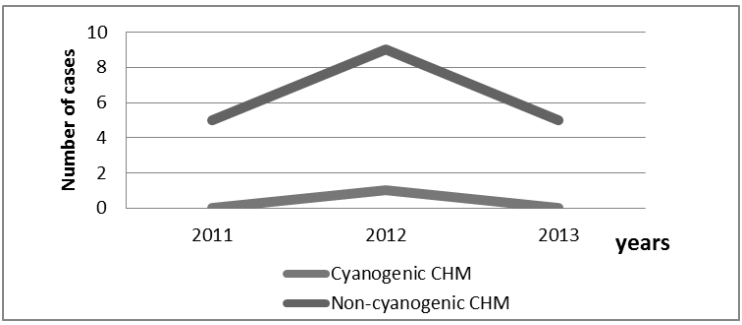

Fig. 8. Distribution on years of the corrective surgery procedure

The results of the anatomical and pathological exam were analyzed in 24 cases of the study group who died in the Department of Pediatric Cardiology of "Sf. Maria" Clinical Hospital for Children of Iasi during 2011-2013 and were confirmed by necropsy with congenital heart defect. The mortality of the cases diagnosed with congenital cardiovascular malformations was the maximum in the first year of life, when 96% of total number of deaths of the children with congenital heart defect was recorded. In the first week of life, 8% of the deaths of the children with congenital heart defect took place, and in the first month, half of deaths recorded in the first year (42%) (tab. II).

Regarding the sex of the deceased children with CHM, we notice a slight predominance of the males (13 cases - 54%). Congenital heart malformations that are the most implicated in tanatogenesis were represented by transposition of great arteries (40%), Fallot Tetralogy (25%) and atrioventricular canal defect (20%). One should note that, of the 24 deaths, a third was associated to chromosomal syndromes: Down syndrome (6 cases) and Edwards's syndrome (2 cases).

TABLE II

Death distribution in children with CHM according to the age the death took place

	Weeks	Months			Year
	0-1	1	1-3	3-12	>1
Number of cases	2	10	7	4	1
Percentage	8	42	29	17	4

DISCUSSION

Romania has got a prematurity indicator higher than many EU countries, the percentage of the babies born before 28 weeks and under 1,000 grams is approximately 1 – 1.5% of the total of living new-born. The figures vary depending on the geographical areas (Moldova and Muntenia having a higher prematurity rate). Besides the prematurity problem, the frequent occurrence of congenital heart malformations appears in this group of patients (3).

The pathology was dominant in the cardiovascular conditions diagnosed at a pediatric age in the Department of Pediatric Cardiology of „Sf. Maria" Clinical Hospital for Children of Iasi during 2011-2013, including only the group that had a low weight at birth, which resulted after anamnesis or hospital discharge note from neonatology department.

Upon analyzing the background environment, we notice a significant difference between urban and rural environment, control during pregnancy, early diagnosis of congenital heart disease and permanent monitoring being less often.

Different teratogens possible factors were tracked in 32 cases, representing 11.8% of the total CHM of the study group. Of the factors that intervened during pregnancy, we mention viral infectious factors (noticed in cases with DSA), chronic medication during some metabolic and neurological diseases, chronic alcohol consumption (met in cases with PCA and Fallot tetralogy). Thus, prevention by limiting exposure in collectivities, forbidding toxic substances consumption and a careful monitoring of the chronic diseases during pregnancy is very important (5, 11).

Most of the children included in the study, at birth were grade I (43%) premature, followed by dismature (25%) and a clinical state with APGAR score of 6-8 (80% of the cases) which demonstrates that the baby's sufferance is obvious since birth.

The analysis of the distribution on sexes shows a slight increase of the male children (55.71%) compared to female ones (44.29%).

Regarding the frequency of the different types of CHM in new-born with a low weight at birth; we recorded an elevated percentage of the atrial septal defect (43.10%) and a smaller percentage for patent *ductus arteriosus* (23.28%), which has the highest frequency in literature (2).

Dominant symptomatology was intense or moderated cyanosis with onset immediately after birth and/or decompensation of cardiacs globals with tachycardia, tachypnea, breathing in circulation, pulmonary rales, hepatomegaly. The weight deficit is an alarm sign suspecting congenital heart malformations: within non-cyanogenic congenital heart malformations, this one occupies 60% of the cases while in cyanogenic cardiac heart diseases the frequency is much more elevated (86%) (9).

Diagnose sensibility of the echocardiographical investigation in the studied cardiovascular malformations was much higher than electrocardiography and X-ray, but the results of the echocardiographical exam were interpreted only in the clinical and anamnesical context and in correlation with the results of EKG exam and X-ray (7).

The most frequent complications were the recurrent respiratory infections, heart failure, pulmonary hypertension and endocarditis (complication of ventricular septal defect in 2 cases, of patent ductus arteriosus - 1 case, atrioventricular canal defect - 1 case) (10, 12, 13).

Chromosomal malformations associated to congenital heart malformations have a different distribution compared to those mentioned in literature: ostium secundum atrial septal defects is associated with malformations of conjunctive tissue: Marfan and Ehlers Danles, aortic stenosis associated with Turner syndrome, Fallot tetralogy is frequently associated with Di George syndrome, atrioventricular canal defect with ADPKD. Hence, the genetic consultation during pregnancy is always indicated and very important in quantifying the postnatal risks (4, 6).

The death occurred in a number of 24

(8.8%) children; 8 (2.6%) of them having genetic syndromes, 12 (4%) being linked to pulmonary association and respiratory infections. The mortality of the cases was at maximum in the first year of life and none was operated (8).

CONCLUSIONS

In our study new-born patients with low weight at birth, diagnosed with CHM, represents an elevated percentage 44.13% of the total of hospitalized cases for cardiovascular diseases in the Department of Pediatric Cardiology of "Sf. Maria" Clinical Hospital for Children of Iasi, with a slight predominance of the males and of Grade I premature patients.

Under structural aspect, the cardiovascular malformations, analyzed in the descending order of the frequency, were: atrial septal defects, patent ductus arteriosus, ventricular septal defects, pulmonary valve stenosis, aortic stenosis, coarctation of the aorta, atrioventricular canal defects, tetralogy Fallot, transposition of great arteries, truncus arteriosus, sole functional ventricle.

Etiopathogenesis is pretty limited, but we have noticed the frequent association of Down syndrome with ostium secundum atrial septal defects and Edwards syndrome with ventricular septal defects, fact that leads to the increase of mortality of these cases.

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ERYTHEMA MULTIFORME - ETIOPATHOGENIC, CLINICAL AND THERAPEUTIC ASPECTS

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ERYTHEMA MULTIFORME - ETIOPATHOGENIC, CLINICAL AND THERAPEUTIC ASPECTS (Abstract): **Aim**: To present the epidemiological, etiopathogenic, clinical and therapeutic aspects in *Erythema multiforme* (EM). **Material and methods:** This is a 3-year retrospective study based on medical records of patients with EM admitted to the Dermatology Clinic. Forty patients were included in this study. The obtained data allowed the classification of patients according to their distribution by sex, age group, area of residence, etiology, clinical aspects, and type of administered treatment. **Results:** The prevalence of EM during the 3 study years was 0.4%. EM prevailed among the rural population, more frequently in women. The minimum age at which EM was diagnosed was 12 years and maximum age 78 years, with a peak incidence between 20-40 years old (37.5% cases). In 42.50% of the cases EM was drug-induced, and in 17.50% of cases it was caused by infection with herpes simplex virus (HSV). There were 18 cases of erythemotous–papular EM (45%), 14 cases of erythematous - vesiculobullous EM (35%) and 8 cases of recurrent EM (20%). All patients received treatment with antihistamines and nonspecific desensitizing agents. Systemic corticotherapy was used in 22 cases. Three patients received treatment with acyclovir. **Conclusions:** *Erythema multiforme* is a rare skin condition, easily diagnosed based on its characteristic clinical appearance, but remains a challenge for the physician in terms of establishing its causal agent. **Keywords:** ERYTHEMA MULTIFORME, STEVENS-JOHNSON SYNDROME, LYELL'S SYNDROME.

Polymorphic erythema or erythema multiform is part of the group of cutaneous – mucosal hypersensitivity reactions, a disease with multiple etiologies, with typical cutaneous and/or mucous clinical manifestations, the episode being self-limited, sometimes recurrent (1). The etiology and pathogenesis are not fully elucidated yet. Its various clinical manifestations range from the simple papular form (the characteristic lesions being erythematous papules, acral in distribution and symmetrical), and erythematous-vesiculobullous form (lesions are erythematous plaques with a vesicle or bulla arranged centrally that can develop a pathognomonic "target-like" or "cockade " appearance) to the severe bullous form (severe damage to the mucous membranes,

the damage to the skin membranes varying in extent and severity). The papular form is also called *Erythema multiforme* minor, and the other two forms, erythematous – vesiculobullous and severe bullous, are included into the *Erythema multiforme* major. Among the precipitating factors are medications and infectious agents. Recurrence may be common, most patients experiencing 1-2 recurrences per year. This is explained by the fact that in some cases EM can be associated with a persistent antigenic stimulation leading to repeated manifestations of the disease. In the medical literature EM is often confused with Stevens – Johnson syndrome (SJS) and Lyell's syndrome (toxic epidermal necrolysis = TEN). It was only in 1993 that a consensus regarding the classification of these three conditions was reached: SJS was separated from the EM and included in TEN, SJS and TEN being considered variants of the same disease, different only by the degree of severity (<10% of body surface area affected corresponds to SJS, and > 30% to TEN) (2).

The not completely understood pathogenesis and multiple etiology are other factors that make the knowledge of this disease to be of particular interest in medical practice.

MATERIAL AND METHODS

For this retrospective, open-label study we studied a group of patients admitted with a diagnosis of *Erythema multiforme* to the Dermatology Clinic of the Iasi "St. Spiridon" Emergency Hospital in the interval January 1, 2006 – December 31, 2008. The aim was to gather data on clinical manifestations, investigation methods and therapies used. Included in the study were 40 patients (some patients with multiple admissions during the 3 study years). The data obtained allowed the classification of patients according to their distribution by sex, age group, area of residence, etiology, clinical aspects, association with other diseases, type of administered treatment and its efficiency. To interpret the data different statistical concepts were used: arithmetic mean, percentage distribution.

RESULTS

During the interval January 1, 2006 – December 31, 2008 a total of 11,204 admissions (of which 5,311 day admissions) were recorded. Of these, 45 admissions were for the diagnosis and treatment of erythema multiforme, corresponding to a number of 40 patients. The prevalence of *Erythema multiforme* over the 3-year study period was 0.4%.

The distribution by area of residence revealed that EM was more common among the rural population (65% vs. 35%), corresponding to an urban/rural patient ratio of 1: 1.86.

There was a higher rate of EM among female patients, the male: female ratio being 1: 1.35 (fig. 1). According to the literature men are more frequently affected than women, more precisely with a male/female ratio ranging from 3:2 to 2:1.

The minimum age at which the occurrence of EM was recorded was 12 years and the maximum age 78 years. Our data were in agreement with literature data according to which all ages are affected, with a peak incidence between the 2nd and 4th decade of life, about 20% of patients being children and adolescents. In our study the peak incidence was indeed between 20-40 years, 37.5% of cases, but the highest percentage of all cases, 27.5%, was recorded in the 30 - 40 year age group. Fifteen percent of our patients were children and adolescents aged

less than 20 years, close to the 20% mentioned in the literature. However we found a significant percentage of cases in patients older than 50 years (32.5%), although EM is considered rare under the age of 3 and above 50 years (fig. 2).

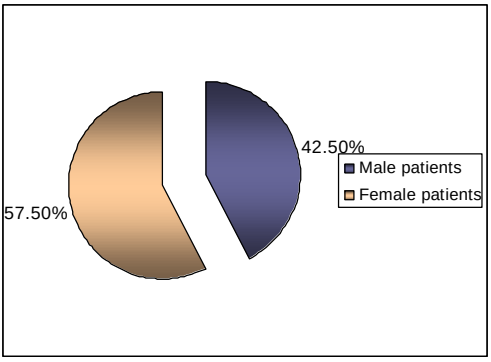

Fig. 1. Distribution of patients by gender

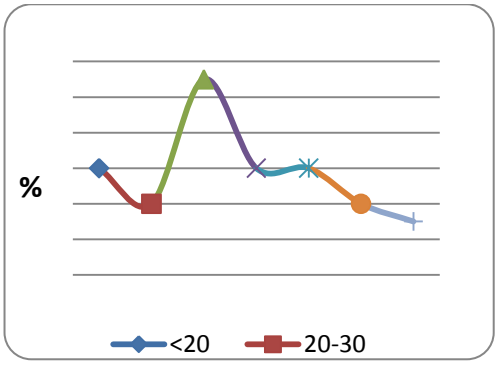

Fig. 2. Distribution by age groups

The onset was sudden in most cases, with rapid, progressive extension of lesions. Eight of the 40 patients had prodromes with constitutional symptoms (moderate fever or subfebrility, headache, asthenia, nausea, vomiting, etc.), some similar to a viral syndrome prodrome. As a result of this, some patients decided to self-medicate to relive these symptoms, making it difficult to differentiate the possible etiology of EM in these cases.

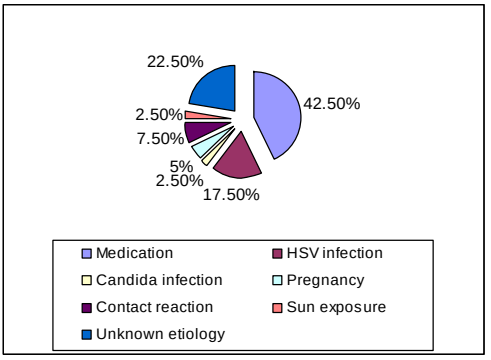

Fig. 3. Etiology of EM

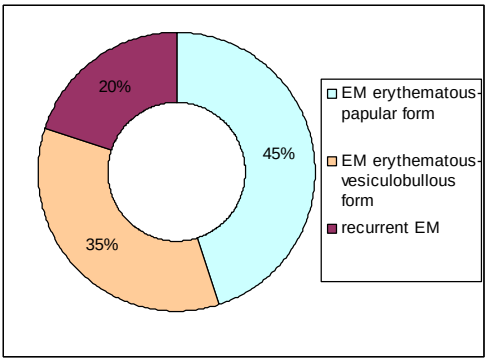

Fig. 4. Clinical forms of EM

As to the medication administered before the disease onset, of the 45 admissions 17 patients confirmed taking various drugs, including antibiotics, NSAIDs, antipyretics, combinations of NSAIDs and other compounds (acetylsalicylic acid in combination with paracetamol and caffeine), sedatives, estrogen receptor modulator, antifungal agents. In several cases the patients took more medicines simultaneously, making it more difficult to establish the one responsible for EM.

Another factor considered to be responsible for the occurrence of EM is herpes simplex virus (HSV) infection. From patient history we found that 7 patients showed signs of labial herpes simplex infection. Of these 7 patients, 4 were diag-

nosed with recurrent EM, 3 patients presenting the erythematous-vesiculobullous form and 1 the erythematous-papular form. These 4 patients presented signs of EM for 8 months to 13 years. Bacterial and fungal infections were also detected: due to beta hemolytic streptococcus (2 cases of streptococcal pharyngitis), candida infection (4 cases of oral candidiasis) infection with dermatophytes (*tinea pedis* = 4 cases, onychomycosis = 1 case). Other possible causes of EM resulting from patient history were: solvents, detergents and other irritant solutions, printing ink, sun exposure, pregnancy (fig. 3).

Of the total of 45 admissions, 2 patients had a check-up admission one month after the first presentation to the clinic, while other 2 patients with recurrent EM had 3 and 2 admissions, respectively, for distinct EM episodes, thus totaling 40 EM patients with 43 admissions. Twenty-two patients presented manifestations of EM erythematous-papular form, while 21 patients EM erythematous-vesiculobullous form. Of these, 8 patients were diagnosed with recurrent EM. One of the 2 patients with multiple admissions had 1 episode of erythematous- papular EM and 2 episodes of erythematous-vesiculobullous EM, while the other patient had 1 episode of EM erythematous-papular form and 1 episode of erythematous-vesiculobullous EM. Thus of the total of 43 admissions, 18 cases had erythematous- papular EM, 14 cases erythematous-vesiculobullous EM and 8 cases recurrent EM, totaling 40 patients (fig. 4). Of the 8 patients with recurrent EM 4 had symptoms of erythematous-papular type and the other 4 of erythematous-vesiculobullous type. In 4 cases (3 with erythematous-vesiculobullous form and 1 case with erythematous-papular form) the etiology was herpetic, in one case a contact reaction and in the other 3 cases the causal factor was not detected. Special attention deserved the 2 EM pregnant women (pregnancy weeks 28 and 31, respectively). One of these women was diagnosed with EM erythematous-papular form, and other with EM erythematous-vesiculobullous form. No other causal factor other than pregnancy could be related to the occurrence of this disease.

Typical acral distribution (fig. 5, 6) was present in 42 of the 43 cases, oral mucosa was affected in 7 cases (fig. 7), lesions on the trunk were present in 9 cases and genital lesions were found in 3 cases.

Fig. 5. Target-like lesions on palms

Fig. 6. Target-like lesions on soles

The lesions occurred progressively, symmetrically, with centripetal spreading, which is consistent with literature data. Acral involvement, another important feature in the diagnosis of EM, was present in 98% of cases; most commonly affected being the back of the hands, palms and soles in 70, 59 and 40% of the cases, respectively.

Pruritus was present in 12 cases, and pain in 6 cases, of which 3 with oral and genital mucosal involvement and 3 with the erythematous-vesiculobullous form. Burning sensation was present in 4 cases.

Twenty-two cases of minor EM and 21 cases of major EM were recorded.

Thirty-one (77.50%) of the 40 study patients presented associated diseases, the most common being *tinea pedis*, oral candidiasis, chronic venous insufficiency, essential hypertension, dyslipidemia.

All patients received treatment with antihistamines and nonspecific desensitizing agents (Ca + vitamin D_3, vitamin C). These were used as adjuvant agents in the treatment of EM which in this study consisted of topical therapy (dermatocorticoids) and systemic therapy (systemic corticosteroids). Systemic corticosteroid therapy was used in 9 cases with EM erythematous-papular form and in 13 cases with erythematous-vesiculobullous EM. The recommended dose was generally 0.5-1 mg/kg/day of prednisone or equivalent dose of methylprednisolone for a short period of time (10 days). Also used were: betamethasone (6 cases), dexamethasone (3 cases) and hydrocortisone hemisuccinate (5 cases). Treatment with acyclovir was administered in 3 cases of recurrent EM of herpetic etiology.

All 43 cases had a favorable outcome, the lesions disappearing after treatment. Of the eight cases of recurrent EM one patient had six episodes of EM over 8 months. In the other cases the episodes occurred about once a year. Adherence to treatment was good, given that the treatment was short-term, and the prognosis was favorable in all cases.

Fig. 7. Oral mucosal involvement

DISCUSSION

In the present study EM had some features consistent with literature data (incidence of less than 1%, more frequent in second to fourth decades of life, onset with prodromal symptoms in 20% of cases, favorable, self-limited course).

The 6 cases in children and adolescents under 20 years of age accounted for 15% of the cases, close to the percentage of 20% reported by other authors (3). A significant percentage (32.5%) of patients was diagnosed at an age older than 50 years, although EM is considered rare under the age of 3 and over 50 years.

Regarding the etiology of EM the drug factor was involved in a considerable percentage of cases, 42.50% (most commonly involved being the antibiotics – especially amoxicillin, and NSAIDs). According to several authors the drugs involved in the etiology of this disease are primarily the sulfonamides, followed by anticonvulsants

and other types of medication (antibiotics, antifungal agents, NSAIDs, and others) (4, 5, 6, 7, 8). In the literature there are numerous case reports of EM due to the use of drugs, but there is no test that can accurately demonstrate the association between a single case and a specific drug. In our study patients various drugs have been were administered concomitantly, making it difficult to differentiate the possible etiology of EM in these cases.

HSV infection, another reported etiologic factor associated with EM minor (9, 10) was found in only 7 cases, being associated with only one case of EM minor the erythematous–papular form. Other infectious causes are the infections with streptococcus, mycoplasma, dermatophytes, cytomegalovirus (CMV) and Epstein Barr virus (EBV) (11, 12, 13). Virus-drug interactions have been reported as a trigger factor for the occurrence of EM, these including CMV infection and terbinafine treatment (14) and EBV infection and amoxicillin treatment (15). In the present study the presence of beta-hemolytic streptococcal infection (2 cases of streptococcal pharyngitis), candida infection (4 cases of oral candidiasis), dermatophyte infection (*tinea pedis* 4 cases, 1 case of onychomycosis) was detected.

The clinical manifestations were key to diagnosis, cases of EM erythematous-papular form and erythematous- papulovesicular form, recurrent EM and EM during pregnancy being recorded. Physical examination was sufficient to establish the diagnosis in all cases. In EM major the mucous membranes can be also involved: oral mucosa showing *bullae* that progress to painful erosions, genital mucosa by vulvitis or balanitis, and conjunctival mucosa by conjunctivitis or corneal ulceration. These can lead to complications such as dehydration and fluid and electrolyte imbalances, secondary bacterial infections, ocular complications (corneal ulcers, anterior uveitis, corneal opacities, etc.). Scars (especially on mucosal lesions), and strictures (esophageal, lower respiratory tract, urethral, vaginal, anal) are less common. Oral mucosal involvement was found in 16% of patients and genital mucosal involvement in 7% without complications.

For the diagnosis of EM laboratory tests are not highly specific. However, the results of routine investigations were consistent with the data in the literature, revealing inflammatory syndrome, mild leukocytosis, mild anemia, and hypereosinophilia in most cases.

With regard to the treatment of EM, mainly used was corticoid therapy, both systemic and topical. Although the literature states that its use is controversial being even contraindicated in the mild forms of disease and the most severe may increase the risk of complications without improving the prognosis, in this study the use of corticosteroids did not lead to complications, with favorable outcome in all cases. Treatment with acyclovir was administered in 3 cases of recurrent EM of herpetic etiology.

CONCLUSIONS

Erythema multiforme is a rare skin condition, easily diagnosed based on the characteristic clinical appearance, but remains a challenge for the physician with respect to establishing the causal agent, in the literature most cases being considered idiopathic. In the present study the drug factor was incriminated in almost half of the cases (42.50%), but we cannot say with certainty that there is a cause- effect relationship

between administered drug and disease occurrence. The presence of prodromal symptoms in a significant number of cases (20%) and self-medication in these cases before presentation to the doctor make impossible the accurate detection of the trigger factor. The therapeutic goals were EM cure and prophylaxis for the prevention of recurrences. In cases of EM major interdisciplinary collaboration is important as ophthalmology, ENT, gynecology, urology, gastroenterology consultations may be required.

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DIRECT ACTING ANTIVIRAL (DAA) THERAPY IN REAL WORLD HCV COMPENSATED CIRRHOSIS

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PATIENT FIRST: DIRECT ACTING ANTIVIRAL (DAA) THERAPY IN REAL WORLD HCV COMPENSATED CIRRHOSIS (Abstract): **Aim:** Given the effectiveness of direct acting antiviral (DAA) therapy in patients with chronic hepatitis C virus (HCV) infection, we aimed to describe the demographic, clinical and biological characteristics of our patients who received treatment with direct acting antivirals during December 2015-October 2016 and September 2017-January 2018. **Material and Methods:** We performed a prospective study of 186 patients with chronic hepatitis C who have undergone treatment with paritaprevir/ombitasvir/ritonavir and dasabuvir with or without ribavirin during December 2015 - October 2016 (group 1) and September 2017 - January 2018 (group 2). The inclusion and exclusion criteria were established according to the national protocol. Demographic, clinical and biological data were recorded before starting treatment. All patients underwent upper gastrointestinal endoscopy and abdominal ultrasound. The determination of HCV genotypes and subtypes was realized by the Abbott RealTime HCV assay. The presence of mild or advanced fibrosis, cirrhosis, the viral necro inflammatory activity and the presence of nonalcoholic steatohepatitis (NASH) were assessed by FibroMax non-invasive liver tests: ActiTest, FibroTest and NashTest. **Results:** The mean age was 58.7 years for the first group and 59.6 for the second group, with the predominance of female gender in both cases. Genotype 1b was present exclusively in the first group. Median HCV RNA at baseline was 1 264 123.180 IU/mL for the first group and 1 678 718.97 IU/mL for the second group. No patients were HBV or HIV co-infected. Most patients were naive to interferon therapy. The comorbidity rate was increased in both groups: 28.82% *vs.* 49.3% had cardiovascular diseases, 23.41% *vs.* 14.66% had diabetes mellitus (with or without insulin requirement), 2.7% *vs.* 10.6% had thyroid dysfunction, 1.8% *vs.* 0% had dermatological disease, 0.9% *vs.* 5.33% had psychiatric disorders, 1.8% *vs.* 1.33% had a history of cured neoplasm, 1.8% *vs.* 2.66% had pulmonary diseases and 0.9% *vs.* 1.33% had hematological diseases. No patient had a history of treated hepatocellular carcinoma. In both groups, the maximum number of drugs administered simultaneously with the antiviral therapy was 4. The necro inflammatory activity assessed by ActiTest highlights a high rate for severe activity for both groups - 66.6%. The prevalence of concomitant NASH assessed by NashTest (N2-severe inflammation) was 50% in group 1 and 53.3% in group 2. There were slight differences between the two groups in terms of biological constants, but without any statistical significance. **Conclusions:** The partial results of our study show a higher prevalence of HCV infection among people born

between 1946 and 1964 (baby boom generation), especially women. In this geographical area, genotype 1b was found exclusively. Most patients, naïve to interferon therapy, associate numerous comorbidities and simultaneous treatments that may decrease adherence to therapy due to the possibility of significant drug-drug interactions, requiring optimal therapeutic management and close monitoring. **Keywords**: DIRECT ACTING ANTIVIRALS, HEPATITIS C VIRUS INFECTION, COMORBIDITIES.

Hepatitis C virus (HCV), discovered in 1989, is an enveloped positive-sense single-stranded RNA virus recognized as the main causative agent of chronic liver disease, cirrhosis and hepatocellular carcinoma (1, 2). The global prevalence of HCV estimated by WHO is approximately 2.5% (185 million people), with a higher rate in Africa and Asia compared to North America, Europe and Australia (1,3,4). HCV related liver failure is the main indication of liver transplantation in the United States, Western Europe and Japan (2,5,6).

In the presence of humoral and cellular immune response directed against viral proteins, 70-100% of patients remain HCV-RNA positive secondary to acute infection due to the immune system's ability to circumvent the virus (7,8,9). Heterogeneity of HCV genome arises as a result of DNA-dependent RNA polymerase (NS5B) that causes viral replication to mis-incorporate ribonucleotides with a substitution rate of about one million more than the replacement rate for the human genome (10).

Most patients are asymptomatic or have nonspecific manifestations: fatigue, myalgia, arthralgia and weight loss. Evolution is slowly progressive to liver cirrhosis, hepatocarcinoma or death (9, 11).

Interferon (INF) has been the standard treatment for about 25 years. It was first approved in HCV patients in 1991, although its antiviral activity was first described in 1957. Under interferon monotherapy, the SVR rate increased from 5 to 20% and up to 40-80% with pegylated interferon plus ribavirin (12).

Knowing the replicative cycle of hepatitis C virus, the role of structural and non-structural proteins and their localization in the cell allowed the discovery of compounds with specific antiviral activity.

The main targets are the NS5A replication complex (daclatasvir, ledipasvir, ombitasvir, elbasvir, velpatasvir), the NS5B polymerase (sofosbuvir, dasabuvir) and the NS3/4A protease (simeprevir, paritaprevir, asunaprevir, grazoprevir) (10,13).

The main goal of antiviral therapy is to obtain suppression of the virus replication 12 weeks after the end of antiviral therapy. Clinical trials conducted so far describe high tolerability and safety profile with RVS being achieved in > 95% of patients. The therapeutic failure may occur secondary to the selection of resistant mutants that cause the recurrence of viral replication (14-16).

There are currently several regimens available with an equivalent safety profile: sofosbuvir/ribavirin, sofosbuvir/ simeprevir +/- ribavirin, sofosbuvir/daclatasvir +/- ribavirin, sofosbuvir/ledipasvir +/- ribavirin, ombitasvir/paritaprevir/ritonavir/dasabuvir +/- ribavirin, sofosbuvir/velpatasvir +/- ribavirin and elbasvir/grazoprevir +/- ribavirin (17).

The regimens available in Romania are represented by ledipasvir/sofosbuvir and paritaprevir/ombitasvir/ritonavir/dasabuvir. Currently, limited availability and in-

creased costs of direct-acting antivirals require prioritization of therapy in patients with advanced liver disease.

Given the effectiveness of direct oral antiviral therapy in patients with chronic HCV infection, we aimed to describe the demographic, clinical and biological characteristics of our patients with chronic HCV infection who received treatment with paritaprevir/ ombitasvir/ritonavir/dasabuvir with or without ribavirin during December 2015-October 2016 and September 2017-January 2018.

MATERIAL AND METHODS

We performed a single-center prospective cohort study of 186 patients with chronic hepatitis C who have undergone treatment with paritaprevir/ombitasvir/ritonavir and dasabuvir with or without ribavirin during December 2015 - October 2016 (group 1) and September 2017 - January 2018 (group 2).

According to the national protocol, inclusion criteria during December 2015 - October 2016 were: genotype 1b interferon naive or experienced patients with compensated cirrhosis - F4 (Child Pugh score≤6), detectable HCV-RNA levels, absence of alcohol consumption or other intravenous/intranasal illicit substances in the last 3 months, absence of significant drug-drug interaction.

During September 2017 - January 2018, inclusion criteria were: interferon naive or experienced patients with advanced fibrosis (F3) or compensated cirrhosis - F4 (Child Pugh score≤6), detectable HCV-RNA levels, absence of alcohol consumption or other intravenous/intranasal illicit substances in the last 3 months, absence of significant drug-drug interaction; interferon naive or experienced patients with mild fibrosis - F2 with mixt cryoglobulinemia, chronic kidney disease, non-Hodgkin lymphoma, hemophilia and thalassemia major.

Patients with hepatocellular carcinoma (METAVIR score F2, F3 or F4) could be treated if they had a transplant indication or if relapse was not found (CT/MRI) more than 6 months after ablation or resection.

Patients with decompensated cirrhosis (Child Pugh score >6) and those with dysplastic nodules were excluded.

Demographic, clinical and biological data (gender, age, weight, height, body mass index, status after interferon therapy, comorbidities, concomitant medication, complete blood count, INR, total bilirubin, AST, ALT, GGT, glucose levels, creatinine, AFP) were recorded before starting treatment.

All patients underwent upper gastrointestinal endoscopy and abdominal ultrasound.

The determination of HCV genotypes and subtypes was realized by the Abbott Realtime HCV assay. The presence of mild or advanced fibrosis, cirrhosis, the viral necro inflammatory activity, and NASH was assessed by FibroMax non-invasive liver tests: ActiTest, FibroTest and NashTest.

To present our data, we used univariate descriptive statistic tools.

In the first group were included 111 patients with compensated liver cirrhosis (Child Pugh score ≤6). The diagnostic of cirrhosis was based on clinical, biological and imagistic (abdominal ultrasound) criteria. All patients had a METAVIR score according to FibroTest F3 (26.12%), F3-F4(2.7%) or F4(70.27%). In the second group were included 75 patients with advanced fibrosis or compensated cirrhosis (score Child Pugh <=6) with a METAVIR score according to FibroTest F3(41.3%), F3-F4(6.6%) or F4(52%) (tab. I).

TABLE I.
Demographic, clinical and biological characteristics (1 month prior to DAA therapy)

	Group 1` Mean (SD) / Number (%)	Group 2 Mean (SD) / Number (%)	p value
Total	111	75	p=0.03
age	58.7 (8.35)	59.6 (9.6)	p=0.01
female gender	61 (54.9%)	46 (60.52 %)	p=0.3
BMI	27.45 kg/m2 (3.75)	27.38 kg/m2 (4.37)	
genotype	1b (100%)	not required	p=0.004
HCV-RNA	1264123.180 (1835368.49)	1678718.97 (299474686)	
Fibromax (METAVIR score)			
F4	78 (70.27%)	39(52%)	
F4/F3	3 (2.7%)	5 (6.6%)	
F3	29 (26.12%)	31 (41.3%)	
F2	-	0	
hemoglobin	14.3 g/dl (1.76)	14.37 g/dl (1.44)	p=0.1
platelets	154126.12/mm3 (66031.13)	173373.33/mm3 (77401.85)	p=0.004
INR	1.26 (1.29)	1.1 (0.13)	p=0.1
ALT	106.96 U/L (73.59)	99.64 U/L (58.37)	p=0.7
AST	96.3 U/L (62.64)	82.79 U/L (41.4)	p=0.5
GGT	89.32 U/L (68.48)	90.76 U/L (75.68)	p=0.3
BT	1.05 mg/dl (0.45)	0.9 mg/dl (0.4)	p=0.3
glucose	114.68 mg/dl (35.09)	120.46 mg/dl (69.58)	p=0.03
creatinine	1.17 mg/dl (4)	0.76 mg/dl (0.12)	p=0.06
albumin	4.45 g/dl (2.68)	4.15 g/dl (0.39)	p=0.01
AFP	15.5 ng/ml (20.28)	21.03 ng/ml (34.69)	p=0.08
Comorbidities			
➢ cardiovascular diseases	32 (28.82 %)	37 (49.3 %)	
➢ diabetes + insulin	18 (16.21 %)	7 (9.33 %)	
➢ diabetes - insulin	8 (7.2 %)	5 (5.33 %)	
➢ psychiatric disorders	1 (0.9%)	4 (5.33%)	
➢ neurologic diseases	0	1 (1.33%)	
➢ thyroid dysfunction	3 (2.7%)	8 (10.6 %)	
➢ dermatologic diseases	2 (1.8%)	0	
➢ hematological diseases	1 (0.9%)	1 (1.33 %)	
➢ asymptomatic cryo-globulinemia	not tested	3 (4 %)	
➢ pulmonary diseases	2 (1.8 %)	2 (2.66%)	
➢ rheumatologic diseases	1 (0.9%)	1 (1.33%)	
➢ cured cancer	2 (1.8%)	1 (1.33%)	
Upper gastrointestinal endoscopy			
esophageal varices	20 (18.01 %)	12 (16 %)	

The mean age was 58.7 (SD=8.35) years for the first group and 59.6 (SD=9.6) for the second group, with the predominance of female gender in both cases. Genotype 1b was present exclusively in the first group. For patients who received antiviral treatment between September 2017 to January 2018, HCV genotyping was not performed because it was not cost-effective.

RESULTS

Median HCV RNA at baseline was 1,264,123.180 IU/mL (SD=1,835,368.49) for the first group and 1,678,718.97 IU/mL (SD=2,994,746.86) for the second group. No patients were HBV or HIV co-infected. Most patients were naive to interferon therapy. The comorbidity rate was increased in both groups: 28.82% *vs.* 49.3% had cardiovascular diseases (hypertension, arrhythmia, valvular diseases), 23.41% *vs.* 14.66% had diabetes mellitus (with or without insulin requirement), 2.7% *vs.* 10.6% had thyroid dysfunction, more commonly autoimmune thyroiditis, 1.8% *vs.* 0% had dermatological disease (vitiligo, lichen planus), 0.9% *vs.* 5.33% had psychiatric disorders (depression, anxiety, cognitive impairment), 1.8% *vs.* 1.33% had a history of cured neoplasm (colon, kidney, urinary bladder), 1.8% *vs.* 2.66 had pulmonary diseases (asthma, bronchiectasis, pulmonary fibrosis) and 0.9% *vs.* 1.33% had hematological diseases (systemic lupus erythematosus, myeloproliferative syndrome). No patient had a history of treated hepatocellular carcinoma. In both groups, the maximum number of drugs administered simultaneously with the antiviral therapy was 4. The necro inflammatory activity assessed by ActiTest highlights a high rate for severe activity for both groups - 66.6%. The prevalence of concomitant NASH assessed by NashTest (N2-severe inflammation) was 50% in group 1 and 53.3% in group 2. There were slight differences between the two groups in terms of biological constants, but without any statistical significance.

DISCUSSION

The prevalence of HCV infection in adult patients (over 15 years old) in Romania was reported at 3.2%, equivalent to 595 000 anti-HCV positive individuals with a viremic prevalence rate of 91.3% (19). Previous data suggest that the high-risk group for HCV infection in Romania is the Baby Boom generation (people born between 1946 and 1964) (20). The age distribution of our patients met the same characteristics.

The analysis of the nucleotide sequences of the HCV RNA single-stranded molecule allowed the identification of 7 major genotypes and 67 subtypes with different and complex geographic distribution (21, 22). The distribution of HCV “epidemic subtypes” is probably influenced by historical and contemporary immigration patterns.

The global prevalence of genotype 1 is forecasted at 42.2% (83.4 million individuals), with a higher rate in East Asia (more than ⅓ of cases). According to the Epidemiological National Multicenter Study (ENMS) for genotyping of HCV in Romania conducted by Grigorescu (20) between October 2015 and March 2006, genotype 1, subtype b is predominant (97.72%) in Romanian patients with chronic HCV infection. More recent epidemiologic studies show a slightly lower prevalence (92.6%) (19). Genotypes 1a (5.4%), 3 (0.8%) and 4 (1.2%) were recently introduced because of

injecting drug use (19,23). In our study sample, even not fully representative for Romanian population, HCV genotype 1b was exclusively present for those tested between December 2015- October 2016. According to the expert committee of the National House of Health Insurance, during September 2017- January 2018, genotyping was not mandatory due to lack of cost-effectiveness.

Given the relatively silent nature of HCV infection, the degree of addressability of undiagnosed patients is lower compared to those with other infections with more prominent clinical signs and symptoms. Even if the optimal timing for the initiation of antiviral therapy is unknown, lack of funds in our country has led to a gradually upscale of HCV treatment.

At present, in our country, the access to antiviral therapy with paritaprevir/ ombitasvir/ritonavir/dasabuvir is limited to patients with advanced fibrosis (METAVIR score F3) or compensated cirrhosis (METAVIR score F4 and Child Pugh score <=6) who are either treatment-naive or interferon-experienced, with or without coinfection with HBV or HIV and to patients with mild fibrosis (METAVIR score F2) interferon-naive or interferon-experienced with mixt cryoglobulinemia, chronic kidney disease, non-Hodgkin lymphoma, hemophilia and thalassemia major. Patients with hepatocellular carcinoma (METAVIR score F2, F3 or F4) may be treated if they have a transplant indication or if relapse is not found (CT/MRI) more than 6 months after ablation or resection.

According to the *"Summary of Product Characteristics as authorized by European Medical Agency",* administration of paritaprevir/ombitasvir/ritonavir/dasabuvir is not indicated in patients with decompensated liver cirrhosis (Child Pugh score B or C) (17).

Currently in Romania, the only treatment available for patients with decompensated liver cirrhosis (only Child Pugh score B) is represented by a single-tablet fixed-dose combination of sofosbuvir and ledipasvir with or without ribavirin.

In our study, although in group 2 were included patients with a lower degree of fibrosis, we didn't find statistically significant differences compared to group 1 regarding the severity of hepatic disease (baseline biological constants, comorbidities, esophageal varices, necro inflammatory activity and concomitant presence of nonalcoholic steatohepatitis). Patients with less advanced fibrosis should be included in the antiviral treatment to improve outcomes, prevent disease progression and reduce the incidence of hepatocellular carcinoma.

In august 2017, after gaining increased efficacy in phase 3 trials ENDURANCE and EXPEDITION (99% SVR12 rate for genotypes 1,2,4,6 and 91-96% SVR12 rate for genotype 3), the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved the use of pan genotypic combination of direct acting antiviral agents glecaprevir (NS3/4A protease inhibitor)/pibrentasvir (NS5A inhibitor) to treat patients with compensated liver disease, with or without cirrhosis (24). This fixed dose oral combination (300 mg/120 mg) has the possibility to reduce by 4 weeks the time for cure in non-cirrhotic patients with genotype 1-2, 4-6, even in those with severe kidney disease or HIV coinfection (25).

The real-world prioritization is to treat all patients with advanced disease.

Prioritization of antiviral therapy should

also be done in those who associate extra-hepatic comorbidities. In our cohort, the most prevalent comorbidities were cardiovascular diseases followed by diabetes, thyroid dysfunctions and psychiatric disorders. In these patients, there is an increased risk of significant drug-drug interactions, especially with concomitant administration of proton pump inhibitors, thyroid hormones and dihydropyridine derivates. This risk is higher in elderly patients (> 65 years), in patients with advanced liver cirrhosis and with a combination of at least two direct-acting antivirals (26). In patients undergoing treatment with paritaprevir/ombitasvir/ritonavir/dasabuvir and concomitant ambulatory medication, the risk of significant drug-drug interaction is estimated at 66.3% (26).

All patients with chronic HCV infection regardless of degree of fibrosis should receive antiviral therapy. Early initiation of DAA may improve the outcome and lower the costs.

Cost-effectiveness analysis are needed to identify different ways to redirect resources to minimize the inequality of distribution of therapy with DAA and to achieve the WHO's objective to eradicate HCV by 2030.

CONCLUSIONS

The partial results of our study show a higher prevalence of HCV infection among people born between 1946 and 1964 (baby boom generation), especially women. In this geographical area, genotype 1b was found exclusively. Most patients, naïve to interferon therapy, associate numerous comorbidities and simultaneous treatments that may decrease adherence to therapy due to the possibility of significant drug-drug interactions, requiring optimal therapeutic management and close monitoring.

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ASSOCIATED FACTORS OF EJECTION FRACTION IN INSULIN-TREATED PATIENTS WITH TYPE 2 DIABETES

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ASSOCIATED FACTORS OF EJECTION FRACTION IN INSULIN-TREATED PATIENTS WITH TYPE 2 DIABETES (Abstract). **Aim of the study**: to evaluate the relation between ejection fraction (EF), diabetes characteristics and cardiovascular risk factors. **Material and methods**: We carried out a cross-sectional study in 171 patients with insulin-treated type 2 diabetes hospitalized at the Sf. Spiridon Emergency Clinical Hospital, Iasi. All patients were evaluated for asymptomatic organ damage and cardiovascular risk factors of hypertension and diabetes metabolic control. Global ejection fraction (EF) was evaluated through 2-D echocardiography. **Results and discussion:** In the studied group the ejection fraction had significant negative correlations with the duration of the disease (p=0.007) and the presence of microalbuminuria (p=0.001). There were some differences between the categories realized by grouping the patients according to the presence of hypertension and/or previous myocardial infarction. In patients without personal history of cardiovascular disease EF was correlated only with LDLc levels. In the hypertensive patients without myocardial infarction it was correlated with diabetes duration, Hb A1c and LDLc. In those patients with both conditions, EF had significant correlations with Hb A1c and microalbuminuria. **Conclusions:** These results emphasized that the determinants associated with heart failure in patients with type 2, insulin-treated diabetes, differ according to the presence of high blood pressure and myocardial infarction. **Keywords:** ARTERIAL ESSENTIAL HYPERTENSION, DIABETES MELLITUS, HEART FAILURE

Metabolic syndrome is a frequent association in hypertensive patients because insulin resistance is the main trigger of metabolic abnormalities and is related to lifestyle characteristics (1). High blood pressure is associated with primary lifestyle risk factors, and induces a large range of injuries in cardiovascular and renal system since the earliest stages (1, 2, 3, 4). Metabolic syndrome is acknowledged as the clustering of risk factors (obesity, insulin resistance, dyslipidemia and hypertension) associated with the subsequent development of cardiovascular disease and type 2 diabetes (8, 9, 10, 11, 12). The metabolic syndrome is a known risk factor for cardiovascular morbidity and mortality (1, 2); moreover, some of the antihypertensive drugs, such as beta-blockers and diuretics, have metabolic side effects that could inter-

fere and worsen the preexistent anomalies (5, 6, 7).

Diabetes mellitus (DM) and congestive heart failure (HF) are frequently associated. The presence of DM in HF patients determines an increased number of adverse events compared with patients without DM. Recent guidelines regarding glycemic control emphasized the importance of the individualization of therapy and targets depending of patient diseases and the risks associated with hypoglycemia. This balance in establishing therapeutic targets may be particularly relevant in patients with DM and HF (8).

The aim of the study was to evaluate the relation between ejection fraction (EF), diabetes characteristics and cardiovascular risk factors.

MATERIAL AND METHODS

During January 2012 and December 2013, we carried out a cross-sectional study in 171 patients hospitalized at the Sf. Spiridon Emergency Clinical Hospital, in Iasi. The inclusion criteria were the presence of insulin-treated type 2 diabetes. The exclusion criteria were type 1 diabetes, type 2 diabetes treated with non-insulinic agents, patients' refusal.

The information obtained from patients was directly registered in a well-structured questionnaire filled in as part of direct interview. The questions here comprised referred to the following aspects: demographic data, previous myocardial infarction, history of high blood pressure and duration of diabetes. The diagnosis of arterial hypertension was assessed in the presence of values higher than 140/85 mmHg, or normal ones in treated patients for high BP.

All patients underwent physical examination, laboratory investigations and diagnostic tests having as aim to search for asymptomatic organ damage and cardiovascular risk factors. A complete 12 lead ECG was registered to identify patients with previous asymptomatic myocardial infarction (MI). Global ejection fraction (EF) was evaluated through 2-D echocardiography.

We examined the relation between EF, diabetes characteristics and cardiovascular risk factors.

Continuous data was expressed as medium and standard deviation. The Shapiro-Wilk test was used to evaluate the normal distribution of the analyzed data. The chi-square test was used to determine significant differences between various categories formed out of the frequency data. Association between variables was estimated by Pearson correlation coefficient. Stepwise multiple regressions were used to identify the variables with a predictive role in ejection fraction.

The study was conducted after obtaining written informed consent in accordance with the Declaration of Helsinki. We paid full respect to the confidentiality and intimacy terms when maneuvering the data and records keeping of the participating subjects to the study.

RESULTS

We conducted a cross-sectional study on a sample of 171 patients with insulin-treated type 2 diabetes. The mean duration of the disease was 9.56 years and almost three quarters had a poor glycemic control. The criteria associated to metabolic control which were analyzed were: LDLc, HDLc, Hb A1c, microalbuminuria and blood pressure. Most of the patients had abnormal levels of these parameters, indicating a poor metabolic control (tab. I).

82.5% of the patients had high blood pressure (BP) and 24.6% had had myocardial infarction in their medical history. All normotensive patients had not had a previous myocardial infarction, while 42 (29.8%) of hypertensive ones experienced it ($p<0.001$).

The ejection fraction (EF) was also evaluated by 2-D Echocardiography, having a mean value of 54.82%. However a high proportion of the participants had cardiac failure (35.1%) (tab. I).

In the sample studied the ejection fraction had significant negative correlations with the duration of the disease ($p=0.007$) and the microalbuminuria ($p=0.001$)(fig. 1).

However, there were noticed some differences between the categories realized by grouping the patients according to the presence and/or the association of hypertension and previous myocardial infarction (tab. II).

In patients without personal history of cardiovascular disease, EF was related only with LDLc levels. In the hypertensive patients without myocardial infarction it was related with diabetes duration, Hb A1c and LDLc, while in those with both conditions, EF had significant correlations with Hb A1c and microalbuminuria (tab. II).

In the multivariate analysis, we included as potential predictors of EF the following parameters: diabetes duration, microalbuminuria, HDLc, LDLc, HbA1c.

When analyzing the entire sample, the predictors of EF were only diabetes duration and microalbuminuria ($R=0.33$, $R^2= 0.11$).

TABLE I.

Background characteristics

	Mean
Age (mean ± SD)	58.40 ±11.052 y.o.
Gender – males (N, %)	84 (49,1)
Smokers (N, %)	21 (12,3)
Diabetes duration (mean ± SD)	9.56±6.52 years
High BP (N, %)	141 (82,5)
Myocardial infarcts (N, %)	42 (24,6)
Ejection fraction (mean ± SD)	54,82±12,50
Hb A1c %(mean ± SD)	8.6409±2.46
LDLc mg/dl (mean ± SD)	156.64±53.99
HDLc mg/dl (mean ± SD)	38.68±10.93
Microalbuminuria mg/dl(mean ± SD)	63.43±36.87
Polineuropathie (N, %)	123 (71,9)
Cardiac failure (N, %)	60 (35,1)
HbA1c>7% (N, %)	126 (73,7)
LDLc>100 mg/dl (N, %)	135 (78,9)
HipoHDLc (N, %)	147 (87)

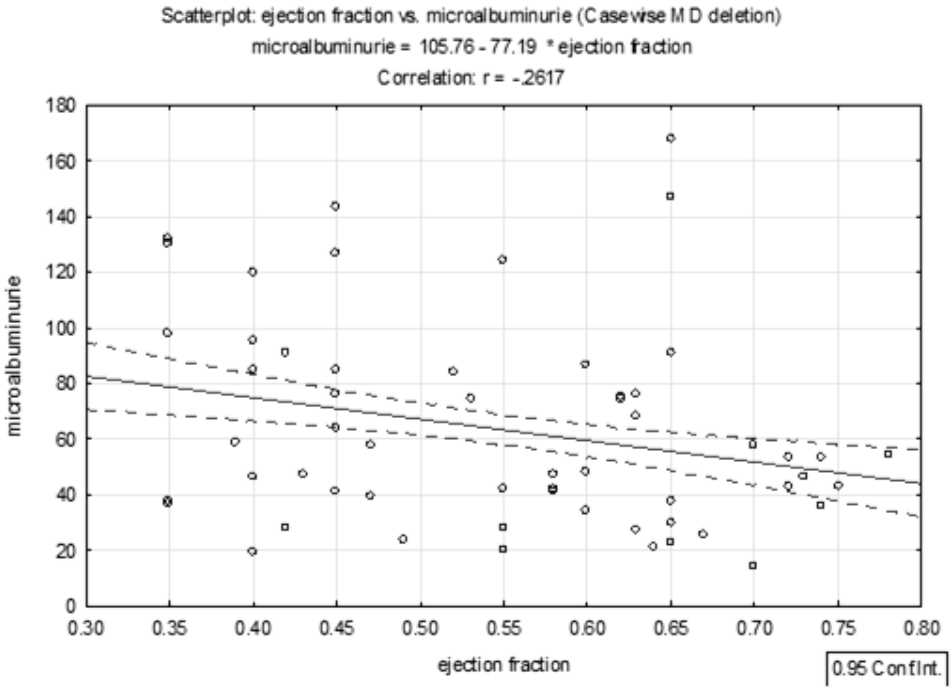

Fig. 1. Association between ejection fraction and microalbuminuria

TABLE II

Significant correlations between the ejection fractions and characteristics of diabetes

Ejection fraction		**Diabetes duration**	**Age**	**Hb A1c**	**LDLc**	**HDLc**	**Micro albuminuria**
Total sample	r	**-.207****	-.08	.087	.010	.02	**-.262****
	p.	**.007**	.27	.256	.900	.79	**.001**
Patients without high BP and MI (N=30)	r	-.118	-.05	.200	**-.47****	-.04	-.221
	p.	.533	.78	.288	**.008**	.83	.240
Patients with high BP and without MI (N=99)	r	**-.338****	-.09	**.232***	**-.218***	-.04	-.194
	p.	**.001**	.36	**.021**	**.030**	.63	.054
Patients with high BP and with MI (N=42)	r	-.110	.24	**-.467****	.260	.27	**-.392***
	p.	.487	.11	**.002**	.096	.07	**.010**

However, we identified different predictors of EF, when analyzing on the subgroups determined by the presence of high BP and previous myocardial infarction. In diabetics without cardiovascular diseases, only one predictor was identified: LDLc (R=0.47, R^2=0.22). Hypertensive diabetics with MI had as predictors of EF, diabetes duration and microalbuminuria (R=0.30, R^2=0.09). The participants with high blood pressure, but without MI had as predictors of EF both LDLc and Hb A1c (R=0.65, R^2=0.42) (tab. III).

TABLE III
Multiple linear regression coefficients and associated p-values for ejection and specific characteristics

		Standardized Coefficients	p.	95% Confidence Interval for B	
		Beta		Lower Bound	Upper Bound
Total sample					
1	(Constant)		.000	.568	.641
	microalbuminuria	-.262	.001	-.001	.000
2	(Constant)		.000	.598	.687
	microalbuminuria	-.262	.000	-.001	.000
	diabetes duration	-.207	.005	-.007	-.001
Patients without high BP and MI (N=30)					
1	(Constant)		.000	.170	.533
	LDL	.476	.008	.000	.003
Patients with high BP and with MI (N=42)					
1	(Constant)		.000	.552	.629
	microalbuminuria	-.256	.002	-.001	.000
2	(Constant)		.000	.573	.673
	microalbuminuria	-.257	.002	-.001	.000
	diabetes duration	-.163	.047	-.006	.000
Patients with high BP and without MI (N=99)					
1	(Constant)		.000	.693	1.155
	Hb A1c	-.467	.002	-.064	-.016
2	(Constant)		.000	.680	1.083
	Hb A1c	-.641	.000	-.077	-.032
	LDL	.488	.001	.001	.002

DISCUSSION

Diabetes is currently among the most challenging threats to public health. It is estimated that 382 million people worldwide have diabetes and the majority will likely die from cardiovascular disease (9).

In patients with type 2 diabetes, HbA1c is used to evaluate the long term glycemic control and represents an indicator of development of micro vascular and macrovascular complications. Even if the improvement of glucose control significantly reduces the risk of micro vascular complications, most of the trials failed to show if there is also a decrease of risk for atherosclerotic diseases. Despite this, the value of HbA1c is still used as a surrogate measure of anti diabetic drugs potential to lower cardiovascular risk. Some authors considered that this assumption is no longer acceptable (10).

Cardiovascular diseases are the main causes of death and disability among people with type 2 diabetes. It is estimated that 65% of diabetics would die due to heart disease or stroke. Also, they are two to four times more likely to have atherosclerotic cardiovascular diseases than adults without diabetes (11).

In our sample, 24.6% had myocardial infarction in their medical history. However a higher proportion of the participants had cardiac failure (35.1%). The incidence of HF in patients with ischemic heart disease was 35.7% in non diabetic patients, and 45.7% in diabetic patients (12). Several studies have suggested that diabetics, with or without previous cardiovascular disease,

have up to five-fold increased risk of heart failure (13).

At least 3 mechanisms are involved in inducing HF in diabetic patients: associated diaseases or favoring coronary atherosclerosis or through diabetic cardiomyopathy (14).

In the sample we studied, the ejection fraction had significant negative correlations with the duration of the disease and the levels of microalbuminuria and the predictors of EF were only diabetes duration and microalbuminuria.

Other studies, such as UK Prospective Diabetes Study (UKPDS), demonstrated a strong association between the incidence of HF and HbA1c levels in diabetic patients (15). A large trial, conducted in 48,858 diabetic patients, showed that the increase of Hb A1c with 1% was associated with a 12% increased risk of hospitalization for the same patients (16).

In our study, Hb A1c was significantly associated with EF in patients with high blood pressure even if myocardial infarction was present or not. In normotensive patients, there were no correlations between Hb A1c and EF. However, in these patients diabetes duration was a significant predictor of left ventricular systolic function.

Other studies demonstrated that the onset of HF may be better correlated with the duration of the disease. It is considered that the development of HF may be more closely related to the duration of DM than to glycemic control (14).

In patients without personal history of cardiovascular disease or high blood pressure, EF was correlated only with LDLc levels. In the hypertensive patients without myocardial infarction it was correlated with Hb A1c and microalbuminuria, while in those with both conditions, EF had significant correlations with diabetes duration, Hb A1c and LDLc.

In another study, performed in a well characterized population of patients with anterior MI and mild residual left ventricular dysfunction or preserved ejection fraction, diabetes is associated with worse functional class and a greater risk of developing congestive heart failure in the following year (13). In our study, the predictors of EF in patients with previous MI were: diabetes duration, microalbuminuria, HDLc, LDLc, HbA1c.

CONCLUSIONS

The results of our study suggested that there would be some differences between determinants associated to heart failure in type 2 insulin-treated diabetics, according to the presence of high blood pressure and myocardial infarction. Future research will clarify the extent of this association and its relevance for clinical practice.

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DIFFERENTIATION OF RHEUMATOID ARTHRITIS FROM HEPATITIS C-RELATED ARTHROPATHY: CASE REPORT

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DIFFERENTIATION OF RHEUMATOID ARTHRITIS FROM HEPATITIS C-RELATED ARTHROPATHY: CASE REPORT (Abstract): Chronic virus C hepatitis records high prevalence, almost 170 million people worldwide being infected. Systemic involvement is frequent and the implication of the osteoarticular system raises various problems in properly diagnosing and treating it. Rheumatoid arthritis is the most frequent type of inflammatory polyarthritis, with a prevalence of 0.8% in the general population. The rheumatoid factor recorded high values at virus C hepatitis patients (19-80%) even in the absence of articular manifestations, its sensitivity and specificity being reduced for the rheumatoid arthritis diagnosed simultaneous with virus C hepatitis. We report a case of chronic virus C hepatitis patient which, after 30 years of evolution, presents the onset of senile rheumatoid polyarthritis. The authors discuss the usefulness dosage of anti-cyclic citrullinated peptide antibodies for establishing the differential diagnosis between rheumatoid arthritis and hepatitis C-related arthropathy and the particularities of the specific treatment when there is a hepatitis C virus associated infection. **Keywords**: VIRUS C HEPATITIS, RHEUMATOID ARTHRITIS, HEPATITIS C–RELATED ARTHROPATHY, RHEUMATHOID FACTOR, ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES.

The prevalence of virus C hepatitis (VCH) infection reaches alarming levels, worldwide being recorded almost 170 million cases (1, 2). The prevalence is maximum at the age group of 30–49 years, the infection is chronic at 80% of the patients and the progression rate towards end–stage liver disease is of 10–20%, for a period of 20 years of observation (3). Rheumatoid arthritis (RA) is the most frequent type of inflammatory arthritis, with a prevalence of 0.8% in the general population, 1.3 million adults being diagnosed with RA in the USA. Without treatment, 20 – 30% of the patients diagnosed will evolve towards a major degree of disability in the next 3 years (4, 5). The relationship between the two affections is two-way, as far as epidemiology, pathogenesis and treatment response are concerned; the traditional serological marker for RA, the rheumatoid factor (RF), was detectable at 70 – 80 % of the patients with RA, but it can have higher values also at patients with VCH, in a per-

cent of 19-80%, even in the absence of joint manifestations (6). On the other hand, serum anti–hepatitis C virus (HCV) antibodies were present in RA patient at the following levels: 5.2% and 7.6% (7, 8). We report a case of an elderly patient, which came with polyarthralgias, after more than 30 years of evolution of chronic hepatitis with HCV, at which imagistic and immunologic investigations establish the diagnosis of senile RA.

CASE PRESENTATION

An 83-year-old women, with antecedents of VCH from the age of 50 and with arterial hypertension associated with left bundle branch block from the age of 58, presents pain, tumefaction and functional incapacity at the level of metacarpophalangeal (MCP) and proximal bilateral interphalangeal (IP) joints, cervicalgia, weight loss and progressive asthenia during the past 3 months. The patient is a former nurse, has not smoked or used alcohol, and from the clinical examination resulted normal body mass index (21.5 kg/m^2), the affected joints of both hands being swollen and painful, the limitation of the cervical spine for anterior, posterior and lateral flexion, hepatomegaly with high consistency and 2nd degree splenomegaly.

Hematological and biochemical biological tests were within the normal limits, except for the increase of gamma-glutamyl transferase (ɣGT) up to the value of 89 U/L (reference range <61U/L) and moderate hepatocytolisis (ALAT 136 U/L, ASAT 134 U/L).

The abdominal ultrasound pointed out the enlargement of the right liver lobe, with micronodular hyperechoic structure and splenomegaly; the abdominal computed tomography excluded the presence of liver tumor formations. From the radiography of the hands results bilateral risarthrosis associated with clips of MCP articulations, proximal and distal IP but also periarticular osteoporosis and erosions, suggestive for the begging of RA (fig. 1). Imagistic investigations are completed by cervical spine computed tomography which excludes the presence of osteolytic lesions at this level and articular ultrasound which underlines the erosions from the II and III MCP joints accompanied by inflammatory reaction and synovitis (fig. 2, 3).

Fig. 1. Bilateral radiography of the hands showing periarticular osteoporosis and erosions

Fig. 2. Cervical spine computed tomography showing degenerative lesions

The immunological report revealed positive tests for HCV, RF increased at 70.9 UI (normal range < 16 UI), anti-cyclic citrullinated peptide (ACCP) antibodies with pathological values of 34 UI (normal range < 12 UI), cryoglobulins and antinuclear antibodies absent. The treatment was started with hydroxycloroquine in dose of 400mg/day in association with non-steroid anti-inflammatory drugs (NSAID) during hyperalgesic periods. The general state and the painful articular syndrome improved in only 3 weeks.

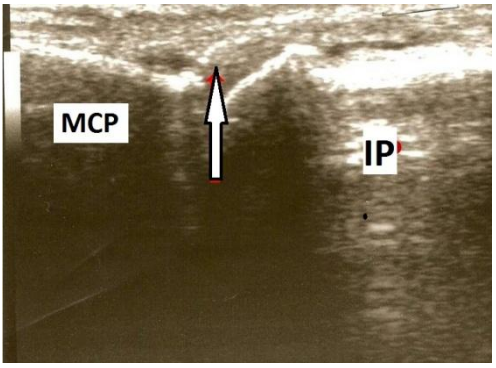

Fig. 3. Articular ultrasound of MCP and IP joints showing erosions, inflammatory reaction and synovitis (arrow)

DISCUSSION

Chronic HCV infection is accompanied by various extrahepatic manifestations which were included by Ferri C et al. in 2007 in the so-called HCV syndrome (9), and the articular modifications are among the most frequent ones. Most studies reported polyarthralgias of variable frequency (20–83%) (1). One of the big prospective studies, which included 1614 patients with chronic hepatitis C, mentioned the prevalence of polyarthralgias of 23% (10). Arthritis are less frequent and they are characterized by symmetric polyarthritis which can be mistaken for RA or by mono or oligoarthritis, intermittent, without erosive–destructive features. Mono or oligoarticular affectation is more frequent at large joints and is associated to the presence of cryoglobulins, while symmetric polyarthritis has an evolution similar to that of RA (11-14). HCV-related arthropathy can be clinically similar to RA, many patients experiencing the diagnosis criteria stated by American College of Rheumatology (ACR) (15).

In this patient's case, the main differential diagnosis reported to senile RA and HCV associated arthropathy. The differentiation consisted in the dosage of the ACCP antibodies, which recorded higher levels and the radiographies of the hand which pointed out periarticular erosions, rare in HCV arthropathy. The comparative immunological studies at patients with chronic hepatitis C virus proved the superiority of ACCP antibodies in RA diagnosis. ACCP antibodies were scarcely positive at patients with HCV and arthralgia (5.7%), absent at the secondary Sjögren syndrome (0%) but they were present at 78% of the patients with RA (1). A previous study used for detecting ACCP antibodies a second generation ELISA kit (cut off = 20U/ml) and reported a prevalence of 6.9% (2/29) in patients with HCV and mixed crioglobulinemia and 0% (0/50) in patients without crioglobulinemia (16). In a different group of 39 patients with HCV, 31 of which with associated arthropathy, compared with 30 patients with RA criteria, ACCP antibodies were present in 77% of the patients with RA and in no patients with HCV (13). Classically, 50% of the patients with VCH present mixed crioglobulines and 24% arthralgia (1). Referring to the primary Sjögren syndrome, the

percentages are small, less than 10 % of the patients with primary Sjögren syndrome presenting ACCP antibodies (17).

VCH was associated with many other extrahepatic manifestations as vasculitis, glomerulonephritis, thyroiditis and sialoadenitis, but poliarthritis were most cited (10). Fadda et al. examined 302 patients with VCH for arthritis (8% of the patients with crioglobulinemia presented non-erosive oligoarthritis, 15% of the patients with crioglobulines experienced polyarthritis and 30% of them presented erosions at radiological investigations) (18). Among the self-antibodies produced by the organism in VCH, RF is one of the most frequent (30%-70%), sometimes present at high levels (12). Unlike RF, ACCP antibodies were not mentioned as autoimmune markers and did not record high levels at patients with VCH, included in the published studies (1, 6, 13, 16, 19-21). Lu et al. (19) also studied the diagnostic role of anti-agalactosil Ig G antibodies in differentiating RA from the other similar arthropathies, present in VCH, hepatitis B virus or primary Sjögren syndrome. They pointed out that the anti-agalactosil antibodies' sensitivity and specificity is lower than that of ACCP antibodies (81% sensitivity and 98.4% specificity), which remain the golden standard in differentiating RA from other arthropaties associated to viral hepatitis and primary Sjögren syndrome, regardless of the presence or the absence of RF. In the study conducted by Ezzat et al. (20), which included 30 patients with RA and 22 with HCV–related arthropathy, the sensitivity of ACCP antibodies was comparable to that of RF (83.3% vs 90%), but their specificity was by far higher (95.4% *vs.* 18.2%).

The apparition of ACCP antibodies in RA is the result of the migration of inflammatory cells at the level of joints where the enzyme protein – arginil deaminase (PAD) transforms the arginin amino-acid in citrulline, followed by apoptosis of articular cells (22). Up to this moment, there have been used 3 generations of tests to point out the anti-citrulline antibodies, the first generation including only antiperinuclear factor (APF) antibodies and anti-keratin antibodies (AKA). In the case of second and third generation tests, sensitivity was increased by using some combinations of synthetic cyclic citrullinated peptides (23).

In our case, the immunological investigation was completed by the imagistic assessment which helped completing the RA diagnosis criteria according to the present guides (15). The pain from the level of the cervical spine being intense, vertebral computed tomography excluded the presence of metastases, and the abdominal computed tomography was performed to underline the possible liver tumor formations. Articular symptoms could also be the expression of paraneoplastic rheumatoid syndrome, associated to hepatocarcinoma, although liver tumors are, more frequently, accompanied by various hematological disorders, hypoglycemia, myositis and neurological syndromes.

The presence of chronic VCH limits the treatment options of the associated RA. Considering the age too, we chose for our patient the hydroxcloroquine treatment associated with NSAID; the improvements were noticeable after the first weeks of treatment. Other authors also mention the possibility of using hydroxcloroquine and sulfasalazine in patients with moderate liver disorders. Cyclosporine, with antiviral effect as well, but also tumor necrosis

factor (TNF) inhibitors, rituximab and abatacept were used (3, 24).

CONCLUSIONS

This case as well as the previous reports in the literature emphasized the particularities of the RA in comparison with hepatitis C-related arthropathy and describe the utility of ACCP as laboratory diagnostic tool.

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NEWS

TARGETED ANTIFUNGAL PROPHYLAXIS IN HEART TRANSPLANT RECIPIENTS

Tissot *et al* conducted a retrospective analysis to identify the risk factors for the development of invasive fungal infection (IFI) after heart transplantation (HTx) and implement a new antifungal prophylaxis policy. For this purpose, clinical characteristics of HTx recipients hospitalized during a seven years period were recorded and risk factors associated with IFI were identified using Cox regression analysis (Period 1). Based on the results of the first analysis, all recipients at high risk for IFI received targeted caspofungin prophylaxis (Period 2 of the study, lasting 3 years). During Period 1, 10% of patients developed IFI after transplantation. The use of posttransplant extracorporeal membrane oxygenation was the strongest predictor for IFI by multivariate analysis, while *Aspergillus* colonization and renal replacement therapy were significant predictors by univariate analysis. Antifungal prophylaxis was administered to 17% of patients at high risk for IFI in period 1 versus 100% in Period 2. Only 4% of the patients developed IFI during Period 2. Antifungal prophylaxis was associated with a reduction in IFI incidence and mortality. Extracorporeal membrane oxygenation is an important risk factor for IFI after transplantation and requires targeted administration of antifungal prophylaxis (Tissot F, Pascual M, Hullin R, *et al*. Impact of Targeted Antifungal Prophylaxis in Heart Transplant Recipients at High Risk for Early Invasive Fungal Infection. *Transplantation*. 2014 Feb 11).

Teodora Vremeră

BARRIERS OF ANTIAGGREGANT TREATMENT

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BARRIERS OF ANTIAGGREGANT TREATMENT (Abstract): In the present study we **aimed** to evaluate side effects of antiplatelet therapy in order to establish correlations with medication type, doses and association with other therapies. **Material and methods**: in the study we prospectively evaluated a cohort of patients who received antiplatelet therapy for different pathologies. We included patients with acute coronary syndromes, valvular disease complicated with supraventricular arrhythmias (especially atrial fibrillation), carotidal critical stenosis, neurologic disease (ischemic or thrombotic), and peripheral artery disease. **Results and discussions**: The study included 125 patients (85 males and 40 females), aged between 45 and 85, and admitted in the 1st Cardiology Department, St Spiridon Hospital, between January 2012 and December 2013, who received antiplatelet therapy for different pathologies. All the patients included in the study received platelet antiaggregant therapy with Clopidogrel in association or not with Aspirin or low weight molecular heparin. Side effects reported (possibly correlated with antiplatelet therapy) were: macroscopic hematuria (7 cases), cutaneous ecchymosis (7 cases), purpuric lesions (9 cases), gingival bleeding (12 cases), upper gastrointestinal bleeding (6 cases), and hemoptysis (2 cases). **Conclusions:** Hemorrhagic events under the treatment with antiplatelet agents are rare in comparison with the large number of patients treated. Clinical manifestations are very different depending on the drug and also on the drug-associations used. Hemorrhagic accidents may sometimes be very serious, determining the specific therapeutic measures. **Keywords:** ANTIAGGREGANT TREATMENT, CLOPIDOGREL, ASPIRIN, HEMORRHAGE, SIDE EFFECTS.

Atherothrombosis accounts for 22.3 % of all causes of mortality worldwide as reported by the World Health Organization in 2001 (1). Cardiovascular disease (CVD) was defined as coronary heart disease (myocardial infarction (MI), angina pectoris, coronary insufficiency), cerebrovascular disease (stroke, transient ischemic attack), congestive heart failure, and intermittent claudication. This definition of CVD covers all the manifestations of athero-thrombosis. According to this analysis, more than 60% of men and women over 40 years of age will develop atherothrombotic disease at some point in their lives (1). For patients older than 50 years of age, the development of atherothrombotic disease reduces life expectancy by 8 to 12 years (1).

The role of platelets in athero-thrombosis is a very important factor to be considered in

preventing cardiovascular death, MI and stroke (2). After plaque rupture or vessel damage, platelets undergo three processes: adhesion, activation, and aggregation, and then a thrombus is formed. An agent that would inhibit these platelet processes would therefore definitely be beneficial and effective in preventing atherothrombotic events (3). Antiplatelet agents such as aspirin, triflusal, clopidogrel, and ticlopidine generally act on the platelet activation process while GIIb/IIIa inhibitors impede platelet aggregation by blocking activated fibrinogen receptors (3). They are widely used for primary and secondary prevention of cardiovascular and cerebrovascular diseases (4). However, use of either aspirin or clopidogrel is associated with bleeding adverse events (5).

Clopidogrel is a potent anti-platelet drug of the thienopyridine group that inhibits adenosine dinucleotide phosphate (ADP) receptors. It is considered superior to aspirin for secondary prevention after cardiovascular events, it is widely administered following cardiac catheterisation and stent implantation or bypass and its indications are expanding (5, 6). Combined aspirin and clopidogrel therapy is even more effective in preventing vascular events and associated mortality, but that regimen may significantly increase the risk for bleeding (7, 8).

The aim of this study was to assess the occurrence of antiplatelet therapy side effects in patients with CAD.

MATERIAL AND METHODS

We conducted a prospective cohort study in which we included 125 patients (85 males and 40 females), aged between 45 and 85, who received antiplatelet therapy for different pathologies, admitted in the 1st Cardiology Department, St Spiridon Hospital, between January 2012 and December 2013.

Patients with uncertain acute coronary syndrome criteria, those without clopidogrel therapy, patients with contraindication of platelet antiaggregant therapy (e.g., antecedents of hemorrhagic accidents or known hemorrhagic diatheses, previously known liver disease) as well as patients with psychiatric disorders, clinical severe thrombocytopenia, progressive malignancy or noncompliant ones were excluded.

Patients were initially evaluated following a complete check-up, including past history, clinical examination, blood tests, electrocardiogram and cardiac ultrasound. The final decision regarding study inclusion was taken after having a precise diagnosis and therapeutic scheme. They were followed up for one year, initially at one month and then at 3, 6 and 12 months.

All the patients included in the study received antiaggregant therapy with clopidogrel associated in some patients with aspirin or low weight molecular heparin.

After enrolment, patient age, sex, smoking and drinking habits, previous medications including antiplatelet agents (aspirin, clopidogrel), nonsteroidal anti-inflammatory drugs (NSAIDs), and gastroprotective agents, and past history of endoscopically proven peptic ulcer disease were recorded. During the follow-up period, data concerning hemoglobin, platelet levels, and evidence of bleeding were recorded.

RESULTS

We reviewed the records of 125 patients (68% men, average age 65.3 ± 10.1 years) with baseline characteristics (tab. I).

The most commonly associated co morbidities were hypertension (70.4 %), diabetes mellitus (35.5 %), congestive heart failure (26.6 %), and dyslipidemia (22.7 %).

The majority (77 patients, 61.6% of cases) were diagnosed with acute coronary syndromes (most cases with different forms

of unstable angina) that have satisfied the study inclusion criteria. Thirteen patients (10.4%) received antiaggregant treatment for acute and subacute MI, and 28% of the patients received antiplatelet therapy for other diseases such as: valvular disease (mitral or aortic) complicated with supraventricular arrhythmias (especially atrial fibrillation) (15 patients), carotidean critical stenosis (3 patients), neurologic disease (ischemic or thrombotic), peripheral artery disease (17 patients).

TABLE I
Baseline characteristic of study population

Characteristics	Patients (n=125)
Age, yrs (mean ±SD)	69.3±9.39
Male n, %	66 (52.8%)
Diabetes n, %	51 (40.8%)
Smoking n, %	64 (51.2%)
Blood hypertension n, %	79 (63.2%)
Cholesterol, mg/dl (mean ±SD)	229.5±45.99
Creatinine, mg/dl (mean ±SD)	0.98±0.39

All the patients included in the study received 75 mg of clopidogrel daily. In 77 patients clopidogrel was associated with aspirin 75 mg daily, and seventy-four patients (13.9%) also received low weight molecular heparin (enoxaparin). Anticoagulant treatment was associated for a medium of 7 days in patients with ACD. The majority of patients 67 (53.6%) received double antiaggregation (clopidogrel and aspirin) upon discharge (fig. 1).

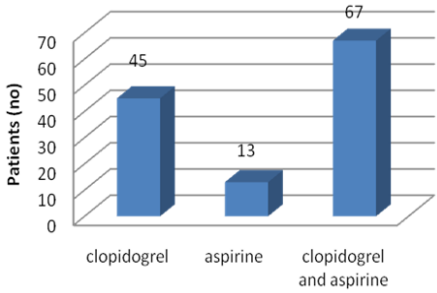

Fig. 1. Treatment received by the patients included in the study upon discharge.

During the follow-up, we recorded 7 (5.6%) cases of hematuria, between 7 and 10 days after the treatment was begun. All the patients were males and had been treated with clopidogrel and aspirin.

Seven patients (5.6%) has developed cutaneous ecchymosis after an average period of one month, four of them had a double antiaggregation, with no platelet count. Six patients (4.8%) had purpuric lesions secondary to clopidogrel treatment. All the skin lesions were reversible after clopidogrel was stopped and replaced with aspirin (tab. II).

TABLE II
Side effects in patients with antiaggregant treatment

Side effect	Clopidogrel (n=45)	Aspirin (n=13)	Clopidogrel and aspirin (n=67)
Macroscopic hematuria n, %	0	0	7 (5.6 %)
Cutaneous ecchymosis n, %	2 (1.6%)	1 (0.8%)	4 (3.2 %)
Cutaneous purpura n, %	5 (4%)	0	1 (0.8%)
Gingival bleeding n, %	4 (3.2%)	0	8 (6.4%)
Upper digestive hemorrhage n, %	0	0	7 (5.6%)
Hemoptysis n, %	2 (1.6%)	0	0

Patients with upper digestive hemorrhages had melena and also received heparin therapy. Upper digestive endoscopy was performed in half of the cases, and acute hemorrhagic gastritis was described in all the cases. Antiaggregant treatment was stopped and a proton pump inhibitor was added as treatment.

Two smoking male patients were diagnosed with haemoptysis, all of them on clopidogrel. Bronchoscopy was normal and they did not repeat the bleeding after clopidogrel was changed to aspirin.

DISCUSSION

Although some studies demonstrated that clopidogrel increases the risk of bleeding (4, 6) our findings suggested that hemorrhagic adverse events are rare in patients on antiaggregant therapy. These results may have important implications in antiplatelet therapies for patients with or at risk for cardiovascular disease.

Antiplatelet therapies are effective in reducing ischemic events, yet are likely to increase bleeding risk (7). Use of more potent antiplatelet therapies is likely to further reduce the risk of ischemic events, while simultaneously increasing the risk of bleeding (6). While these agents are effective in reducing thrombotic complications of atherosclerosis (ASC), their use increases the likelihood that patients will develop a bleeding complication (8). The reported risk of bleeding during hospitalization for ACS varies between 1 and 10% and is influenced by patient co morbidities, age, sex, rates of treatment with revascularization or invasive procedures such as angiography, intra-aortic balloon pumps or coronary artery bypass grafting (CABG) and the antiplatelet/antithrombotic treatment regimen used for treatment (9).

Prior studies have consistently identified female gender, older age, lower body mass index, low creatinine clearance, and the use of percutaneous interventions as risk factors for bleeding complications (9, 10). In order to better understand an individual's risk of bleeding, there have been several attempts to develop standardized predictive models for bleeding risk; unfortunately, the majority of these bleeding scores have had relatively poor predictive power (10).

Recently, Subherwal et al. (11) published the CRUSADE Bleeding Score using clinical criteria available at presentation such as baseline hematocrit, creatinine clearance, heart rate, sex, systolic blood pressure, diabetes, prior vascular disease, and the presence of cardiac heart failure upon presentation. Current ACC/AHA guidelines for the treatment of patients with ACS recommend dual antiplatelet therapy due to numerous studies demonstrating a reduction in ischemic complications (12). The addition of an additional antiplatelet agent does increase the incidence of bleeding, results which were also confirmed in our study.

In the CURE trial of patients with ACS randomized to clopidogrel plus aspirin versus aspirin alone, the rate of major bleeding in patients assigned to clopidogrel plus aspirin was 3.7%, while the rate in the patients assigned to aspirin alone was 2.7% (RR=1.38; p=0.001) (13). Bleeding risk was also significantly higher among patients treated with aspirin and clopidogrel who required CABG. Post hoc analysis of the CURE trial demonstrated that the bleeding risk was especially high when CABG was performed within 5 days of the last dose of clopidogrel (13).

Patients who suffer from bleeding com-

plications or require transfusions are often treated less aggressively with evidence-based medications placing them at higher risk of future ischemic events. The Prospective Registry Evaluating Myocardial Infarction: Events and Recovery (PREMIER), a registry of 2.498 patients with acute MI, found that patients with bleeding during the index MI hospitalization were less likely to be treated with antiplatelet therapy and other agents during the first 6 months after discharge (14). These findings offer insight into the current treatment of patients with bleeding complications and may help explain why patients with bleeding complications are at a significantly higher risk for ischemic events.

Bleeding events which occurred during antiplatelet therapy are relatively rare when compared to the large number of patients treated with an antiaggregant drug in mono or dual therapy. Bleeding side effect can manifest in different territories and disappear with cessation of treatment. Association between double antiaggregation and heparin therapy increased the risk of upper digestive bleeding.

CONCLUSIONS

Our study did not demonstrate an increased risk of bleeding in patients treated with clopidogrel versus aspirin, but the double antiaggregation was associated with more hemorrhagic adverse events.

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NEWS

A NEW ASSAY FOR PATHOGEN GENOTYPING

A group of researchers developed a new assay for pathogen detection and amplicon genotyping, based on restriction endonuclease digestion. In the first stage, target DNA is hybridized to immobilized complementary oligonucleotide probes carrying a molecular marker (horseradish peroxidase-HRP). In the second stage, the target-probe duplex is cleaved by the restriction enzyme at the corresponding restriction site and the HRP marker released into solution is quantified colorimetrically. This assay was tested for MRSA detection, using the *mec*A gene as a target. The detection limit for the target oligonucleotide and the PCR amplicon was approximately 1 nM. The sequences of target oligonucleotides were altered in order to prove the following: mutation to the restriction site reduced the signal to zero, mutations of the sequences that flanked the restriction site reduced restriction to 50-80% of the positive control, and a minimum of 16-bp target-probe hybrid is required for a significant cleavage. The assay is able to detect the *mec*A amplicon from an unpurified PCR mixture with limits of detection similar to standard qPCR. Detection of amplicons is not affected by the presence of excess heterologous genomic DNA and because it uses two biorecognition steps, the test has a very high specificity. The study concluded that the assay is efficient, low-cost, fast (can be completed in less than 1 hour) and can also be used for analysis of different amplicons from an unpurified mixture (Smith MW, Ghindilis AL, Seoudi IA et al. A New Restriction Endonuclease-Based Method for Highly-Specific Detection of DNA Targets from Methicillin-Resistant *Staphylococcus aureus*. PLoS One. 2014;9(5):e97826. doi: 10.1371/journal.pone.0097826).

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THYROID DYSFUNCTION AND ISCHEMIC HEART DISEASE – CLINICAL CORRELATIONS, PROGRESSIVE IMPLICATIONS AND IMPACT ON THE PROGNOSIS

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THYROID DYSFUNCTION AND ISCHEMIC HEART DISEASE – CLINICAL CORRELATIONS, PROGRESSIVE IMPLICATIONS AND IMPACT ON THE PROGNOSIS (Abstract): Thyroid dysfunctions are associated with systolic and diastolic heart dysfunction, hypertension, rhythm disorders, etc. Clinically significant hyperthyroidism and hypothyroidism may have an impact on the patients with ischemic heart disease. **Objectives:** Investigation of the risk of developing ischemic heart disease, of the evolution and prognosis in relation to the entire spectrum of thyroid dysfunctions. **Materials and methods:** All participants included in the study were selected from among subjects with heart disorders who were controlled with concern to the thyroid hormonal condition and who hadn't been treated previously for thyroid functional disorders. Based on these criteria we defined a study group made out of 791 subjects, divided into five lots based on the level of thyroid hormones. Once the group was formed, we conducted evaluations of the cardiovascular and thyroid status at 6 and 12 months, respectively. **Results:** In the witness lot, during monitoring 49% of the patients showed an ischemic heart disease. The main risk factors were: heart frequency of over 80 beats/min (RR=1.83), age over 60 (RR=1.47), female sex (RR=1.21) and values of triglycerides over 160 mg/dl (RR=1.23). In the group of patients with overt clinic hyperthyroidism, during monitoring 46.1% showed ischemic heart disease. The main risk factors were: heart frequency over 80 beats/min (RR=2.41), age over 60 (RR=1.67), high level of LDL-cholesterol (RR=1.53) and female sex (RR=1.31). Among the patients with overt clinical hyperthyroidism, during monitoring 53.3% showed ischemic heart disease. The main risk factors identified were: heart frequency over 80 beats/min (RR=2.01), age over 60 (RR=1.42), high levels of triglycerides (RR=1.42) and LDL-cholesterol (RR=1.32), as well as the presence of hypertension in the health records (RR=1.31). **Conclusions:** Thyroid dysfunction is a common clinical condition with a key role in the regulation of the cardiovascular system and may contribute to the evolution of the ischemic heart disease and which should be taken into consideration when patients with heart disease are treated. In this light, thyroid function needs to be evaluated for all patients with a risk for ischemic heart disease.
Keywords: HYPERTHYROIDISM, HYPOTHYROIDISM, ISCHEMIC HEART DISEASE.

Hyperthyroidism is present at 1.3% of the population (clinically overt in 0.5% and subclinically overt at 0.7%) while hypothyroidism is present at 4.6% (with clinical

significance at 0.3% and subclinical significance at 4.3%) (1).

Thyroids disorders are associated with systolic and diastolic heart dysfunction, hypertension, rhythm disorders, etc. Clinically significant hyperthyroidism and hypothyroidism may have an impact on patients with ischemic heart disease. As recently shown in numerous studies, even the subclinical hyperthyroidism may be an independent risk factor for the mortality caused by cardiovascular disorders (2). Previously, subclinical hypothyroidism was admitted as an independent risk factor for atherosclerosis and heart failure at older women (3). However, the results of these studies are sometimes controversial (4, 5, 6, 7).

The study tried to determine through a retrospective analysis (case study – control) and a subsequently prospective perspective the correlation between the thyroid status, the cardiovascular risk factors and mortality at adult patients.

MATERIAL AND METHODS

All participants included in our study were selected from among subjects with cardiovascular disorders who were subject to a control of thyroid hormone status in the 3rd Medical Clinic of "St. Spiridon" Emergency University Hospital, "Gr. T. Popa" University of Medicine and Pharmacy, Iaşi and who hadn't been previously treated for thyroid functional disorders. The study lots were constituted from 2006 to 2012.

We included among the subjects men and women, with an age varying from 22 to 86, with an average of about 60 years old, based on the serum values of free triiodothyronine (FT3), free tetraiodo-thyronine (FT4) and the thyroid-thyrotropin stimulating hormone (TSH). All patients were subject to a complete medical evaluation, laboratory exams, electrocardiogram and echocardiography. The exclusion criteria included: age < 18; hypophysary/hypothalamic disorders, pregnancy, administration of specific thyroid treatment prior to the inclusion in the study. Based on these criteria we defined a study lot made out of 791 patients who were divided into five groups based on the level of thyroids hormones: a) HYPER subclinic – 81 patients with subclinical hyperthyroidism (10.2%); b) HYPER – 165 patients with hyperthyroidism (20.9%); c) hypo subclinical – 75 patients with subclinical hypothyroidism (9.5%); d) hypo – 170 patients with hypothyroidism (21.5%); e) Witness group – 300 patients with cardiovascular modifications without thyroid dysfunction (37,9%). Once the study lot was formed we conducted evaluations off the cardiovascular status at 6 and 12 months.

The analysis was made by means o the *SPSS 13.0* package (SPSS Inc, Chicago, Illinois, USA).

RESULTS

At the witness group, during monitoring 49% of the patients showed ischemic heart disease. The main risk factors were: heart frequency of over 80 beats/min (RR=1.83), age over 60 (RR=1.47), female sex (RR=1.21) and values of triglycerides over 160 mg/dl (RR=1.23). The treatment with antiarrhytmic drugs (RR=0.88) and beta blockers (RR=0.79) administered prior to the inclusion in the study proved to be a protective factor (tab. I, fig. 1).

Among the patients with subclinical hyperthyroidism, 28.4% showed ischemic heart disease at the beginning of the study. The main risk factors identified were: the presence of hyperglycemia (RR=1.65) and

hypertension (RR=1.42) in antecedents and high values of triglycerides (RR=1.55). In the context of decompensation of thyroid disease, the treatment with beta blockers (RR=0.79), ACEIs (angiotensin converting enzyme inhibitors) (RR=0.61) and/or antiarrhytmic drugs (RR=0.48) shows a protective factor in the development of ischemic heart disease (tab. I, fig. 1).

In the group of patients with clinically overt hyperthyroidism, during monitoring 46.1% showed ischemic heart disease. The main risk factors were: heart frequency over 80 beats/min (RR=2.41), age over 60 (RR=1.67), high level of LDL-cholesterol (RR=1.53) and female sex (RR=1.31). The treatment with ACEIs, beta blockers and antiarrhytmic proved a protective factor for this group (tab. I, fig. 1).

The main risk factors leading to ischemic heart disease for the 52% of the patients with subclinical hypothyroidism included: heart frequency over 80 beats/min (RR=3.83), obesity (RR=2.55), presence of hypertension in the personal records (RR=1.97), age over 60 (RR=1.67), female sex (RR=1.61) and a high level of LDL-cholesterol (RR=1.57). In the case of the patients who, prior to the first evaluation, received beta blockers (RR=0.61) and/or antiarrhytmic drugs (RR=0.63), the medication proved to be a protective factor in the development of ischemic heart disease (tab. I, fig. 1).

Among the patients with clinically overt hypothyroidism, during monitoring 53.3% showed ischemic heart disease. The main risk factors identified included: heart frequency over 80 beats/min (RR=2.01), age over 60 (RR=1.42), high levels of triglycerides (RR=1.42) and LDL-cholesterol (RR=1.32), as well as the presence of hypertension in the personal records (RR=1.31). The treatment with ACEIs (RR=0.95), antiarrhytmic drugs (RR=0.68) and/or beta blockers (RR=0.42), administered prior to the admission in the group is a protective factor for the development of ischemic heart disease in this study group (tab. I, fig. 1).

TABLE I

Risk factors in the development of ischemic heart disease

	Witness (n=147/300)		**Subclinical hyper-thyroidism (n=23/81)**		**Clinical hyper-thyroidism (n=76/165)**		**Subclinical hypo-thyroidism (n=39/75)**		**Clinical hypo-thyroidism (n=91/170)**	
	RR	**IC95%**	**RR**	**IC95%**	**RR**	**IC95%**	**RR**	**IC95%**	**RR**	**IC95%**
Female sex	1.21	0.8-1.8	0.67	0.3-1.6	1.31	0.8-2.2	1.61	0.5-5.1	0.63	0.4-0.9
Age ≥ 60	1.47	1.2-1.9	1.04	0.5-2.1	1.67	1.2-2.4	1.67	1.0-2.8	1.42	1.0-2.0
BMI ≥ 25 Kg/m^2	1.18	0.9-1.6	1.08	0.5-2.4	1.11	0.7-1.7	2.55	1.1-6.1	1.15	0.8-1.6
Hypertension	1.12	0.9-1.4	1.42	0.6-3.2	1.10	0.7-1.6	1.97	1.2-3.3	1.31	0.9-1.9
Diabetes Mellitus	1.13	0.8-1.5	1.65	0.5-6.1	1.15	0.8-1.7	1.02	0.6-1.7	1.02	0.7-1.5
HF ≥ 80 beats/min	1.83	1.4-2.4	1.07	0.5-2.2	2.41	1.7-3.4	3.83	1.7-8.6	2.01	1.4-2.9
LDLc ≥ 130mg/dl	1.15	0.9-1.5	1.31	0.6-2.7	1.53	1.1-2.1	1.57	1.0-2.6	1.32	0.9-1.8
Tg ≥ 160mg/dl	1.23	0.9-1.6	1.55	0.7-3.4	1.09	0.7-1.7	1.29	0.8-2.0	1.49	1.1-2.0
ACEIs	1.04	0.8-1.4	0.61	0.8-1.8	0.37	0.3-0.5	1.00	0.6-1.8	0.95	0.7-1.3
Beta blocker	0.79	0.6-1.0	0.79	0.3-2.0	0.29	0.2-0.4	0.61	0.4-0.9	0.42	0.3-0.5
Antiarrhytmic	0.88	0.6-1.3	0.48	0.1-3.1	0.61	0.4-0.8	0.63	0.4-1.0	0.68	0.5-0.9

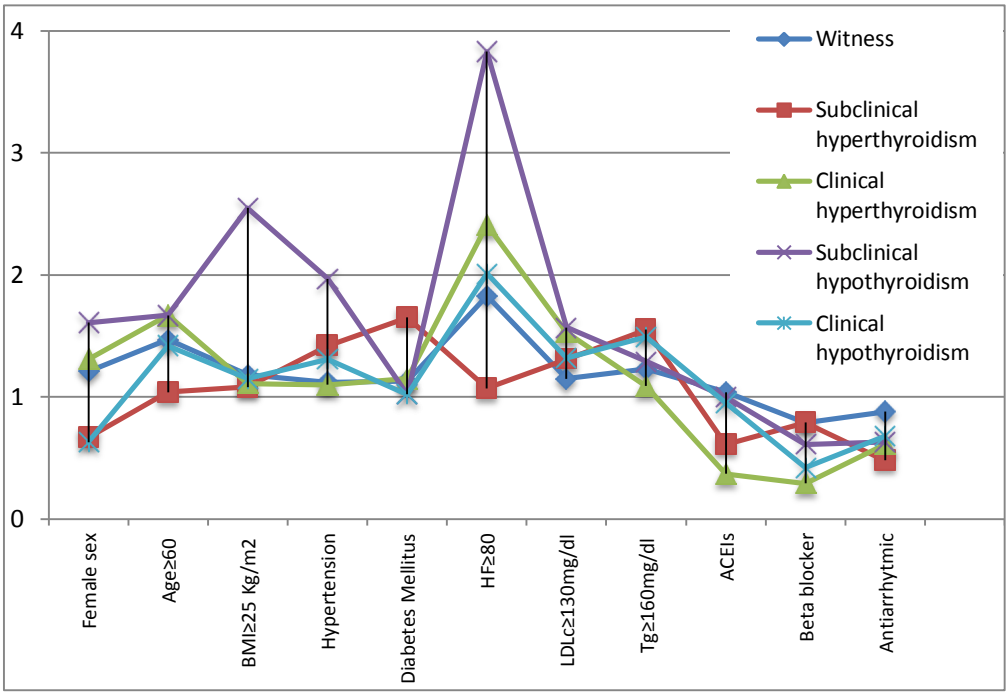

Fig. 1. Relative risk of ischemic heart disease on study lots

During the study, in the case of the witness lot, out of the 147 cases of ischemic heart disease 2 fatal events are recorded (1.4%) and 37 non-fatal events (25.2%), statistically significant proportions (p= 0.001). At the patients with antecedents of ischemic heart disease, the relative risk of developing an unfavorable event is 1.85 times higher (RR=1.85; IC95%: 1.38÷2.48) (tab. II, fig. 2).

Among the patients with subclinical hyperthyroidism who, at the beginning of the study, showed ischemic heart disease, at 15 (65.2%) were recorded non-fatal events during monitoring (p=0.002), with a relative risk 2.3 times higher (RR=2.30; IC95%: 1.45÷3.63) (tab. II, fig. 2).

At the lot with clinically overt hyperthyroidism, 28 non-fatal events are recorded (36.8%) 76 patients who, at the beginning o the study, showed in the personal pathological records ischemic heart disease (p=0.229), showing a relative risk 1.25 times higher (RR=1.25; IC95%: 0.89÷1.75) (tab. II, fig. 2).

At the lot with subclinical hypothyroidism, 7 non-fatal events are recorded (17.9%) at the 39 patients with ischemic heart disease in the records (p=0.001). At this study lot, the relative risk of an unfavorable event in the context of the presence of heart disease in the records was 2.9 times higher (RR=2.90; IC95%: 1.43÷5.87) (tab. II, fig. 2).

At the lot with clinically overt hypothyroidism, 3 fatal events (3.3%) and 46 non-fatal events (50.5%) are recorded, statistically significant proportions (p=0.001), with a relative risk 1.11 times higher (RR=1.11; IC95%: 0.85÷1.45) (tab. II, fig. 2).

TABLE II

Proportion of patients with ischemic heart disease

Ischemic heart disease	Witness		HYPER subclinic		HYPER		Hypo subclinic		Hypo	
	n	%	n	%	n	%	n	%	n	%
Beginning of the study	147	49.0%	23	28.4%	76	46.1%	39	52.0%	91	53.3%
End of the study										
Fatal events	2	1.4	0	0	0	0	0	0	3	3,3
Non-fatal events	37	25.2	15	65.2	28	36.8	7	17,9	46	50,5
p	0.001		0.002		0.229		0.001		0.001	

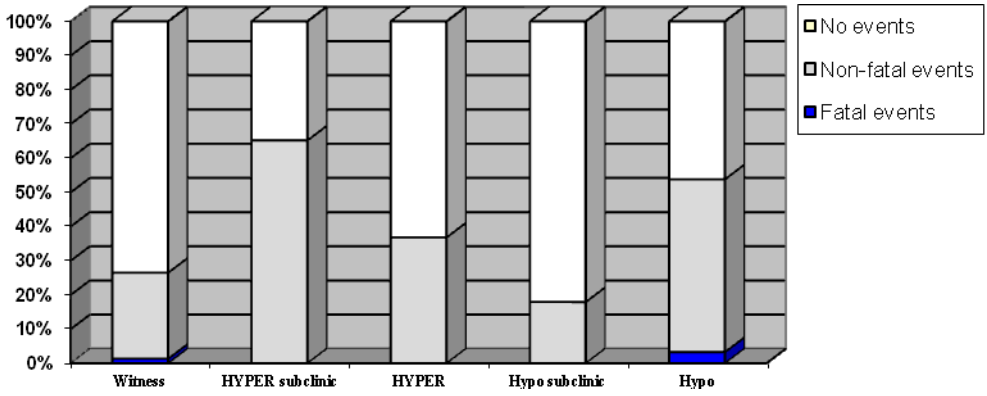

Fig. 2. Proportion of fatal/non-fatal events during monitoring of ischemic heart disease

DISCUSSION

Hyperthyroidism, through the cardiac hyperkinetic status it determines and thus through the increased myocardium oxygen requirement may worsen or decompensate ischemic heart disease. This mechanism is most frequently met at older patients. However, acute myocardial ischemia is rarely met in hyperthyroidism, in the absence of an already developed heart disorder (8). There are nonetheless, numerous case off hyperthyroidism which determined coronary spasm with the production of an acute myocardial infarction (8, 9).

Taking into consideration the fact that at the patients with hyperthyroidism an increase of the flow-mediated vasodilatation dependent on the endothelium was demonstrated, it is somehow paradoxical that thyrotoxicosis may induce a vasospasm, given the peripheral vasodilatation effect of thyroxine. *In vitro* studies showed an increase of contractility of smooth muscle tissues upon stimulation with various vasoconstrictor agents in case of a hyperthyroid status (10). The hyperthyroid status is associated with an adrenergic hyper stimulation which may have as an effect coronary spasm. Myocardial infarction at hyperthyroid patients may appear if the

coronary spasm is maintained long enough.

Generally, the prognosis of patients with coronary spasm associated to hyperthyroidism is favorable. Studied demonstrated the disappearance of anginous symptoms once the euthyroid status was reestablished at these patients (8).

The association between clinically overt hypothyroidism and ischemic heart disease was noticed in several studies (11). Less evident is the role of subclinical hypothyroidism, much more frequently met among patients with cardiovascular disorders. However, it was demonstrated that subclinical hypothyroidism is an independent risk factor which doubles the risk of myocardial infarction for women (12).

In a study that included patients with symptomatic ischemic heart disease, the prevalence of hypothyroidism was of 11.5%. Hypothyroidism was 4 times more frequent at women with ischemic heart disease than at men (23.4% vs. 6.9%) (11). At the level of the general population, out of which the study lot was selected, the prevalence of hypothyroidism was of 6.8% for men and of 13.8% for women. The prevalence of hypothyroidism for women was similar both under 55 years old and for above this age, which leads to a recommendation to investigate the thyroid function for all women with ischemic heart disease, regardless of their age (13).

The importance of hypothyroidism as a cardiovascular risk factor was assessed based on its association with high levels of total or LDL cholesterol. In the study conducted by Mayer O. (13), it was noticed that women with untreated hypothyroidism have higher levels of total or LDL cholesterol. Surprisingly, no similar association was found in case of men and this discrepancy couldn't be explained based on the difference in the statin therapy.

Increased serum levels of homocysteine, an important predictor for coronary disorder, were found in both men and women with hypothyroidism. Wald and coll. showed that an increase of 4-5μmol/L of homocysteine determines an increase by 32% of the risk of coronary events and by 59% of the risk of cerebrovascular events (14). The increase of homocysteine in hypothyroidism is due to a reduction of the glomerular filtration rate and a decrease of the activity of methylenetetrahydrofolate reductase, a key enzyme in the remethylation of homocysteine at methionine.

As in the case of homocysteine, an association was found between hypothyroidism, both clinical and subclinical, and the increase of reactive C protein (CRP) as well as endothelial dysfunction, evaluated through the endothelium dependent vasodilatation (15, 16). A very important aspect is that this endothelial dysfunction may be improved by the treatment of hormonal substitution with levothyroxine (16).

Taking into consideration this correlation between hypothyroidism and ischemic heart disease, the next question that arises is whether the treatment of hormonal substitution may reduce the coronary risk. Several placebo controlled trials, as well as a review showed that the levothyroxine administered to patients with hypothyroidism leads to a decrease of the total or LDL cholesterol level, failing to influence the HDL cholesterol or triglycerides (17, 18, 19).

In the recent years, several biomarkers, such as CRP and homocysteine gained an important role in the stratification of risk for patients with an alleged ischemic heart disease However, we shouldn't omit the importance of the thyroid function, which should bee evaluated for the patients at risk

for ischemic coronary disease. This could help to exclude secondary dyslipidemia. Taking into account the reverse association revealed between the serum level of FT3 and the prevalence off ischemic heart disease, as well as the correlations between the levels of FT4 and the mortality of any cause, their dosing is a rather inexpensive test which could prove useful in the stratification off coronary risk (20, 21).

CONCLUSIONS

Thyroid dysfunction is a common clinical issue with a key role in the regulation of the cardiovascular system and may contribute to the evolution of ischemic heart disease and should be take into account when patients with coronary disorders are treated. In this light, the thyroid function should be evaluated for all patients with a risk of ischemic heart disease.

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NEWS

DIABETES AND PERIODONTITIS

Diabetes mellitus is emerging as a global epidemic, whose complications impact significantly on quality of life, longevity and healthcare costs. The escalating human and economic burden across both the developed and developing world necessitates a multidisciplinary approach, including adjunctive measures to managing diabetes and its complications. The onset of diabetes is preceded by inflammation, which leads to pancreatic beta-cell dysfunction and apoptosis, as well as impacting on the development of insulin resistance and ultimately diabetes. It is logical that comorbidities that contribute to systemic inflammation are likely to increase the risk of developing diabetes, and impact on diabetes control and the development of diabetes complications, ultimately affecting diabetes-associated morbidity and mortality. Inflammatory periodontal diseases are the most common chronic inflammatory conditions of humans worldwide. Periodontal disease is a microbially initiated chronic inflammatory disease, in which dysregulated immune-inflammatory processes are responsible for the majority of host tissue destruction, and ultimately tooth loss. There is increasing evidence that systemic inflammation results from the entry of oral microbial agents and their virulence factors into the circulation. This is evidenced by elevated serum levels of C-reactive protein and other acute-phase reactants and raised biomarkers of oxidative stress. It is therefore biologically plausible that non-resolving chronic inflammation derived from periodontal disease impacts on diabetes control (elevated HbA1C) and complications, as well as beta-cell function, insulin resistance and development of type 2 diabetes. New researches are needed because a strong evidence base exists from epidemiological studies that periodontitis and diabetes are directly associated, and that periodontal interventions provide beneficial effects on diabetes outcomes (Iain L. C., Robert Genco. Diabetes and periodontal Diseases. *J Periodontol* 2013; 84(4 Suppl.): S106-S112).

Doina Butcovan

A CROSS-SECTIONAL STUDY REGARDING THE IMPACT OF END-STAGE RENAL DISEASE ON QUALITY OF LIFE

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A CROSS-SECTIONAL STUDY REGARDING THE IMPACT OF END-STAGE RENAL DISEASE ON QUALITY OF LIFE (Abstract): The number and severity of physical and psychological symptoms reported by chronic hemodialysis (HD) patients are significant and increasing; with a clear need to clarify which symptom intervention is the most needed. Measurements of quality of life (QoL) have shown, for many decades, an impairment of both physical and psychological aspects in both chronic kidney disease (CKD) pre-dialysis and in end stage renal disease (ESRD) patients. This cross-sectional study was conducted assess the quality of life of a Romanian hemodialysis population and the impact of several clinical and biochemical factors. **Material and methods:** A total of 102 patients (41 males, 61 females) with a mean age of 52.5 ± 12.0 years, who were treated with HD three times per week in our dialysis center were included in the study. All subjects completed the Short Form Health Survey Questionnaire (SF-36). Clinical and biochemical parameters were extracted from the EUCLID® electronic database. **Results:** Our measurement showed a deteriorated QoL in our population, all of the included subjects presenting with much lower scores in both physical and mental components than the reference values of SF-36. We did not found any statistic significant correlation between hemoglobin (Hgb) levels or dialysis adequacy and different domains of the SF-36. The only significant association was found between age and the physical component of the SF-36, implying that older patients perceive a more degraded quality of life. **Conclusion:** HD patients experience a great burden from physical and psychological symptoms of the disease, perceiving an important impairment in their quality of life, especially regarding the physical component. **Keywords:** QUALITY OF LIFE, END STAGE RENAL DISEASE, DIALYSIS, SF-36

End stage renal disease has become a worldwide health problem and it is associated with increased mortality and morbidity (1). The ERA-EDTA Registry (http:// www.era-edta-reg.org) collects individual and aggregated data from national and/or regional renal registries in Europe (2). According to ERA-EDTA Registry Annual Report 2010 the expected remaining life spam for patients after initiation of renal replacement therapy (RRT) is 10.7 years for patients' age between 40-44 years and 5.6 years for patients' age between 60-64 years (3).

It is well known the fact that patients with ESRD present with major life disturbances caused by a multitude of factors: severe cardiovascular and metabolic complications, treatment, dietary and fluid restriction, the dependency of the dialysis machine and the stringency of the dialysis schedule (4). All of these imply an important psychological, social, familial and professional impact, potential losses and lifestyle changes for the ESRD patients.

Health related quality of life (HRQoL) is a concept developed for the first time in 1946 when the constitution of the World Health Organization (WHO) defined heath as 'a state of complete physical, mental and social well being and not merely the absence of disease or infirmity' (5). Nowadays, this definition has been extended also to the effects of a person health (including the effects of both disease and its treatment) on physical, cognitive and social functioning in day to day life (6).

ESRD patient's high burden of the disease is also experienced by most individuals suffering from different chronic illnesses. The association between deteriorated quality of life and non-renal chronic diseases was reported in various medical specialties: diabetes (7), cardiovascular diseases (8), pulmonary diseases (9), neurologic diseases (10), rheumatologic diseases (11), all of these studies showing a disrepair in different domains of the quality of life when a long term illness is instituted.

The same results were found in the renal population, both chronic kidney disease pre-dialysis stages and ESRD population on different RRT (hemodialysis or peritoneal dialysis (PD)). Impaired quality of life and the relationship between illness perception (IP) and perceived quality of life were reported previously by our group (12) in a Romanian HD population without significant co morbidities. This study found that illness perception could be interpreted as a powerful predictor of QoL in ESRD patients.

Since improvement of the quality of life has become a major treatment goal in ESRD patients, the aim of this study was to evaluate the QoL of a well-established cohort of Romanian patients receiving HD treatment and to analyze the impact of several clinical and biochemical parameters on this psychosocial measurement.

STUDY DESIGN

We performed a cross-sectional questionnaire analysis in HD patients in order to determine the prevalence of severity of the psychological and physical symptoms and to evaluate the quality of life in these patients.

PATIENTS AND METHODS

We included a total of 102 stable patients (41 males and 61 females) with a mean age of 52.5 ± 12.02 years who were receiving HD treatment three times weekly in *Fresenius Nephrocare* Iasi dialysis unit. All subjects were informed about the purpose of the study and gave written consent prior to participation. All subjects completed the Short Form Health Survey Questionnaire in order to assess the QoL.

The data were analyzed using *SPSS version 22.0* for Mac. Frequencies and descriptive statistics of the demographic and symptoms data of the study population were calculated. We used independent t-test for independent variables to compare the means and to observe the influence of independent variables on the dependent variables. ANOVA was used to assess the

relationship between QoL and continuous variables (age, hemoglobin (Hgb), Kt/v).

INSTRUMENTS

The SF-36 adapted for Romanian population was used to assess eight dimensions of the QoL: physical functioning, social functioning, role-functioning emotional, role-functioning physical, vitality, bodily pain, mental health and general health perception. Two summary score were obtained: one for the physical component (assessing physical functioning, role-functioning physical, vitality, bodily pain and general health perception) and one for the mental component (assessing social functioning, role-functioning emotional, mental health, vitality, and general health perception). Score ranged from 0 to 100, a higher score indicating a better perceived quality of life.

RESULTS

A summary of demographics and clinical data is shown in table I (n=102). The mean age of the sample was 52.51 years (±12.02). All included subjects were Caucasian and the majority was constituted by female (59.8%). The mean Hgb level was 10.9 g/dl (±1.06). On average, patients received adequate dialysis as indicated by a mean Kt/V of 1.47 (±0.27). The mean scores for the SF-36 Physical Component Score (PCS) and Mental Component Score (MCS) were 51.87 (±19.21), respectively 61.38 (±16.71) suggesting a decreased perceived quality of life among the analyzed population.

TABLE I

Baseline characteristics of the included patients

Variable	Statistics
Mean age ± SD years	52.51±12.02
Male, n(%)	41(40.2)
Ethnicity (Caucasian), n(%)	102(100)
Mean hemoglobin ± SD, g/dl	10.99(1.06)
Mean Kt/V ± SD	1.47(0.27)
PCS	51.87(19.21)
MCS	61.38(16.71)

SD=standard deviation; PCS=physical component summary;
MCS=mental component summary;

Table II summarize the mean score for each of the eight domains of the SF-36 that were evaluated. The lowest score was registered by the role-functioning physical domain with a value of 36.61(±36.21) suggesting that ESRD patients seem to perceive the highest burden from the physical restriction of the disease.

Also, we analyzed the influence of Hgb level on PCS and MCS. There were no significant differences between the three groups in the general scores of QoL (p=0.237) (tab. III, IV). The analysis was conducted with One Way ANOVA.

TABLE II
Mean scores for each domain assessed by the SF-36

SF-36 domain	Score, mean (SD)
Physical functioning	54.63(28.52)
Role-Physical	36.61(36.21)
Bodily Pain	68.77(23.64)
General Health	45.20(21.90)
Vitality	54.41(19.87)
Social Functioning	61.95(28.07)
Role-Emotional	78.84(36.44)
Mental Health	66.39(20.79)
Physical Component Summary (PCS) score	51.78(19.21)
Mental Component Summary (MCS) score	61.38(16.71)

TABLE III
Mean scores of Hgb levels in relation to PCS score

Hgb level	N	Mean (SD)
<10	16	47.57(17.93)
10-11.5	58	50.54(18.47)
>11.5	28	56.77(21.03)

p=0.237

TABLE IV
Mean scores of Hgb levels in relation to MCS score

Hgb level	N	Mean (SD)
<10	16	58.19(15.26)
10-11.5	58	60.90(17.25)
>11.5	28	64.20(16.51)

p=0.494

We did not found any significant correlation between Kt/V and the general scores of the SF-36, suggesting that an adequate dialysis does not have an impact on quality of life in our population (table V, VI).

TABLE V
Mean scores of Kt/V in relation to PCS score

Kt/V	N	Mean (SD)
<1.4	44	51.74(20.54)
>1.4	58	51.81(18.32)

p=0.277

TABLE VI
Mean scores of Kt/V in relation to MCS score

Kt/V	N	Mean (SD)
<1.4	44	61.68(17.96)
>1.4	58	61.15(15.86)

p=0.561

The analysis showed that the only statistical significant influence (p=0.051) was found regarding age and the physical component of the SF-36 (tab. VII), indicating that the elderly patients report a more deteriorated quality of life, especially in the physical component. There were no significant differences between the two age groups in patients mental component score (p=0.098) (tab. VIII).

TABLE VII
Mean scores of age in relation to PCS score

Age (years)	N	Mean (SD)
<45	27	53.96(16.99)
>45	75	51.00(20.00)

p=0.051

TABLE VIII
Mean scores of age in relation to MCS score

Age (years)	N	Mean (SD)
<45	27	62.82(13.17)
>45	75	60.86(17.87)

p=0.098

The analysis regarding the influence of Kt/V and age on QoL was conducted with Independent samples t-test.

DISCUSSION

Dialysis care has increased in importance throughout the world because of the growing prevalence of patients receiv-

ing RRT and its related mortality and morbidity and high social and financial costs. The multidimensional aspects of quality of life of these patients have been reported previously in literature and include not only a general perception, but also specific aspects of physical, psychological and social functioning.

Our primary objective was to assess HRQoL in our study group sample. According to our results, and consistent with other literature data (13), there appears to be an important impairment to QoL in our population. All of the eight domains that were evaluated were below the reference values established by the SF-36 questionnaire, the most heavily affected components being role-physical functioning, vitality and physical functioning. The worst SF-36 score was for the general health section (PCS=51.78) implying that patients on RRT have a negative self-assessment of current health.

Our secondary objective was to identify several clinical and biochemical parameters that could be significant predictors of HRQoL.

Regarding the dialysis parameters, our results are both conflicting and consistent with other studies. We found that our population presented a mean Kt/V of 1.47, but no statistical significant association between the general scores of SF-36 and Kt/V, suggesting that dialysis adequacy had no impact on quality of life on our population. These data support the results found by the HEMO study (14), a randomized controlled trial (RCT) that was designed to test the hypothesis that a higher dose of dialysis could modify different outcomes and did not found any significant differences between the two dosage groups regarding mortality, hospitalization or other secondary endpoints including quality of life. In contrast a cross-sectional survey performed on 128 HD patients with an average Kt/V level greater than 1.3 showed that dialysis adequacy was significantly associated with a better HRQoL (15).

The KDIGO Clinical Practice Guideline for Anemia in Chronic Kidney Disease 2012 (16) recommends for HD patients that erythropoiesis-stimulating agents (ESA) therapy should be started at a Hgb level between 9-10 g/dl (level of evidence 2B). Since this guideline is also suggesting that individualization of therapy can be necessary as some patients may present improvements in quality of life at Hgb concentration above 11.5 g/dl, if they are prepared to accept that risk (level of evidence not graded), in our study we divided our population according to their Hgb levels into three groups: <10 g/dl, between 10 and 11.5 g/dl and >11.5 g/dl. We did not found any statistic significant association between Hgb level and HRQoL, in contrast to a RCT that showed a more important improvement in the SF-36 scores in a higher Hgb group compared to a lower Hgb group (17).

Consisting with previously reported data (18,19) demonstrating that SF-36 scores are negatively associated with age and that QoL seems to be deteriorated in elderly HD patients, our study found a statistical borderline negative association between age and QoL general physic component score (p=0.051).

CONCLUSION

It seems that hemodialysis patients experience a great burden from physical and psychological symptoms of the disease, perceiving an important impairment in their quality of life, especially regarding the physical component. Our findings are not

alone in suggesting the imperative need to design effective interventions, such as the implementation of different types of programs for physical activity that are stimulated by social support networks (for example creating patient groups or involving family members for carrying out various activities).

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NEWS

SEVERE ADVERSE EVENTS CAUSED BY MSSA ANTIMICROBIAL TREATMENT IN BONE AND JOINT INFECTION

Because the safety of prolonged antimicrobial therapy, recommended in methicillin-susceptible Staphylococcus aureus (MSSA) bone and joint infection (BJI), and risk factors for severe adverse events (SAE) were unknown in clinical practice, Valour and co. conducted a retrospective cohort study (2001-2011) analysing antimicrobial-related SAE (defined according to Common Terminology Criteria for Adverse Events) in 200 patients with MSSA BJI. The patients were admitted in a reference regional centre with acute (66%) or chronic arthritis (7.5%), osteomyelitis (9.5%), spondylodiscitis (16%), or orthopaedic device-related infections (67%) and received anti-staphylococcal therapy for 26.6 weeks. Thirty patients (15%) had 38 SAE, including 10 hematologic reactions, 9 cutaneo-mucosal reactions, 6 acute renal injuries, 4 hypokalaemia, and 4 cholestatic hepatitis. The most frequently implicated antimicrobials were anti-staphylococcal penicillins (ASP) (13 SAE/145 patients), fluoroquinolones (12/187), glycopeptides (9/101), and rifampin (7/107). To determine risk factors for the occurrence of antimicrobial-related SAE were used Kaplan-Meier curves and stepwise binary logistic. They found that only age (p=0.006) appeared to be independent risk factors for SAE. Daily dose (p=0.014) and obesity (p=0.018) were associated with occurrence of SAE only in patients receiving ASP or rifampin. The high rate of SAE and their determinants highlighted the importance of the management and follow-up of BJI, with a particular attention to be paid in elderly, especially for ASP dosages, and in rifampin dose adjustment in obese patients. (Valour F, Karsenty J, Bouaziz A et al. Antimicrobial-related Severe Adverse Events during Treatment of Methicillin-Susceptible Staphylococcus aureus Bone and Joint Infection. *Antimicrob Agents Chemother*. 2013 Nov 18. [Epub ahead of print]).

Simona Tucaliuc

ANNEXINS, CALCIUM-DEPENDENT PHOSPHOLIPID BINDING PROTEINS IN IRREDUCIBLE HEART FAILURE

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ANNEXINS, CALCIUM-DEPENDENT PHOSPHOLIPID BINDING PROTEINS IN IRREDUCIBLE HEART FAILURE (Abstract): **Aim:** To analyze the expression and distribution of annexins V and VI in intact human hearts and in dilated cardiomyopathy (DCM) in patients with irreducible heart failure. **Material and methods:** The study included nine patients with DCM and irreducible heart failure. By immunoblotting and indirect immunofluorescence, the amount and location of annexins was determined using samples of left ventricular (LV) myocardium collected during orthotopic heart allotransplantation. Samples of LV myocardium from 9 individuals who died of craniocerebral trauma served as controls. **Results:** Quantitative analysis showed an increased level of both annexins in the myocardium of DCM patients compared with normal myocardium: 122 ± 5% ($p < 0.05$) and 119 ± 5% ($p < 0.05$) for annexins V and VI, respectively. In the intact heart annexin V was located at the sarcolemma and intercalated discs, while in the myocardium with heart failure mainly in the interstitium. In normal hearts, annexin VI was located at the sarcolema, including *T*-tubules, *Z*-lines and intercalated discs. In heart failure, annexin VI was also located in the interstitium. **Conclusions:** Redistribution of annexins V and VI in the cardiomyocytes in the interstitial space may have important functional consequences and indicates the role of myocardial protein expression disturbances in the etiology and/or pathogenesis of heart failure in patients with DCM. **Keywords:** ANNEXINS, CARDIOMYOCYTES, HEART FAILURE, DILATED CARDIOMYOPATHY.

Annexins constitute a family of proteins characterized by their ability to bind phospholipids in a calcium-dependent manner. Although the biological role of annexins remains unknown, they are involved in various intra and extracellular processes, including mitogenic signal transduction, differentiation, transmembrane events, calcium flux, and extracellular matrix binding (2, 4 , 5, 6, 9, 10, 11). The annexin family proteins are present in all animal tissues, and annexins V and VI are the major cardiac proteins (2, 4, 6). Annexin V forms a voltage-dependent calcium channels and modulates the activity of protein kinase C and phospholipase A2 (3, 7, 12,

13). Annexin VI was located at the sarcolemma and intercalated discs of myocytes in rats, bovines and guinea pigs (1, 10, 11). Annexin VI overexpression in transgenic mice heart was demonstrated in dilated cardiomyopathy with disturbed cardiac contractility or intracellular calcium turnover imbalance. However, the potential role of annexins in heart failure can not be considered without establishing their precise distribution in human heart with or without heart failure. The purpose of this study was to study the expression and distribution of annexins V and VI in the healthy human heart and in patients with dilated cardiomopathy and irreducible heart failure.

MATERIAL AND METHODS

The study included nine DCM patients (8 men, 1 woman, mean age 29 ± 8 years) with irreducible heart failure. To determine the amount and location of annexins samples of left ventricular myocardium from excised hearts collected during orthotopic heart allotransplantation. Samples of left ventricular myocardium from 9 adults (7 men, 2 women, mean age 33 ± 8 years) who died of craniocerebral trauma served as controls.

Quantitative determination of annexins

Annexins V and VI content in myocardial samples was determined by immunoblotting using membrane fractions of normal and DCM human myocardium isolated according to the method of Rannou et al. (8) or Seint-Beuve et al. (1997) The LV tissue sample (150 mg) was homogenized with a homogenizer, Polytron PT knife 10 (3 times for 5 seconds each and 30 seconds pauses) in 10 ml buffer containing 0.3 M sucrose, 20mM HEPES, 1 mM NaN3, pH7.4, and protease inhibitors (1.1 mcrM leupeptin; 0.7 mcrM aprotinin, 0.7 mcrM pepstatin A; 120mcrM phenyl methane sulfonyl fluoride, 1 mM diisopropyl fluorophosphate, 1 mM iodoacetamide). All procedures were performed on ice. The homogenate was centrifuged for 45 minutes, 41.000g. The precipitate was resuspended and then homogenized in a buffer (pH6.8) consisting of 0.3 M sucrose, 30mm imidazole, 1 mM sodium azide, 100 mM NaCl and protease inhibitors. The 10% polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate was carried out by the Laemmli method (1970). Electrical transfer of proteins to nitrocellulose membrane (Hybond ECL, Amersham) was performed according to the method of Towbin et al. (1979) in Tris-Glycine buffer system (PH8), over one hour at a voltage of 100V. Immunodetection of proteins on the membrane was performed using specific antibodies as previously described (Sambrook, Fritsch, Maniatis, 1989, Molecular Cloning A Laboratory Manual). Nonspecific interactions were blocked by incubating the membrane in phosphate saline buffer with 5% skim milk and 0.1% Tween 20, pH7.4. Then, the membrane was incubated for one hour in the primary antibody solution (1:5000) at room temperature, in blocking buffer and washed three times, 15 minutes each, in phosphate saline buffer, followed by incubation in secondary antibody (peroxidase conjugated anti-rabbit IgG) (Sigma). Membrane signal was detected by chemiluminescence reaction using the ECL kit (Western blotting detection reagents, Amersham, England) and radiological Fudgy Film (Germany). The bands were quantitatively analyzed by scanning densitometry. The results are presented as mean ± standard error. Accuracy of differences between the control and experimental groups was calculated using the Student

two-sample T-test, and the differences were considered accurate when close to $p < 0.05$. Antibodies to annexins V and VI (primary antibody) were obtained by immunizing rabbits with recombinant human annexins; these were highly specific.

Immunofluorescence histochemistry

Cellular localization of the studied proteins was done by the indirect immunofluorescence method. To do this, cryosections were fixed in a 1:1 mixture of acetone-methanol for 20 minutes at 20 ° C, processed in a 5% bovine serum albumin solution in phosphate buffer, for 30 minutes, with primary antibodies: mouse sarcomeric α-actinin or actin monoclonal antibody (Sigma) (1:10000 in phosphate buffer, 37° C) and rabbit annexin V or VI antibodies in suitable dilution (1:50 in phosphate buffer, 37° C). After triple washing in phosphate buffer, the sections were incubated successively for 30 minutes at room temperature with secondary antibodies: mouse immunoglobulin antibody conjugated with *Alexa green* fluorochrome (594nm), and rabbit immunoglobulin antibody (1:2000) conjugated to *Alexa red* (488nm). Sections were analyzed with an Opton fluorescence microscope (Germany).

RESULTS

Immunoblotting

By this method bands of annexin V (36cDa) and annexin VI (68cDa) were detected in the LV membrane fractions both in normal myocardial samples and in those from patients with heart failure (fig. 1).

The signals of both annexins were stronger in the myocardium with heart failure. Quantitative analysis indicated a definite increase in the level of both annexins in the myocardium of patients with DCM, compared with normal myocardium: 122 ± 5% ($p < 0.05$) and 119 ± 5% ($p < 0.05$) for annexins V and VI, respectively.

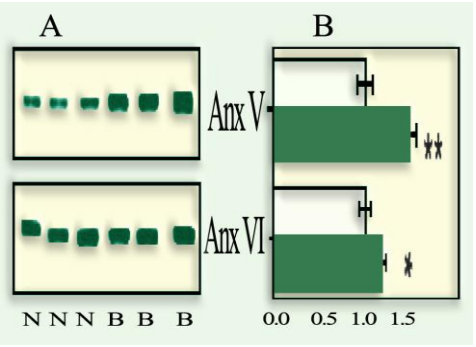

Fig. 1. Imunoblott-analysis of annexins V and VI in normal and DCM myocardium. A relative number of annexins V and VI in the healthy hearts (bright columns) and in the myocardium of patients with DCM, (dark columns). The levels are marked * $p = 0.05$, ** $p < 0.05$.

Immunofluorescence investigation

By indirect immunofluorescence in cardiac tissue cryosections differences in cellular localization of the studied annexins between normal and DCM hearts were detected (fig. 2).

Comparison of actin and annexin V location indicated that in the normal heart annexin V is located at the sarcolemma and intercalated discs. On longitudinal sections one can see that annexin V is located near the Z-lines, also labeled by actin (fig. 2, A-C).

On the cross-sections of normal myocardium notable were the invaginations – T- tubules containing annexin V, implying that annexin V was located on/near-T tubules. On the cross sections of DCM myocardium annexin V was absent in T-tubules and poorly stained the sarcolemma. On the longitudinal section of the DCM myocardium annexin V stained less brightly in sar-

colemma and intercalated discs. Comparison of annexin V and alpha-actin distribution showed that in the myocardium with heart failure, annexin V is mainly located in the interstitium, between concentrations of myocytes, where alpha-actin was not labeled (fig. 2, DF). Like annexin V, in normal hearts annexin VI is located in the sarcolemma, including T-tubules, Z-lones and intercalated discs. The cross-ribbed structure of cardiomyocytes stained with annexin VI was observed in the myocardium of patients with heart failure. In longitudinal sections of myocardium with heart failure it was seen that annexin VI is also present in the interstitium between myofilament bundles, highlighted by alpha-actinin staining (fig. 2, F).

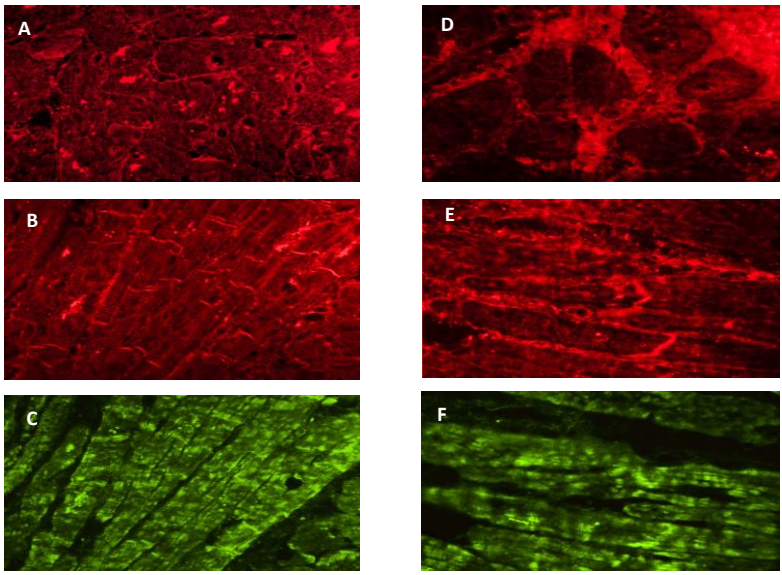

Fig. 2. Location of annexins V and VI in normal hearts (A-C) and myocardium of patients with DCM and heart failure (D-F). Immunofluorescence of cross sections (A) and longitudinal sections (B) of normal and heart failure myocardium (D and E), labeled with antibodies to annexins V and VI (50mm band).

RESULTS AND DISCUSSION

Our data showing increased annexin V and VI levels in the left ventricular myocardium of patients with DCM compared to healthy individuals are consistent with studies in patients with terminal heart failure, in which increased levels of mRNK and annexins V and VI were found. This demonstrates that the accumulation of mRNK and protein results from transcriptional regulation. Data in the literature show increased annexin V levels in the myocardium of patients with DCM and ICC and severe heart failure. However, we found an increase in annexin VI undetected by the authors of these studies. A similar increase in the amount of annexins V and VI was found in hypertensive rats with early-stage heart failure, while in compensatory hypertrophy the amount of annexin

V remained unchanged, which allows us to assume that the expression of ansexins varies with the severity of cardiac disease. To elucidate the consequences of annexin regulation in heart failure we studied their distribution. Our results showed that in the healthy myocardium the subcellular location of anexins V and VI is at the sarcolemma, intercalated discs and T-tubules, linked to the terminal cisternae of sarcoplasmic reticulum, in the complex where excitation-conjugation coupling takes place (12, 13). Comparison of annexin V and actin distribution showed that in the myocardium of patients with heart failure annexin V is mainly located in the interstitium between myocyte concentrations in areas corresponding to fibrous tissue. This redistribution is the clearest feature/ indication of a failing heart. In experiments on mice the redistribution of annexin V is a rare finding in end-stage heart failure and is absent in compensatory myocardial hypertrophy (13), although the location of annexin V in normal cardiac tissue is the same in both biological species. The ability of annexin V to bind to extracellular matrix proteins (12, 14) probably contributes to the development of "reactive" interstitial fibrosis and structural reorganization of cardiac ventricles in heart failure. Decrease of membrane-bound annexin V in myocytes can lead to a local change in Ca2 + concentration leading to the activation of protein-kinase C (10), phospholipase A2 (11), and signaling pathways (12), which contributes to the contractile cardiac dysfunction in CMD patients with terminal heart failure. Annexin VI is a negative inotropic factor, its activity being related to the regulation of the cytoplamic intracellular calcium flux by slowing the removal of calcium during diastole. All the above mentioned clearly indicate the role of annexins V and VI in the emergence and progression of heart failure in patients with DCM.

CONCLUSIONS

In the CMD patients with irreducible heart failure the amount of annexin V and VI in the myocardium increases; moreover, annexin V is mainly located in the areas of fibrosis. The ability of annexin to bind to extracellular matrix proteins indicates its participation in ventricular remodeling in heart failure. Redistribution of annexins V and VI in the cardiomyocytes in the interstitial space may have important functional consequences and indicates the role of myocardial protein expression disturbances in the etiology and/or pathogenesis of heart failure progression in patients with DCM.

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NEWS

VERAPAMIL IN SHORTENING TB TREATMENT

Tuberculosis (TB) is one of three leading causes of death due to a single pathogen. The increased prevalence of multidrug-resistant TB is due to low adherence to drug treatment caused by side effects and long time therapy. One of the mechanisms of resistance to anti-TB drugs is intervention of mycobacterial efflux pumps that reduce the effectiveness of antibiotics. The inhibition of these pumps enhances drug efficacy. Verapamil that is a calcium channel blocker used in treatment for high blood pressure also is an efflux pump inhibitor. A recent study in mice conducted by Gupta and colleges showed that Verapamil used in combination with isoniazid and rifampicin accelerates bacterial clearance by 10-fold after two months of treatment and the bacteriological cure is achieved after four months of therapy instead of the six. These findings supported future studies on efflux pump inhibitors to shorten TB treatment duration and increase compliance of treatment. (Gupta S, Tyagi S, Almeida DV, Maiga MG, Ammerman NC, Bishai WR. Acceleration of Tuberculosis Treatment by Adjunctive Therapy with Verapamil as an Efflux Inhibitor. *American Journal of Respiratory and Critical Care Medicine*, 2013; 130711121718008 DOI: 10.1164/rccm.201304-0650OC).

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THE ROLE AND INTERACTIONS OF GHRELIN CONCERNING THE NUTRITIONAL AND INFLAMMATORY STATUS

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THE ROLE AND INTERACTIONS OF GHRELIN CONCERNING THE NUTRITIONAL AND INFLAMMATORY STATUS **(**Abstract): Ghrelin is an important neuroendocrine peptide having as main purpose the stimulation of growth hormone (GH) secretion. It is also an important regulator of the long-term energy balance and short-term nutritional intake. Ghrelin has several other biological actions, among which the capacity to regulate gastrointestinal motility, to modulate the reproductive and stress axes **as well as** the glucose metabolism, and other well-defined actions within the cardiovascular and renal physiology. Due to its numerous effects, ghrelin is considered on one hand a potential target in the treatment of obesity and on the other, a therapeutic option in other dysfunctions and illnesses. **Keywords**: GHRELIN, GHRELIN-O-ACYL-TRANSFERASE, INFLAMMATION, OBESITY, CACHEXIA.

The term "ghrelin" originates from the Proto-Indo-European language, where "ghre" means "to grow" and "relin" means "release". The gene for ghrelin is located on chromosome 3p26-p25, encoding a protein of 117 amino acids named pre-proghrelin (1). This protein is processed post-transcriptionally into a 28 amino acid and 3,3 kDa peptide, synthesized especially in X/A-like cells of the gastric mucosa and to a smaller degree in the entire organism. X/A-like cells are a type of endocrine cells situated in the middle and lower regions of the gastric oxyntic glands that represent about 20-30% of all gastric endocrine cells, therefore occupying the second place within the gastric endocrine cell populations (2).

There are two isoforms of mARN for pro-ghrelin, one for ghrelin and the other one for des-Gln^{14}-ghrelin precursors, and they are produced in the rat stomach by an alternative splicing mechanism (3). Des-Gln^{14}-ghrelin is found in limited amounts only in the stomach, which proves that the physiologically important peptide is actually ghrelin, being the one released in the general circulation (3).

In the serum, ghrelin is found in 2 forms: non-acylated ghrelin, predominant in the plasma and acylated ghrelin. The latter is produced post-translationally by esterification of the hydroxyl group of the third amino acid residue, serine, by n-octanoic fatty acid, increasing the hydrophobicity of the molecule. This change allows acylated ghrelin to bind to the growth hormone secretagogue receptor 1a

(GHS-R1a), manifesting thereby its biological activities including growth hormone secretion, appetite regulation, modulation of insulin secretion, and stimulation of adiposity (4).

The enzyme responsible for ghrelin acylation was found in humans in 2007 as the forth member of the membrane-bound O-acyl-transferase family and was named GOAT (ghrelin-O-acyl-transferase). It is a well-conserved protein across vertebrates with a similar sequence in humans, rat and mouse (74.5% identity and 98.4% similarity). The endocrine cells expressing GOAT are found in the murine stomach, especially in the middle portion of the oxyntic glands, a localization which corresponds to the X/A-like cells that secrete ghrelin (5).

In rats and mice, GOAT has also been found in the plasma, which could suggest an extracellular ghrelin acylation. GOAT concentration after 24 hours of fasting increases significantly and can lead to an increase in acylated ghrelin concentration, enhancing the orexigenic effect of the latter. This represents a supplementary mechanism, apart from the gastric ghrelin mRNA expression increase as a response to fasting, to the decrease in ghrelin concentration in the X/A-like cells and increase of plasma levels of ghrelin in mice, as well as in rats.

Ghrelin has a wide distribution in human tissues and the distribution of GOAT follows the same pattern (6), allowing therefore active ghrelin to manifest its effects. GOAT expression is lower than that of ghrelin, without a statistical correlation between the expression of ghrelin and GOAT mRNA. The mRNA for GOAT is mostly found in the pancreas, stomach and pituitary, where the endocrine, paracrine and autocrine effects of ghrelin have been described.

The importance of GOAT and ghrelin in human physiology was demonstrated by knock-out studies on mice under severe caloric restriction (60%). The absence of ghrelin with the consecutive decrease in growth hormone leads after 7 days to sever hypoglycemia in the hours that precede feeding (7).

Ghrelin receptor has been known long before the discovery of ghrelin in 1999. The cells of the anterior pituitary present a receptor that, once activated, stimulates growth hormone secretion. This receptor has been named the growth hormone secretagogue receptor (GHS-R). It is encoded by a gene localized on chromosome 3q26.2. There are two types of GHR: GHS-R1a with a more limited distribution and GHS-R1b, with inhibitory effects on the GHS-R1a signaling, much more widely distributed in tissues (8). GHS-R1a is a G-protein coupled receptor that contains 366 amino acids and 7 transmembrane domains.

Ghrelin's actions on the energy balance are mediated partially through its effects on the centers of appetite, such as the central melanocortin system. Ghrelin activates GHS-R localized in the pituitary and the neurons that contain Growth Hormone Releasing Hormone (GHRH) in the hypothalamic arcuate nucleus, thereby stimulating the secretion of GH. Activating the GHS-R situated on the arcuate and ventromedial neurons leads to an increase in the expression and release of orexigenic peptides neuropeptide Y (NPY) and agouti related-peptide and to the decrease of the expression of pro-opiomelanocortin, with its anorexigenic effects. These effects lead to a decrease in the signaling taking place on the melanocortin-4 receptor and an increase in signaling in the Y1 receptor, re-

sulting in the accentuation of food-seeking behavior and in the decrease of basal metabolic rate (9).

Ghrelin and other hormones

The stimulatory effect of ghrelin on GH secretion is specific, potent, dose-dependent and synergistic with that of GHRH. In humans, ghrelin induces a significant and durable increase in the level of circulating GH, an effect more potent than that produced by GHRH itself.

Central regulation of feeding is based on ghrelin and leptin, which signal the nutritional status and the level of energy deposits to the hypothalamic feeding centers. Ghrelin is orexigenic when administered both centrally as well as peripherally and is one of the humoral signals regulating the appetite. Leptin on the other hand is an anorexigenic hormone secreted by the adipose tissue, involved in thermogenesis and the control of several neuroendocrine functions of the hypothalamic-pituitary-adrenal axis.

Knock-out mice for ghrelin as well as knock-out mice for GHS-R are not anorexic dwarfs as expected; their size, growth rate, food intake, body composition, reproduction, behavior and tissue pathology are not different from those of the wild types (10). Fasting leads to an identical decrease in serum leptin and insulinemia in mutant mice as well as in the wild ones, demonstrating that ghrelin does not have a direct regulatory effect on leptin or insulin. Knock-out mice for ghrelin present normal responses to starvation and diet-induced obesity (10). As in wild mice, exogenous ghrelin administration stimulates appetite (10). Insulin-like Growth Factor 1 (IGF1) level and weight of the mature GHS-R null mice are slightly reduced when compared to the wild ones, which demonstrates the effect of ghrelin as an amplifier of GH pulsatility and the supposed role to maintain a certain set-point of IGF1 in the anabolic metabolism. Therefore, ghrelin is not critically necessary for the viability, fertility, growth, appetite, mineral bone density and adipose accumulation and probably is not a direct regulator of leptin and insulin and consequently, ghrelin antagonists are unlikely an anti-obesity solution (10).

The fasting ghrelin level is strongly associated to insulinemia (negative correlation), insulin resistance (negative correlation) and the high density lipoprotein cholesterol levels (positive correlation). Ghrelin suppression induced by food intake is correlated to the postprandial increase in insulin (11).

Ghrelin level is similar in men and women (11) and does not vary with the menopausal status or hormonal substitution therapy, demonstrating that ghrelin level is not regulated by estrogens in women.

Ghrelin and obesity

In humans, ghrelin concentrations double preprandially and decrease to basal values one hour postprandially. In negative energy balance situations such as hypocaloric diets, chronic physical exercises, cancer anorexia, anorexia nervosa and Prader-Willi syndrome, ghrelin concentrations are increased (12). In the obese, ghrelin concentrations are reduced, which can be a result of the important caloric intake, while a weight loss in the obese leads to an increase in ghrelin concentrations. Although ghrelin is increased in patients under certain diets, gastric bypass surgery reduces ghrelin concentrations, suggesting that the size of the stomach can be directly related to ghrelin concentra-

tions.

Ghrelin plasma concentrations evolve in a divergent manner in obesity and anorexia nervosa after dietary intervention, suggesting that ghrelin is a good marker of the nutritional status (13).

Resistance to insulin and hyperinsulinemia are inversely correlated to the ghrelin concentration, which can represent a part of the feedback mechanism that regulates body weight in humans.

A study that has followed the dynamics of ghrelin in obese patients that were subject to a diet for 6 months and in obese patients operated through gastric bypass has shown that the weight loss induced by diet of 17% of the initial weight was associated to a significant increase in ghrelin, demonstrating its role in long-term regulation of body weight. This role is supported by studies on murine models that prove that continuous ghrelin administration increases in a durable manner the body weight (14). Not only does the caloric intake increase, but with the administration of exogenous ghrelin there is also a decrease in the metabolic rate and of the catabolism of fat (14), leading to modifications of all the energy regulation systems that may eventually lead to weight gain. Blocking the cerebral action of ghrelin leads to a reduction in food intake, which suggests that endogenous ghrelin is necessary to maintain a normal appetite. It appears that the increase in ghrelin levels contributes to the adaptive responses that limit weight gain.

In operated patients with a weight loss of 36% of the initial weight, ghrelin level is significantly lower when compared to the obese subjects that represented the controls, as well as when compared to normal-weight subjects. Normal ghrelin fluctuations related to meals and the diurnal secretion rhythm are suppressed after gastric bypass (15). This suggests the role of ghrelin decrease in the weight loss and appetite reduction in subjects operated by gastric bypass, effects that cannot only be explained by the gastric restriction created through the surgical intervention. These patients have a diminished sense of hunger and they spontaneously reduce their intake of high-caloric foods, although they are still capable to appreciate the taste of food. The explication that the authors propose (15) for the decrease in ghrelin levels could be an override inhibition: normally, the absence of nutrients in contact with the gastric mucosa stimulates ghrelin secretion. In the case of gastric bypass, the permanent absence of food in the stomach and duodenum leads to a continuous stimulatory signal that eventually suppresses the production of ghrelin by a process named override inhibition.

Another study (16) has investigated the evolution of ghrelin in obese and anorexia nervosa patients, in obese and normal-weight type 2 diabetics, as well as ghrelin evolution in response to the oral glucose uptake test in normal subjects and to a test meal in diabetics. Ghrelin concentrations were higher in patients with anorexia nervosa and lower in the obese when compared to normal-weight control subjects. Tschop et al. (17) have reported that fasting ghrelin is negatively correlated to the percentage of body adiposity, fasting insulin and leptin concentrations. The study conducted by Shiiya et al. (16) has proven that fasting ghrelin in normal subjects, in those with anorexia nervosa, obesity or type 2 diabetes is negatively correlated to the body mass index of each group. Oral or intravenous glucose administration in normal subjects leads to a decrease in serum

levels of ghrelin, while the administration of a similar volume of water does not, proving that the effect on ghrelin concentration is not related to gastric expansion.

Ghrelin and cachexia

Cachexia is a constellation of symptoms that lead to the wasting syndrome, an increase in the metabolic rate that favors the loss of lean and fat mass and a paradoxical decrease in appetite, persistent in spite of the depleted energy stores. Cachexia appears in the pathophysiological context of various chronic illnesses, such as cancer, cardiac failure, chronic kidney disease and acquired immunodeficiency syndrome. Cachexia is a negative prognostic factor for the underlying illness. In this context, a promising pharmacologic agent is represented by the ghrelin receptor analogue. Ghrelin binds to the GHS-R1a in the cerebral appetite regulatory centers, leading to the increase in expression of Y neuropeptide and agouti-related peptide during short-term treatment. It also presents anti-inflammatory properties, which is even more important when taking into consideration the fact that another factor producing cachexia is the inflammation induced by the initial pathology. In animal studies, the efficacy of GHS-R1a agonists was demonstrated in the treatment of cancer-induced cachexia, chemotherapy and chronic renal disease. Human studies using ghrelin or ghrelin receptor analogues in cancer or cardiac diseases have proven an improvement in appetite and body weight during short-term treatment.

Cachexia is a common characteristic in malignancies, found in up to 85% of the patients with several types of cancer and contributing to over 20% of deaths by cancer, underlining thereby the necessity of an efficient treatment. Weight loss presented by these patients can be severe, with the loss of up to 75% of the lean mass, although even the smallest degrees of weight loss and anorexia are associated to the worsening of prognosis, of the response to chemotherapy and an increased morbidity. Loss of appetite can be a sign of particular importance seeing as in a study on patients with cancer in terminal phases it was proven that the presence of nausea and vomiting is associated to a 68% decrease in survival and contributes to a substantial decrease in the quality of life (18). As in most illnesses associated with cachexia, the presence of underlying inflammation plays an important role in producing cancer cachexia and can represent a target of ghrelin action. Proinflammatory cytokines including IL-1β, IL-6 and TNF-α can be produced by the tumor cells and as well by the host as a response to the tumor. Up to 50% of cancer patients present inflammatory signs at diagnosis and the associated increase in cytokines is involved in the appearance of anorexia, at least partly through its action on the melanocortin central system.

The level of ghrelin was already demonstrated to be increased with approximately 25 % compared to normal values in a variety of cancers associated with cachexia, including pulmonary, breast, colon and prostate neoplasia. Although there may be ghrelin secreting neoplasias, the levels of acyl-ghrelin are 50% higher in cancer patients with cachexia than those without cachexia and 80% higher than in non-cancer patients (19). The presence of anorexia despite higher concentrations of acyl-ghrelin suggests either the presence of a certain resistance to the orexigenic properties of ghrelin in the context of cancer cachexia, or due to the exaggerated anorexi-

genic effects of other processes. The increase of these values can be associated with a saturation of ghrelin receptors, making the pharmacological doses inefficient. Moreover, there is the possibility that these high levels of ghrelin produce unwanted effects such as tumor growth.

The majority of the interventions that administered ghrelin in models of cancer-induced cachexia have shown the improvement of food intake and weight gain, especially due to increase in lean mass. The attempts to treat cancer cachexia with ghrelin have required supraphysiological doses, which have had positive effects on appetite, suggesting that the effect of ghrelin on the appetite regulation centers is not saturated in cancer cachexia. The use of ghrelin and other GHS-R1a agonists on animal models of cancer cachexia has shown a 20-37% increase in food intake, a 10% increase in body weight and a reversal of the losses of lean and fat masses. In humans, the long-term treatment using a GHS-R1a agonist called RC-1291 for a 12-week period has lead to weight gain and increase of the lean mass when compared to placebo (20). However, when measuring the quality of life – important criteria in the treatment of any advanced cancer – there have been no differences between the group treated with the agonist and the placebo group. The problem that is raised is that of the risk of tumor growth that could be stimulated by the increase in GH secretion as a response to GHS-R1a agonists of ghrelin. The study conducted by Garcia (20) has reported a significant growth in the level of IGF1 and IGF1-binding protein 3 in subjects that received the ghrelin receptor analogue compared to the placebo group, without it being clear which type of cancer is more at risk of being developed due to high levels of IGF1. In acromegalic patients with chronic high levels of IGF1, the only more frequently encountered neoplasia is the colonic one.

Another study conducted on 31 patients with progressive, non-responsive gastrointestinal cancers (21) that received low or high doses of ghrelin during an 8-week period has shown that subjects that received high doses reported an increase in appetite, although food intake remained the same. The group receiving high doses of ghrelin presented a decrease in fat mass and a tendency to increase the lean mass, as well as a positive energy balance. Subjects in both groups had a decrease between 9-28% of IGF1, proving that the effect of ghrelin on the body weight can be achieved without an increase in food intake or IGF1 levels.

Ghrelin treatment after the gastric tumor resection has determined a decreased weight loss, although this can be partially due to the replacement of the normal endogenic secretion of ghrelin in the stomach.

Another problem is the possibility that a certain neoplasia might express GHS-R that could respond to the treatment with ghrelin. In a study lead by Wang et al. (22), 51-80% of various types of gastrointestinal cancers expressed GHS-R, but it is unclear whether ghrelin can actually induce tumor growth by stimulating these receptors.

CONCLUSIONS

Ghrelin is a recently-discovered hormone with essential effects on the nutritional status. The understanding of its physiology can help explain the disturbances found in several nutrition-related pathologies and can support the development of therapeutic possibilities using ghrelin or its analogues.

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OBESITY - A RISK FACTOR FOR CARDIOVASCULAR DISEASES

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OBESITY - RISK FACTOR FOR CARDIOVASCULAR DISEASES (Abstract): Infantile and juvenile obesity is a medical, social and cultural problem, which reaches epidemic proportions worldwide. In Romania, according to the World Health Organization, one out of four Romanians is obese. As far as infantile obesity is concerned, Romania ranks third in Europe. Infantile obesity has a major impact on all body systems and is associated with risk factors for cardiovascular diseases and accelerated atherosclerotic processes, including hypertension, atherogenic dyslipidemia, atherosclerosis, metabolic syndrome and functional and structural cardiac changes. **Aims:** To study the cardiovascular complications of the obesity at children and adolescents. **Material and methods:** Our study was conducted on a group of 188 obese and overweight patients that were hospitalized in the Pediatric Cardiology Department, the "Sfânta Maria" Children's Hospital of Iaşi between 1 January 2006 and 1 January 2011. The patients' evaluation was based on: anamnesis, clinical criteria and paraclinical criteria. **Results:** The distribution of cases by age group was the following: 51% of patients were aged 7-13 years, 42% were teenagers and only 6% of patients were aged between 2-6 years. As far as the patients' origin is concerned, we noted that 58% of them lived in urban areas. In the study group, 21% of the subjects had borderline hypertension, while 16% of them suffered from definite hypertension. The echocardiogram that was performed on all the patients included in the study revealed hypertrophic cardiomyopathy in 12% of the patients. **Conclusions:** Child obesity has a major impact on all the big body systems and it is known to contribute to significant increases in the mortality and morbidity rates. **Keywords**: OBESITY, CARDIOVASCULAR COMPLICATIONS, HYPERTENSION, METABOLIC SYNDROME.

Infantile and juvenile obesity is a medical, social and cultural problem, which reaches epidemic proportions worldwide.

Children whose body mass index are equal to or exceeds 95 percent are considered obese, whereas those whose body mass index ranges between 85 and 95 percents are referred to as overweight (1, 3).

The worldwide prevalence of overweight and obesity in children and adolescents increased considerably from 4.2% in 1990 to 6.7% in 2010. The infantile obesity prevalence is expected to reach 9.1% (about 60 million) in 2020. According to the data collected in 144 countries in 2010, about 43 million pre-school children (of whom 35 million in developed countries) were obese or overweight, while 92 million ran an overweight risk. The infantile obesity prevalence was 8.5% in Africa in 2010, and it is expected to reach 12.7% in 2020. This prevalence was low in Asia, in 2010, as it reached a mere 4.9%, yet the number of concerned children was higher (18 mil-

lion). In America, between 16 and 33% of the children and adolescents are obese (1,2).

In Romania, according to the World Health Organization, one out of four Romanians is obese. As far as infantile obesity is concerned, Romania ranks third in Europe.

Infantile obesity has a major impact on all body systems and is associated with risk factors for cardiovascular diseases and accelerated atherosclerotic processes, including hypertension, atherogenic dyslipidemia, atherosclerosis, metabolic syndrome and functional and structural cardiac changes (1).

Infantile obesity causes the occurrence of the atherosclerotic disease in the child's vascular structures, which affects especially the aorta and coronary arteries.

Owen et al. argue in their study that the body mass index during childhood is closely related to later cardiovascular disease risks (1, 4).

MATERIAL AND METHODS

Our study was conducted on a group of 188 obese and overweight patients that were hospitalized in the Pediatric Cardiology Department, the "Sfânta Maria" Children's Hospital of Iaşi between 1 January 2006 and 1 January 2011.

The patients' evaluation was based on:

- Anamnesis: which included questions related on the patients' age, gender, origins, family history of various diseases (close family diagnosed with obesity, hypertension, diabetes mellitus), personal physiological history (small weight at birth, food received during the first months of life, diversification) and personal pathological history (genetic syndromes, endocrine diseases, dyslipidemia, diabetes mellitus). Emphasis has been placed on the child's feeding habits, on investigating possible psychological causes of hyperphagia (separation from parents of the latter's leaving abroad to work), and also on the child's lifestyle (practicing different forms of physical activity).
- Clinical criteria: anthropo-metric data (weight, body mass index), arterial tension and heart rate measurement, pulse determination, identification of symptoms of headache, tinnitus, dizziness.
- Paraclinical criteria: haematological checks (glycemia, total lipids and cholesterol dosing, lipid profile), electrocardiogram, Holter monitoring of arterial tension and Holter – EKG monitoring (of patients with high arterial tension values or altered electrocardiogram), echocardiogram, ophthalmological examination, genetic examination, endocrine examination, neuropsychiatric and psychological examination, consult nutritional diseases.

RESULTS

The distribution of cases by age group was the following: 51% of patients were aged 7-13 years, 42% were teenagers and only 6% of patients were aged between 2-6 years (fig. 1, 2).

As far as the patients' origin is concerned, we noted that 58% of them lived in urban areas, which is attributed to sedentary as well as eating fast food.

Here is the case distribution over the five years of study: 24% of the cases in 2006, 16% in 2007, 30% in 2008, 10% in 2009 and 20% in 2010 (fig. 3, 4).

Study of anthropometric indices (weight, height, body mass index) showed that 63% of patients in the study were obese (BMI greater or equal than percentile 95) while 37% were overweight (BMI of between 85

and 95 percentiles). When case distribution according to gender was considered, we noted a higher obesity rate in the female sex (59%) than in the male sex (41%).

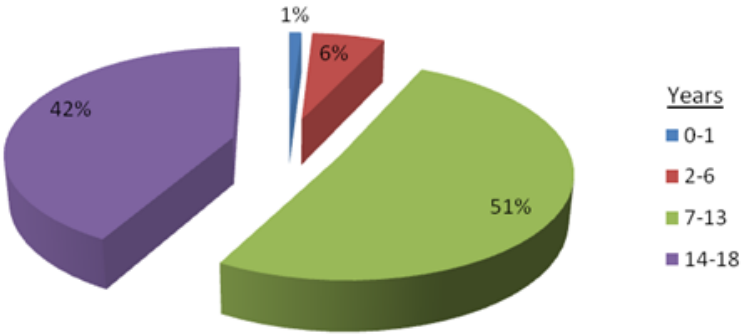

Fig. 1. Case distribution on age groups

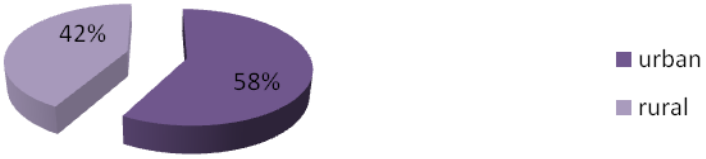

Fig. 2. Case distribution to the patients' origin

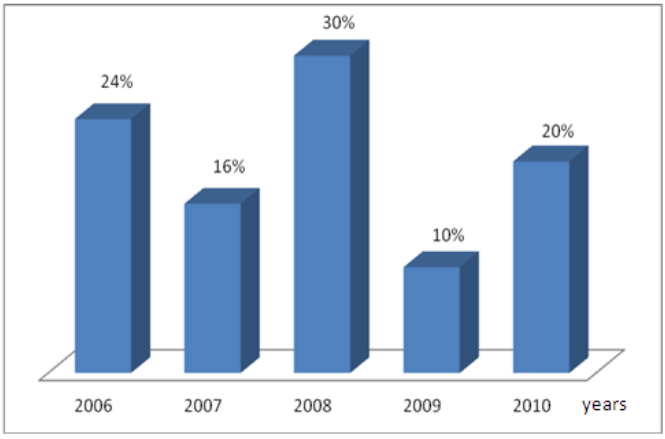

Fig. 3. Case distribution over the years studied

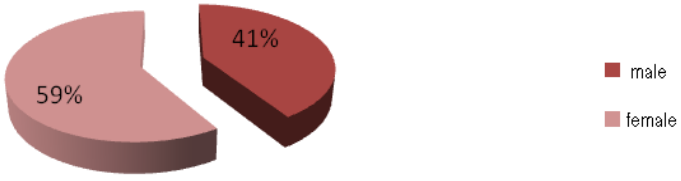

Fig. 4. Case distribution according to sex

The analysis of the data gathered during anamnesis revealed the 33% of the patients had a personal history of various diseases, and 34% of the subjects had immediate family members suffering from arterial hypertension, diabetes mellitus or obesity.

35% of the patients came to the hospital since they experienced specific symptoms such as dyspnoea on effort, tinnitus, headache, dizziness or high arterial tension values detected in the outpatient clinic.

In the study group, 21% of the subjects had borderline hypertension, while 16% of them suffered from definite hypertension (fig. 5).

Another criterion considered was modification of the ECG. Of the 188 patients included in the study group, 10% had sinus tachycardia, 2% had extrasystolic arrhythmia, 5% had sinus arrhythmia, 7% had atrioventricular conduction diseases and 6% had biventricular overstrain.

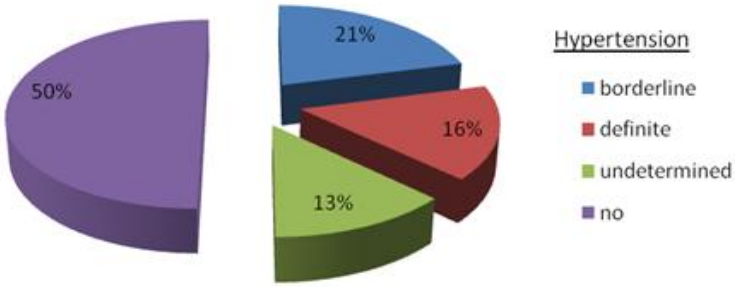

Fig. 5. Case distribution depending on hypertension presence

The ophthalmological examination performed revealed fundus of the eye changes in 5% of the cases, of whom 4% had retina vessels with increased spasticity and only 1% suffered from 1st or 2nd degree hypertensive retinopathy.

One of the diagnosis criteria was the detection of connections between hypertension and fundus of the eye changes. Of the 188 patients, 5% exhibited such connections, 6% suffered only from hypertension and had no fundus of the eye changes, whereas 89% of them did not exhibit such an association.

The echocardiogram that was performed on all the patients included in the study revealed hypertrophic cardiomyopathy in 12% of the patients (fig.6).

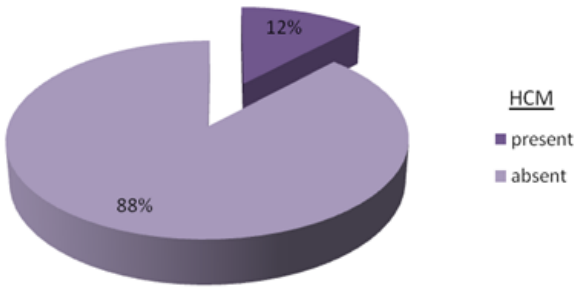

Fig. 6. Case distribution according to the presence of HCM

8% of the patients in the study group associated hypertension and high cholesterol levels, 16% of them had high blood pressure values yet their cholesterol levels were low and 5% of the patients had high cholesterol levels but no hypertension (fig. 7).

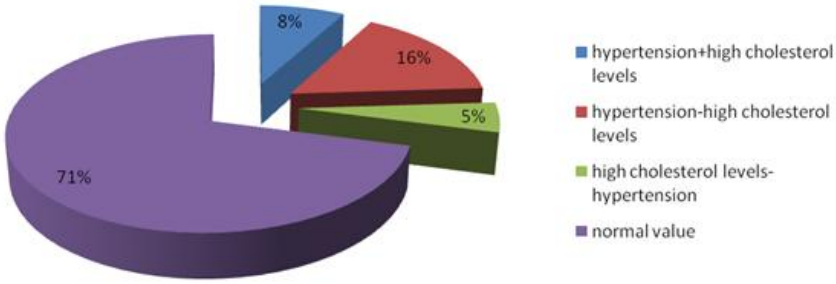

Fig. 7. Case distribution according to the association between hypertension and high cholesterol levels

4% of the children in the study group associated high blood pressure values, high cholesterol levels and hypertrophic cardiomyopathy (fig. 8)

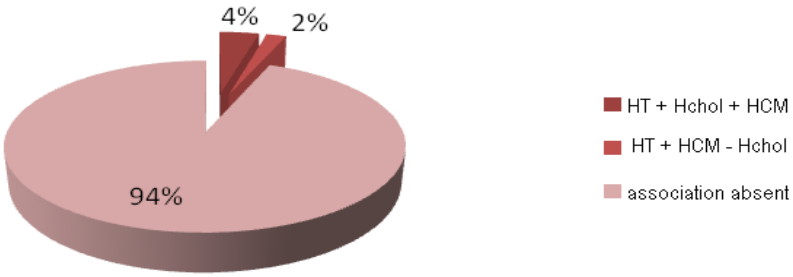

Fig. 8. Presence of an association between HT + high cholesterol levels +HCM

The psychological and neuro-psychiatric exams carried out revealed that 1% of the 188 studied patients came from over-protecting families, 4% had suffered psychological trauma in their personal history (parents' divorce/separation), 2% of the subjects suffered from the Italia syndrome, 18% encountered social and family integration difficulties and 14% had a luminal intellect.

DISCUSSION

Child obesity has a major impact on all the big body systems and it is known to contribute to significant increases in the mortality and morbidity rates (1,5).

The data gathered so far suggest that atherosclerotic cardiovascular processes commence in early childhood and are influenced over a person's life by both genetic and modifiable risk factors such as exposure to harmful environmental factors, including obesity (1,6).

According to literature, the obesity rate is higher in pre-school and adolescent children (1,2). The infantile obesity rate has increased twice in pre-school children aged between 2 and 5 and in adolescents be-

tween 12 and 19 years of age, and 3 times in 6 to 11 year-old children, over the last three decades. Although the obesity rate is higher in females, the male sex has a higher risk of cardiovascular diseases (1).

High blood pressure values (AT>95%) were detected in 35.4% of the overweight children by a study conducted in Europe (1,8). The data collected by a study conducted in India, on a group of 25000 of school children aged between 5 and 16 years revealed a similar percentage (1,7). In our study, high blood pressures were identified in 10.10% of the normal weight children, in 17.34% of the overweight children and in 18.32% of the obsess children (1).

Hypertension and obesity are connected most of the times and they put double pressure on the left ventricle, thus leading to the occurrence of both dilation and muscular hypertrophy (13).

In left pathological ventricular hypertrophy, the increase is not proportional, and the ventricular function is altered, the coronary vessel dilating capacity is reduced and the process regression is impossible (12). Obese subjects are 4 times more likely to suffer from compensatory left ventricular hypertrophy associated with low ejection fraction, reduced myocardial contractility and the need of the left atrium to have more force to fill the left ventricle (11). Concentric remodelling was the most common in obese children, whereas concentric hypertrophy was especially characteristic of those who also suffered from hypertension (13).

In their study, Li et al. performed echocardiograms of 467 young people and reported that a high body mass index during childhood, obesity and systolic hypertension during adulthood, as well as the cumulated burden of obesity and systolic hypertension from childhood up to adulthood are predictive factors of left ventricle muscle mass increase in young adults (10). Ventricular hypertrophy has higher prevalence in obese (33.5%) and overweight (12.4%) children as compared to normal weight children (3.5%). In a study, the patients with borderline hypertension were found to have higher left ventricle muscle mass values than those with normal tension values (13). Obesity, hypertension and concentric hypertrophy are independent predictors of diastolic dysfunction. 52% of the obese children in our study had hypertension, which means that their body mass index, left ventricle dimensions and left atrium muscle mass index were also high. These subjects also exhibited diastolic function changes.

42% of the patients had concentric remodeling (muscular dilation) and 32% of them suffered from concentric hypertrophy, which supports a strong association between hypertension and ventricular remodeling. Previous studies have shown that an excess of body mass is associated with high blood flow values and eccentric hypertrophy. Nevertheless, only 6% of the subjects in our study suffered from such conditions, as most of them exhibited muscular dilation and concentric hypertrophy (13).

On the other hand, cardiovascular diseases, be they inborn or acquired, cause increased obesity prevalence, given the limited physical activity recommended by the cardiologist or preferred by the family or even the patient. A vicious circle is thus created in which obesity worsens heart conditions and lack of physical activity due to heart conditions accentuates obesity (14).

CONCLUSIONS

Our research conducted on the study

group described above enabled us to conclude that high overweight and obesity rates are especially characteristic of the 7-13 years age group. The incidence of obesity is much higher in the urban (58%) than in the rural areas, which is due to the unhealthy fast food available in urban areas. The incidence of obesity is higher in the female (59%) than in the male sex. 37% of the patients suffered from hypertension, 21% of whom had borderline hypertension and 16% suffered from definite hypertension. Changes in the fundus of the eye associated to hypertension were revealed in 5% of the patients, while hypertrophic cardiomyopathy was detected in 12% of the study subjects. 8% of the subjects associated hypertension and high cholesterol levels, and 5% had high cholesterol levels but no hypertension. Only 4% of the subjects associated high blood pressure values, high cholesterol levels and hypertrophic cardiomyopathy.

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RESEARCH ON AROMATASE GENE (CYP19A1) POLYMORPHISMS AS A PREDICTOR OF ENDOCRINE THERAPY EFFECTIVENESS IN BREAST CANCER

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RESEARCH ON AROMATASE GENE (CYP19A1) POLYMORPHISMS AS A PREDICTOR OF ENDOCRINE THERAPY EFFECTIVENESS IN BREAST CANCER (Abstract): Single nucleotide polymorphisms (SNPs) of the CYP19A1 gene have shown the ability to modify its activity, but no association has been established with aromatase inhibitor (AI) efficiency in hormone receptor positive breast cancer (BC). **Material and methods**: The study included blood samples from 53 patients (p) with BC and 1 control (male DNA); 22p. (investigational group) were administered an AI and followed up. **Results**: Homozygous (hh) – CC and TT –, and heterozygous (hz) – TC – genotypes of the rs10046 SNP were balanced. Response to treatment or progression were not affected (p=0.630) in patients with T allele 1**;** local relapse occurred significantly more rarely and overall survival (OS) was superior (p=0.046). For rs4646, genotypes were mainly hhGG (57%), with no implication to study parameters (p>0.05). For rs727479 SNP, major genotypes were hhTT (40%) and hzTG (45%). Therapeutic response was better in patients with T allele 2 (p=0.040), T allele 1 was associated with reduced local recurrence (p=0.047) and TT genotype with better OS (p = 0.008). In rs700518 SNP, genotype was mostly hzGA (49%). Local recurrence rates were reduced in the presence of G allele 1 (p=0.047) and GG homozygosity. **Conclusions**: At this stage, the study was purely exploratory and hypothesis generating. Genotyping of at least three of the four CYP19A1 SNPs evaluated (except rs4646) may have an impact in clinical practice, providing better criteria for patient selection, prognosis and therapeutic decision in BC.
Keywords: BREAST CANCER, AROMATASE INHIBITORS, CYP19A1, GENETIC POLYMORPHISMS.

Breast cancer (BC) is the most common female neoplasia (crude incidence 109.8/ 100000/year in the EU), and the leading cause of cancer death (38.4/ 100000/year), mostly by metastatic disease. Treatment options in BC are systemic chemotherapy (aggressive disease but good performance status), targeted biological agents such as trastuzumab and lapatinib (HER-2/*neu*-positive tumors) and hormone therapy (non-visceral metastases); about 50-55% of breast tumors are hormone-dependent (1).

Estrogens exert their physiological effect after binding of two specific nuclear proteins: estrogen receptors (ER) alpha (predominant in the mammary gland and reproductive tract) and beta. Estrogen-receptor complexes (ERCs) form homodimers and

interact with specific elements in promoter regions of responsive genes in target cells, and further with transcription factors, such as co-activator proteins of the steroid receptor (SRC, p160), initiating the production of mRNA and increased protein synthesis in the endoplasmic reticulum. Resulting enzymes, receptors, and secretory factors are involved in regulation of various cell functions, growth and differentiation. Thus, estrogen induces and/or promotes proliferation of certain target cells (mammary epithelium, but also estrogen-dependent malignant cells), as demonstrated on several experimental BC cell lines (MCF-7, T47D, ZR-75-1) (2).

For these reasons, endocrine therapy is a major therapeutic option in ER-positive BC patients. While tamoxifen remains the standard therapy for premenopausal women, third generation aromatase inhibitors (AI) (non-steroidal: anastrozole, letrozole, or steroidal: exemestane) are the preferred first-line treatment in postmenopause (3, 4).

Aromatase (also known as estrogen synthase) is a 58-kDa monoxigenase belonging to the cytochrome P450 superfamily of enzymes, which converts androgens (testosterone and androstenedione) to estrogens (estradiol and estrone, respectively). Its activity is modulated through tissue-specific promoters (PI.1, PI.3, PI.4, PI.6, PI.7, PII), hormones, cytokines; it increases with age, body weight, alcohol, decreases due to smoking; it may be induced by clonidine, dexamethasone, insulin, diethylstilbestrol, phenylephrine, and inhibited (besides AI) by clomiphene, dopamine, antifungal drugs, oxycodone, paclitaxel, tamoxifen or trastuzumab. The gene that encodes aromatase is called CYP19, has two transcriptional variants (A1 and A2) and is located on chromosome 15q21.1. Mutations could lead to both inhibition and stimulation of its expression (5).

MATERIAL AND METHODS

Our study included a total of 53 histologically-confirmed BC patients, from which informed consent was obtained for the collection of peripheral blood to assess the four selected polymorphisms of the aromatase gene (CYP19A1). Full details of each sample (collection date, blood volume, anticoagulant, name, code number) were recorded for DNA collection. The patients had a mean age at diagnosis of 55.09 years (range 36-72 years). All were hormone receptor-positive (ER expression: 5-95%, progesterone receptor, PR: 0-100%), and more than half (54.5%) were Her2/*neu* negative; as such, 68.2% of cases were assigned to the luminal A and B molecular subtypes (1-4). Radiotherapy indication, schedule and dosage were not modified in any way by participating in the study. Therapy was well tolerated in all cases, and there were no major toxicities. Average survival for the whole patient sample was 4.95 years (range 1.6 to 17.4 years). CYP19A1 single nucleotide polymorphism (SNP) genotyping was performed by allelic discrimination in the Taqman system using the Applied Biosystems 7500 Real-Time PCR System. To assess potential clinical significance of SNPs considered of interest, we followed the response to hormone therapy (including an aromatase inhibitor, AI) and other anticancer therapies, and survival parameters (overall survival, OS; disease-free survival, DFS; time to progression, TTP) for a group of 22 investigational patients diagnosed with BC in the records of the "St. Spiridon" Hospital Oncology Outpatient facility. Significance of correlations between dif-

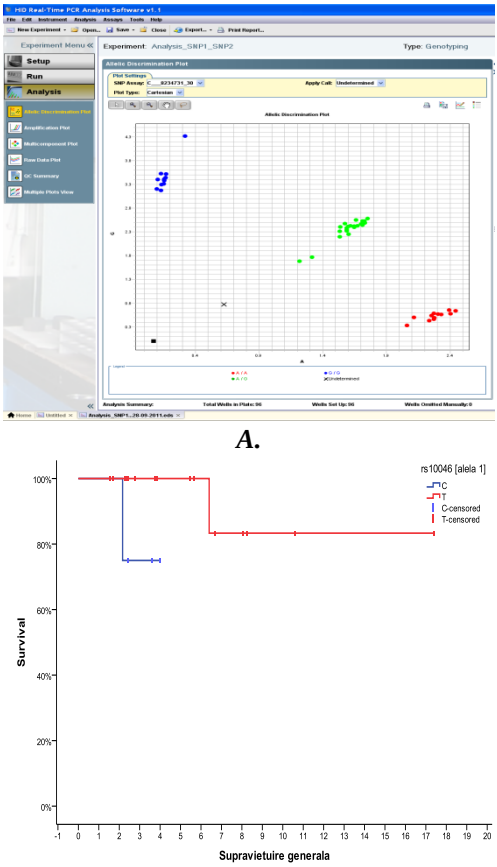
ferent SNPs, clinical parameters, and those involving time-dependent variables, was evaluated by chi-square tests and two-sided log-rank tests, respectively.

RESULTS

A. Genotyping of the rs10046 polymorphism (CYP19A1 c.1531C> T). Of the 53 BC patients and 1 control (DNA from a male) genotyped for this SNP, 14 were homozygous (hh) CC genotype (26.5%), 14 hhTT (26.5%), and 25 heterozygous (hz) TC (47%), as well as the control. Frequencies were identical, 50% (53/106) for C and T alleles, and their allelic distribution in the study group was very well balanced (fig. 1.A).

The distribution of alleles in the investigational group (22 patients) was also balanced: 22.7% with hhTT genotype (5/22), as compared with 18.2% for the hhCC genotype (4/22), and 59.1% for hzTC (13/22). T allele was present in 52.3% of studied haplotypes, while the ancestral allele C in the remaining 47.7%.

Response to treatment was not significantly influenced by any of the genotypic variants (p=0.630), although the presence of T allele (especially homozygous) appeared to associate with a lower rate of progression after first line treatment. When AIs were administered, local recurrence occurred significantly less frequently in patients who had a T allele 1, and nodal recurrence in those with T allele 2 (p=0.047); pattern of metastasis was not altered by genotype. The response to second line treatment also appeared better (p=0.503) for homo-and heterozygous T variants, this allele showing a trend for negative prediction of relapse in this situation (p=0.082). DFS did not correlate with this SNP, but TTP is potentially improved in patients with a T allele 1 (p=0.070). On the other hand, a T allele 2 (and possibly hhTT genotype) was prognostic for relapse as unresectable metastatic disease, meeting statistical significance criteria in patients not receiving an AI (p=0.046), while the presence of T allele 1 predicted a superior OS (p = 0.046) in patients treated with an AI (fig. 1.B).



A.

B.

Fig. 1.A. Allelic discrimination plot for rs10046 SNP (hhCC: blue, upper left; hhTT: red, lower right; hzTC: green, middle). **B.** Significant OS improvement (p=0.046) for T allele 1.

B. Genotyping of the rs4646 polymorphism (CYP19A1 c.1673T> G). Of the 53 patients analyzed, 30 were hhGG (57%), 6 hhTT (11%) and 17 had hzTG profile (32%). The control (DNA from a male) had the GG genotype. Allele frequencies in the study group were 27% for T allele (29/106), and 73% for the ancestral G allele (77/106) (fig. 2), which has been previously associated with increased ER levels in postmenopausal women. In the investigational group, rs4646 SNP allele distribution was extremely unbalanced, 63.6% of patients presenting hhGG genotype (14/22); no patient in this subset was genotyped as hhTT. T allele was present in 18.2% of studied haplotypes, and the ancestral G allele in 81.8%. Response to first line treatment and relapse rates were not affected by rs4646 SNP (p=0.933, p=0.746). Second line endocrine therapy had better results in genotype TG patients (p=0.050) treated with an AI, but OS, DFS and TTP did not correlate with this SNP (p>0.050).

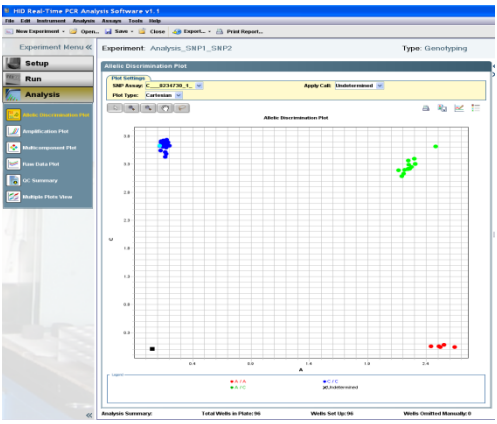
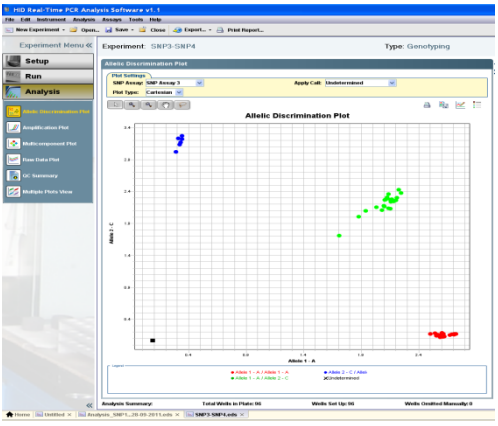
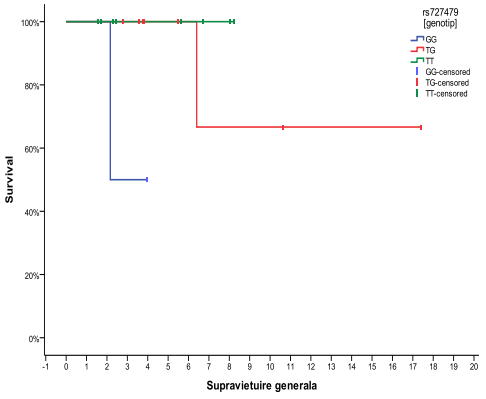


Fig. 2. Allelic discrimination plot for rs4646 SNP (hhGG: blue, upper left; hhTT: red, lower right; hzTG: green, middle).

C. Genotyping of the rs727479 polymorphism (CYP19A1 418 c.145 G> T). Fig. 3.A. shows the allelic distribution plot for the 53 patients: 8 were hhGG (15%), 21 hhTT (40%), and 24 (plus the control) hzTG (45%). Allele frequencies in the study group were 38% for G (40/106), and 62% for the ancestral T allele (66/106), and in the 22-patient study subgroup: 65.9% T, 34.1% G; 63.6% hhTT genotype (14/22), and only 9.1% hhGG (2/22).



A.



B.

Fig. 3.A. Allelic discrimination plot for rs727479 SNP (hhGG: blue, upper left; hhTT: red, lower right; hzTG: green, middle). **B.** Association of hhTT genotype with better OS (p=0.008)

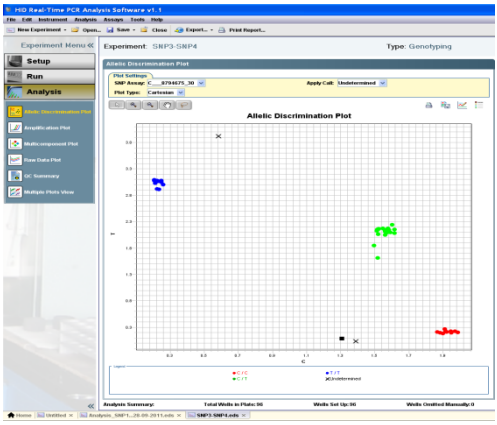
Therapeutic response (first line) was significantly better in patients with a T allele 2 (p=0.040), apparently higher in those treated with an AI (p=0.099); most cases with progressive disease were genotyped GG or TG. The same relationship with the relapse rates has seen a trend towards significance (p=0.099).

T allele 1 was associated with reduced local recurrence (p=0.047) and TT genotype with the much less frequent appearance of visceral metastases (p=0.013) in patients treated with upfront AI. DFS had a trend of positive correlation (p=0.072) with the presence of T allele 2 in patients treated with AIs. The T allele 2 and the hhTT genotype have anticipated a better DFS (p=0.011, and p=0.040 respectively), possibly a longer TTP (p=0.052) after second line treatment, and were strongly associated with better OS (p=0.003, and p=0.008) (fig. 3.B).

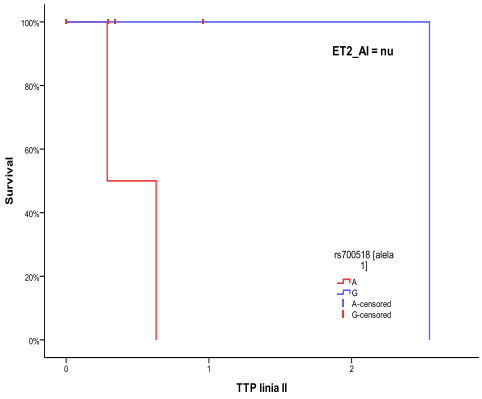
D. Genotyping of the rs700518 polymorphism (CYP19A1 c.1673T> G). A balanced distribution of alleles was observed in the main group (53 patients+1 control). The hhAA genotype was identified 14 times (26.5%), while the opposite GG appeared 13 times (24.5%); most patients (26/53) were genotyped as hzGA (49%), as was the control. At the allelic level, 50.9% of cases (54/106) were A, and 49.1% (52/106) were G (fig. 4.A).

The alleles of SNP rs700518 (A and G) were perfectly equal in the investigational subgroup. 22.7% patients (5/22) had hhAA genotype, 22.7% (5/22) hhGG, and 54.5% (12/22) hzGA.

In this group, treatment response was not influenced by rs700518 genotype (p=0.686), but local recurrence rates were low in the presence of a G allele 1 (p=0.047) and/or GG homozygosity in patients treated with upfront AI. DFS did not correlate with polymorphism, while TTP appeared higher in patients with a G allele 1 (p=0.070), regardless of the use of AI. Statistical significance was reached for TTP improvement in the presence of a G allele 1 (p=0.035), but particularly in patients not treated with an AI (p=0.046). This also appeared to influence OS, but not clearly enough (p=0.083) (fig. 4.B).



A.



B.

Fig. 4.A. Allelic discrimination plot for rs700518 SNP (hhAA: blue, upper left; hhGG: red, lower right; hzAG: green, middle). **B.** Significant TTP improvement (p=0,046) in patients not treated with an AI, in the presence of G allele 1.

DISCUSSION

In the molecular and genomic era, BC is a topic of urgent research. Currently, the role of stem cells, circulating tumor and endothelial cells, and that of nucleic acids, mRNA and miARN in progression and metastasis are under scrutiny; multiple multigene "signatures" have been developed, functional imaging methods for hormone receptors and HER-2/*neu* were imagined, interactions of estrogen signaling pathways with mechanisms of angiogenesis, apoptosis or EGFR/HER were highlighted (6-12).

Although AIs yielded superior clinical outcomes as compared to tamoxifen in terms of response rates, time to progression and (for letrozole) survival, also with a favorable toxicity profile, the results are relatively inconsistent (13). Also, to our knowledge there are currently no direct comparative studies between the 3 commercially available AIs. Moreover, in current clinical practice, the indication for use of AIs in these patients is generally guided by only two clinical-pathological criteria: postmenopausal status and hormone receptor positivity (with little relevance to their quantification, given the mechanism of action different from SERMs). Thus, the ability to discriminate between AI-responsive and non-responsive patients is low, but genetic and epigenetic factors that affect metabolism, efficacy and safety of medication may be of particular importance (14).

The role of the CYP450 system was clearly proved important in the metabolism of tamoxifen – although this information has not been incorporated into routine practice, 7-10% of women with ER-positive BC harbor CYP2D6 mutations involved in resistance to tamoxifen. While AIs are gaining momentum as first-line endocrine treatment in BC, the role of CYP19A1 – although essential in the metabolism of AIs – is much less studied so far. Polymorphisms (single nucleotide polymorphisms, SNPs) of the CYP19 gene have demonstrated the ability to change aromatase activity (and that of its inhibitory drugs) in postmenopausal women (5,15,16). However, although some studies have investigated the association between CYP19 SNPs and the risk of BC (17,18), to date, only one study (16) analyzed the CYP19 SNPs in relation to prognosis of BC patients treated with letrozole. No association between CYP19 genotype and efficiency of AIs is evident at this time. Associations of CYP19 variants with BC risk targeted only certain SNPs, found in most studies in this field. These include repeat polymorphic variants $[TTTA]_n$, of which allele $[TTTA]_7$ was observed with a relatively high frequency in BCs of the Caucasian population. Also, the silent exon 3 version Val80Val (rs700518) was observed in incomplete linkage disequilibrium with $[TTTA]_7$. Other candidate alleles related to the risk and the evolution of BC, although inconsequently, were: $[TTTA]_7$delTCT, $[TTTA]_{>10}$, $[TTTA]_8$, $[TTTA]_{10}$, $[TTTA]_{11}$ and $[TTTA]_{12}$. Associations were observed between some of these alleles and serum concentrations of circulating metabolites associated to estrogen, which justify genotyping of the region affected by the above-mentioned repeats also for their association with treatment response. Most studies have considered two SNPs of the 3'-UTR non-translated region (rs10046 and rs4646), and one located in the intronic region (rs727479), seemingly the most obvious candidates for an analytical study (19,20). Corresponding SNP alleles $[TTTA]_7$ and $[TTTA]_{<9}$ were associated with low estrogen levels, while the

$[TTTA]_8$, $[TTTA]_{>9}$, rs727479, rs10046 and rs4646 were associated with a high level of the hormone. The Val80Val allele (rs700518) was associated with an increased expression of aromatase.

In this context, CYP19 SNP genotyping may be regarded as a potential predictive factor for response to AI treatment of breast tumors. Identifying the prevalence and location of these alterations or simple genetic variants may allow individualization of systemic therapy based on tumor characteristics, and also host genetics, influencing therapeutic efficacy and safety in BC. The limits of this study lie in the small number of cases, and the results and methods used need additional research and external validation. These results should be therefore looked upon with caution, and p-values are given only for illustration of the apparently emerging correlations.

CONCLUSIONS

At this stage, our research was purely exploratory and hypothesis-generating from a clinical point of view, while it was mainly aiming to perfect and standardize the laboratory techniques used. However, we may conclude that genotyping of at least three of the four polymorphisms of the CYP19A1 gene evaluated by us (except rs4646), and possibly others, might have an impact on clinical practice, by providing better criteria for patient selection, prognosis and therapeutic decision in locally advanced, hormone receptor-positive BC.

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NEWS

ROLE OF MASP-1 IN LECTIN PATHWAY ACTIVATION

The activation of lectin pathway of complement is thought to occur due to recognition of pathogens by MBL (mannan-binding lectin) in complex with MASP-2 (MBL-associated serine protease), followed by cleavage of C4 and C2. Related proteases such as MASP-1 and MASP-3 are central to alternative pathway function in mice, by activation of profactor D and factor B. Also, MASP-1 plays a role in MASP-2 convertase generation, through C2 auxiliary cleavage. A study by Degn *et al*. on a patient deficient in MASP-1 and MASP-3, shows that MASP-1 is crucial for complement activation, since it was able to restore the nonfunctional lectin pathway which the patient had. MASP-1 increased lectin pathway activity through direct activation of MASP-2. MASP-1 and MASP-2 can associate in the same MBL complex and such complexes can be found in serum, meaning that MASP-1 and MASP-2 act analogous to C1r and C1s of the classical pathway. Degn SE, Jensen L, Hansen AG et al. Mannan-Binding Lectin-Associated Serine Protease (MASP)-1 Is Crucial for Lectin Pathway Activation in Human Serum, whereas neither MASP-1 nor MASP-3 Is Required for Alternative Pathway Function (*The Journal of Immunology*, 2012; 189 (8): 3957 DOI: 10.4049/jimmunol.1201736).

Teodora Vremeră

DYADIC ADJUSTMENT IN HIV SERO-CONCORDANT AND SERO-DISCORDANT COUPLES

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DYADIC ADJUSTMENT IN HIV SERO-CONCORDANT AND SERO-DISCORDANT COUPLES (Abstract): **Aim**: The HIV infected population in Romania is at an age when engaging in a serious relationship is more and more an issue. Dyadic adjustment is the process which most couples go through, in their attempt to form a steady relationship. It is represented by important changes that appear in one's perception of himself and the significant other. Dyadic adjustment can be measured by a series of parameters – couple's satisfaction, couple's consensus, couple's cohesion and expressing emotions. **Material and methods**: We conducted a study on 60 young men and women (30 male, 30 female). Of these, 45 are in evidence at the Iași Regional Center (22 male, 23 female), the other 15 being their uninfected parteners (8 male, 7 female). They were evaluated using the Dyadic Adjustment Scale, developed by Graham B. Spanier. **Results**: Most couples enrolled in this study (50%) were sero-concordant (both partners were HIV-positive –15). 26% of couples were made-up an HIV-positive female and a non-infected male, and 24% were made-up of a non-infected female and an HIV-positive male. The median age was 23 years, 33.4% of them came from rural areas. Average schooling level was 6 primary classes, with extremes between two classes of primary and higher education. Only 50% of patients had a job, while the rest had no stable job and were not employed, 33.4% came from broken families or foster care. 100% of HIV infected patients are in active therapy, poliexperimented. Most patients have 5-6 treatment regimens (45%), with a maximum of 8-9 regimens. Most of the couples enrolled (83.3%) were not married, as our study evolved only 5 married couples. The relationship length varied between 4 moths and 15 years. The Dyadic Adjustment Scale revealed high scores in couple's consensus and cohesion for both concordant and discordant couples. In couples where both partners are HIV-positive, the DAS revealed average scores for all four areas of dyadic adjustmet. In Couples where one partner is HIV-positive, and the other is not infected, low scores were seen in emotional expression and in the couple's satisfaction. **Conclusions**: The HIV infection affects an individual's life and his adjustment to a couple in the sense that a series of factors changes the couple's dynamic – physical and emotional changes specific to the infection and AIDS, ARV treatments' side-effects, the care for the uninfected partner, and preventing HIV transmission through intercourse. All these elements lower the level of dyadic adjustment and increase the level of stress in individuals that are part of an HIV couple. **Key words**: HIV, SIDA, SIDE-EFFECTS, INFECTED COUPLES.

While recent scientific efforts have resulted in a series of discoveries and advances in understanding and controlling the virus that causes AIDS, this progress has

had limited impact on the majority of HIV infected people and populations living in developing countries. The social and economic conditions that nurture the spread of the virus have to be confronted as essential elements in local and global efforts to stem its spread and create effective solutions to halt the epidemic. They must also be considered in supporting and improving the quality of life of the infected people (1, 12).

The specifics of the adolescent and youth HIV infected population in the Moldova area have been widely discussed. If in the past taking care of the HIV infected person was the duty of the infectious diseases doctor and the patient's family, today the idea of a whole team of specialists working together is becoming increasingly popular. Also the focus has shifted from "taking care" of the patient to helping and supporting him in living a full and normal life. The psychologist's role is more clearly defined, as well as the implications of the social worker. And more importantly, a large number of doctors from different fields are becoming more and more involved and open-minded about treating this type of patient (5, 6).

The mentality of the population in general is also changing. The speed of this change differs from country to country, and in Romania accepting new ideas is not done at a fast pace. However, people are becoming increasingly more aware of different aspects that involve their everyday safety and this includes correct information about being infected with HIV and developing AIDS. This reflects in engaging in sexual contact and forming a couple with an HIV-infected person.

Even though the F type HIV that is the main cause of infection in our country has, so far, allowed a longer than expected life span, the young infected people have grown up with the knowledge that the disease they have is a chronic one, that allows them to do sometimes less than a healthy individual. However, the social and psychological support that they have had since childhood has so far created strong personalities, that can take care of themselves and may in fact have a normal life, going through all the stages of social development (6, 10, 16).

For adolescents and youth, it is difficult to build a life on a foundation that is still unsure and filled with past and current frustrations about discrimination and being different.

Growing up with AIDS has not been and is not an easy task. This explains many young people's desire to live their life on a more accelerated rate than post persons do.

One of the main characteristics of HIV infected youth is their tendency to fall in love and want to get married early, their acute desire to have children, and enjoy all the positive aspects of life "as soon as possible"(1, 4, 15).

Dyadic adjustment is the process which most couples go through, in their attempt to form a steady relationship. It is represented by important changes that appear in one's perception of himself and the significant other. Dyadic adjustment can be measured by a series of parameters – couple's satisfaction, couple's consensus, couple's cohesion and expressing emotions.

MATERIAL AND METHODS

The study was conducted on 60 young men and women (30 male, 30 female). Of these, 45 are in evidence at the Iaşi Regional Center (22 male, 23 female) as being infected with HIV/AIDS, the other 15 being their uninfected partners (8 male, 7 female).

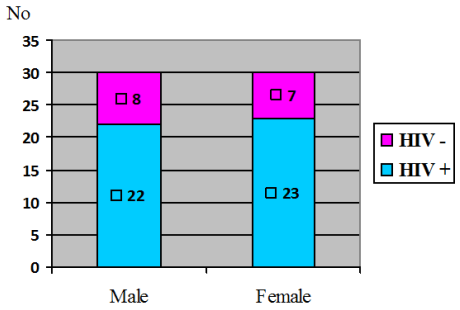

Fig. 1. The subjects conducted study

They were evaluated using the Dyadic Adjustment Scale, developed by Graham B. Spanier. The Dyadic Adjustment Scale (DAS) is regarded as the most used evaluation tool of marital adjustment. It is made up of 32 items that can be evaluated by one or both partners. For each item the subject must choose the answer closest to his/her opinion, from a list of items (fig. 1).

The DAS includes 4 subscales: (a) Dyadic consensus, (b) Dyadic satisfaction, (c) Dyadic satisfaction, (d) Emotional expresivity. It is used in contouring the quality of the dyadic relationship. It can be used for married couples as well as for couples living together or part of a short term relationship.

RESULTS AND DISCUSSION

This study was conducted on both sero-concordant and sero-discordant couples. Most couples enrolled in this study (50%) were sero-concordant (both partners were HIV-positive –15). 26% of couples were made-up an HIV-positive female and a non-infected male, and 24% were made-up of a non-infected female and an HIV-positive male (fig. 2).

The median age was 23 years, 33.4% of them came from rural areas. Average schooling level was 6 primary classes, with extremes between two classes of primary and higher education.

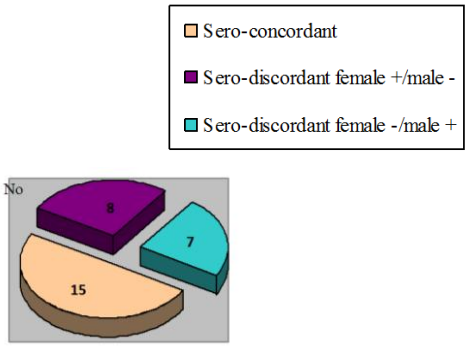

Fig. 2. The sero-concordance of the subjects

Only 50% of patients had a job, while the rest had no stable job and were not employed, 33.4% came from broken families or foster care.

100% of HIV infected patients are in active therapy, poliexperimented. Most patients have 5-6 treatment regimens (45%), with a maximum of 8-9 regimens.

Most of the couples enrolled (83.3%) were not married, as our study involved only 5 married couples. The relationship length varied between 4 moths and 15 years.

The Dyadic Adjustment Scale revealed high scores in couple's consensus and cohesion for both concordant and discordant couples.

In couples where both partners are HIV-positive, the DAS revealed average scores for all four areas of dyadic adjustment.

In couples where one partner is HIV-positive, and the other is not infected, low scores were seen in emotional expression and in the couple's satisfaction.

CONCLUSIONS

Engaging in a relationship is difficult for both healthy and infected individuals. Putting another's whishes and needs before your own is a challenge, especially in time

of illness.

The HIV-infected population in Romania is going through the same processes as their peers. The age of 22-24 is defined by self-development, self-affirmation and love. Regardless of their HIV status, young people naturally go through these stages.

The present study sheds a light on the difficulties faced by an HIV infected man or woman, part of a couple. It shows that communication and expressing one's emotions and concerns is a vital part of forming a steady and durable relationship. Consensus is important, as many decisions should be made by both partners. The couple's cohesion and satisfaction are closely linked and they depend on each individual's power to express their thoughts and feelings.

It is vital to have in mind that times change, and the small HIV-infected children from 1989 are now adolescents and young people with different needs that have to be sought to, both medically and psychologically.

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ENDOCARDITA INFECTIOASA PLURIVALVULARA CU *ENTEROCOCCUS FAECALIS*

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PLURIVALVULAR WITH ENTEROCOCCUS FAECALIS INFECTIVE ENDOCARDITIS (Abstract): Infective endocarditis (IE) is a relatively rare disease that can take part of sepsis and may be a diagnostic and therapeutic challenge. Polyvalvular location of EI is rare. We present a patient of 60 years diagnosed with plurivalvular infective endocarditis by Enterococcus faecalis with septic complications, visceral, dragged, hospitalized for a psychiatric pathology, who raised the problems of diagnosis and treatment, without being able to make assessments on the entrance gate. For help in diagnosing this case proved that cardiac ultrasound studies recommended that a screening method for patients with bacteremia, but the presence of vegetation is the major criterion for diagnosis of EI. Blood cultures and echocardiography led to the diagnosis of infectious endocarditis. Also abdominal ultrasound confirmed the clinical suggestion of gravity, the septic multiviscerale release by splenic abscess image objectivity. Under vigorous antibiotic treatment and symptomatic treatment in combination evolution was to worse, death occurring after 15 days of hospitalization, toxic-septic shock being irreversible. Our case is particular by locating polyvalvular vegetation, the germ involved, the court altered the immunological and disseminator septic complications that have limited therapeutic options and made predictable fatal diagnosis. **Key words**: INFECTIVE ENDOCARDITIS, ENTERO-COCCUS FAECALIS

Cu toate progresele diagnostice şi terapeutice, EI continuă să rămână o provocare sub multiple aspecte, fiecare pacient constituind un motiv de reevaluare a cunoştinţelor în acest domeniu.

În decursul anilor s-a constatat că nici incidenţa, nici mortalitatea n-au scăzut, polimorfismul bolii pretând în continuare la erori de diagnostic, iar comportamentul social dezorganizat şi dezinformat sanitar menţine gradul de risc crescut ca incidenţă şi pronostic (1, 2).

Cazul prezentat ilustrează majoritatea problemelor discutate în diagnosticul şi tratamentul EI, în cadrul ghidurilor elaborate la nivel mondial, ghiduri ce se bazează pe opiniile experţilor rezultate din experienţa raportărilor unei patologii cu incidenţă scăzută, cu lipsa studiilor randomizate şi număr limitat de metaanalize (3,4).

Prezentăm cazul pacientului G.S. de 60 ani, sex masculin, ce a fost internat în Clinica a III-a Medicală Cardiologică, a Spitalului Clinic de Urgenţe „Sf. Spiridon" Iaşi, pentru febră, frison, dispnee, edeme gambiere, scădere ponderală (16 kg în aproximativ 6 luni), astenie marcată.

Boala a debutat insidios, cu 6-7 luni an-

terior, cu sindrom astenic, scădere ponderală progresivă şi episoade de edeme gambiere remise spontan; cu 4-5 săptămâni anterior adăugându-se febra moderată, un sindrom dispeptic frust, precum şi dispneea progresivă de efort, fiind trimis clinicii noastre pentru evaluare, investigaţii suplimentare şi tratament de specialitate.

Antecedentele eredo-colaterale nu au sugerat nici un element semnificativ, iar dintre antecedentele personale s-a remarcat doar o internare la Spitalul Clinic de Psihiatrie Nr. 7, Iaşi, cu 6 luni anterior, pentru o tulburare de personalitate anxioasă de tip impulsiv.

Starea generală la internare era gravă, cu adinamie, inapetenţă, caşexie. Examenul obiectiv a evidenţiat un pacient febril, cu febră de tip septic, palid, cu pliu cutanat persistent, hipocratism digital, mase musculare atrofice, polipneic (22 resp./min), tahicardic (FC=94 bătăi/min), cu un şoc apexian în spaţiul VII intercostal pe linia axilară anterioară, auscultator cardiac cu - suflu sistolic mitral şi tricuspidian, precum şi suflu diastolic aortic, TA=110/60 mmHg, edeme gambiere, hepatomegalie din spaţiul VI medioclavicular drept până la 6 cm sub rebord, cu reflux hepato-jugular şi splenomegalie de gradul I.

Explorările paraclinice iniţiale au relevat: anemie (Hb = 6,7 g%), leucocitoză uşoară (10.600 elem/mm^3) cu neutrofilie (77%), VSH: 107/120 mm, PCR pozitivă, hemocultură (1) pozitivă cu *Enterococcus faecalis*, uree = 66 mg%, creatinină = 2,47 mg%, Fe seric = 11µg%. Radiografia pulmonară a obiectivat mărirea diametrului transvers al cordului. Ecocardiografia transtoracică efectuată de la internare a decelat prezenţa cardiomiopatiei dilatative cu modificări de cinetică parietală segmentară şi prezenţa în mai multe incidenţe a unor formaţiuni vegetante flotante la nivelul valvelor mitrală (23/26 mm), aortică (13/11 mm), tricuspidiană (21/17 mm), cu regurgitări secundare severe şi elemente de hipertensiune pulmonară secundară severă (fig. 1, 2).

Fig. 1. Formaţiuni vegetante la nivelul VM,VT(A4C)

Fig. 2. Formaţiuni vegetante la nivelul VM, VA(A3C)

Un prim diagnostic de etapă a orientat spre o endocardită infecţioasă plurivalvulară cu *Enterococcus faecalis*, introducându-se tratament antibiotic în asociere cu Levofloxacină şi Amoxicilină + acid clavulanic (*Enterococcus faecalis* fiind sensibil conform antibiogramei) şi medicaţie simpto-

matică, formulă terapeutică sub care pacientul a continuat să facă febră, să prezinte dispnee, cu sindrom edematos persistent, tendință la degradare hemodinamică şi astenie marcată, motiv pentru care s-au continuat investigațiile.

Explorările paraclinice ulterioare au arătat progresia sindromului anemic (Hb= 5,9 g%), leucocitoză marcată (26.500/mm3), cu creşterea neutrofilelor, tombocitopenie (38.000/mm^3), VSH repetat cu valori mari (peste 100 mm/h), agravarea sindromului de retenție azotată (uree = 107 mg%, creatinină = 3,75 mg%), hepatocitoliză (TGO = 1376 UI/l, TGP = 676 UI/l), Quantiferon TB negativ, HIV negativ, hemoculturi (2) cu *Enterococcus faecalis* cu acelaşi profil de sensibilitate pe antibiogramă (izolat ca în prima hemocultură).

Ecografia abdominală a confirmat sugestia clinică a gravității, prin diseminări septice multiviscerale, prin obiectivarea imaginilor de abcedări splenice (Fig. 3,4).

Sub tratament susținut cu antibioterapie în asociere cu terapie simptomatică evoluția a continuat să se agraveze, decesul survenind după 15 zile de la internare, prin şoc toxico-septic ireversibil.

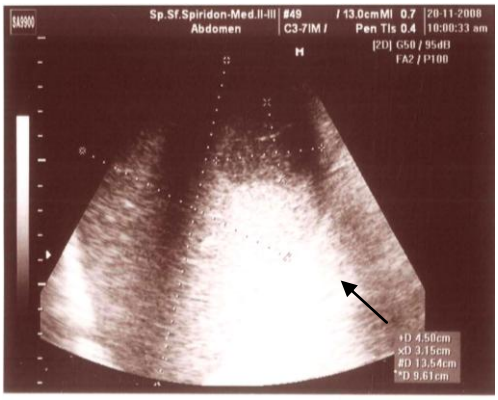

Fig. 3. Abcese splenic

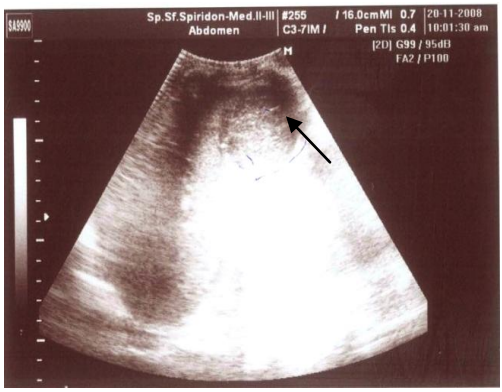

Fig. 4. Abces splenic

DISCUȚII

Incidența EI variază de la o țară la alta, fiind de aproximativ 3-10 cazuri/100.000 persoane pe an. Acest lucru poate reflecta mai degrabă diferențe metodologice între studii decât o variație adevărată (5, 6, 7, 8). De notat că în aceste studii incidența EI a fost foarte scăzută la pacienții tineri, dar creşte dramatic cu vârsta, incidența maximă fiind de 14,5 cazuri/100.000 persoane pe an la pacienții între 70 şi 80 de ani. În toate studiile epidemiologice asupra EI, raportul între incidența EI la sexul masculin față de sexul feminin este 2:1, raport sex ratio greu de explicat, în condițiile în care femeile au un prognostic mai prost şi suferă intervenții chirurgicale mai puțin frecvent decât bărbații (9).

EI pe cordul drept este estimată la 5-10% din cazurile de EI (5, 10, 11). Chiar dacă poate să apară la pacienți cu un stimulator cardiac permanent, defibrilator *cardioverter* implantabil, cateter venos central sau boli cardiace congenitale această situație este cel mai frecvent observată la pacienții cu abuz de droguri intravenoase. Incidența exactă a EI în abuzul de droguri intravenoase este necunoscută însă date recente arată o creştere a numărului spitalizărilor în EI asociată cu consumul de droguri

(12). Această boală apare mai frecvent la pacienții cu abuz de droguri intravenoase care sunt HIV pozitivi, în mod particular la cei cu imunodepresie avansată (11, 12, 14). Lezarea valvelor inimii drepte prin injectarea de materiale particulare asociată cu igiena defectuoasă la locul injectării, administrarea de soluții contaminate şi imunitatea scăzută sunt câteva din ipotezele fiziopatologice care stau la baza apariției EI pe cordul drept în abuzul de droguri intravenos (13). Deşi valva tricuspidă este locul uzual de infecție în abuzul de droguri intravenoase, infecția valvei pulmonare şi a valvulei lui Eustachio pot fi de asemenea observate, iar EI a inimii stângi nu este neobişnuită la acest grup de pacienți.

Cazul expus a prezentat o endocardită infecțioasă plurivalvulară cu *Enterococcus faecalis,* cu complicații septice viscerale, la un pacient tarat, spitalizat pentru o patologie psihiatrică, care a ridicat probleme de diagnostic şi tratament, fără a putea face aprecieri asupra porții de intrare. De un real ajutor în diagnosticul acestui caz s-a dovedit a fi ecografia cardiacă pe care studiile o recomandă ca metodă de screening pentru pacienții cu bacteriemie, dar care prin prezența vegetațiilor constituie criteriu major de diagnostic al EI.

Hemoculturile şi ecocardiografia reprezintă baza în diagnosticul endocarditei infecțioase. Ecocardiografia transtoracică trebuie realizată inițial, la prima suspiciune de sindrom infecțios cu afectare valvulară. EI dată de enterococ este produsă în 90% din cazuri de *Enterococul faecalis* şi mult mai rar de *Enterococul faecium* sau alte specii. Enterococii sunt înalt toleranți la antibioticele bactericide şi eradicarea lor necesită prelungirea terapiei (până la 6 săptamâni) cu combinații sinergice bactericide de inhibitori ai peretelui celular cu aminoglicozide, de asemeni pot fi rezistenți la multe antibiotice, inclusiv beta-lactamine (prin intermediul modificării PBP5 şi uneori prin producerea betalactamazelor), aminoglicozide şi vancomicină (15). Rezistența la vancomicină şi la beta-lactamine e observată în principal la *E. faecium.* Deoarece rezistența la ambele medicamente e rară, vancomicina se foloseşte în rezistența la beta-lactamine şi viceversa. Au fost obținute rezultate variabile cu linezolid, quinupristină, dalfopristină, daptomicină şi tigeciclină (16).

Mortalitatea intraspitalicească a pacienților cu endocardită infecțioasă variază de la 9,6 la 26%, dar diferă considerabil de la pacient la pacient. Identificarea pacienților cu risc crescut de deces poate schimba evoluția bolii şi îmbunătăți prognosticul, dar prezența complicațiilor septice diseminative este defavorabilă atât prin excluderea posibilității de corecție chirurgicală valvulară cât şi prin posibilitatea redusă de limitare a proceselor septice (17, 18, 20).

Chiar dacă embolii splenici sunt des întâlniți, ei rareori constituie un abces. Febra recurentă sau persistentă şi bacteriemia sugerează diagnosticul şi aceşti pacienți ar trebui evaluați prin tomografie computerizată sau IRM pe lângă ecografie. Tratamentul constă în antibioterapie adecvată. Splenectomia, indicată în cazurile de ruptură splenică sau abcese mari care răspund greu la terapia antibiotică, ar trebui făcută înainte de chirurgia valvulară, aceasta având prioritate în schimb în cazurile urgente. Drenajul percutanat este o alternativă la pacienții cu risc înalt chirurgical (19,21).

În literatură se citează influența asupra prognosticului în endocardita infecțioasă a 4 factori: caracteristicile pacientului, prezența sau absența complicațiilor cardiace sau non-cardiace, tipul de microorganism şi evaluarea ecocardiografică. Astfel se con-

sideră că persistența infecției și insuficiența renală sunt predictori ai mortalității la pacienții care necesită intervenție chirurgicală de urgență. Cum era și de așteptat, pacienții care au indicație de operație, dar care nu pot fi supuși acesteia din cauza riscului operator au cel mai rău prognostic (19).

Cazul prezentat reprezintă particularitate prin localizarea multivalvulară a vegetațiilor, prin germenele implicat, prin terenul alterat imunologic și prin complicațiile septice diseminative care au limitat posibilitățile terapeutice și a făcut predictibil diagnosticul infaust.

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NOUTĂȚI

DESCIFRAREA UNUI CIRCUIT NEURONAL CARE MEDIAZĂ APETITUL

Neuronii hipotalamici care exprimă AgRP, neuropeptidul Y şi GABA stimulează apetitul şi creşterea în greutate, prin integrarea diferitelor semnale hormonale, nutriționale şi neuronale.Ablația experimentală a acestor neuroni la şoareci determină blocarea senzației de foame. Se apreciază că scăderea semnalelor GABA de către neuronii ce exprimă AgRP de la nivelul nucleului parabrahia (PBN) determină inhibarea apetitului.Neuronii glutamatergici şi neuronii serotoninergici caudali controlează excitabilitatea nucleului PBN şi inhibă senzația de foame. Inactivarea genetică a semnalelor glutamatergice de către receptori glutamici de tip N-metil D-aspartat la nivelul PBN previne înfometarea. În aceste condiții, PBN poate fi perceput drept sediul integrării semnalelor provenite din diverse regiuni cerebrale, pentru a asigura modularea bidirecțională a saţietății şi greutății corporale (Wu Q., Clark M.S., Palmiter R.D. Deciphering. A neuronal circuit that mediates appetite. *Nature* 2012; 594/97) .

Carmen Lăcrămioara Zamfir

EVALUAREA COMPARATIVĂ A SCORURILOR PROGNOSTICE ÎN EMBOLIA PULMONARĂ

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COMPARATIVE EVALUATION OF PULMONARY EMBOLISM PROGNOSTIC SCORES (Abstract): **Aim:** Pulmonary embolism is a life threatening disease and one of the main causes of in-hospital mortality.There are multiple risk stratification scoring systems for the forecast of the outcomes in patients with pulmonary embolism. **Material and methods:** We conducted a prospective observational cohort study to evaluate the comparative validity of four prognostic models: pulmonary embolism severity index (PESI), simplified PESI score, PESI risk classes and shock index, for predicting short-term mortality in acute pulmonary embolism. The PESI and PESI - simplified scores and shock-index were calculated. PESI scores were segregated into risk class (I-V) obtaining PESI classes. Shock index was dichotomized into 0 (for value <1) versus 1 (for value >1) risk groups. We determined the area under Receiver Operating Characteristic curve, the sensitivity, specificity, likelihood ratio (LR+, LR-) for PESI score, simplified PESI score, PESI risk classes and shock index, to compare the ability of these scoring tools. **Results:** The cohort consisted of 362 subjects. The mortality was 21.54%. The sensitivity, specificity and likelihood ratios were different between scores but the area under *Receiver Operating Characteristic* curve showed values in range 0,6-0,7. **Conclusions:** The assessed prognostic scores performed comparably for identifying pulmonary embolism patients at short-term risk of mortality, even for PESI score segregated in risk classes and dichotomized version of shock index. **Key words**: PULMONARY EMBOLISM, MORTALITY, PROGNOSTIC SCORE

Embolia pulmonară (EP) reprezintă un domeniu de patologie cu incidenţă ridicată (1 milion de persoane anual în Statele Unite ale Americii), marcat de morbiditate şi mortalitate crescută, respectiv 100000 de subiecţi anual şi de costuri ale tratamentului bolii de 1 bilion de dolari anual (1,2).

Având în vedere morbi-mortalitatea prin EP considerăm utilă o evaluare obiectivă complexă a scorurilor prognostice disponibile la acest moment privind riscul de deces precoce la aceşti subiecţi.

Scopul studiului nostru constă în evaluarea puterii diagnostice pentru predicţia riscului de mortalitate în spital, în EP, a următoarelor scoruri clinice: scorul PESI (Pulmonary Embolism Severity Index), clasele de risc PESI (Clasa_PESI), scorul PESI simplificat (PESI_S), respectiv indexului de şoc (Index_soc). Astfel am realizat evaluarea relaţiei dintre valorile scorurilor clinice (PESI, Clasa_PESI, PESI_S, Index_soc) şi mortalitate, la pacienţii cu EP; calcularea pragului de diagnostic, a puterii

diagnostice, a sensibilității, specificității, raportului de probabilitate negativ, respectiv pozitiv pentru fiecare dintre scorurile clinice evaluate; analiza comparativă a puterii diagnostice în predicția riscului de mortalitate în EP a celor patru scoruri clinice.

MATERIAL ŞI METODE

Am realizat un studiu de cohortă prospectiv, pe un lot de 362 pacienții asistați în Unitatea Primire Urgențe a Spitalului Clinic Județean de Urgență ,,Sf. Spiridon" şi internați, în perioada 2004-2010, în Clinica I Medicală Cardiologie ,,C.I.Negoiță" cu diagnosticul de embolie pulmonară.

Pentru pacienții incluşi în studiu s-a evaluat prognosticul pe termen scurt, respectiv, mortalitatea pe parcursul internării. Criteriile de includere în studiu au fost: pacienți cu vârsta între 16 şi 95 ani şi diagnosticul de embolie pulmonară (confirmat ecocardiografic, computer tomografic, scintigrafic sau necroptic). Criterii de excludere au avut în vedere prezența semnelor de sindrom posttrombotic la prima internare şi lipsa consimțământului informat.

Suplimentar față de protocolul de diagnostic al emboliei pulmonare, la fiecare pacient s-au aplicat patru scoruri prognostice pentru riscul de mortalitate, specifice acestei condiții patologice: scorul PESI, clasele de risc PESI, scorul PESI simplificat (tab. I, II) şi indexul de şoc.

TABELUL I

Scorurile PESI (3) şi PESI – simplificat (4)

	PESI original	PESI simplificat
Vârsta	Exprimată în ani	>80 ani = 1 punct
Sexul masculin	+10	
Cancer	+30	= 1 punct
Insuficiență cardiacă	+10	= 1 punct
Afecțiune respiratorie cronică	+10	= 1 punct
Puls > 110/ minut	+20	= 1 punct
Tensiunea arterială sistolică<100mmHg	+30	= 1 punct
Frecvență respiratorie > 30/minut	+60	
Status mental alterat	+20	
SaO2<90 mmHg	+20	
Temperatură<36 grade Celsius	+20	

TABELUL II

Clasele de risc PESI (5)

Interpretare	Clasa de risc EP - PESI
<65	Clasa I
66 - 85	Clasa II
86-105	Clasa III
106-125	Clasa IV
>125	Clasa V

Indexul de şoc reprezintă un scor definit prin raportul dintre frecvenţa cardiacă şi valoarea tensiunii arteriale sistolice (6). În studiul nostru am utilizat o reprezentare dihotomică a indexului de şoc: 0 pentru o valoare subunitară a raportului şi1 pentru o valoare supraunitară a acestuia.

Din punct de vedere a implicaţiilor etice, toţi pacienţii incluşi în studiu, au semnat consimţământul informat referitor la utilizarea datelor personale şi medicale, anexat foii de observaţie, la momentul internării.

Datele studiului au fost gestionate cu ajutorul unei baze de date de tip Microsoft EXCEL, iar prelucrarea statistică s-a realizat cu ajutorul programelor dedicate SPSS 16.0. şi MedCalc 12.1. Pragul de semnificaţie statistică a fost ales p=0,05.

Comparaţiile au fost realizate cu ajutorul testului neparametric Mann-Whitney, având în vedere că datele studiate nu au fost variabile de tip continuu şi nu au respectat principiul normalităţii distribuţiei, sau a testului Chi-pătrat (Exact Fisher) pentru variabilele de tip dihotomic.

S-a aplicat analiza tip receiver operating characteristic (ROC) pentru caracterizarea fiecărui scor clinic studiat, din punct de vedere al pragului de diagnostic, puterii diagnostice (aria de sub curba ROC), sensibilităţii, specificităţii, valorii predictive negative şi pozitive, cât şi pentru realizarea de comparaţii între scorurile clinice. În scopul realizării unei analize obiective a scorurilor clinice, având în vedere că structura grupului de studiu în cercetarea noastră nu a fost echivalentă din punct de vedere al prevalenţei EP cu populaţia generală, s-a preferat folosirea raportului de probabilitate pozitiv/negativ (Likelihood Ratio: LR+, LR-) utilizării valorilor predictive, prezentând avantajul de a nu fi dependent de prevalenţa bolii. LR este util în calcularea probabilităţii posttest, fiind data probabilitatea pretest a unui test diagnostic.

REZULTATE

Urmărirea prospectivă a celor 362 de pacienţi cu embolie pulmonară a demonstrat o mortalitate în spital de 21,5% (78 de pacienţi au decedat).

1. Evaluarea scorurilor de risc la subiecţii supravieţuitori comparativ cu decedaţii. Evaluarea diferenţelor între supravieţuitori şi decedaţi din punct de vedere al scorului PESI, claselor PESI şi scorului PESI simplificat a arătat valori semnificativ statistic mai ridicate la decedaţi faţă de supravieţuitori (Mann Whitney p<0,001). De asemenea, evaluarea diferenţelor între supravieţuitori şi decedaţi din punct de vedere al indexului de şoc, a arătat o frecvenţă semnificativ statistic mai ridicată a valorii 1 a scorului la decedaţi faţă de supravieţuitori (Fisher Exact Test, p<0,001).

2. Analiza ROC individuală a scorurilor de risc. Analiza ROC pentru scorul PESI a stabilit o valoare de 0,691 (p<0,0001) pentru aria de sub curba, reprezentând puterea diagnostică sau eficacitatea testului. Valoare de 120 a scorului PESI, a fost determinată ca fiind valoarea prag, semnificativă pentru discriminarea între absenţa, respectiv prezenţa riscului de mortalitate la pacienţii cu EP. La nivelul valorii prag s-au stabilit valori ale sensibilităţii testului de 43,59, ale specificităţii de 90,85, ale valorilor raportului de probabilitate LR+ de 4,76, respectiv LR- de 0,62.

Analiza ROC pentru clasele PESI a stabilit o valoare de 0,693 (p<0,0001) pentru aria de sub curbă, reprezentând puterea diagnostică sau eficacitatea testului. Valoare >4 a scorului, a fost determinată ca fiind valoarea prag, semnificativă pentru discriminarea între absenţa, respectiv pre-

zența riscului de mortalitate la pacienții cu EP. La nivelul valorii prag s-au stabilit valori ale sensibilității testului de 35.9, ale specificității de 92.96, ale valorilor raportului de probabilitate LR+ de 5.10, respectiv LR- de 0,69.

Analiza ROC pentru scorul PESI simplificat a stabilit o valoare de 0,623 (p=0,0003) pentru aria de sub curbă, reprezentând puterea diagnostică sau eficacitatea testului.Valoare > 0 a scorului PESI_S, a fost determinată ca fiind valoarea prag, semnificativă pentru discriminarea între absența respectiv prezența riscului de mortalitate la pacienții cu EP. La nivelul valorii prag s-au stabilit valori ale sensibilității testului de 92.31, ale specificității de 29.93, ale valorilor raportului de probabilitate LR+ de 1.32, respectiv LR- de 0,26.

Analiza ROC pentru Index_soc a stabilit o valoare de 0,690 (p<0,0001) pentru aria de sub curbă, reprezentând puterea diagnostică sau eficacitatea testului. Valoare > 0 a scorului, a fost determinată ca fiind valoarea prag, semnificativă pentru discriminarea între absența, respectiv prezența riscului de mortalitate la pacienții cu EP. La nivelul valorii prag s-au stabilit valori ale sensibilității testului de 43.59, ale specificității de 94.37, ale valorilor raportului de probabilitate LR+ de 7.74, respectiv LR- de 0,6.

3. Analiza ROC comparativă a scorurilor de risc. Pentru toate cele patru scoruri diagnostice evaluate, valoarea ariei de sub curba ROC se încadrează în intervalul 0,6 – 0,7, corespunzător unei eficiențe diagnostice relativ slabe (fig. 1).

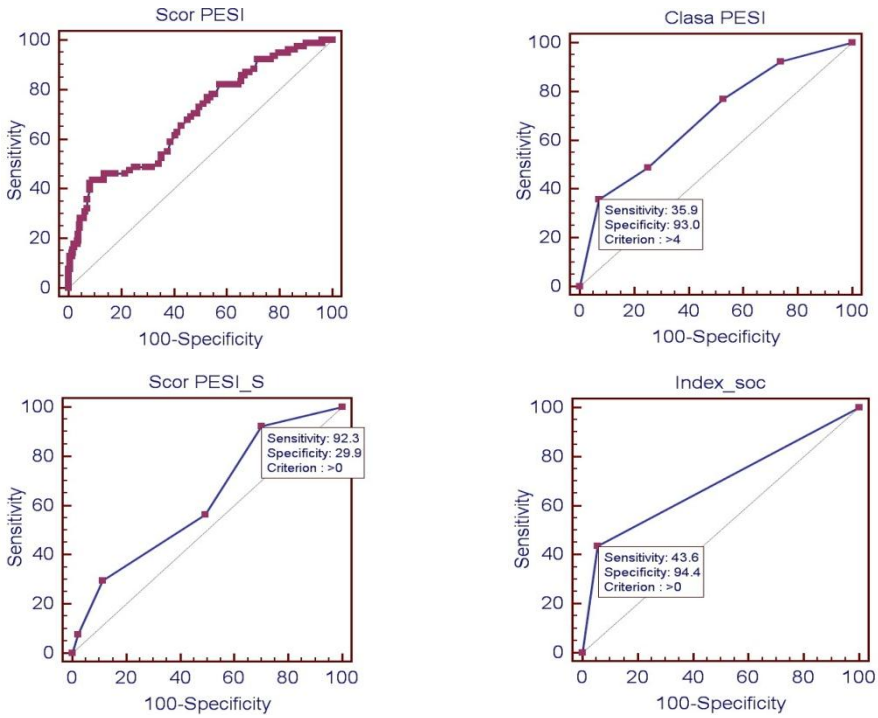

Fig. 1. Curba ROC pentru scorul PESI, clasele PESI, scorul PESI simplificat şi indexul de şoc, evaluări pentru lotul inclus în studiu.

Comparația eficacității celor patru scoruri de risc evidențiază următoarele elemente: eficacitatea scorului PESI nu este semnificativ diferită de eficacitatea claselor PESI şi a indexului de şoc, diferențele între ariile de sub curba ROC fiind nesemnificative statistic (p=0,783, respectiv p=0,978); eficacitatea claselor PESI nu este semnificativ diferită de eficacitatea indexului de şoc, diferența între ariile de sub curba ROC fiind nesemnificativă statistic (p=0,927); eficacitatea scorului PESI simplificat este semnificativ mai mică decât eficacitatea scorului PESI, claselor PESI şi a indexului de şoc, diferențele între ariile de sub curba ROC fiind semnificative statistic (p=0,0059, p=0,0051, p=0,0246); scorurile PESI, clasele PESI, şi indexul de şoc se remarcă prin valori reduse ale sensibilității (<50), dar prin valori mari, peste 90 ale specificității. Spre deosebire de acestea, scorul PESI simplificat are o valoare peste 90 a sensibilității, dar o specificitate foarte redusă, de aproximativ 30.

Din punct de vedere a raportului de probabilitate, indexul de şoc prezintă valoarea cea mai mare a LR+ (7.74) generând modificări moderate ale probabilității diagnosticului pozitiv. Clasele PESI şi scorul PESI prezintă valori mai mici ale LR+ (5.1, respectiv 4.76), generând modificări mici ale probabilității diagnosticului pozitiv.

PESI simplificat prezintă cea mai mică valoare a LR+ (1.32) fiind inferior celorlalte scoruri în generarea modificărilor de probabilitate pentru diagnosticul pozitiv.

Din punct de vedere al raportului de probabilitate negativ, pentru cele patru scoruri clinice, situația se prezintă astfel: scorul PESI simplificat prezintă cel mai bun raport de probabilitate LR-, fiind cel mai valoros dintre cele patru teste din punct de vedere al generarii modificărilor de probabilitate pentru diagnosticul negativ. Valoarea sa (0,26) se situează la limita dintre intervalul corespunzător generării modificărilor mici (0,5-1) şi cel corespunzător generării modificărilor moderate (0,5-0,2) ale probabilității diagnosticului negativ. Valorile LR- pentru PESI, clasele PESI şi indexul de şoc nu diferă semnificativ între ele (0,62, 0,69, respectiv 0,6), situându-se în zona caracteristică unui grad foarte mic de generare a modificărilor probabilităților diagnosticului negativ, fiind inferioare din acest punct de vedere scorului PESI simplificat.

DISCUȚII

Similar datelor din literatura de specialitate, studiul nostru arată o rată înaltă a mortalității intraspitaliceşti la pacienții diagnosticați cu embolie pulmonară, respectiv 21.54%. Mortalitatea după diagnosticul EP a fost raportată ca fiind de 25% (7). De asemenea, rezultatele studiului nostru sunt în concordanță cu rezultatele altor cercetări publicate referitoare la scorurile de evaluare a prognosticului în embolia pulmonară. Astfel, Chang şi colaboratorii demonstrează, similar rezultatelor noastre, că segregarea scorului PESI în clase de risc nu afectează semnificativ puterea prognostică a testului obținând o valoare a ariei de sub curba ROC de 0,684, apropiată de cea din studiul nostru (0,691) (8). Alte studii au obținut o putere diagnostică mai bună pentru scorul PESI. Astfel, în 2007, Jiménez evaluează comparativ scorul PESI şi scorul Geneva, obținând o valoare a ariei de sub curba ROC de 0,76 pentru PESI (9). În 2011 sunt publicate rezultatele unui studiu care evaluează comparativ indexul de şoc şi scorul PESI simplificat, arătând o eficiență mai bună a scorului PESI simplificat (10). Rezultatele noastre sunt diferite din acest punct de vedere, scorul PESI simplificat

dovedind eficiență diagnostică mai mică, comparativ cu celelalte trei scoruri dar, fiind cel mai bun din punct de vedere al raportului de probabilitate negativ (LR-).

În plus, folosind o variantă dihotomică a indexului de şoc, am obținut rezultate comparative cu celelate teste.

CONCLUZII

Din punct de vedere al eficacității diagnostice în evaluarea riscului de mortalitate, în studiul nostru, scorul PESI, clasele PESI, scorul PESI simplificat şi indexul de şoc se caracterizează prin valoari comparabile ale ariei de sub curba ROC în intervalul 0,6 – 0,7, corespunzătoare unei puteri diagnostice relativ slabe. Eficacitatea scorului PESI simplificat este semnificativ mai mică decât eficacitatea scorului PESI, claselor PESI şi a indexului de şoc.

Scorurile PESI, clasele PESI, şi indexul de şoc se remarcă prin valori reduse ale sensibilității (<50) dar prin valori mari, peste 90 ale specificității iar scorul PESI simplificat are o valoare peste 90 a sensibilității dar o specificitate foarte redusă, de aproximativ 30.

Indexul de şoc prezintă valoarea cea mai mare a LR+ (7.74) putând genera modificări moderate ale probabilității diagnosticului pozitiv, iar scorul PESI simplificat prezintă cel mai bun raport de probabilitate LR-, fiind cel mai valoros dintre cele patru teste din punct de vedere al generarii modificărilor de probabilitate pentru diagnosticul negativ.

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THE EVALUATION OF HEPATOPROTECTIVE EFFECT OF SILYMARIN, *PHYLLANTHUS NIRURI* EXTRACT AND CHOLINE COMBINATION

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THE EVALUATION OF HEPATOPROTECTIVE EFFECT OF SILYMARIN, *PHYLLANTHUS NIRURI* EXTRACT AND CHOLINE COMBINATION (Abstract): The high incidence of liver diseases due to the use of xenobiotics and unhealthy lifestyle is associated with increased need for hepatoprotective product administration. **Aim:** The hepatoprotective effect of the combination silymarin / *Phyllanthus niruri* extract / choline was investigated in comparison with silymarin alone in patients with hepatic steatosis, in an open clinical study. **Material and methods:** 101 patients with non-alcoholic hepatic steatosis confirmed by high values of serum transaminases and ultrasonography were included in the study. The patients were divided into two groups, group A received 150 mg silymarin three times daily, and group B a hepatoprotective complex (120 mg silymarin / 225 mg *Phyllanthus niruri* extract / 60 mg choline) three times daily, for 6 months. The hepatoprotective effects were assessed through the evolution of the following laboratory parameters: aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase, serum bilirubin and serum cholesterol at 3 and 6 months after the initiation of the hepatoprotective treatment. **Results:** Both products were effective in the normalization of liver function, but the combination silymarin / *Phyllanthus niruri* extract / choline showed significantly better results than silymarin alone, considering the normalization of the hepatic parameter values after 3 and 6 months. The risk for hepatoprotective failure was significantly lower with the combination silymarin / *Phyllanthus niruri* extract / choline *versus* silymarin alone, the number needed to treat (NNT) being 3.7 after 3 months and 2.5 after 6 months. **Conclusions:** The combination silymarin / *Phyllanthus niruri* extract / choline induced a better liver protection than silymarin alone. **Keywords***: PHYLLANTHUS NIRURI*, SILYMARIN, CHOLINE, HEPATIC STEATOSIS, HEPATOPROTECTIVE.

Liver diseases register an increasing prevalence in Europe. The increased intake of alcohol, xenobiotics, junk-food combined with an unhealthy lifestyle and, finally, the infections caused by hepatitis viruses contribute to this *status quo* (1, 2). In Romania, non-alcoholic hepatic steatosis alone is diagnosed in 20% of the patients presenting for an internal medicine consultation, according to a survey published in 2008 (3, 4). The warnings about the complications associated with non-alcoholic steatosis (5) indicate the need for treatment of patients and the correction of risk factors

(6, 7).

The patients with liver diseases are advised to use a large variety of hepatoprotective products, apart from the specific antiviral treatment in case of viral hepatitis. Of the herbal products, those containing silymarin standardized extracts from *Cardui mariae fructus* have a privileged position among the hepatoprotective products. A recent systematic review with meta-analysis of randomized and controlled clinical trials evaluating the effect of silymarin on biochemical indicators in patients with liver disease have found a statistical significant effect of silymarin in decreasing aspartate transaminase (AST) and alanine transaminase (ALT), but not gamma-glutamyl transferase (GGT) (8). The decrease in hepatocyte oxidative stress and lipid metabolism regulation are considered the main effects of silymarin in steatosis (9).

The aim of this study was to investigate the hepatoprotective effect of the combination silymarin / *Phyllanthus niruri* extract / choline in comparison with silymarin alone, to assess the contribution of *Phyllanthus niruri* (an ayurvedic herb) extract and choline (an essential nutrient for the optimal liver functioning) to the hepatoprotective effect of silymarin.

MATERIALS AND METHODS

Study population. Patients aged 18 to 80 years old, diagnosed with non-alcoholic hepatic steatosis confirmed by high values of serum transaminases and ultrasonography, were enrolled in this clinical study at "Gen. Dr. Aviator Victor Anastasiu" National Institute of Aeronautical and Space Medicine, Bucharest, Romania. The inclusion criteria were: patients with abnormally elevated level of at least one of the enzyme AST, ALT or GGT, according to the method used by the laboratory of the medical unit. Abnormal were considered the levels of AST above 36 IU / L in women and 59 IU / L in men, ALT above 52 IU / UL in women and 72 IU / L in men and of GGT higher than 73 IU / L in both genders. The exclusion criteria were patients who followed antiviral therapy or candidates for specific antiviral therapy for hepatic viral infections.

The patients randomly received either silymarin 150 mg three times / day for 6 months (food supplement that contains 150 mg silymarin / tablet) (group A) or a combination of 120 mg silymarin / 225 mg *Phyllanthus niruri* extract / 60 mg choline three times daily (food supplement) (group B). All the patients were advised to eat a healthy diet and avoid alcoholic (ethanolic) beverages and highly processed foods. Physical exercise programs were indicated for the overweight and obese patients. The patients were assessed at baseline and after 3 and 6 months.

Evaluation criteria and outcome measures. At every time points, the patients were assessed for: AST, ALT, GGT, alkaline phosphatase, serum bilirubin, and serum cholesterol. In addition, blood sugar level and blood pressure were measured to investigate the impact of 225 mg of *Phyllanthus niruri* extract / dose on these parameters. Blood sugar and blood pressure were considered because non-clinical studies described for *Phyllanthus niruri* a decrease in blood sugar, comparable with that induced by glibenclamide (10, 11, 12) and blood pressure, comparable with the one induced by hydrochlorothiazide (13); but the experience from well-organized studies in human subjects is sparse. Side effects that could occur following the administration of both products have been monitored.

Statistical analysis.

The results were subjected to statistically interpretation using *EpiInfo* software; Z-test was used to determine the significance of the differences between the results in the two groups. The relative risks (RR) of not obtaining the normalization of laboratory parameters with silymarin / *Phyllanthus niruri* extract / choline *versus* silymarin administration and the NNT value were calculated.

RESULTS

The clinical study was conducted between March 2015 and December 2015. One hundred and one patients met the inclusion criteria and have completed the 6-month hepatoprotective treatment. The patients were randomly divided into 2 groups: 50 patients (28 M / 22 F) in group A (silymarin group) and 51 patients (35 M / 16 F) in group B (silymarin / *Phyllanthus niruri* extract / choline). The initial characteristics of the groups (demographic data and laboratory parameters) are summarized in first table. Initially, the groups were generally homogenous. Significant statistical differences were recorded only for ALT, which was significantly higher in the group B ($p<0.001$) and patient age which was significantly greater in group A ($p=0.04$).

TABLE I

Demographic data and laboratory parameters at baseline

Parameters	Group A (n= 50)	Group B (n= 51)	*p* value
Demographic data			
Sex, Male / Female (%Male / %Female)	28 / 22 (56 / 44)	35 / 16 (68.63.31.37)	0.19
Age, years mean ± SD (range)	58.72 ± 10.46 (37 - 81)	53.71 ± 12.45 (25 - 77)	0.04
Body weight, Kg mean ± SD (range)	84.11 ± 11.94 (58 - 114)	82.29 ±1 2.74 (68 - 118)	0.39
Para-clinical parameters			
AST, IU / L mean ± SD (range)	68.76 ± 30.26 (30 - 208)	68.94 ± 29.93 (39 - 180)	0.98
ALT, IU / L mean ± SD(range)	99.52 ± 26.72 (64 - 173)	126.92 ± 47.62 (68 - 280)	<0.001
GGT, IU / L mean ± SD (range)	73.72 ± 30.03 (28 - 152)	72.75 ± 34.09 (30 - 180)	0.88
Alkaline Phosphatase, U / L mean ± SD (range)	70.94 ± 23.33 (37 - 165)	67.20 ± 22.20 (37 - 145)	0.41
Serum Bilirubin, mg / dL mean ± SD (range)	1.11± 0.28 (0.8 - 2.2)	1.06 ± 0.18 (0.7 - 1.5)	0.35
Serum cholesterol, mg / dL mean ± SD (range)	223.54 ± 33.40 (164 - 295)	230.39 ± 31.20 (175 - 311)	0.29
Systolic Blood Pressure, mm Hg mean ± SD (range)	134.72 ± 13.22 (110 - 170)	130.92 ± 9.72 (112 - 148)	0.10
Diastolic Blood Pressure, mm Hg mean ± SD (range)	82.08 ± 9.37 (60 - 105)	81.75 ± 9.37 (60 - 100)	0.86
Blood Glucose, mg / dL mean ± SD (range)	108.62 ± 21.14 (82 - 197)	103.71 ± 15.74 (65 - 142)	0.19

n – number of patients, SD – standard deviation

Comparing to the baseline, at 3 months, significantly lower levels were recorded in group B than in group A for serum ALT, AST, bilirubin, alkaline phosphatase and cholesterol. Only for GGT was found no significant decrease in group B *versus* group A.

At 6 months, all the monitored parameters of the liver function were significantly lower in group B *versus* group A (fig. 1-6, tab. II).

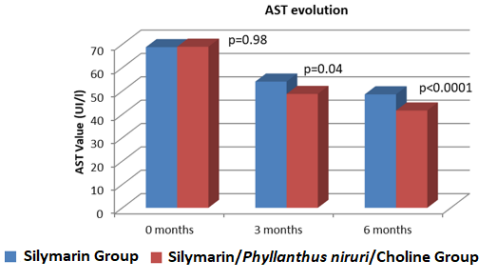

Fig. 1. Mean AST levels at baseline, after 3 and 6 months

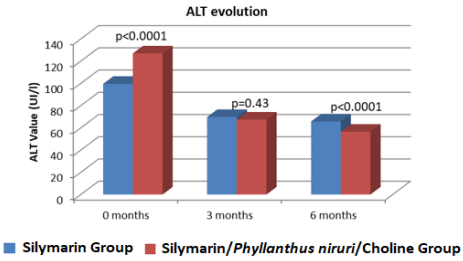

Fig. 2. Mean ALT levels at baseline, after 3 and 6 months

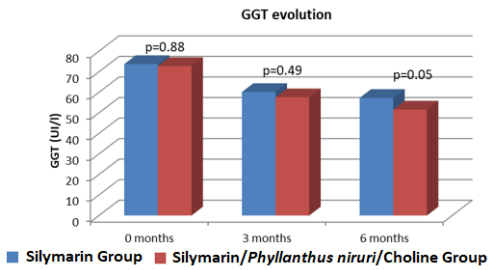

Fig. 3. Mean GGT levels at baseline, after 3 months and 6 months

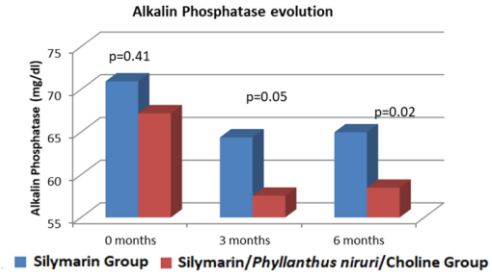

Fig. 4. Mean alkaline phosphatase levels at baseline, after 3 months and 6 months

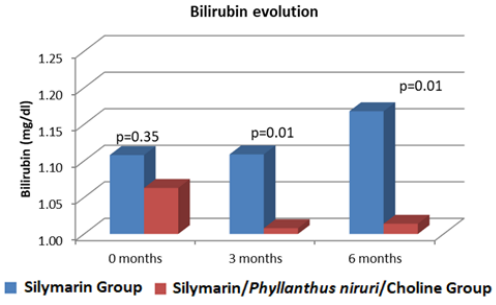

Fig. 5. Mean serum bilirubin levels at baseline, after 3 and 6 months

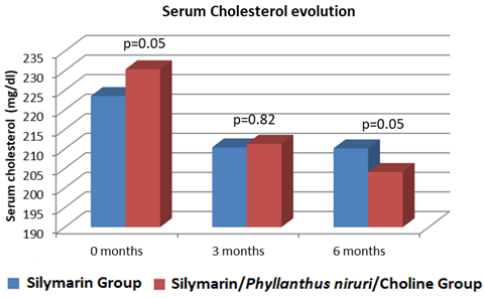

Fig. 6. Mean alkaline phosphatase levels at baseline, after 3 and 6 months

TABLE II

The evaluation of laboratory parameters after 3 and 6 months of hepatoprotective product administration

Laboratory parameter	3 months			6 months		
	Group A (n=50) mean ± SD (range)	Group B (n=51) mean ± SD (range)	*p*-value	Group A (n=50) mean ± SD (range)	Group B (n=51) mean ± SD (range)	*p*-value
AST, UI / L	54.02 ± 14.8 (30 - 112)	48.74 ± 9.82 (30 - 75)	0.04	48.56 ± 8.34 (34 - 70)	41.63 ± 9.05 (23 - 69)	<0.0001
ALT, UI / L	69.7 ± 16.57 (32 - 110)	67.36 ± 12.80 (43 - 108)	0.43	65.68 ± 9.84 (47 - 88)	56.43 ± 11.18 (37 - 93)	<0.0001
GGT, UI / L	60.12 ± 17.04 (25 -9 2)	57.76 ± 16.80 (21 - 98)	0.49	57.26 ± 14.94 (28 - 83)	51.58 ± 13.99 (27 - 80)	0.05
Alkaline phosphatase, U / L	64.38 ± 18.46 (29 - 98)	57.55 ± 16.28 (30 - 102)	0.05	64.98± 12.39 (40-88)	58.48± 14.52 (23-98)	0.02
Serum bilirubin, mg / dL	1.11 ± 0.25 (0.8 - 2.1)	1.01 ± 0.13 (0.8 - 1.4)	0.01	1.17 ± 0.30 (0.9 - 2.1)	1.01 ± 0.12 (0.8 - 1.3)	0.02
Serum cholesterol, mg / dL	210.41 ± 20.10 (172 - 241)	211.33 ± 16.87 (185 - 258)	0.82	210.14 ± 13.94 (169 - 230)	204.11 ± 11.39 (180 - 230)	0.05
Systolic Blood Pressure, mg Hg	129.36± 7.95 (115-145)	128.06± 6.51 (115 - 140)	0.37	128.54 ± 7.80 (110 - 140)	126.92± 7.99 (110 - 145)	0.30
Diastolic Blood Pressure, mg Hg	80.20± 5.80 (70-90)	80.06 ± 6.53 (60 - 95)	0.91	80.44 ± 6.95 (60 - 90)	78.92± 5.60 (65 - 90)	0.23
Blood sugar, mg / dL	104.12 ± 11.71 (82 - 139)	100.88 ± 10.07 (80 - 131)	0.14	102.6 ± 9.48 (85 - 127)	101.59 ± 6.11 (90 - 118)	0.52

n – number of patients, SD – standard deviation

The results are more relevant when considering the rate of hepatic enzyme normalization. At baseline, in both groups, all patients had abnormal level of at least one of the ASL, ALT or GGT enzymes, but after 3 months 10 (20%) patients in group A and 24 (47.1%) patients in group B had normal hepatic enzyme levels. Thirteen (26%) patients in group A and 34 (66.7%) patients in group B had normal hepatic enzyme levels after 6 months (fig. 7, tab. III).

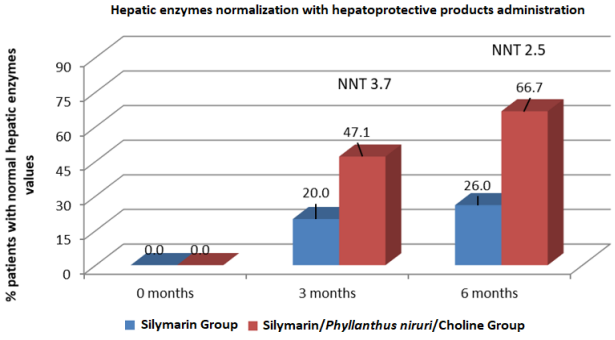

Fig. 7. The rate of hepatic enzyme normalization after 3 and 6 months of treatment with hepatoprotective products

TABLE III

Comparison of the risk for hepatoprotective treatment failure between the two treatment groups - intention-to-treat approach

	Group A (n=50)	Group B (n=51)	*p* - value	RR (95% CI)	NNT
Patients uncured after 3 months n, (%)	40 (80%)	27 (53.9%)	0.0058	0.66 (0.49 to 0.89)	3.696
Patients uncured after 6 months n, (%)	37 (74%)	17 (33.3%)	0.0002	0.45 (0.30 to 0.69)	2.459

RR – risk ratio, NNT – number needed to treat

No statistically significant differences in blood sugar level and blood pressure were recorded between the two groups (tab. II) and no side effects were reported in both groups.

DISCUSSION

Silymarin standardized dry extracts from *Cardui mariae fructus* are used in Europe at least since 1974 in liver diseases such as toxic liver damage, chronic inflammatory liver disease, cirrhosis and are also clinically proven to be effective in non-alcoholic steatosis (14, 15). Silibinin, the main component of flavonolignan complex silymarin, stimulates the protein biosynthesis and induces stabilization of the hepatocyte membranes, exerts antifibrotic effect and prevents the cellular damage and the secretory failure associated with various hepatotoxins (15).

Phyllanthus niruri is an ayurvedic herb, traditionally used for the effect on kidney stones and hepatoprotective effect. Aerial parts are rich in lignanic compounds such as: phyllanthin and hypophyllantin responsible for hepatic tissue regenerating effects (16). *Phyllanthus niruri* has an efficient antitoxic effect, confirmed by acetaminophen, carbon tetrachloride, and thioacetamide-experimentally induced hepatotoxicity models (16, 17, 18, 19). Similar hepatoprotective activities both for silymarin and *Phyllanthus niruri* have been recorded in paracetamol-induced liver toxicity (20). The hepatoprotective effect of *Phyllanthus niruri* was clinically demonstrated and explained by the increase in physiological antioxidants, reduced lipid peroxidation in hepatocyte membrane and counteracting of the destructive effects of free oxygen radicals in the liver (21). The combination between silymarin and *Phyllanthus niruri* demonstrated synergistic hepatoprotective effect in a non-clinical study in rats with carbon tetrachloride-induced hepatotoxicity. A superior effect in normalizing the blood biochemical parameters was recorded for the combination of silymarin with *Phyllanthus* in comparison with silymarin or *Phyllanthus* alone (22). Another non-clinical research (23) supported the beneficial effects of the combination of silymarin and *Phyllanthus*; more efficient hepatoprotective effects were recorded for this combination compared with silymarin alone. The study considered the prevention of liver injury and the normalization of transaminase values in carbon tetrachloride - and paracetamol-induced hepatotoxicity models.

Choline is a source of methyl for the hepatic transmethylation reactions and it is also an essential component of cell membranes and plasma lipoproteins. Choline deficiency is associated with liver injuries (hepatic steatosis and high level of alanine

aminotransferase) that have been recorded both in choline-free parenterally fed patients (24) and in the choline-poor diet in post-menopausal women (25); these effects are reverted by choline dietary supplementation. Currently, choline is the single nutrient with specific liver health claim approved by the *European Food Safety Authority* "maintenance of normal liver function" (26).

The current study investigated the hepatoprotective effect of the combination silymarin / *Phyllanthus niruri* extract / choline in comparison with silymarin alone.

The study confirmed the hepatoprotective effect assessed by the normalizing effect on serum transaminases for silymarin alone and the combination silymarin / *Phyllanthus niruri* extract / choline. However, the combination silymarin / *Phyllanthus niruri* / choline was significantly more efficient than silymarin alone in normalizing the AST, ALT and GGT levels after 3 and 6 months of administration. A brief assessment revealed that the results obtained after 6 months with silymarin alone have been already obtained after 3 months with the combination silymarin / *Phyllanthus niruri* extract / choline, even though a lower dose of silymarin was used in the combination product. Results with statistical significance favorable for silymarin / *Phyllanthus niruri* / choline combination were also obtained for alkaline phosphatase, serum cholesterol and serum bilirubin.

The study also aimed to investigate if *Phyllanthus niruri* extract influences the glycemic profile and blood pressure. In both groups, a slight decrease of blood sugar and blood pressure was recorded. No statistically significant difference between groups in the levels of these parameters was recorded. On the other side, most patients had quite normal levels of these parameters at the inclusion in study: higher rate of abnormal levels at inclusion was observed for the blood sugar levels. Twenty-one (42%) patients in group A and 16 (31.4%) patients in group B presented blood sugar levels above normal ranges at baseline. After 6 months of hepatoprotective treatment, 12 (24%) patients in group A and 6 (11.8%) patients in group B presented higher blood sugar levels. No significant difference in blood sugar levels was found between groups ($p=0.52$) and no influence of *Phyllanthus niruri* on blood sugar levels could be demonstrated. The results are not in agreement with the results of non-clinical studies that demonstrated the hypoglycemiant effect (10, 11, 12) and the hypotensive effect mediated by the diuretic effect of *Phyllanthus niruri* (13, 27). In our study, no statistically significant differences were recorded between group B, receiving silymarin / *Phyllanthus niruri* extract / choline combination and group A, receiving silymarin alone.

No side effects were reported, and this study confirmed the safety use of the combination silymarin / *Phyllanthus niruri* / choline.

CONCLUSIONS

The results of our clinical study indicate that the combination silymarin / *Phyllanthus niruri* extract / choline achieved a better liver protection than silymarin alone in patients with hepatic steatosis. Significantly better results were also obtained after 6 months of administration.

The combination silymarin / *Phyllanthus niruri* / choline showed a safety profile like silymarin alone since no adverse reactions were recorded.

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NEWS

PLACENTA ACCRETA FOLLOWING HYSTEROSCOPIC LYSIS OF ADHESIONS CAUSED BY ASHERMAN'S SYNDROME: A CASE REPORT AND LITERATURE REVIEW

Asherman's syndrome is defined as partial or complete obstruction of the uterine cavity primarily caused by intrauterine procedures and infections. Hysteroscopic adhesiolysis is commonly used to treat Asherman's syndrome. Although the frequency of placenta accreta is known to increase with pregnancy after hysteroscopic adhesiolysis, precise data remain unknown. We report a case of placenta accreta following hysteroscopic lysis of adhesions caused by Asherman's syndrome and IVF treatment and review the literature on placenta accreta following hysteroscopic adhesiolysis. It is necessary to consider placenta accreta as a complication of pregnancies after hysteroscopic adhesiolysis for Asherman's syndrome, particularly in those conceived using IVF. The incidence of placenta accreta is significantly higher in IVF pregnancies than in spontaneous pregnancies [16]. Esh-Broder et al. reported that the rate of placenta accreta in spontaneous pregnancies was 12 / 752 (1.2 / 1000), while that in IVF pregnancies was 30 / 24,441 (16 / 1,000). The higher rate of placenta accreta in IVF pregnancies may be due to the change in endometrial environment and morphological and structural changes to the endometrium due to the IVF treatment protocol (stimulation protocol). Intrauterine adhesions (IUA) severity is associated with greater reduction in fertility thereby increasing the likelihood that affected patients will need to undergo IVF to have a successful pregnancy. The extent of endometrial loss increases with IUA severity, which likely confers a greater risk of placenta accreta. For this reason, doctors should be aware that IVF pregnancy following hysteroscopic adhesiolysis involves a greater risk of placenta accreta (Yuko Sonan, Shigeru Aoki, Kimiko Enomoto, Kazuo Seki, Etsuko Miyagi. Placenta Accreta following Hysteroscopic Lysis of Adhesions Caused by Asherman's Syndrome: A Case Report and Literature Review. *Case Reports in Obstetrics and Gynecology*, 2018, https://doi.org /10.1155/2018/6968382).

Emil Anton

PREVALENŢA SINDROMULUI METABOLIC ÎNTR-O POPULAŢIE DE LUCRĂTORI ÎN TRANSPORTURI - STUDIU PROSPECTIV

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THE METABOLIC SYNDROME'S PREVALENCE IN A POPULATION OF TRANSPORT WORKERS (Abstract): The metabolic syndrome is a problem which regards public health this phenomenon being explined by the world –wide obesity epidemy.The metabolic syndromes prevalence varies with the studied populations.The unhealthy lifestyle which is defined by peoples sedentary character and unhealthy diets is being significantlly asociated with the metabolic syndromes prevalence growth. **Material and methods:** The study was performed on 836 individuals with ages between 25 and 65 years, who have a questionnare based interview.Their blood pressure was measured, some anthropometric measures were taken and several laboratory test were made. The diagnosis of metabolic syndrome was established according to the consensual deffinition that was published in October 2009. **Results and conclusions:** The metabolic syndrome is a compilation of clinical and biological elements which cause the individ to have a higher cardiovascular risk. The metabolic syndrome increased prevalance in the study is significantlly asociated with the growth of the obesitys and hypertensions prevalance**.** The lifestyle of the individuals from the study is based on unhealthy diets and sedentarism. **Key words:** METABOLIC SYNDROME, HYPERTENSION, OBESITY, SEDENTARISM, PREVALENCE

Sindromul metabolic (SM) este o constelaţie de factori clinico-biologici care conferă indivizilor un risc cardiovascular crescut (1).

Studiile populaţionale au arătat că SM dublează riscul evenimentelor cardiovasculare (2), iar la pacienţii nondiabetici creşte de 5 ori riscul de diabet zaharat tip 2 (3).

Hipertensiunea arterială este cea mai frecventă componentă individuală a SM fiind prezentă la 50-76% dintre cazuri.

De-a lungul timpului s-au acumulat multe cunoştinţe despre incidenţă, prevalenţă şi s-au adus unele informaţii nu numai cu privire la etiopatogenia SM, la originea sa multifactorială, ci şi în legătură cu factorii agravanţi ca: sedentarismul, disfuncţia endotelială etc (4, 5, 6, 7).

MATERIAL ŞI METODE

Studiul a fost realizat pe un lot de 836 subiecţi, lucrători în transporturi, care au răspuns la un interviu pe bază de chestionar, s-a determinat tensiunea arterială şi s-au efectuat măsurători antropometrice. Pentru subiecţii cu TA≥140/90 mmHg şi /sau

PT>94 cm s-a extins protocolul de studiu cu teste de laborator care au inclus glicemia à jeun, HDL-colesterolul, trigliceridele.

REZULTATE ŞI DISCUŢII

1. Repartiția pe grupuri de vârstă

Cel mai tânăr participant a avut vârsta de 25 de ani iar cel mai vârstnic 65 ani, media de vârstă pentru lotul de studiu fiind de 42,93 ani.

Cei mai mulți pacienți (35,4%) s-au încadrat în categoria de vârsta 35-44 de ani, cei mai puțin reprezentați au fost între 54-65 de ani (15,79%), celelalte categorii având o pondere de 26,32%(45-54 de ani) şi 22,4% (25-34 de ani).

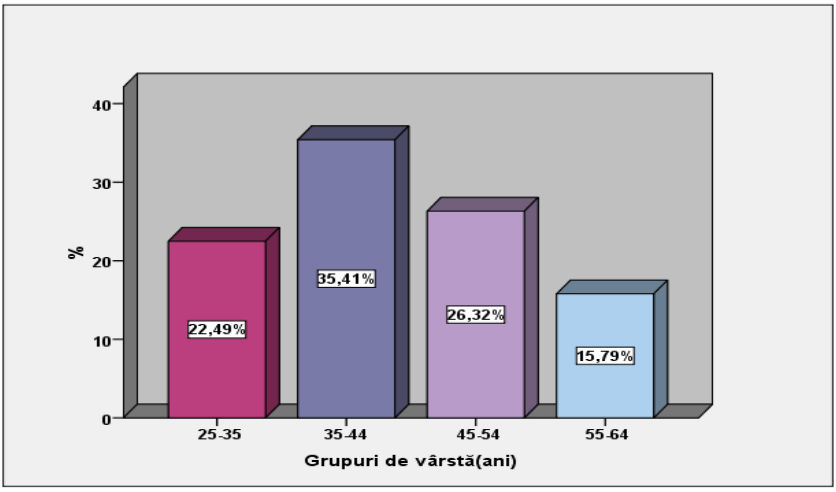

Fig. 1. Repartiția pe grupuri de vârstă

2. Prevalența sindromului metabolic

La nivelul întregului lot SM a fost prezent la 28,23% din subiecți şi la 64,47% din hipertensivi (fig. 2). S-a constatat că prevalența SM la lotul studiat este mai mare decât în populația generală, iar valorile prevalenței SM cresc cu vârsta fapt corelat parțial cu creşterea prevalenței HTA şi a anomaliilor glicemice (tab. I).

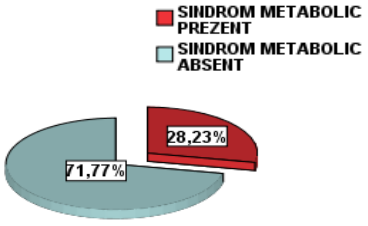

Fig. 2. Prevalența sindromului metabolic în lotul de studiu

TABELUL I

Prevalența SM pe grupuri de vârstă

Sindromul metabolic	Total cazuri	grupuri de vârstă(ani)							
		25-34		35-44		45-54		55-64	
Prezent	236	Nr.	%	Nr.	%	Nr.	%	Nr.	%
		28	11,9	72	30,5	76	32,2	60	25,4
Absent	600	160	26,7	224	37,3	144	24,0	72	12,0
Total	836	188	22,5	296	35,4	220	26,3	132	15,8

3. Prevalența obezității

Pentru cuantificarea obezității am utilizat măsurarea perimetrului taliei (PT) care conform definiției trebuie să fie mai mare de 94 cm. În ansamblul studiului procentul obezilor este semnificativ crescut 60,77% fapt explicabil prin caracteristicile lotului (sedentarism şi diete necorespunzătoare). Pentru o prezentare cât mai detaliată am încadrat subiecții în trei categorii funcție de PT (PT≤94 cm; PT 95-101 cm; PT≥102 cm).

S-a constatat că pacienții cu PT ≥102 cm (35,89%) au fost în număr aproape egal cu cel al pacienților fără obezitate (39,23%), dar în structura lotului s-au înregistrat 24,88% cu PT între 95-101 cm (fig. 3).

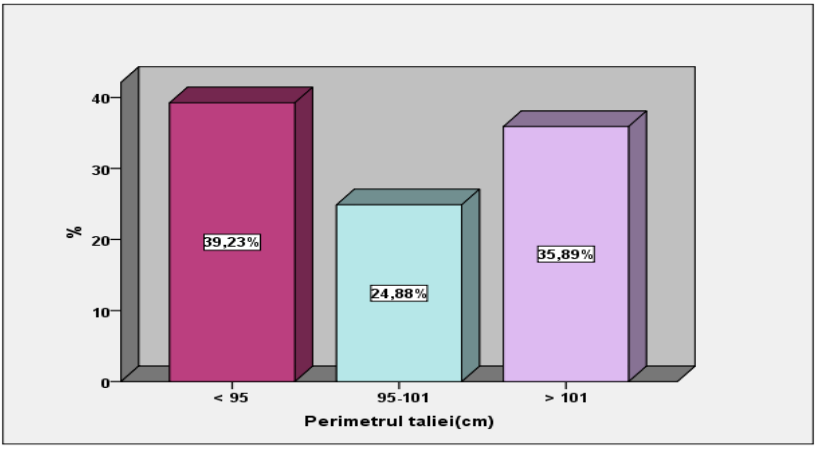

Fig. 3. Repartiția perimetrului taliei

4. Prevalența hipertensiunii arteriale

Din cei 836 pacienți care au răspuns la ancheta clinico-epidemiologică hipertensiunea arterială a fost prezentă la 36,4%. Luând în considerație faptul că lotul studiat a avut vârsta sub 65 de ani putem remarca o prevalență crescută a HTA comparativ cu datele din literatură, unde HTA se înregistrează la aproximativ 40% din populația adultă (fig. 4).

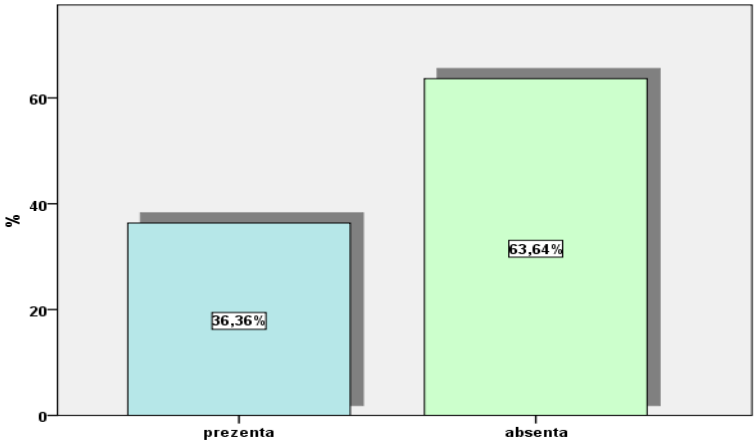

Fig. 4. Prevalența HTA în lotul de pacienți incluşi în studiu

CONCLUZII

Identificarea sindromului metabolic are valoare în contextul depistării factorilor de risc cardiovascular, talia hipertensivă fiind elementul de orientare.

Există limite în depistarea elementelor sindromului metabolic

Cooperarea interdisciplinară între rețeaua primară, şi cea de specialitate este obligatorie iar medicul de familie – are un rol important în supravegherea pacienților purtători ai factorilor de risc, coordonarea screening-ului pentru complicații şi educarea pacienților.

Sindromul metabolic este o constelație de factori clinico-biologici care conferă indivizilor un risc cardiovascular crescut.

Prevalența sindromului variază în funcție de populațiile studiate.

S-a dovedit că prevalența SM are valori crescute la lucrătorii în transporturi comparativ cu populația generală.

Hipertensiunea arterială se înregistrează, la peste 50 % dintre pacienții cu obezitate.

Cercetările au dovedit existența unei relații directe între stilul de viață nesanogen şi valorile ridicate ale prevalenței obezității, HTA şi SM.

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STUDIU TRANSVERSAL DE EVALUARE A UNOR FACTORI DE RISC IMPLICAŢI ÎN APARIŢIA MALNUTRIŢIEI LA SUGAR

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CROSS - SECTIONAL STUDY TO EVALUATE RISK FACTORS IN INFANTS' MALNUTRITION (Abstract): Malnutrition is a major health problem in our country by maintaining a high number of infants with poor nutritional status. Various studies have highlighted the role of infant's malnutrition in the development of adult diseases. Methods: We made a cross-sectional study during six months (October 2010 - March 2011) on a group of 63 infants admitted in Pediatric Recovery Department-Children's Hospital, Iasi; we evaluated the presence of risk factors for malnutrition. The data were processed using SPSS 16 and EpiInfo 3.5.2. (December 2010). Results: The infants were predominantly female (52,4%); the most affected age group was 5-24 weeks (84,11%). Most of them came from rural areas (79,4%), from families with low socioeconomic income (95,2%), mothers with a low educational status (63,4%), housewives (88,9%). We noted the presence of previous diseases in 71,4% infants. Only 12 infants received breast milk for a short time (three weeks), the other 51 infants have been bottle-fed since birth. Complementary food was incorrect in 68,42% cases. With a proper diet the mean weight gain was 895,68 g and the Z score values (weight for age, height for age, weight for height) have improved during an average of 34,15 days; positive correlation between these factors is strong. Conclusions: Low socio-economic income, rural areas, low maternal educational level, diet errors, small infants with multiple previous diseases are the main risk factors in malnutrition's occurrence. We consider particularly important to solve social problems too, not only the medical, because when the infant returns in the same disadvantaged family there is an increased risk for malnutrition to recur. **Keywords**: MALNUTRITION, RISK FACTORS, Z SCORE, INFANT NUTRITION.

Malnutriţia reprezintă un indicator important în aprecierea stării de sănătate a populaţiei având în vedere corelaţiile care s-au stabilit între diverse afecţiuni ale adultului şi nutriţia inadecvată din perioada de sugar (1).

În plus, datele din literatură subliniază prezenţa unor efecte negative pe termen lung şi prin afectarea dezvoltării cerebrale. Astfel aproximativ 50% dintre copiii afectaţi de malnutriţie în primul an de viaţă vor prezenta afectarea comportamentului verbal şi socio-afectiv în perioada de adult (2,3).

Studiile desfăşurate până în prezent au evidenţiat că mortalitatea infantilă este determinată în peste 50% din cazuri de mal-

nutriţie (4,5), iar estimările efectuate subliniază posibilitatea reducerii acesteia cu cel puţin o treime dacă malnutriţia ar fi eliminată (4,6).

MATERIAL ŞI METODĂ

Am efectuat un studiu retrospectiv, pe o perioadă de 6 luni (octombrie 2010 – martie 2011), pe un lot de 63 sugari, a căror vârstă la internare a fost cuprinsă între 5 şi 48 de săptămâni, internaţi în Secţia Recuperare Pediatrică a Spitalului Clinic de Urgenţă pentru Copii „Sfânta Maria" Iaşi, la care am evaluat prezenţa unor factori de risc în apariţia malnutriţiei.

Criteriul de includere în studiu a fost prezenţa malnutriţiei protein-energetice (Protein Energy Malnutrition – PEM) apreciată prin determinarea indicatorilor antropometrici recomandaţi de OMS (greutatea pentru talie, talia pentru vârstă, greutatea pentru vârstă) (7,8).

Pentru respectarea normelor deontologice ale cercetării, includerea copiilor în lotul de studiu a fost realizată după obţinerea, în prealabil, a consimţământului informat al aparţinătorilor legali ai acestora. Am redactat astfel, într-un limbaj accesibil, un formular de consimţământ informat care conţine date despre scopul, metodologia şi riscul redus al studiului (prelevarea probelor este non-invazivă şi nu provoacă discomfort), precum şi beneficiile ce rezultă prin participarea la acesta, garantând confidenţialitatea rezultatelor obţinute (9).

De asemenea, din foile de observaţie au fost preluate toate informaţiile referitoare la factorii ce pot favoriza apariţia malnutriţiei: mediul de provenienţă, nivelul socio – economic al familiei, date referitoare la mamă (stare civilă, nivel educaţional, ocupaţie, antecedente obstetricale), date referitoare la alimentaţia administrată anterior internării (tipul, durata şi modul de administrare al alimentaţiei, utilizarea unei alimentaţii corecte/incorecte), date despre antecedentele personale patologice.

Pentru evaluarea statusului nutriţional, atât la internare cât şi la externare, s-au determinat greutatea şi talia. Valorile greutăţii şi taliei pentru vârstă, precum şi ale greutăţii pentru talie au fost calculate, la internare şi la externare, cu ajutorul tabelelor de referinţă recomandate de Centers for Diseases Control (CDC)/National Center for Health Statistics (10).

Ulterior acestea au fost interpretate cu ajutorul scorului Z, ce exprimă valoarea antropometrică în număr de deviaţii standard inferioare sau superioare valorii mediane de referinţă (7).

La subiecţii incluşi în studiu, pe toată durata internării, au fost recomandate regimuri dietetice adaptate gradului de severitate a malnutriţiei. De asemenea au fost monitorizate evoluţia clinică şi sporul ponderal.

Toate datele de anamneză, cele antropometrice, de laborator precum şi valorile calculate ale scorului Z au fost introduse într-o bază de date în Excel. Aceasta a fost importată şi prelucrată cu ajutorul programului *SPSS 16.0* şi *EpiInfo 3.5.2*. (17 Decembrie 2010).

REZULTATE

Analizarea distribuţiei sugarilor ce au alcătuit lotul de studiu pe grupe de vârstă (5-12 săptămâni, 13-24 săptămâni, 25-36 săptămâni, 37-48 săptămâni) a evidenţiat prezenţa PEM în special la cei cu vârsta între 5 – 24 săptămâni (53 sugari - 84,11%) (fig. 1). Diferenţa între această grupă de vârstă şi grupele de vârstă 25 - 36, respectiv 37 – 48 săptămâni este semnificativă statistic ($p < 0,01 \text{ X } 10^{-5}$), la fiecare dintre acestea înregistrându-se numai câte 5 sugari (7,93%).

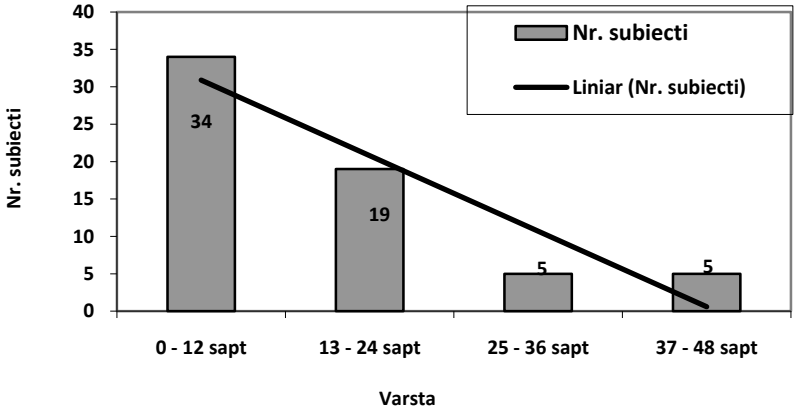

Fig. 1. Distribuţia subiecţilor incluşi în studiu pe grupe de vârstă

Au predominat sugarii de sex feminin (52,4% versus 47,6% sex masculin) însă diferenţa nu a fost semnificativă statistic (p = 0,50).

Majoritatea sugarilor cu PEM luaţi în studiu au provenit din mediul rural (50 sugari – 79,4%) (fig. 2), aproximativ jumătate dintre ei fiind din familii organizate (32 sugari – 50,8%, p = 0,007 X 10^{-2}), cu 1 – 2 copii (31 sugari – 49,20%, p = 0,09 X 10^{-5}**)** şi cu un nivel socio – economic scăzut în majoritatea cazurilor (60 sugari – 95,2%, p = 0,01 X 10^{-5}) (fig. 3). Corelaţiile între nivelul socio-economic, statusul marital şi paritatea mamei nu au demonstrat asociaţii statistic semnificative (r = - 0,1, respectiv r = 0,2).

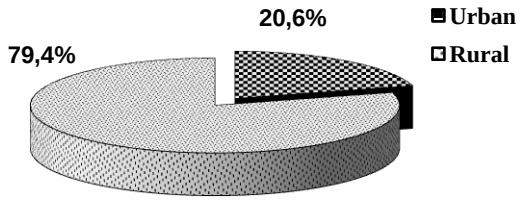

Fig. 2. Frecvenţa subiecţilor incluşi în studiu după mediul de proveniență

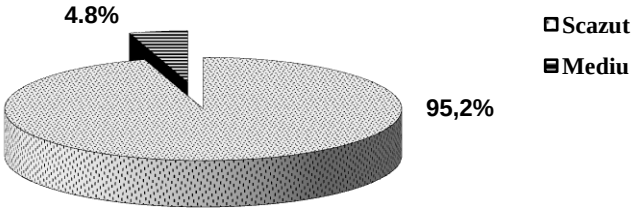

Fig. 3. Distribuţia subiecţilor incluşi în studiu în funcţie de nivelul socio-economic

Cei mai mulţi dintre sugari (40 - 63,4%, p = 0,01 X 10^{-5}) au avut mame cu nivel educaţional scăzut (analfabet şi elementar) (fig. 4), casnice (56 - 88,9%, p< 0,01 X 10^{-5}).

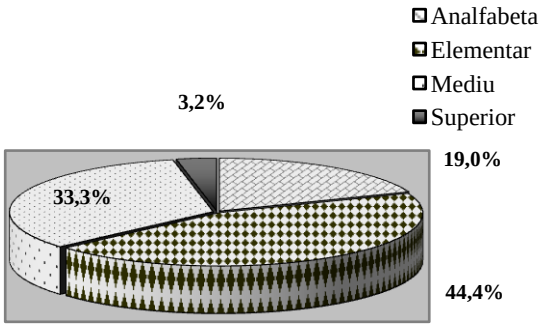

Fig. 4. Distribuţia nivelului educaţional al mamelor subiecţilor incluşi în studiu

Din antecedentele personale fiziologice am remarcat că marea majoritate au fost născuţi în maternitate (56 – 88,9%, p < 0,01 X 10^{-5}), pe cale naturală (44 – 69,8%, p = 0,008 X 10^{-3}) şi peste jumătate din cazuri au avut o vârstă gestaţională mai mică de 37 de săptămâni (35 - 55,55%, p = 0,001), o greutate la naştere sub 2500g şi o talie la naştere mai mică de 47 cm (37 - 58,74%).

Anamneza efectuată la internarea în secţie a relevat prezenţa antecedentelor patologice la un număr semnificativ de sugari (45 - 71,4%) (p = 0,01 X 10^{-4}).

Analizând tipul de alimentaţie administrată sugarilor anterior internării am remarcat că 80,96% (51 sugari) au fost alimentaţi artificial de la naştere, cu biberonul (p = 0,01 X 10^{-5}), în timp ce numai 12 sugari (19,04%) au primit alimentaţie naturală pentru foarte scurt timp (în medie 3 săptămâni), ulterior fiind alimentaţi artificial. Din cei 19 sugari care ar fi trebuit să primească anterior internării o alimentaţie diversificată (complementară), în 13 cazuri (68,42%) s-a efectuat o diversificare incorectă.

Durata medie de spitalizare pentru lotul luat în studiu a fost de 34,15 zile (cu variaţii între 13 – 69 zile), timp în care s-a obţinut un spor ponderal mediu de 895,68 g, între cei doi factori existând o corelaţie pozitivă puternică (r = 0,9).

Studiul scorului Z atât la internarea în secţie cât şi la externare a evidenţiat îmbunătăţirea valorilor medii ale acestuia de la – 4,35 la – 3,54 în ceea ce priveşte greutatea pentru vârstă, de la – 2,93 la – 2,30 referitor la talia pentru vârstă şi de la – 1,53 la – 0 ,70 în cazul greutăţii pentru talie.

Analiza statistică a evidenţiat existenţa unei corelaţii pozitive puternice (r = 0,87) între nivelul socio - economic şi valorile scorului Z greutate pentru vârstă efectuate la internarea în secţie. De asemenea, am obţinut o corelaţie pozitivă puternică (r = 0,97, respectiv r = 0,9) între tipul de alimentaţie pe durata internării şi valorile la externare ale scorului Z (greutate pentru vârstă, talie pentru vârstă). Între durata medie de spitalizare şi scorul Z al taliei pentru vârstă efectuat la externare corelaţia pozitivă a fost moderată (r = 0,55).

DISCUŢII

Derularea acestui studiu este motivată

de menţinerea unui procent ridicat de sugari cu malnutriţie în ţara noastră (7).

Deşi aproximativ jumătate dintre sugarii luaţi în studiu aparţin unor familii organizate, cu un număr redus de copii, proveniența din mediul rural asociată cu şcolarizarea redusă şi statutul de mamă casnică justifică de ce nivelul socio-economic scăzut reprezintă principalul factor de risc în apariţia malnutriţei (11).

Marea majoritate au fost născuţi în maternitate, predominând naşterea pe cale naturală, dar considerăm că se menţine deosebit de ridicat procentul naşterilor în afara unităţilor spitaliceşti (11,1%), comparativ cu un procent de 2% la nivel naţional (12), explicabil prin nivelul educaţional scăzut şi implicit prin educaţia sanitară redusă.

Peste jumătate dintre sugarii incluşi în lotul de studiu până în prezent au fost născuţi la o vârstă gestaţională mai mică de 37 de săptămâni, cu o greutate mai mică de 2500g şi o talie mai mică de 47cm, ceea ce confirmă datele din literatură potrivit cărora naşterea prematură şi greutatea mică la naştere reprezintă factori favorizanţi în apariţia malnutriţiei (13).

Am remarcat apariţia mai frecventă a PEM la grupa de vârstă 5 – 24 de săptămâni, explicabilă prin erorile de alimentaţie: absenţa administrării sau administrarea pe o durată redusă (3 săptămâni) a alimentaţiei naturale, alimentaţia artificială incorectă prin imposibilitatea procurării unor formule de lapte corespunzătoare vârstei sau prin diluarea excesivă a acestora, diversificarea precoce şi/sau incorectă.

Subliniem astfel necesitatea îmbunătăţirii educaţiei mamelor în ceea ce priveşte importanţa alimentaţiei naturale în primele luni de viaţă, a cunoaşterii tehnicii de preparare a formulelor de lapte precum şi a etapelor de diversificare.

Antecedentele patologice prezente într-un procent ridicat (71,4%), concordant cu studiile care subliniază rolul de factor de risc al acestora în apariţia PEM la sugar (14), au impus spitalizări anterior internării în Secţia Recuperare Pediatrică.

Analiza valorilor medii ale scorului Z greutate pentru vârstă şi ale scorului Z talie pentru vârstă la internare subliniază o afectare importantă a acestor indicatori antropometrici potrivit criteriilor OMS (15).

În acelaşi timp valorile medii ale scorului Z greutate pentru talie la internare evidenţiază o afectare mai redusă, justificată de ritmul mai lent de recuperare a taliei la sugarii ce au avut la naştere valori mici ale acestui parametru.

La externare se remarcă o ameliorare mai accentuată a valorilor scorului Z greutate pentru vârstă şi greutate pentru talie, comparativ cu valorile talie pentru vârstă, argumentată de un spor ponderal mediu considerabil (895,68 g) pe o durată medie de spitalizare de 34,15 zile, durată ce nu se poate asocia cu un spor statural la fel de important.

Îmbunătăţirea valorilor acestor indicatori antropometrici la externare subliniază importanţa administrării unei alimentaţii corespunzătoare vârstei şi greutăţii precum şi a asigurării unor condiţii optime de îngrijire.

CONCLUZII

Nivelul socio-economic scăzut reprezintă principalul factor de risc în apariţia malnutriţiei la sugari, în special la cei proveniţi din mediul rural. Considerăm deosebit de importantă abordarea malnutriţiei şi din punct de vedere social, nu doar medical, deoarece revenirea sugarului în acelaşi mediu familial defavorizat are ca rezultat reapariţia malnutriţiei.

Rezultatele obţinute până în prezent confirmă că şi greutatea mică la naştere constituie un alt factor de risc pentru malnutriţie.

Administrarea unei alimentaţii necorespunzătoare afectează cel mai sever statusul nutriţional la grupa de vârstă 5 – 24 săptămâni.

Antecedentele patologice reprezintă, de asemenea, un factor de risc în apariţia PEM.

Acest studiu subliniază importanţa îmbunătăţirii asistenţei medicale primare, în special în mediul rural, în vederea asigurării unui status nutriţional adecvat sugarilor, pentru a întrerupe astfel cercul vicios malnutriţie – infecţie – malnutriţie.

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RANKL/RANK/OPG MOLECULAR COMPLEX – CONTROL FACTORS IN BONE REMODELING IN PSORIATIC ARTHRITIS

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RANKL/RANK/OPG MOLECULAR COMPLEX – CONTROL FACTORS OF BONE REMODELING IN PSORIATIC ARTHRITIS (Abstract): **Aim**: To investigate the blood levels of bone remodeling factors (RANKL, OPG and Bone-ALP) in psoriatic arthritis patients. **Material and methods:** Included in this study were 27 patients with psoriatic arthritis (CASPAR classification criteria) and 20 healthy subjects. The RANKL , OPG and Bone-ALP levels have been quantified by ELISA method. **Results:** Peripheral blood concentrations were higher in psoriatic arthritis than in the healthy subjects, and a strong negative correlation between RANKL and OPG and between RANKL and Bone-ALP was also found. **Conclusions**: The increase in OPG and Bone-ALP serum level is strongly related to the increase in RANKL serum level in patients with psoriatic arthritis. The increase in OPG and Bone –ALP serum level induces the production of bone matrix parallel to the bone destruction mediated by RANKL. The follow up of the patients with psoriatic arthritis by early RANKL, OPG and Bone-ALP serum level determinations, and of bone metabolism markers (with specific role in bone remodeling), allows an accurate evaluation of disease activity and, in the future, could be a criterion for the initiation of specific, targeted treatment. **Key words**: BONE REMODELING, RANKL/RANK/OPG MOLECULAR COMPLEX, PSORIATIC ARTHRITIS

The recently discovered fact that psoriatic arthritis represents itself a unique pathology increased the concern for studying this disease in the past years.

The psoriatic arthritis is a chronic inflammatory disease accompanying psoriasis, characterized by bone proliferation and resorption, particularly affecting the tendons, the articulations and the enteses (the articular capsular insertion) (1). Psoriatic arthritis, together with rheumatoid arthritis, is one of the most frequent causes for handicap in patients' social and familial activities. In twenty five procents of patients labor inability was found in 6 years, and in 50% of patients in 10 years (2). In order to demonstrate this real problem in the literature, one French study reveals that the costs for the psoriatic arthritis (in France) is estimated to 5,2-6,5 billions euro (3). Concerning all these, an important objective seems to be the early identification of patients with major risk for articular destruction and early therapeutic intervention.

Remodeling, the bone reconstruction, comes immediately after its formation, being more intense in utero. Frost, in 1964, was the first that described the bone remodeling (4).

Bone remodeling is the cellular and mor-

phologic support for the bone turnover; it keeps in good shape the biomechanical status of the skeleton. In adults, in which the growth is finished, the bone mass and structure are kept constant in time, although the bone remodeling is continue, by an exact balance between the resorbtion and formation of bone in the entire skeleton (5,6,7).

In normal conditions, the resorbed bone is replaced by an equal amount of new bone, so that after each cycle of bone remodeling, the total mass of bone stays constant. This remodeling permits the maintenance of the bone mass constant during the normal adult life. A good bone remodeling needs close interactions between the bone cells, bone marrow and the matrix; these interactions also need soluble factors synthesized by the cells or being available within the matrix.

The imbalance of this process have consequences on the bone architecture and the bone mass, and could lead, for a long period of time, to a loss of integrity in the skeleton structure and to fractures, all these being important causes of morbidity and mortality in ageing, or causes of different diseases that sustain this imbalance.

The bone remodeling regulation is done by many factors:

- RANKL (Receptor activator of nuclear factor NF-kB ligand) also known as ODF (osteoclast differenciation factor), OPGL (osteoprotegerin ligand) or TRANCE (TNF related activation induced cytokine)
- RANK (Receptor activator of NF-kB): this receptor is situated on the pre-osteoclasts cellular membrane; it is also known as ODAR (osteoclast differentiation activating receptor).

OPG (osteoprotegerina) is a natural "decoy" receptor of RANKL; it's also known as OCIF (osteoclast inhibiting factor).

These factors regulate the bone remodeling in psoriatic arthritis, rheumatoid arthritis, post-menopausal osteopenia, osteoporosis in inflammatory chronic reumatisms, in gums pathology in which the alveolar bone is resorbed, and in bone metastasis (6,7).

MATERIAL AND METHODS

The trial includes individuals in two groups:

- **Group 1** (patients with psoriatic arthritis diagnosed by the CASPAR criteria - The **Cla**ssification **C**riteria for **P**soriatic **A**rthritis). It includes 27 patients (n=27), 15 men and 12 women, aged between 27 and 50.
- **The healthy group** includes 20 individuals (n=20), 9 women and 11 men, aged below 50 and without any rheumatism, infectious, neoplasic or autoimmune disease.

The two groups have been investigated in CDMT Laboratory in Timisoara between 2004 and 2007.

- **Inclusion criteria**: patients aged 27-50, diagnosed with psoriatic arthritis by CASPAR criteria (The **Cla**ssification **C**riteria for **P**soriatic **Ar**thritis) for at least 36 months, being in an acute stage of the disease and lacking an anti-inflammatory treatment for at least one month before inclusion. A complete clinical general inspection and paraclinical investigations have been done for these patients, with the aim to exclude an associated autoimmune disease, a cancer disease or a systemic infection.
- **Exclusion criteria**: patients with:
  - ankilopoetic spondilitis
  - Reiter Syndrome
  - Other bone diseases diagnosed by a detailed history, the clinical general examination, the paraclinical investigations (Pa-

get disease, Cushing Syndrome, Multiple Myeloma, Hodgkin's Disease etc)

– alcohol consumers

– intestinal inflammatory diseases (Crohn Disease etc)

– malnutrition

– vitamin D deficiency or any treatment that affects the bone metabolism

– patients that received glucocorticoids or methotrexate treatments in the last 12 months

– other diseases that need long term treatment with glucocorticoids (more than 6 months)

– patients that received dietary supplements with Calcium or Vitamin D

– sustained physical activity in the last 12 months

– women aged below 40 in early menopausa of different etiology (chirurgical or medical).

The RANKL and OPG serum levels were quantified by sandwich Elisa immunoenzimatic assay (Enzyme Linked Immuno Sorbant Assay).

THE AIM OF THE STUDY

Evaluation criteria:

- **Bone resorbtion** evaluation by the determination of RANKL serum level, marker of bone resorbtion in bone remodeling.
- **Osteosynthesis** evaluation by the determination of serum level of OPG, marker of bone synthesis in bone remodeling.
- The **correlation of RANKL** (marker of bone resorbtion) and **OPG** (marker of bone synthesis) serum levels.

Statistical analysis

The graphics and the statistical analysis in this study were done by use of Microsoft Office Excel 2007. We used:

– The elements of descriptive statistics: mean and standard deviation (mean ± DS);

– The elements of intererential statistics: the t´ Student test and the Pearson test.

– **The t´ Student test** was used for the comparison of data belonging to the psoriatic arthritis group comparative to the healthy group. The values of the p_1 were calculated (group 1 and healthy group) $p<0.05$ has statistically semnificative value.

– **The Pearson test** awas used for the comparison of data within the same analised group. The values of the r_1 were calculated.Y $r>0.5$ has good correlation (positive or negative)

RESULTS

RANKL serum levels were assessed by enzyme linked immunosorbent assay (ELISA).

In group 1 (psoriatic arthritis), the RANKL serum level was between 36.35 and 68.99 pg/ml (mean was 51.98±10.74 pg/ml; $p_1<0.003$- ES, statistically semnificative compared to the healthy group).

In the healthy group, the RANKL serum level was between 29.65 and 37.14 pg/ml (mean was 32.46±3.03 pg/ml) (Table I, Fig. 1).

TABLE I

RANKL serum levels in psoriatic arthritis comparative to those in the healthy group

Parameter (mean ± DS)	Group 1 (psoriatic arthritis)	Healthy Group
RANKL serum level (pg/ml)	51.98±10.74 p<0,003	32.46±3.03

(Table I. Increased RANKL serum levels: $p_1<0.003$- ES, ES - intense statistical significance comparative to the healthy group)

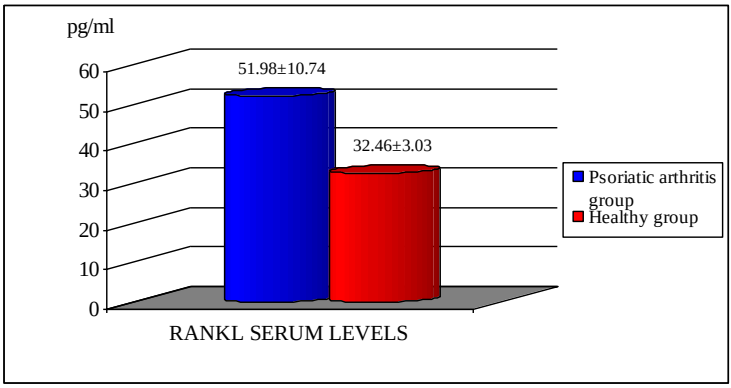

Fig. 1. The comparison of RANKL serum levels in analyzed groups
(Figure 1. Increased RANKL serum levels: $p_1<0.003$- ES in psoriatic arthritis group, ES- intense statistical significance comparative to the healthy group)

After processing the data, the results show an increase in RANKL serum levels in lot 1 comparative to the healthy group, due to the increase in bone destruction: the mean increased with 160.13 %, up to 51.98±10.74 pg/ml.

The **OPG serum levels** were assessed by enzyme linked immunosorbent assay (ELISA).

In group 1 (psoriatic arthritis**),** the OPG serum level was between 33.18 and 48.42 pg/ml (mean was 38.05±4.89 pg/ml; $p_2<0.002$- ES, intense statistical significance comparative to the healthy group).

TABLE II

The OPG serum levels in group 1 (patients with psoriatic arthritis) comparative to those in the healthy group

Parameter (mean ± DS)	Group 1 (psoriatic arthritis)	Healthy Group
OPG serum level (pg/ml)	38.05±4.89 $p_2<0.002$	28.06±1.39

(Table II. Increased OPG serum levels: $p_2<0.002$- ES, ES- intense statistical significance comparative to the healthy group)

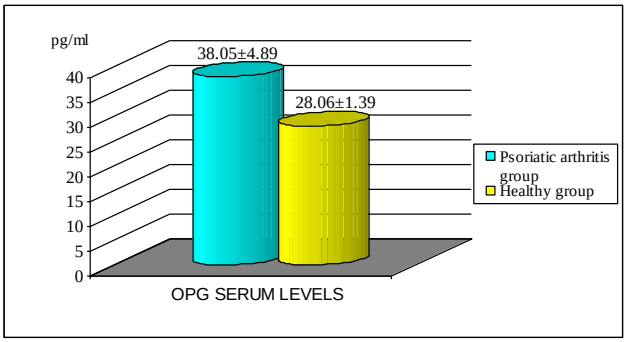

Fig. 2. The comparison between OPG serum levels in analyzed groups
(Figure 2. Increased OPG serum levels: $p_2<0.002$- ES in psoriatic arthritis group, ES- intense statistical significance comparative to the healthy group)

TABLE III

Comparison between the RANKL serum level and the OPG serum level in psoriatic arthritis patients.

Parameter (mean ± DS)	Serum RANKL (pg/ml)	Serum OPG (pg/ml)
Group 1 (n=27)	51.98±10.74 p_1<0,003	38.05±4.89 p_2<0,002 r_2= -0.87

(Table III: Increased RANKL and OPG serum levels in psoriatic arthritis: p_2< 0.002-E S, intense statistical significance in group 1, p_1< 0.003-ES, intense statistical significance in group 1 comparative to the healthy group and in strong negative correlation, r_2= -0.87).

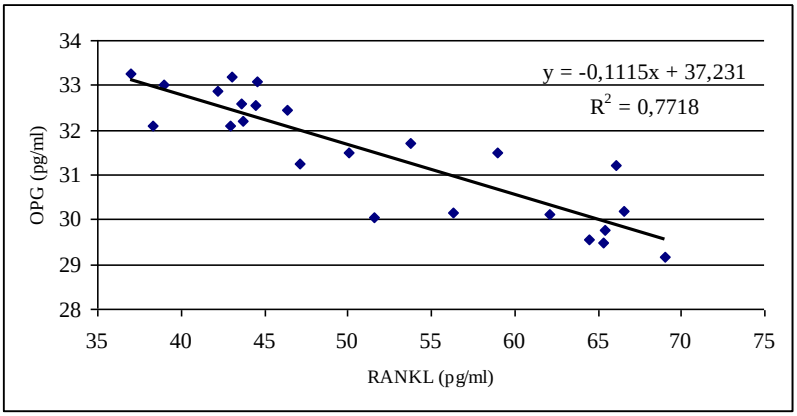

Fig. 3. The correlation between OPG and RANKL serum levels in psoriatic arthritis patients. (Figure 3.The RANKL serum level showing strong negative correlation with OPG serum level, r_2 = -0.87, R_2^2= 0.7718).

In the healthy group, the OPG serum level was between 26.65 and 33.18 pg/ml (mean was 28.06±1.39 pg/ml).

After processing the data the results show an increase in OPG serum levels in group 1 comparative to the healthy group, due to the increase in bone synthesis. The mean increased with 135.60 % up to 38.05±4.89 pg/ml.The data processing showed a strong negative correlation between OPG and RANKL serum levels in psoriatic arthritis patients: r_2= - 0.87 (R_2^2= 0.7718 in group 1).

DISCUSSION

The tendency to developed bone erosions in psoriatic arthritis is well known and manifests radiological as massive loss of articular space, big bone lesions, bone erosions radiologicaly described as “pencil head” and the resorbtion of the distal phalanx(8). The periarticular mineralization is intense and is accompanied by new bone formation in periostatic and ankilotic forms of disease. The presence of bone resorbtion and of new bone formation markers attests the bone remodeling in psoriatic arthritis.

Concerning the fact that the osteoclasts play a role in mediation of bone erosion in rheumatoid arthritis and that the RANKL/ RANK signal is also important in this process, the researchers focused on psoriatic arthritis, too(11).

Studies using anti-RANKL and anti-OPG monoclonal antibodies showed that

RANKL is expressed on the synovial cells, but OPG, which is expressed on the endothelial cells.

These studies demonstrate that RANKL is the key mediator in articular destruction and in bone loss in psoriatic arthritis.

The therapeutic inhibition of RANKL action using osteoprotegerine (OPG), natural "decoy" receptor, completely prevents the bone loss in articular inflammatory diseases and has a benefic effect on the cartilage destruction in all arthritic studied models. The modulation of this system allows the physicians to use a new therapy for stopping the bone loss and to prevent the handicap due to arthritis.

CONCLUSIONS

The final assessment of all data revealed from this study leads to:

– In psoriatic arthritis the RANKL serum level increases due to the activation of osteoclasts and therefore due to bone destruction.

– This increase in RANKL serum level represents a marker for the activity of the disease.

– In psoriatic arthritic patients, the OPG serum level increases more than in normal individuals.

– The increase in OPG serum level represents a marker for the activation of osteosynthesis, which takes place in bone remodeling process in psoriatic arthritis.

– The increase in OPG serum level shows a strong negative correlation with that the increase in RANKL serum level in psoriatic arthritic patients.

– This increase in OPG serum concentration tries to induce the reconstruction of bone matrix, compensatory to the destructive process mediated by RANKL.

– The monitoring of psoriatic arthritis patients by early determination of RANKL and OPG serum levels, bone metabolism markers (they have specific role in bone remodeling), allows a precise evaluation of the disease activity, and, in future, could be a criteria for initializing the specific, targeting therapy.

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NOUTĂȚI

NOI MODALITĂȚI DE DIAGNOSTIC ÎN INFECȚIILE CU *KINGELLA KINGAE*

Kingella kingae este frecvent implicată în infecțiile ostearticulare la copil. Deoarece metodele de izolare clasice sunt dificile, s-a încercat standardizarea metodelor de diagnostic prin tehnici de biologie moleculară. În acest scop s-a folosit ca secventă de interes toxina RTX, genomul bacterian nefiind secvențiat până în prezent. Scopul studiului a fost dezvoltarea unei metode rapide, sensibile şi specifice utilă pentru a stabili diagnosticul etiologic al acestor infecții. Au fost aplicate două metode Real Time PCR (în sistem Taq Man şi SYBR green), ambele având ca scop indentificarea secvențelor nulceotidice rtxA. Sensibilitatea şi specificitatea metodelor a fost evaluată folosind 67 tulpini bacteriene (31 au fost *Kingella kingae).* Apoi, 52 probe pozitive şi/sau negative în cultură şi/sau PCR au fost testate folosind aceste două tehnici. Ulterior s-a folosit tehnica PCR "în cuib" şi secvențierea tulpinilor izolate pentru a confirma prezența *Kingella kingae* în probele luate în lucru. Rezultatele obținute au demonstrat acuratețea metodelor mai sus prezentate în stabilirea diagnosticului etiologic (Lehours P, Freydière A, Richer O et al, The rtxA Toxin Gene of Kingella kingae: a Pertinent Target for Molecular Diagnosis of Osteoarticular Infections. *J Clin Microbiol* 2011; 49: 1245-1250).

Teodora Vremera

ACTIVE DETECTION OF FRAILTY IN THE ROMANIAN ELDERLY POPULATION - THE GFI QUESTIONNAIRE METHOD

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ACTIVE DETECTION OF FRAILTY IN THE ROMANIAN ELDERLY POPULATION - THE GFI QUESTIONNAIRE METHOD (Abstract): The notion of frailty has gradually gained hold in medical discourse through the identification of an elderly population at risk of death or of events affecting their autonomy. This state of vulnerability is a central feature of old age, a new concept in medicine, leading to decreased quality of life, dependency and death. **Aim:** To identify frailty score and factors involved in elderly patients through the Romanian version of the Groningen fragility indicator (GFI) questionnaire among the independently living elderly in Romania. **Material and methods:** Observational study which included 100 persons aged 65 to 74 years, randomly selected from primary care practices in Romania in the interval March-April 2017. **Results**: A moderate to severe degree of frailty was identified in the study cases (mean score 4.08). The mean frailty score was significantly higher at the age of 70 years old compared to age group 60-69 (4.58 *vs*. 3.58, p = 0.036). The study showed that frailty score was increased by the following comorbidities: stroke, COPD, chronic urinary infection, cataracts. **Conclusions**: Frailty score can be easily determined in primary care practices, leading to an early identification of the elderly requiring geriatric assessment and treatment. **Keywords:** FRAILTY DETECTION, STATE OF VULNERABILITY, ELDERLY POPULATION.

Frailty syndrome is reported to affect 7% of the elderly patients aged over 65 years, and 25–40% of those aged 80 years old or over. The borders between age and frailty appear to be so indistinct that it is widely supposed that at a specific age, all people become frail (1). Because frailty is strongly related to adverse outcomes, it is important to identify the persons at risk for frailty and to detect it in an early stage.

The Romanian population is one of the fastest aging populations in Europe, whereas the total population is simultaneously decreasing rapidly. During the last decade, health policy has been directed towards strengthening the role of primary health care, to reduce (unnecessary) hospital admissions and to encourage preventive screening programs (2).

The Romanian elderly population has been confronted with dramatic changes in social security (pension cuts and limited free access to health care services) as well as in the quality of health care (waiting lists, lack of personnel and facilities, co-payments). The need for pro-active health

programs such as screening for frailty may be evident (3).

Several studies have proposed instruments to measure frailty. Some are complex, others simple. One of these studies compared the feasibility, reliability and validity of frailty assessment instruments, i.e. the Groningen Frailty Indicator (GFI), the Tilburg Frailty Indicator and the Sherbrook Postal Questionnaire (4, 5). Based on this comparison, the GFI has demonstrated the highest internal consistency and construct validity (5, 6, 7).

The GFI questionnaire can be used to assess frailty in the non-institutionalized Romanian elderly since 2014.

MATERIAL AND METHODS

This observational study was conducted in a series of 100 patients aged 65-74 years old who presented to their primary care physician between March 1st to April 30th, 2017. Inclusion criteria were: age over 65 years, free consent to complete the questionnaire.

The Romanian version of GFI questionnaire was used to determine the overall fragility status using 3 areas: Daily Activity, Health Problems and Psychological Functions. It is also 15 items screening instrument.

The main objective was to identify frailty score in patients in the target group and the secondary objective was considered a personalized approach to elderly patients: risk assessment, prevention and early identification of factors involved in the occurrence of frailty in the elderly: biological, psychological/cognitive, social/familial.

The standard score scale ranges from 0 to 15, a small score signifying robustness, and a high score a state of vulnerability.

The obtained data were statistically analyzed using *SPSS 18* software.

RESULTS

Descriptive indicators revealed a higher number of women (77 patients) versus men (23 patients), M/F sex ratio 1:3. Data on patient age did not reveal significant gender differences in the mean age (69 years; p=0.758). In terms of civil status: 60% married *vs.* 39% divorced/widowed and 1% unmarried.

A score of 4 or more is considered an indicator of moderate to severe frailty.

TABLE I

Descriptive score indicators for frailty depending on the socio-demographic characteristics

Characteristics	N	Average	Standard deviation	Standard error	Confidence interval 95%		Min	Max	Test t-Stud. p
					-95%CI	+95%CI			
Number of patients	100	4,08	2,40	0,240	3,60	4,56	0	11	-
Gender									0,934
Male	23	4,04	3,30	0,687	2,62	5,47	0	11	
Female	77	4,09	2,09	0,238	3,62	4,56	1	11	
Age groups (years)									
60-69	50	3,58	2,46	0,348	2,88	4,28	0	10	**0,036**
70-79	50	4,58	2,25	0,318	3,94	5,22	1	11	
Civil Status									0,660
Married	60	4,17	2,68	0,345	3,48	4,86	0	11	
Unmarried	40	3,95	1,93	0,306	3,33	4,57	1	8	

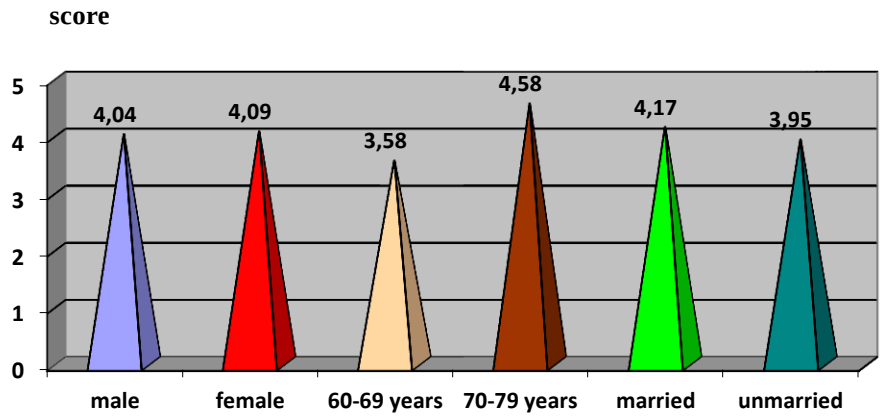

Fig. 1. Average frailty score depending on socio-demographic characteristics

The average score (4.08 ± 2.40) of the study patients revealed moderate to severe frailty. There were no significant gender differences in mean frailty scores (M=4.04 *vs.* F=4.09, p = 0.934).

The mean frailty score was significantly higher in those over 70 years compared to age group 60-69 (4.58 *vs.* 3.58, p = 0.036).

The average frailty score was slightly lower in unmarried, divorced or widowed compared to married patients (4.17 *vs.* 3.95, p = 0.660) without statistical significance (tab. I, fig.1).

The presence of the following comorbidities significantly increased the frailty score: stroke (p=0.036), Parkinson's disease (p=0.019), COPD (p= 0.015), chronic urinary infection (p=0.042), cataracts (p=0.025), lumbar disc disease (p= 0.021).

DISCUSSION

Frailty of the elderly is a complex concept with multifactorial determinations: age, disease/comorbidities, marital status, family and social context. The frailty score increases with age, determining it with the GFI questionnaire being also a good tool for detecting frailty in the general population (8).

In our study the percentage of subjects identified as frail with the help of the GFI questionnaire was high, with a mean and median frailty score of 4.04/4.09 among the old Romanians (*n* = 100; mean age 69 years).

Peters *et al.* (7) reported a median score of 3 for independent-living old persons (*n* = 168) and a score of 6 for persons living retirement or nursing homes. Also, Metzelhin *et al.* (4) and Drubbel *et al.* (6) found a mean score of 3.8 (*n* = 532; mean age 77 years) and 3.2 (*n* = 623; mean age 73 years), respectively, among independent-living old persons. Our findings indicate that independent-living old Romanians are frailer.

The frailty score increases in direct proportion to the deterioration in health. Wong *et al.* (9) recently reported that among community dwelling seniors who are frail 82% have comorbidities. Findings suggest that frailty may be caused by mechanisms that are shared with chronic diseases once the diseases have reached a severe or advanced stage.

These findings agree with our study re-

sults showing that comorbidities like stroke, Parkinson's disease, COPD, chronic urinary tract infection, cataracts, and lumbar disc disease significantly increase the frailty score.

CONCLUSIONS

We conclude that the predictive value of GFI in primary health care is limited, which is in line with other findings, but identifying the frailty status through the questionnaire among elderly in primary care requires complex outpatient geriatric assessment.

The frailty score can be calculated easily in primary care practices, leading to an early identification of frailty status. The benefits of this determination are: prevents medical-social events: comorbidities, addiction, immobilization; early identification of early elderly people requiring geriatric evaluation and/or treatment; personalized and targeted (biopsychosocial) intervention that improves the quality of life.

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WILSON'S DISEASE: A CHALLENGING DIAGNOSIS. CLINICAL MANIFESTATIONS AND DIAGNOSTIC PROCEDURES IN 12 PATIENTS

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WILSON'S DISEASE: A CHALLENGING DIAGNOSIS. CLINICAL MANIFESTATIONS AND DIAGNOSTIC PROCEDURES IN 12 PATIENTS (Abstract): Wilson disease is a neurodegenerative disease of copper metabolism. The genetic defect, localized to chromosome arm 13q, has been shown to affect the copper-transporting adenosine triphosphatase (ATPase) gene (*ATP7B*) in the liver. Our aim was to study the clinical and laboratory characteristics of 12 children and young adults diagnosed with WD and point out the diagnostic difficulties. **Material and method**: We retrospectively analyzed the patients diagnosed with Wilson's disease between 2001 and 2009 diagnosed in Constanţa County Emergency Hospital. Evaluation included detailed physical examination, conventional laboratory testing, genetic analysis, and liver biopsy. **Results**: Patients with hepatic symptoms showed a considerably earlier onset of symptoms and a shorter diagnostic delay before definitive diagnosis than those with neuropsychiatrical symptoms. The mean age at diagnosis was 9.12 +/- 2.59 years (range 5 years-20 years). 10 patients were symptomatic, 6 were referred because of abnormal liver function test results and/or hepatomegaly, 4 had neuropsychiatrical symptoms and 2 received their diagnoses after family screening. Hepatic copper concentration was between 250 and 1200 micrograms/g. 4 patients had liver cirrhosis, five chronic hepatitis and one had massive hepatic necrosis on necropsy. Any person with recurrent hepatic disease and unexplained neurological symptoms should be investigated to have Wilson's disease. **Conclusions**: Detection of WD in children and young adults remains very difficult. The most important investigation is liver biopsy with the assessment of liver copper. Genetic analysis may help in doubtful cases. **Key words**: WILSON'S DISEASE, DIAGNOSTIC PROCEDURES, CLINICAL MANIFESTATIONS

Wilson disease, or hepatolenticular degeneration, is a neurodegenerative disease of copper metabolism (1). In 1912, Wilson first described it as a familial disorder associated with neurological symptoms and cirrhosis. In 1956, Walshe first treated patients with the chelating agent penicillamine (2, 3).

The genetic defect, localized to chromosome arm 13q, has been shown to affect the copper-transporting adenosine triphosphatase (ATPase) gene (*ATP7B*) in the liver[3]. Patients with Wilson disease usually present with liver disease during the first decade of life or with neuropsychiatric illness during the third decade[1]. The diagnosis is confirmed by measurement of serum ceruloplasmin, urinary copper excretion, and hepatic copper content, as well as the detection of Kayser-Fleischer rings (1, 2).

Wilson's disease may exhibit a variety of clinical symptoms, the most common being liver disease and neuropsychiatric disturbances (4, 5, 6). Wilson's disease typically begins with a presymptomatic period, during which copper accumulation in the liver causes subclinical hepatitis, and progresses to liver cirrhosis and development of neuropsychiatrical symptoms. The type of hepatic and

neurological symptoms can be highly variable. Wilson's disease may also present as fulminant hepatic failure with an associated Coombs-negative haemolytic anaemia and acute renal failure.

OBJECTIVES

Wilson disease (WD) has a wide spectrum of clinical manifestations. Affected children may be entirely asymptomatic making the diagnosis very difficult. Our aim was to study the clinical and laboratory characteristics of 12 children and young adults diagnosed with WD and point out the diagnostic difficulties in this population.

MATERIAL AND METHOD

Because the disease affects mostly young patients, the median age of our patients was between 8 and 20 years. We retrospectively analyzed the patients diagnosed with Wilson's disease between 2001 and 2009 diagnosed in Constanta County Emergency Hospital. Clinical and laboratory data were collected from 12 consecutive children and young adults with WD. Evaluation included detailed physical examination, conventional laboratory testing, genetic analysis, and liver biopsy.

Clinical manifestations

We found 12 patients, in whom the diagnosis of Wilson's disease was challenging. In this case series, there were 6 patients with recurrent hepatic disease and 4 patients with hepatic disease and unexplained neurological symptoms Liver disease occurred in more than a half of the patients.

Liver disease

Wilson disease manifested as liver disease in children and adolescents, peaking at ages 10-13 years, and as neuropsychiatrical illness in young adults aged 19-20 years.

Neurological and psychiatrically symptoms

We identified 4 adult patients with neurological and psychiatric symptoms at the beginning. Frequent early symptoms included : difficulty speaking, excessive salivation, ataxia, masklike facies, clumsiness with the hands, and personality changes.

The most common presenting neurological feature was asymmetric tremor, occurring in approximately half of individuals with Wilson disease. The character of the tremor is variable and may be predominantly resting, postural, or kinetic.

In our case series, most adult patients who presented with neuropsychiatrical manifestations have also cirrhosis.

Late manifestations (now rare because of earlier diagnosis and treatment) included : dystonia, spasticity, grand mal seizures, rigidity, and flexion contractures, encountered in more than a half adult patients.

We describe four distinct diagnostic categories based on patients' major neurological findings : parkinsonian, pseudosclerotic, dystonic and choreoathetotic.

Ophtalmological examination

Kayser-Fleischer rings are formed by the deposition of copper in the Descemet membrane in the limbus of the cornea. The color ranged from greenish gold to brown ; when well developed, rings was readily visible to the naked eye or with an ophthalmoscope set at +40. When not visible to the unaided eye, the rings were identified using slit-lamp examination or gonioscopy.

Kayser-Fleischer rings were observed in up to 90% of adults with symptomatic Wilson disease with neurological manifestations. In children with neurological Wilson's disease the Kayser-Fleisher ring was present in only 60% of cases.

Musculo-skeletal manifestations

Musculo-skeletal involvement is a common feature of Wilson disease. More than a half of our patients exhibited osteopenia on conventional radiological examination.

The arthropathy of Wilson disease is a degenerative process that resembles premature osteoarthritis. Symptomatic joint disease occurred at 3 patients, usually late in

the course of the disease, frequently after age 25 years. The arthropathy generally involved the spine and large appendicular joints, such as knees, wrists, and hips.

Hematological findings

Hemolytic anemia was a recognized complication of the disease at one patient.

Renal disease

The frequency of renal manifestations was variable. Clinically, some patients resembled those with Fanconi syndrome, demonstrating defective renal acidification and excess renal losses of amino acids, glucose, fructose, galactose, pentose, uric acid, phosphate, and calcium. In one patient who presented with Fanconi Syndrome-like manifestations, the dignosis was delayed for five years, until the ataxia became apparent. Urolithiasis, found in one patient with Wilson disease, may be the result of hypercalciuria or poor acidification.

RESULTS

The mean age at diagnosis was 9.12 +/- 2.59 years (range 5 years-20 years). 10 patients were symptomatic, 6 were referred because of abnormal liver function test results and/or hepatomegaly, 4 had neuropsychiatrical symptoms and 2 received their diagnoses after family screening.

All patients presented with hepatic disease. 4 patients also had neurological involvement. Hepatic copper concentration was between 250 and 1200 microg/g. 4 patients had liver cirrhosis, 5 chronic hepatitis, and 1 had massive hepatic necrosis on necropsy.

5 patients had both Kayser-Fleischer ring and decreased serum ceruloplasmin levels, 6 had urinary copper excretion after penicillamine challenge >1600 microg/24 hours, and 2 had liver copper content >250 microg/g dry weight. Of the remaining 6 patients, 5 had both low serum ceruloplasmin $<$ or $=20$ mg/dL and increased urinary copper excretion, >75 microg/24 hours before, or $>$ 1000 microg/24 hours after penicillamine challenge. In 1 patients the diagnosis was confirmed by genetic analysis.

Hepatic involvement manifested in four ways : acute hepatitis, chronic active hepative hepatitis, cirrhosis and fulminant hepatitis. Recurrent episodes of liver disease ocurred in a variable interval (months to years), until neurological symptoms developed. At one patient, the fulminant liver injury leads to sudden release of copper in the bloodstream and hemolytic anemia with negative Coomb's test. This was a life threatening condition and conducted to death. In 1 patient the diagnosis of Wilson's disease was established because they were brothers and sisters of known patients.

The most common presenting neurological feature was asymmetric tremor, occurring in approximately half of individuals with Wilson disease. The character of the tremor is variable and may be predominantly resting, postural, or kinetic.

The patient in the parkinsonian group was distinguished by paucity of expression and movement.

The patient in the pseudosclerotic group had tremor resembling multiple sclerosis.

The patients in the dystonic group were characterized by hypertonicity associated with abnormal limb movements.

The patients in the choreic group were predominantly characterized by choreoathetoid abnormal movements associated with dystonia.

The majority of patients diagnosed with neurological Wilson's disease presented also with psychiatric features : emotional lability, impulsiveness, disinhibition, and self-injurious behavior.

Although Kayser-Fleischer rings are a useful diagnostic sign, they are no longer considered pathognomonic of Wilson disease unless accompanied by neurological manifestations. They may also be observed in patients with chronic cholestatic disorders, such as partial biliary atresia, primary bil-

iary cirrhosis, primary sclerosing cholangitis, and cryptogenic cirrhosis.

Approximately 90% of all patients with Wilson disease have ceruloplasmin levels of less than 20 mg/dL (reference range, 20-40 mg/dL).

Ceruloplasmin levels was also decreased in 10-20% of *WD* gene heterozygotes, who do not develop Wilson disease and do not require treatment

The urinary copper excretion rate was greater than 100 mg/d (reference range, <40 mg/d) in most patients with symptomatic Wilson's disease.

Hepatic copper concentration

A liver biopsy with sufficient tissue revealed levels of more than 250 mcg/g of dry weight was performed in 2 patients. Special collection vials were used to help avoid contamination.

Genetic diagnosis : Linkage analysis has been used in 3 patients and to screening of family members for an identified mutation detected in the index patient. Using this method we identified 2 asymptomatic siblings.

Imaging Studies

Computed tomography (CT) scan revealed hypodense regions in the basal ganglia (caudate nucleus, putamen, globus pallidus) in all adults patients diagnosed with neurological Wilson's disease. The cranial lesions observed on CT scan were typically bilateral and were classified into two general categories, as it follows :

(1) well-defined, slitlike, low-attenuation foci involving the basal ganglia particularly the putamen

(2) larger regions of low attenuation in the basal ganglia, thalamus, or dentate nucleus

Brain MRI appears to be more sensitive than CT scanning in detecting early lesions of Wilson disease. MRI studies have identified focal abnormalities in the white matter, pons, and deep cerebellar nuclei in 4 patients These lesions, measuring 3-15 mm in diameter, were typically bilateral, appearing with low signal intensity on T1-weighted images and with high signal intensity on T2-weighted images, representing cell loss and gliosis.

Hepatic biopsy

The earliest changes detectable with light microscopy included glycogen deposition in the nuclei of periportal hepatocytes and moderate fatty infiltration. The lipid droplets, which are composed of triglycerides, resembling the steatosis induced by ethanol, were also described in 9 patients who underwent this procedure.

No correlation was found between specific mutations and the disease phenotypic expression. Chelating therapy was well tolerated, and the outcome was satisfactory.

DISCUSSIONS

The presence of Kayser-Fleischer rings and ceruloplasmin levels of less than 20 mg/dL in a patient with neurologic signs or symptoms suggest the diagnosis of Wilson's disease. If a patient is asymptomatic, exhibits isolated liver disease, and lacks corneal rings, the coexistence of a hepatic copper concentration of more than 250 mg/g of dry weight and a low serum ceruloplasmin level is sufficient to establish a diagnosis.

Both the sensitivity and the specificity of urinary copper are suboptimal for use as a screening test ; however, it may be useful to confirm the diagnosis and to evaluate the response to chelating therapy.

One aspect of our study was to discriminate between the age at symptom onset and at diagnosis. Patients with hepatic symptoms showed a considerably earlier onset of symptoms and a shorter diagnostic delay before definitive diagnosis than those with neuropsychiatrical symptoms. Family screening was effective in diagnosing patients with Wilson's disease at an early, and most often asymptomatic or pre-symptomatic, stage of

disease. By family screening, patients were diagnosed an average of 4 years earlier, with an excellent long-term outcome.

The wide spectrum of symptoms and of age at onset in patients with Wilson's disease raised the question whether these features are determined by the type of the ATP7B mutation. The ATP7B mutational analysis showed no clear genotype–phenotype correlations 5.6. However, previously published studies on genotype–phenotype correlations have shown variable results. A recent meta-analysis showed an association of the ATP7B H1069Q mutation with a late neurological presentation in Wilson's disease (7) although the underlying studies are quite (8, 9, 10). Most reports on mutations other than H1069Q show no clear-cut phenotype–genotype correlations (12, 13, 14).

A substantial proportion of the patients developed side effects. This was especially true in D-penicillamine treatment, where severe side effects were recorded in more 30% of all treated patients. In the light of our data, and especially considering that effective alternative drugs available, the role of D-penicillamine as a standard treatment for Wilson's disease must be questioned (15, 16, 17, 18). In our study cohort, development and worsening of neurological symptoms were reported while under treatment with all three drugs (penicillamine, Trientine and Zinc) but most frequently (13.8%) for D-penicillamine. This may be partly as a result of the longer summarized follow-up period for D-penicillamine. Nevertheless, in our opinion, D-penicillamine should not be the drug of choice for patients with neurological symptoms.

CONCLUSIONS

Any person with recurrent hepatic disease and unexplained neurological symptoms should be investigated to have Wilson's disease. Liver disease occurs in half of the patients. It may be manifested in four ways : acute hepatitis, chronic active hepatitis, cirrhosis and fulminant hepatitis. Recurrent episodes of liver disease may occur in a variable interval (months to years) until neurological symptoms develop. Ultimately, all patients develop cirrhosis. The fulminant liver injury may lead to sudden release of copper in the bloodstream and hemolytic anemia with negative Coomb's test. This may be a life threatening condition if not promptly corrected.

The best way to monitor efficacy is to measure serum nonceruloplasmin-bound copper. This is measured by the following formula :

Total serum copper (mcg/dL) - 3[ceruloplasmin (mg/dL)].

The reference range is <15 mcg/dL.

An adjunctive way to monitor efficacy is to measure urinary copper excretion. Urinary chelator levels usually measure 200-500 mcg/day. Urinary zinc levels usually measure <75 mcg/day.

Detection of WD in children and young adults remains very difficult. The most important investigation is liver biopsy with the assessment of liver copper. Genetic analysis may help in doubtful cases.

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LINKING THEORY TO PRACTICE: UNDERUSE OF CHRONIC ORAL ANTICOAGULATION IN ATRIAL FIBRILLATION

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LINKING THEORY TO PRACTICE: UNDERUSE OF CHRONIC ORAL ANTI-COAGULATION IN ATRIAL FIBRILLATION (Abstract): The effectiveness of chronic oral anticoagulation in preventing thromboembolic events related to atrial fibrillation has already been proven. Nevertheless, a suboptimal use of oral anticoagulants is documented in real-world practice. **Aim:** To characterize individuals diagnosed with atrial fibrillation in need of chronic oral anticoagulation for thromboembolic prophylactic purposes, focusing on primary reasons of inadequate anticoagulation (lack of recommendation from the attending physician, inappropriate dosing and/or presence of contraindications). **Material and methods:** Patients presenting atrial fibrillation on admission or having been previously diagnosed with at least one episode of atrial fibrillation were included. Parameters like the type of atrial fibrillation (newly diagnosed, paroxysmal, persistent, permanent), associated thromboembolic and bleeding risk factors, as well as the long-term use of oral anticoagulants were assessed. **Results:** The study group comprised 90 subjects (mean age 75.83 ± 8.75 years, 40% men). All patients associated high thromboembolic risk according to CHA_2DS_2-VASC score. In one third of the patients no objective reason justifying the lack of anticoagulation was found. A reduced glomerular filtration rate (<60 mL/min/1.73 m^2) was noted in 34.4% of the study group. **Conclusions:** Our study confirmed the underuse of oral anticoagulants in patients with atrial fibrillation. If pathological conditions predisposing to high bleeding risk were excluded, the socioeconomic status represented the main argument when establishing the need for oral anticoagulation. Additional strategies to promote public awareness are needed in patients with atrial fibrillation requiring antithrombotic treatment. **Keywords:** ATRIAL FIBRILLATION, ORAL ANTI-COAGULATION, THROMBOEMBOLIC RISK, BLEEDING RISK.

The cardioembolic risk associated with atrial fibrillation represents a major healthcare concern. It has been estimated that among these patients the rate of developing thromboembolic events (ischemic stroke/transient ischemic attack/arterial embolism) ranges from 1 to 30% according to associated risk factors (1, 2). If the risk of stroke in patients with atrial fibrillation is estimated at 2.8% in persons aged 60 to 69 years, the risk increases to 23.5% in persons aged 80 to 89 years (3). Less clinically overt in short-term outcome, but equally disabling, conditions like cognitive

impairment and vascular dementia are more frequent associated with atrial fibrillation, having as a possible mechanism the formation of micro emboli and transient cerebral hypoperfusion (4).

As for any other prophylactic treatment, difficulties are encountered regarding patient's acceptance of a treatment not free of serious complications. The fear of bleeding complications associated with anticoagulants derives from the possible altered pharmacokinetics due to drug/food/alcohol interactions, low body mass index and altered body distribution of adipose tissue, polypragmasy, renal impairment, cognitive decline and balance disorders, which may increase the risk of falling (3, 5).

Despite a major worldwide interest in enhancing adherence to and acceptance of long-term oral anticoagulation in high-risk thromboembolic conditions, such as atrial fibrillation, current data sustain an underuse of chronic oral anticoagulation in up to 40-50% of the patients considered suitable candidates for anticoagulation (6, 7). Moreover, maintenance of anti-coagulants seems to raise as much difficulties as the decision to initiate the treatment. For instance, a therapy discontinuation rate of 30% was reported during the first year of treatment with vitamin K antagonists (VKAs), increasing to a rate up to 50% within the next two years (3, 8, 9).

MATERIAL AND METHODS

The study group is part of a larger group of patients evaluated during a four-year PhD research. Although the primary objective of the investigation was to assess the impact of several cardiac biomarkers in various types of heart failure, the study design and results being described in detail elsewhere (10), concomitant data revealed the underuse of oral anticoagulants in patients with atrial fibrillation. Hence, we conducted a subgroup analysis of a prospective observational study involving patients with atrial fibrillation aiming to perform a descriptive analysis of the reasons explaining the decreased rate of oral anticoagulation in clinical practice.

The study group included patients presenting to the 2nd Internal Medicine Clinic of the Iasi "Sf. Spiridon" County Clinical Emergency Hospital, diagnosed with atrial fibrillation on admission or who had been previously diagnosed with at least one episode of atrial fibrillation. The patients with valvular atrial fibrillation (prosthetic heart valves) were excluded from the study, due to differences in the management of anticoagulation therapy (higher VKAs doses are required corresponding to a higher bleeding risk; discontinuation of chronic anticoagulation involves an increased risk of mortality due to prosthetic valve thrombosis) (11).

According to the 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS, atrial fibrillation was defined as: newly diagnosed (first diagnosed, irrespective of the duration of the arrhythmia), paroxysmal (self-terminated, in most cases within 48 hours, but some episodes may continue up to 7 days), persistent (lasts longer than 7 days) and permanent (accepted by the patient and physician, no rhythm control interventions required) (12).

The eligibility for anticoagulation was established according to the presence of high thromboembolic risk factors, assessed by the CHA_2DS_2-VASC score. Age $\geq$ 75 years and prior embolic events (stroke, transient ischemic attack, or thromboembolism) were assigned 2 points each, and

factors like congestive heart failure, hypertension, diabetes, vascular disease, age 65-74 years, and sex (female) were assigned 1 point each. A CHA_2DS_2-VASC score of 1 or more for men and 2 or more for women indicates the necessity to anticoagulated the patient (12).

Long-term use of chronic oral anticoagulants, dosage and type of anticoagulants were recorded (vitamin K antagonist – acenocoumarol or direct-acting oral anticoagulants (DOACs) – apixaban, dabigatran, rivaroxaban). For the group of patients treated with acenocoumarol, INR (International Normalized Ratio) level was determined in serum samples in order to evaluate the effectiveness of the treatment with vitamin K antagonists. The therapeutic INR range was considered 2.0-3.0.

The bleeding risk was assessed using the HAS BLED score, which includes parameters like associated hypertension, abnormal renal/liver function (1 point each), stroke, bleeding history or predisposition, labile INR, elderly people (≥ 65 years), drugs/alcohol concomitantly (1 point each). A HAS BLED score ≥ 3 indicates an increased risk of developing the hemorrhagic complications of anticoagulant treatment (12).

The study design was approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy Iasi and the Ethics Committee of the "Sf. Spiridon" County Clinical Emergency Hospital. from Iasi. All participants were informed about the study protocol and signed the informed consent.

Data were expressed as means ± standard deviations (SD) or medians and interquartile ranges (IQR), whichever was appropriate.

RESULTS

Our study included 90 patients. The mean age was 75.83 ± 8.75 years (IQR, 51-92 years), and there was a female predominance (60% versus 40% male patients). Most of the patients (53.33%) were in the 60-79 years age range. The mean glomerular filtration rate was 88.5 ± 38.89 ml/min/1.73m^2.

The distribution of patients according to the type of atrial fibrillation revealed the preponderance of permanent atrial fibrillation subgroup (fig. 1).

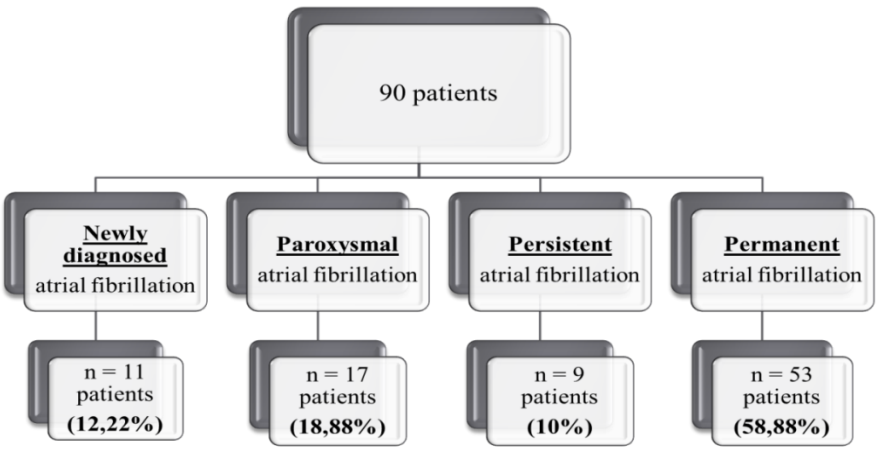

Fig. 1. Distribution of patients according to the type of atrial fibrillation

All patients associated high thrombo-embolic risk according to CHA_2DS_2-VASC score. The median CHA_2DS_2-VASC score was 5 (IQR 2-8) and the median HAS BLED score was 2 (IQR 1-6). A low prevalence of anticoagulant use was found, even in the presence of multiple embolic risk factors (fig. 2).

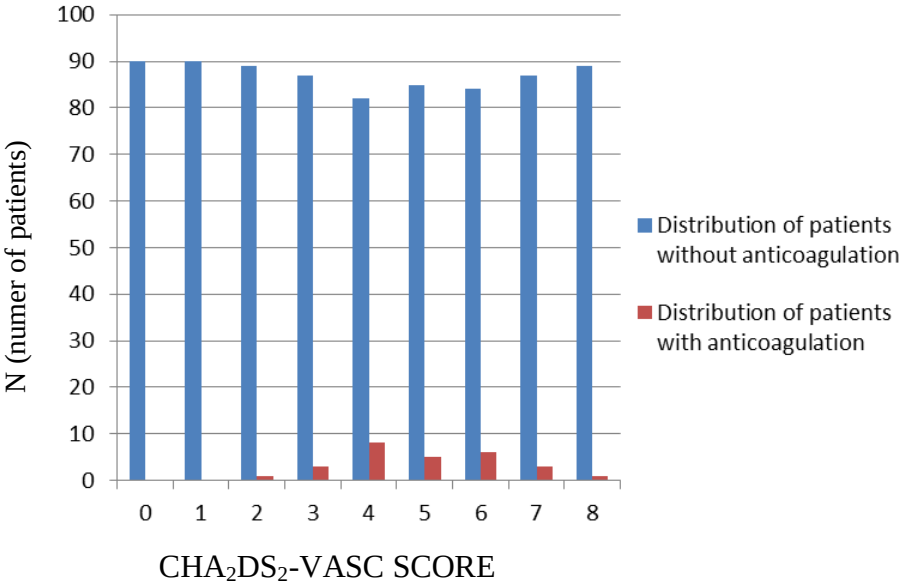

Fig. 2. Distribution of patients receiving chronic oral anticoagulation according to the CHA_2DS_2-VASC score (at admission)

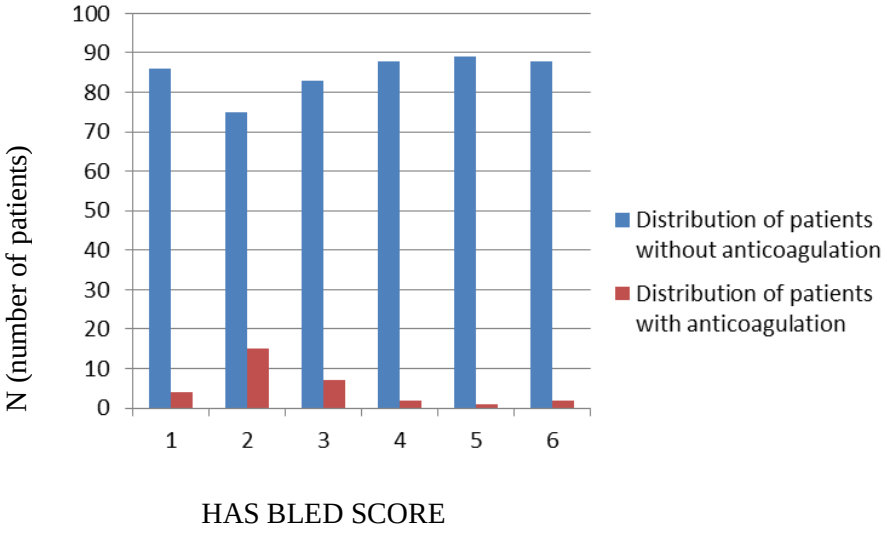

Fig. 3. Distribution of patients receiving chronic oral anticoagulation according to the HAS BLED score (at admission)

We identified 32.21% of the patients who were receiving anticoagulation treatment on admission, principally with acenocoumarol (fig. 3). The most relevant bleeding risk factors included age over 80 years (41.12%), alcohol abuse (22.22%), single or dual antiplatelet therapy (16.66%) or acenocoumarol overdose (6.66%). A reduced glomerular filtration rate (< 60 mL/min/1.73m^2) was recorded in 34.4% of the study patients.

Only 12.21% of the total number of patients (3.33% – therapeutic INR; 8.88% – DOACs) was properly anticoagulated. Nearly all patients treated with VKAs (acenocoumarol) had an INR level out of the therapeutic range, with the distribution of values shifted towards lower INR. No objective reason justifying the lack of anticoagulation was found in one third of the patients (35.56%) (tab. I).

TABLE I

Antithrombotic management from admission to discharge

ATRIAL FIBRILLATION						
	Admission %	**Comments**	**%**	**Discharge**	**Comments**	**%**
No treatment	51.11	First diagnosis	12.22	7.77	Social conditions	2.22
		Previous hemorrhage	3.33		Hemorrhage	1.11
					Death	4.44
VKAs - Acenocoumarol	23.33	Therapeutic INR	3.33	27.77	-	-
		Out of therapeutic range	20			
DOACs	8.88	-	-	47.77	-	-
Antiplatelet therapy	16.66	Single antiplatelet therapy	15.55	16.66	Associated atherosclerotic pathology	16.66
		Dual antiplatelet therapy	1.11	0	-	-

VKAs, vitamin K antagonists; DOAC, direct-acting anticoagulants;
INR, International Normalized Ratio

DISCUSSION

We present a study involving patients with atrial fibrillation, high thromboembolic risk and inadequate anticoagulation. Irrespective of numerous studies that emphasize the importance of therapeutic anticoagulation, multiple physician-related factors and patient-related factors cause interferences with guideline-recommended antithrombotic treatment (2-7).

It is well stated that the benefits of chronic oral anticoagulation in atrial fibrillation are higher precisely for the groups of patients who associate higher bleeding risks, mainly elderly people (3, 13). Thus, the decision to anticoagulate a patient often requires "a leap of faith" from the attending physician who has to predict possible complications and maximize safety of antithrombotic treatment. Balancing the risk of developing ischemic stroke against the bleeding risk requires to evaluate the CHA_2DS_2-VASC and the HAS BLED scores.

In our study, all patients associated an increased risk of embolic events according

to the CHA_2DS_2-VASC score, thus requiring a long-term administration of oral anticoagulants. A significant percentage of patients had subtherapeutic acenocoumarol anticoagulation (INR < 2).

The results of this research agree with the reported data, the majority of the patients included lacking the antithrombotic protection. Similar results, at a much larger scale, were reported by Xian Y *et al.* (14) in a study involving 94,474 patients with atrial fibrillation and acute ischemic stroke. 83.6% of those patients were not receiving therapeutic anticoagulation.

The main reasons associated with no anticoagulation were represented by an advanced age (> 80 years), increased risk of trauma and associated hemorrhages. Poor adherence to oral anticoagulants and a significant variability in INR ranges have been found to be related to chronic alcohol consumption and social conditions. Furthermore, the socio-economic status of patient had a major impact regarding financial limitations when in need to buy direct-acting anticoagulants. The difficult access to health services, clinical laboratory or general practitioner contributed to a suboptimal INR surveillance.

After proper identification and correction, when possible, of factors that reduced compliance, the rate of oral anticoagulation at discharge increased to 75.54% (VKAs – 27.77%; DOACs – 47.77%) (tab. I).

Our study has limitations which have to be pointed out. As stated previously, we conducted a subgroup analysis of a larger research that, according to the initial study design, included patients with heart failure, irrespective of the presence or absence of atrial fibrillation. Thus, only a limited number of patients with atrial fibrillation were suitable for the current study. Further research is needed to proper analyze larger groups of patients and to consider the results representative for the North-East Region of Romania.

CONCLUSIONS

Our results confirm the underuse of oral anticoagulants in patients with atrial fibrillation. If pathological conditions predisposing to high bleeding risk are excluded, the socioeconomic criteria represent the main argument when establishing the need for oral anticoagulation. Additional strategies to promote public awareness are necessary in patients with atrial fibrillation requiring antithrombotic treatment. Identifying potentially modifiable bleeding risk factors is of major interest in order to optimize the rates of oral anticoagulation for atrial fibrillation.

It is also important to underline in medical practice the no utility place for antiplatelets in atrial fibrillation patients, adding no benefit but higher hemorrhagic risk. Further dissemination of ESC and EHRA guidelines among practitioners is mandatory.

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NEWS

SHOULD WE USE DENTAL IMPLANTS EVERY TIME?

Nowadays, dental implants have become more common treatment for replacing missing teeth and aim to improve chewing efficiency, physical health, and esthetics. Although the survival rate of dental implants over a 10-year observation has been reported to be higher than 90% in totally edentulous jaws, the clinical outcome of implant treatment is challenged in compromised (bone) conditions, as are frequently present in elderly people. The biomechanical characteristics of bone in aged patients do not offer proper stability to implants, being similar to type-IV bone (Lekholm & Zarb classification), in which a decreased clinical fixation of implants has been clearly demonstrated. However, the search for improved osseointegration has continued forward for the new evolution of modern dental implants. This represents a continuum of developments spanning more than 20 years of research on implant related-factors including surgical techniques, implant design, and surface properties **(**Hamdan S. Alghamdi. Methods to Improve Osseointegration of Dental Implants in Low Quality (Type-IV) Bone: an Overviewhttp://dx.doi.org/ 10.3390/jfb9010007).

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TRATAMENTUL RECUPERATOR AL COXITEI LA PACIENŢII CU SPONDILITĂ ANCHILOZANTĂ. OBSERVAŢII PE UN LOT DE 30 PACIENŢI INTERNAŢI ÎN CLINICA DE REUMATOLOGIE ŞI RECUPERARE MEDICALĂ IAŞI

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COXITIS REHABILITATION TREATMENT IN PATIENTS WITH ANKYLOSING SPONDYLITIS (Abstract): The study aims to assess and compare the efficiency of two different kinetic programmes, in order to improve the treatment of patients with ankylosing spondylitis (AS). **Material and method**: Our study included two samples (control and cases) of 30 patients admitted for AS and unilateral coxitis in the Clinic of Rheumatology and Medical Rehabilitation Iasi, between 2008-2009. Certain paramentres such as: age, body mass index, disease evolution, Schober test, index-earth test, inspir-expir index, BASDAI activity index, BASFI functional index, Health Assessment Questionaire (HAQ), pain, morning ankylosing inflammatory tests, and general state of health were assessed at every patient. **Results**: No variation with statistical significance were registered between the two samples. The Pilates method used in the rehabilitation treatment of patients with AS could have a favourable effect on indices regarding mobility, as well as patients' perception about their disease. **Key words**: ANKYLOSING SPONDYLITIS, PILATES METHOD, REHABILITATION

Spondilita anchilozantă este o boală inflamatorie cronică, care vizează în principal scheletul axial, dar şi articulaţiile periferice, caracteristica majoră fiind afectarea precoce a articulaţiilor sacroiliace. Face parte din grupul spondilartropatiilor seronegative, ca entitate reprezentativă a spondilartritelor axiale. Prevalenţa spondilartritelor în populaţia generală a fost raportată a atinge 1,9% (1 2,3,4).

Fiind o boală cu caracter evolutiv şi cu un înalt grad de invaliditate, momentul începerii kinetoterapiei trebuie să fie cât mai precoce (5,6,7). Principiul de bază este încercarea de a preveni sau limita anchilozele şi devierea coloanei şi/sau a articulaţiilor centurilor, predominând programul de kinetoprofilaxie primară şi secundară şi, în mai mică măsură, cel kineto-terapeutic sau de recuperare (8,9,10).

Studiul de faţă îşi propune a evalua şi compara eficienţa a două programe kinetice diferite, în scopul eficientizării şi modernizării tratamentului kinetic la pacienţii cu spondilită anchilozantă.

MATERIAL ŞI METODĂ

În studiu au fost incluşi 30 pacienţi cu diagnosticul de spondilită anchilozantă şi coxită unilaterală, internaţi în Clinica de Reumatologie şi Recuperare Medicală din cadrul Spitalului de Recuperare Iaşi, în perioada 2008-2009.

Urmărind a evalua beneficiile unui nou

program de kinetoterapie, am exclus din studiu pacienţii în stadii avansate de boală, pe cei cu alte afecţiuni articulare, cu patologii care contraindicau efortul fizic susţinut, cu obezitate, pe cei care aveau deja un program kinetic la domiciliu, precum şi pe cei aflaţi sub terapie biologică. Ne-am referit doar la pacienţii în stadii incipiente, când recuperarea poate fi eficientă în a preveni degradarea staticii şi dinamicii.

Cu ajutorul unei fişe de anchetă clinico-epidemiologică, special concepută, au fost urmăriţi, pentru fiecare pacient în parte, principalii parametri clinici şi paraclinici, la internare şi la 10 zile. Pacienţii au fost împărţiţi în două loturi egale numeric şi randomizaţi, astfel încât loturile să fie asemănătoare.

REZULTATE ŞI DISCUŢII

Parametrii luaţi în studiu şi evaluaţi la fiecare pacient au fost: vârsta, indicele de masă corporală (IMC), durata de evoluţie a bolii, rezultatele testului Schober, indicele index-sol, indicele inspir-expir, indicele de activitate a bolii BASDAI, indicele funcţional BASFI, indicatorul de calitate a vieţii HAQ, redoarea matinală, oboseala, durerea, testele de inflamaţie şi starea generală de sănătate evaluată de către medic şi de către pacient. Nu au fost înregistrate variaţii cu semnificaţie statistică între cele două loturi (tab. I).

Pentru evaluarea articulaţiei coxofemurale afectate, a fost luată în consideraţie toată gama de mişcări, notându-se valorile goniometrice pentru fiecare tip în parte (tab. II).

Pentru toţi pacienţii internaţi, tratamentul aplicat a respectat principiile terapeutice clasice: programul educaţional şi igieno-dietetic, tratamentul medicamentos, fizic, masaj şi hidroterapie.

Cele două loturi au practicat exerciţiile clasice în aceeaşi măsură, câte 30 minute/zi. Pacienţii din lotul cu *Pilates* au mai efectuat un program de 30 minute la distanţă de 5 ore de primul, care a conţinut exerciţii pentru ameliorarea posturii, tonifierea musculaturii, creşterea amplitudinii de mişcare şi ameliorarea respiraţiei.

TABELUL I

Parametrii studiaţi pentru cele două loturi de pacienţi cu spondilită anchilozantă

Parametrii studiaţi	Lot martor	Lot Pilates	p
Vârstă (ani)	35,67±3,12	34,42±2,14	NS
IMC (kg/m^2)	24±1,2	23±1,7	NS
Durata bolii (ani)	5,2±2,4	5,7±1,9	NS
Testul Schober (cm)	6,5±1,2	5,8±1,3	NS
Index-sol (cm)	15,3±3,5	13,7±2,8	NS
Expansiune toracică (cm)	6,7±1,8	5,9±2,3	NS
BASDAI	4,5±1,2	3,9±0,3	NS
BASFI	5,3±0,6	5,5±0,2	NS
HAQ	2,3±0,2	2,4±0,2	NS
Redoarea matinală (min)	32,3±3,2	35,2±4,6	NS
Oboseala (VAS)	35,4±9,2	37,1± 7,3	NS
Durerea (VAS)	67,5±4,3	62,8±6,9	NS
VSH (mm/h)	22,3±8,2	20,8±9,5	NS
CRP (mg-dL)	2,45±0,8	2,20±1,1	NS
GH - medic (VAS)	65,3±5,3	62,9±7,2	NS
GH – pacient (VAS)	62,14±4,4	65,6±2,4	NS

Întregul concept al *metodei Pilates* se bazează pe convingerea că, fortifiind această regiune anatomică, se optimizează capacităţile individuale de funcţionare în activităţile zilnice. Pilates denumeşte această regiune "powerhouse", ceea ce sugerează că aceasta

TABELUL II

Evaluarea diverselor tipuri de mişcări la pacienţii cu spondilită anchilozantă, incluşi în studiu

Mişcări	Lot martor	Lot Pilates	p
Flexie	35,5±1,2	34,9±2,1	NS
Extensie	10,3±2,3	11,2±1,5	NS
Abducţie	25,5±1,6	26,1±2,1	NS
Adducţie	15,3±1,7	15,9±2,3	NS
Rotaţie internă	26,7±1,3	25,9±1,4	NS
Rotaţie externă	15,5±2,4	14,8±2,6	NS

este regiunea generatoare şi o delimitează între planşeul pelvin şi cutia toracică.

Reevaluarea s-a făcut la 10 zile după începerea tratamentului. Mobilitatea axială s-a îmbunătăţit în ambele loturi, mai mult în lotul care a efectuat metoda *Pilates*, acest fapt constatându-se din reevaluarea indicelui Schober şi a indicelui index-sol, care, deşi fără semnificaţie statistică deosebită, este totuşi de notat, având în vedere caracterul anchilozant al bolii (tab. III).

În privinţa indicatorului BASDAI şi indicelui BASFI, am înregistrat de asemenea o ameliorare uşoară comparativ cu lotul martor, fapt cu o importanţă deosebită pentru prognosticul evolutiv al bolii. O îmbunătăţire a oricăruia dintre aspectele la care se referă întrebările chestionarelor mai sus menţionate se asociază cu îmbunătăţirea calităţii vieţii.

În privinţa evaluării calităţii vieţii, am utilizat chestionarul HAQ (Health Assessment Questionaire), adaptat pentru pacienţii cu spondilită anchilozantă, pentru aprecierea cât mai exactă a calităţii vieţii acestora. Scorul final al scalei variază între 0 şi 3 (tab. IV).

În privinţa mişcărilor efectuate din articulaţia coxo-femurală afectată, trebuie subliniată ameliorarea îmbucurătoare a mobilităţii, pe toate planurile de mişcare, mai crescută în lotul supus metodei Pilates (tab. V).

Indicele de calitate a vieţii HAQ a avut valori net favorabile în lotul care a aplicat metoda *Pilates*, stabilindu-se o corelaţie cu semnificaţie statistică. Rezultatele implică o complianţă bună a pacienţilor la noua metodă implementată şi impactul semnificativ asupra modului în care aceştia percep afecţiunea de care suferă. Acest lucru s-ar putea datora faptului că pacienţii care au efectuat exerciţii preluate din metoda *Pilates* au fost

TABELUL III

Rezultatele testelor la reevaluarea după 10 zile de tratament pentru pacienţii cu spondilită anchilozantă, luaţi în studiu

Teste / indici	Lot martor la internare	Lot martor la 10 zile	Lot Pilates internare	Lot Pilates la 10 zile	p
Testul Schober (cm)	6,5±1,2	7,1±0,9	5,8±1,3	6,2±0,2	NS
Index-sol (cm)	15,3±3,5	10,2±0,3	13,7±2,8	10,8±0,7	NS
Exp. toracică (cm)	6,7± 1,8	7,2±0,5	5,9±2,3	7,9±0.4	NS
BASDAI	3,8±0,2	3,3±0,2	3,9±0,1	2,1±0,2	NS
BASFI	5±0,4	4,5±0,3	4±0,2	3,1±0,3	NS
HAQ	2,3±0,2	1,2±0,1	2,4±0,2	0,5±0,3	NS

TABELUL IV

Mobilitatea mişcărilor în articulaţia coxo-femurală, după tratamentul cu metoda Pilates

Mobilitatea	Lot martor la internare	Lot martor la 10 zile	Lot Pilates la internare	Lot Pilates la 10 zile	p
Flexie	35,5±1,2	78,4±1,3	34,9±2,1	80,3±1,5	NS
Extensie	10,3±2,3	21.3±1,5	11,2±1,5	22,5±1,4	NS
Abducţie	25,5±1,6	37,3±2,4	26,1±2,1	39,2±2,3	NS
Adducţie	15,3±1,7	21,7±1,8	15,9±2,3	22,9±1,6	NS
Rotaţie internă	26,7±1,3	35,4±1,7	25,9±1,4	37,2±1,8	NS
Rotaţie externă	15,5±2,4	19,5±1,2	14,8±2,6	20,3±1.3	NS

TABELUL V

Rezultatele chestionarului HAQ privind calitatea vieţii pacienţilor

Chestionarul	Lot martor la internare	Lot martor la 10 zile	Lot Pilates	Lot Pilates la 10 zile	p
HAQ	2,3±0,2	1,2±0,1	2,4±0,2	0,5±0,3	< 0,05

încurajaţi în continuu să se implice şi să perceapă progresele pe care le reuşesc pe durata tratamentului.

Markerii de inflamaţie repetaţi la externare s-au îmbunătăţit la majoritatea pacienţilor din ambele loturi, la fel ca şi ceilalţi parametri evaluaţi.

CONCLUZII

Kinetoterapia este esenţială la pacientul cu spondilită anchilozantă; dacă tratamentul medicamentos este absolut necesar, menţinerea funcţionalităţii şi reeducarea funcţională a articulaţiilor interesate are o importanţă fundamentală pentru prevenirea, întârzierea şi corijarea deformărilor articulare, de postură şi ale aliniamentului.

Includerea metodei *Pilates* în tratamentul recuperator al pacienţilor cu spondilită anchilozantă poate avea un efect favorabil asupra atât a indicatorilor obiectivi privind mobilitatea, cât şi a percepţiei pacienţilor despre afecţiunea de care suferă.

Deşi medicaţia contribuie la menţinerea/refacerea amplitudinii de mişcare prin absenţa durerii şi controlul procesului inflamator, metodele şi tehnicile kinetice sunt cele care asigură funcţionalitatea optimă, corectă a segmentelor corpului la pacientul cu spondilită anchilozantă.

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NEW INSIGHTS INTO DIAGNOSIS AND THERAPEUTIC MODULATION OF DRUG-INDUCED HEPATOTOXICITY

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NEW INSIGHTS INTO DIAGNOSIS AND THERAPEUTIC MODULATION OF DRUG-INDUCED HEPATOTOXICITY (Abstract): Drug-induced hepatotoxicity may be the consequence of numerous drugs and chemical compounds, such as antimicrobial agents, anticonvulsant drugs, antineoplastic medication, herbal medicines and dietary supplements. The early and correct diagnosis of the drug-induced liver injury is an important aspect that allows for adequate therapy and the possibility of assessing the severity and prognosis of this pathological condition. Currently, the variations of serum transaminase, alkaline phosphatase and total bilirubin values, represent usual, but suboptimal laboratory parameters for the identification of presence, severity and the evolution of liver damage. Some biomarkers such as: apoptotic index, glutamate dehydrogenase, high mobility group box 1 protein and microRNA-122, are recently considered the valuable candidates in assessing the drug induced liver injury. A lot of active substances: chitosan, quercetin, collagen, citral, have been extensively investigated and proved hepatoprotective, antioxidant and anti-inflammatory effects during the *in vitro* and *in vivo* researches. The use of different compounds based on biocompatible polymers and natural substances represents a generous research direction and provides interesting perspective on development of innovative hepatoprotective agents. **Keywords:** DRUG-INDUCED HEPATOTOXICITY, DIAGNOSIS, BIOMARKERS, HEPATOPROTECTIVE, ANTIOXIDANT.

The purpose of this review is to reveal the classic and modern diagnosing possibilities, and the therapeutic opportunities to modulate the evolution of drug-induced hepatotoxicity. A great variety of chemicals (drugs, recreational agents, ethyl alcohol, toxic compounds, industrial and domestic substances, herbal medicines and dietary supplements) is an important cause of hepatocytes alteration, the majority being responsible for different degree of liver injury (1).

Drug-induced liver toxicity

Drug-induced hepatotoxicity is a frequent cause of hepatic damage, with a wide range of manifestations, the most severe of which is hepatocellular death, leading to

acute hepatic failure (2). Risk factors include idiosyncrasy, age, gender, alcohol consumption, concomitant use of other drugs, pre-existing liver disease, obesity, genetic and environmental factors (3, 4). The liver plays an important role in the biotransformation of drugs or exogenous toxic substances. Pathological status of the liver may disturb the metabolism of various chemical agents, thus affecting the efficacy and the development of adverse reactions, while some their metabolites may directly cause hepatic injury. Drug-induced hepatotoxicity may be determined by the administration of numerous and various drugs and chemical substances such as antimicrobial chemotherapeutic medication, anticonvulsants, antineoplastic drugs, herbal remedies and dietary supplements (5, 6).

There are two main forms of hepatotoxicity: intrinsic and idiosyncratic (7). Hepatic damage may be the result of direct toxicity from the administered drug, one of its metabolites, or the lesion may be due to immune-mediated mechanisms (8).

Basic diagnosis
of drug-induced liver toxicity

Hepatic lesions can be classified into hepatocellular, cholestatic and mixed, being caused by increasing in alanine aminotransferase and alkaline phosphatase concentrations, above the upper limit of normal (9). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase levels and total bilirubin, although not specific for drug-induced liver damage, remain the parameters for the detection and classification of liver lesions. However, serum ALT cannot differentiate drug-induced hepatic cytolysis from necrosis of other causes, such as viral hepatitis. In addition, important elevation of serum ALT may even occur in other cases of hepatic damage due to, for example, the liver accumulation of glycogen in poorly controlled diabetes mellitus; in autophagy of hepatocytes in the context of anorexia nervosa and, also, in hepatic steatosis (10).

In addition, frequent and high increase of ALT, can often be caused by drugs that have little potential, or do not cause clinically important liver damage, such as statins, but it is unclear, whether this increased enzyme activity, really reflects the existence of hepatic lesions. In other situations (such as isoniazid, troglitazone, ximelagatran use), it is possible to occur hepatocyte lesions, including necrosis, but elevated serum ALT levels decrease over time, after continuing treatment, as a result of a so-called adaptive process (11). It appears that, this adaptive adjustment involves altering the regulation of production the metabolic enzymes of drugs and transporters. Changes in the regulation of transporters, have been observed in the human liver during recovery of drug-induced hepatopathy, suggesting that similar adaptive mechanisms may be operative in both rodents and humans. A widely accepted theory is that, patients developing clinically manifested drug-induced hepatotoxicity are a group of people with increased ALT values that cannot adapt to existing lesions (10).

Data from the literature shows that a concomitant increase in serum ALT and bilirubin is a much better predictor of evolution, indicating severe liver damage during a hepatocellular lesion, than the increase in ALT alone. It is considered, however, that bilirubin together with ALT, are not optimal assessment parameters, because during hepatic impairment there must be a very large destruction of functional hepato-

cytes before bilirubin increases. However, the simultaneous existence of elevated levels of these two parameters signifies an increased risk in liver failure (11).

For proper management of idiosyncratic hepatotoxicity, one of the key questions is whether, in a patient with this reaction, it will progress to a serious liver lesion with potentially fatal outcome or will be spontaneously remitted, after discontinuation of the causative agent. A diagnostic algorithm that allows the identification of risk factors and the prediction of subsequent clinical development, will greatly facilitate the appropriate treatment of acute liver injury. The current lack of testing the predictive safety, prior to the administration of a potentially hepatotoxic compound, reinforces the need for rapid identification of a high-risk hepatotoxicity situation, involving additional monitoring (10).

There is a growing need for *in vitro* researches, to predict the risk of drug-induced hepatotoxicity, before initiating studies in human subjects. Such investigations are based on human-derived cell culture studies, to evaluate mitochondrial toxicity, inhibition of transporters, induction of oxidative stress and others involved in pathophysiological mechanisms (11).

Biomarkers in the evaluation of drug-induced liver toxicity

A number of new biomarkers are, currently, being considered, for systematic implementation in future studies, related to drug-induced hepatotoxicity investigation (12). The researches performed in rodent, indicate that the ratio between the caspase fragment of cytokeratin 18 and total serum K18 (called the "apoptotic index"), estimates the relative level of apoptosis, compared to necrosis, during drug-induced liver lesions.

Glutamate dehydrogenase can specifically differentiate the damage of the liver, from the muscular damage, additional highlighting mitochondrial toxicity, as a mechanism of hepatic impairment. It is known that microRNA-122 is a liver specific marker, however, recent data suggesting it may be actively released from the hepatocytes, in the absence of visible toxicity, which reduces the enthusiasm for considering it to be specific for drug-induced hepatotoxicity (13, 14).

Finally, a number of specific molecular structures, associated with liver damage, especially the high mobility box 1 nuclear protein and its various modified forms, are promising biomarkers of innate immune activation, useful to distinguish the so-called benign increasing of aminotransferase, over those that indicate clinically important liver lesions (7). Recently, these new biomarkers are used in early clinical trials, but widespread acceptance of their use, will require extensive in-depth studies.

Therapeutic promises valuable in drug-induced hepatotoxicity

Literature data highlight the fact that a number of natural compounds (of vegetable or animal origin) or some polymers have hepatoprotective effects on lesions induced by medication or toxic agents, antioxidant and anti-inflammatory actions (15, 16, 17).

One of the modern medicine challenges is the use of nanotechnology to optimize pharmaceutical forms of drugs, in order to limit their liver toxicity. The important technological advantages of nanoparticles used as drug delivery systems, are represented by the targeted release of active substance, prolongation of drug action in the target tissue and lowering their adverse

effects.

Various biocompatible and biodegradable polymers, or some natural compounds, have given rise to a great interest from numerous researchers to investigate their potential as drug carriers with possible biomedical applications (18).

Chitosan, a marine polysaccharide made from crustaceans, has long attracted attention, especially as a biocompatible material, due to its anti-tumor, anti-ulcer, immunostimulatory, antioxidant and antibacterial activities. Experimental studies have shown that chitosan exhibits hepatoprotective effects due to the improvement of endogenous antioxidant defense systems and the reduction of lipid peroxidation in the experimental model of acetaminophen-induced hepatotoxicity in rats (19). The use of original nanoparticles based on chitosan proved to have potent antioxidant, anti-hyperlipidemic actions and hepatocytes protective effects on alcohol and fatty regimen (20). Isoniazid and rifampicin both induced (21) experimental hepatotoxicity in rats.

Other pharmacological active agent, the polyphenolic flavonoid compound quercetin, known to possess effective antioxidant, anti-inflammatory properties and a protective activity in different pathological states, evidenced potent effects in prevention of hepatic injuries in both acetaminophen and acrylamide-induced liver toxicity in rats (22, 23). The treatment with nano-formulations based on quercetin showed hepatoprotective and antioxidant influence, against liver cytotoxic injuries induced by aflatoxin B1 in rats, as well as *in vitro* studies performed on hepatocytes cultures (24). Moreover, the association of the flavonoid agent quercetin, demonstrated dose-dependently potentiation of chitosan actions, to improve the biochemical parameters and histological liver architecture, in rats with experimental-induced hepatotoxicity with carbon tetrachloride (25).

Literature data suggests that some types of collagen, such as marine origin (extracted from the skin of some salmon species), have various biological functions, including antihypertensive, anti-ulcer, anti-aging, but also liver protective activities. The antioxidant and hepatoprotective effects of marine collagen, have been confirmed by diminishing the activity of oxidative stress markers, decreasing in serum levels of aminotransferases, improving the lipid profile, reducing the hepatic histological lesions in rats with toxic alcoholic hepatopathy (26). Other researches highlighted hepatoprotective influences of collagen peptides obtained from some fish species skin, demonstrated by reduction in serum liver enzymes level, the intensification in antioxidant enzymes activity in rats with drug-induced liver damages. Additionally, the *in vitro* studies showed a substantial increase in hepatocytes viability in the culture cells pre-treated with these cod skin collagen peptides (27).

It is known that, the monoterpene citrate, an essential oil, the component of lemongrass oriental plant, has many biological effects, such as antibacterial and anti-inflammatory. The researches also demonstrated the citrate significant antioxidant activity, anti-inflammatory effects and hepatoprotective actions on experimentally acetaminophen-induced hepatopathy in mice (objected by decreasing the aminotransferases activity and by improving of liver histopathological lesions) (28, 29).

Other interesting research direction, is using diazeniumdiolate derivatives O(2)-vinyl 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-

diolate (V-PYRRO/NO) or O2-vinyl-[2-(carboxylato)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (V-PROLI/NO), nitric oxide donors, which proved hepatoprotective effects in different experimental models of liver damage, represented by acetaminophen-induced hepatotoxicity, D-glactosamine/endotoxin-induced liver injury and nonalcoholic fatty liver disease in rodents (30, 31).

CONCLUSIONS

Our study revealed that drug-induced hepatotoxicity is the most common cause of liver injury in the clinical practice. Currently, the variations of serum transaminase, alkaline phosphatase and total bilirubin values represent the usual, but suboptimal laboratory parameters for the identification of the presence, severity and the evolution of liver damage. Some biomarkers, such as apoptotic index, glutamate dehydrogenase, high mobility group box 1 protein and microRNA-122, are recently used in assessing the drug-induced liver injury.

The use of different compounds based on biocompatible polymers and natural substances represents a generous research direction and provides interesting perspective on development of innovative hepatoprotective agents.

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CORELAŢII CLINICE ŞI BIOLOGICE PRIVIND VARICELE ESOFAGIENE LA PACIENŢII CU CIROZĂ HEPATICĂ COMPENSATĂ

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3. Disciplina de Asistenţa Primară a Stării de Sănătate şi Epidemiologie

CLINIC AND BIOLOGIC CORRELATIONS CONCERNING ESOPHAGEAL VARICES IN PATIENTS WITH COMPENSATED HEPATIC CIRRHOSIS (Abstract): **Material and methods**: The retrospective study included 126 patients with hepatic cirrhosis (HC) CHILD A class, assisted in Botoşani Hospital, during 2007 and 2008. Patients were clinic, biochemical, hematological, etiological, ultrasonographic, endoscopical examined. Statistical analysis carried out with EXCEL soft and SPSS 16.0 soft, calculating chi^2, p, ROC curve, SPRI and ASPRI values. **Results**: We assessed the sample characteristics using the univariate analysis: age, the presence / absence of esophageal varices, SPRI score (limit value ≥ 15.5), ASPRI score (limit value ≥ 19.3). **Conclusions**: Non-invasive prediction cannot exclude the endoscopic exam in patients with compensated HC, caused especially by the virus C and alcohol consumption. Thrombocytopenia and splenomegaly and Fibroscan exam were the best non-invasive indicators to predict the esophageal varices. **Key words**: ESOPHAGEAL VARICES, NON-INVASIVE PREDICTORS, LIVER CIRRHOSIS, ENDOSCOPIC SCREENING

Formarea, dilatarea şi în final ruperea varicelor esofagiene (VE) reprezintă o complicaţie majoră, dramatică a hipertensiunii portale (HTP) din ciroza hepatică. Posibilitatea de a identifica prin metode non-invazive prezenţa VE este utilă pentru a selecta pacienţii care necesită screening endoscopic (1,2,3). Cu ocazia primului diagnostic, VE sunt prezente la aproximativ 40% din pacienţii cu ciroză hepatică (CH) compensată şi la 60% din cei decompensaţi, cu ascită. La pacienţii fără VE, rata de apariţie a acestora este de 5-10 % pe an şi cu tendinţa de a progresa spre cele de gradul I şi II şi apoi la III şi IV, cu riscul de sângerare (4,5). În prezent, se recomandă evaluarea endoscopică a pacienţilor cu CH, cu includerea acestora în programe preventionale, pentru a reduce riscul sângerării varicelor. O asemenea orientare implica existenţa unor echipamente şi aparatură medicală de înaltă performanţă, cu costuri ridicate. În acest context, a crescut interesul pentru intervenţiile non-invazive, clinice, biologice, imagistice care au putere de predicţie pentru prezenţa VE (6,7,8,9).

Studiul nostru urmăreşte determinarea parametrilor clinici, biologici şi ecografici cu valoare în orientarea diagnostică a prezenţei VE în CH şi aplicabilitatea lor în practica medicală curentă.

TABELUL I
Calcularea indicilor ASPRI, SPRI

Indicator	Calcul
SPRI	Diametrul longitudinal al splinei (cm) / numărul de trombocite (10^6 / ml) x 100
ASPRI	Vârsta (ani): < 30 = 0; 30-39 = 1; 40-49 = 2; 50-59 = 3; 60-69 = 4; ?70 = 5
	ASPRI = vârsta + SPRI

MATERIAL ŞI METODE

Studiul retrospectiv a inclus 126 pacienţi cu CH clasa CHILD A, internaţi în compartimentul de gastroenterologie al Spitalului Judeţean „Mavromati", Botoşani, în perioada 1.01.2007 - 31.12.2008 şi care au fost urmăriţi la intervale de 6 săptămâni, 6 luni şi 1 an. Pacienţii au fost examinaţi clinic complet, concomitent cu efectuarea bilanţului biochimic, hematologic, evaluarea etiologică, examinarea ecografică abdominală cu înregistrarea diametrului longitudinal al splinei. Lotul de studiu a fost evaluat, iniţial, prin endoscopie digestivă superioară cu notarea prezenţei VE, varicelor gastrice şi gastropatiei portale. S-a calculat scorul de gravitate CHILD POUGH selectându-se pacienţii din clasa A. Diagnosticul de CH a fost susţinut de elementele clinice, biochimice şi ecografice. Au fost excluşi pacienţii care au prezentat : sângerare a varicelor în antecedente, tromboză venoasă portală evidenţiată ecografic, alte cauze de splenomegalie, precum şi cei aflaţi în tratament cu antagonişti ai receptorilor beta-adrenergici. ASPRI şi SPRI s-au calculat după formula derivată din combinarea valorilor de vârstă, mărimea splinei şi valoarea trombocitelor (tab. I).

Analiza statistică s-a efectuat cu ajutorul programelor Microsoft Excel si un soft SPSS versiunea 16.0, şi indicatori statistico-matematici uzuali (chi^2, p, curba ROC, valorile prag pentru SPRO şi ASPRI).

REZULTATE ŞI DISCUŢII

1. Caracteristicile lotului de studiu. Cei 126 de pacienţi au avut vârsta medie de 52 (19-85 ani). La endoscopie, VE digestivă

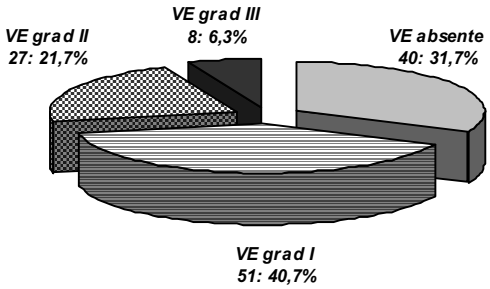

Fig. 1 . Repartiţia varicelor esofagiene

superioară a fost prezentă la 99 (78,5%) – 40,7% gr. I, 21,7% gr. II şi 6,3% III) (fig. 1).

2. Analiza univariată a evidenţiat relaţia prezenţei sau absenţei VE cu diverşi parametri (tab. II). Prin analiza univariată, 6 parametri au fost găsiţi cu semnificaţie statistică pentru : vârstă, trombocitopenie, splenomegalie, prezenţa circulaţiei colaterale abdominale, scorurile SPRI şi ASPRI. Compararea grupurilor definite de prezenţa sau absenţa VE s-a realizat utilizând teste statistice specifice (tab. III).

3. Evaluări statistice. Reprezentarea în diagrame boxplot a repartiţiilor celor doua grupuri (prezenţa şi / sau absenţa VE) în legătura cu valorile trombocitelor, valoarea SPRI şi valoarea ASPRI este ilustrată în figura 2, diagramele 1, 2, 3. S-a constatat că 92,9 % din cazurile cu trombocitopenie medie sau severă au prezente VE. Rezultatele obţinute în urma estimării modelului prin testul Hosmer-Lemeshow arată că *modelul este semnificativ statistic***,** cu o bună concordanţă a modelului cu datele analizate. Se poate spune că aproximativ 60,5% (Nagelkerke R Square) din varianţa iniţială este regăsită în model. Analiza clasificărilor predictive privind prezenţa şi absenţa VE, arată un procentaj global foarte bun al cla-

TABELUL II

Evaluarea pe un lot de 126 pacienţi a diferiţilor parametri cu prezenta sau absenţa varicelor esofagiene, evidenţiată prin analiza univariată

Variabile	Varice esofagiene		Pearson Chi-Square	Odds ratio	P-value
	Prezente	*Absente*			
Sex (bărbaţi : femei)	99 (44:55)	27 (10:17)	0,475	0,73 (0,3-1,76)	0,491
Mediu (urban : rural)	99 (40:59)	27 (15:12)	1,98	1,84 (0,78-4,35)	0,159
Vârsta (ani) < 30	6	1			
30-39	7	1			
40-49	12	4			
50-59	41	4	11,829		**0,037**
60-69	15	11			
? 70	18	6			
Etiologie					
Alcoolică	47	52	0,387	1,31 (0,55-3,11)	0,534
VHC	45	54	0,611	1,41 (0,59-3,4)	0,434
VHB	32	67	0,406	1,36(0,52-3,55)	0,524
VHB+VHD	4	95	2,021	0,33 (0,07-1,6)	0,155
VHC+Alcoolică	15	84	1,09	2,23 (0,47-10,42)	0,296
VHB+Alcoolică	12	87	0,477	1,72(0,36-8,21)	0,49
alte etiologii	2	97	0,554		0,457
Comorbidităţi					
Cardiovasculare	53	46	0,764	0,67 (0,28-1,62)	0,382
Respiratorii cronice	5	94	1,31	0,42 (0,09-1,9)	0,252
Diabet zaharat	21	78	0,094	1,18 (0,4-3,5)	0,759
Obezitate	10	89	0,477	0,64 (0,18-2,24)	0,49
Insuficienţă renală cronică	4	95	0,006	1,09 (0,11-10,22)	0,937
Boli maligne extrahepatice	6	93	0,225	1,67 (0,19-14,56)	0,636
Elemente clinice					
Ascită	51	48	1,781	1,8 (0,75-4,33)	0,182
Icter	12	87	0,477	1,72 (0,36-8,21)	0,49
Circulaţie colaterală abdominală	57	42	8,502	3,87 (1,5-10,01)	**0,004**
Angiectazii	76	23	0,085	1,15 (0,43-3,07)	0,771
Eritem	45	54	0,009	1,04 (0,44-2,45)	0,926
Purpură	7	92	0,404	1,97 (0,23-16,81)	0,525
Trombocitopenie absentă	8	2			
uşoară	39	21	13,984		**0,003**
medie	37	4			
severă	15	0			

sificărilor corecte de 85,2%, indicând o bună adecvanţă a modelului. Se poate, totuşi, observa că are loc o clasificare mai bună pentru cazurile de prezenţă a VE (92,6%), decât pentru cele de absenţă (59,3%) (tab. IV).

4. Analiza prin curba ROC a modelului arată o bună comportare a acestuia cu o arie de 0,825 (interval de încredere 0,74--0,91). De asemenea, prin analiza ROC a parametrilor introduşi s-a stabilit: pentru *scorul SPRI* o arie de 0,699 (cu p=0,002, interval de încredere 0,59-0,79) şi o valoare

TABELUL III

Compararea grupurilor definite de prezenţa şi / sau absenţa varicelor esofagiene

Variabila	Egalitatea dispersiilor - testul Levene		Egalitatea mediilor - testul t		Diferența mediilor			
	F	p-value	t	p-value	Diferența	Eroarea standard	Intervalul de încredere 95% al diferenței	
							Inferior	Superior
Vârsta	0,03	0,864	-1,41	0,162	-4,36	3,10	-10,50	1,77
Valori splenomegalie	0,00	0,969	2,72	0,007	9,54	3,50	2,60	16,48
Valori trombocite	0,07	0,790	-2,64	0,009	-26,25	9,96	-45,96	-6,54
SPRI	6,57	0,012	3,75	0,000	15,08	4,02	7,11	23,04
ASPRI	6,45	0,012	3,64	0,000	14,68	4,03	6,69	22,68

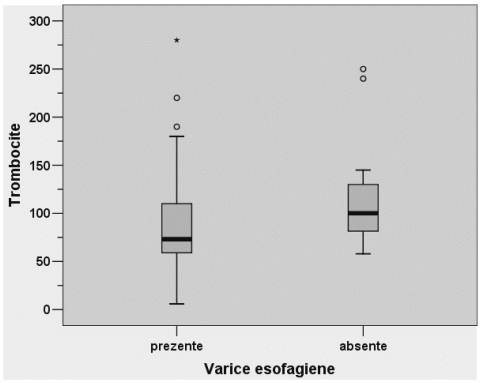

diagrama 1

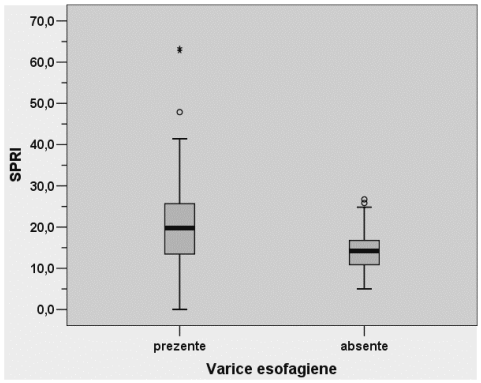

diagrama 2

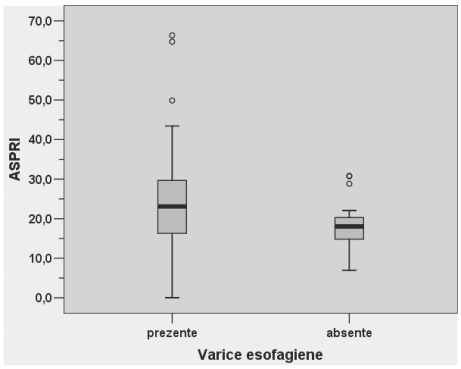

diagrama **3**

Fig. 2. Repartiţia prezenţei / absenţei varicelor esofagiene după valorile trombocitelor (diagrama 1), valoarea SPRI (diagrama 2), valoarea ASPRI (diagrama 3)

prag a SPRI ≥15,5, având puterea cea mai mare de predicţie pentru prezenţa VE (sensibilitate 67%, specificitate 66,7%); pentru *scorul ASPRI* o arie de 0,686 (cu p=0,003, interval de încredere 0,58-0,78) şi o valoare prag a ASPRI≥19,3, având puterea cea mai

TABELUL IV

Prognoza prezenţei / absenţei varicelor esofagiene în cadrul modelului

Valori observate		Valori prognozate de model		
		varice esofagiene		Clasare corectă (%)
		absente	prezente	
Varice esofagiene	absente	16	11	59,3
	prezente	7	88	92,6
Clasare corectă (%)				**85,2**

mare de predicţie pentru prezenţa VE (sensibilitate 64,9%, specificitate 63%).

Trombocitopenia este în relaţie inversă cu VE şi, din analiza valorilor, se poate fixa un prag de 98.000 mm^3 (sensibilitate 66%, specificitate 55%). Pentru *splenomegalie*, aria ROC are o valoare bună, de 0,655 (p= 0,014), cu o valoare prag de 150 mm, când obţinem sensibilitatea 65% şi specificitatea 55%.

Analiza ROC a scorurilor SPRI şi ASPRI diferenţiate după etiologia cirozei hepatice arată că, pentru lotul pacienţilor cu ciroză alcoolică, curba ROC nu este semnificativă şi valoarea prag pentru SPRI diferă la etiologia virală C (≥15,58, sensibilitate 71% şi specificitate 70%) faţă de etiologia virală B (≥17,1, sensibilitate 70%, specificitate 71,4%). Valoarea ASPRI este relativ similară la loturile cu etiologie VHB şi VHC. În mod surprinzător, aici s-a constatat că, pe lângă lotul de pacienţi cu ciroză alcoolică şi la cei cu ciroză VHB curba ROC are o semnificaţie la limită. Puterea de predicţie a indicatorilor ASPRI, SPRI, trombocite, splină este relativ similară dacă se compară ariile ROC.

CONCLUZII

Rezultatele de predicţie pentru VE obţinute sugerează că predicţia non-invazivă nu are o acurateţe suficientă pentru a elimina examenul endoscopic, la pacienţii cu CH compensată. S-a dovedit că grupul de vârstă 50-59 ani, cu prevalenţa cea mai ridicată în rândul lotului studiat şi, de asemenea, vârsta este factor independent de predicţie în analiza multivariată, atestând vechimea infecţiei cu virusul hepatitei C sau a consumului de alcool.

Trombocitele şi splina sunt parametri bine corelaţi cu prezenţa şi/sau absenţa VE, având semnificaţie statistică în analiza uni- şi multivariată, ei caracterizând relevant hipertensiunea portală din ciroza hepatică. Până în prezent, s-au dovedit cei mai buni indicatori non-invazivi în prognozarea prezenţei VE. Studiile efectuate în privinţa valorii scorurilor APRI şi ASPRI au rezultate divergente, posibil datorate diferenţelor de lot studiat.

Ca markeri non-invazivi, examenul Fibroscan are o acurateţe bună, dar şi limite de aplicabilitate pentru pacienţii obezi şi din cauza costurilor ridicate. Studiile în curs se preocupă de compararea performanţelor testelor non-invazive şi anume Fibroscan, Fibrotest şi ale raportului trombocite / diametrul splinei, în aprecierea prezenţei VE. Utilizarea markerilor non-invazivi poate ameliora raportul cost-eficienţă şi îmbunătăţi managementul medical al pacienţilor cu ciroză hepatică, prin limitarea examinării endoscopice la un subgrup de pacienţi consideraţi cu risc, dar, în prezent, Consensul Baveno IV, care recomandă screening-ul endoscopic ca fiind cea mai bună metodă pentru detectarea VE, la toţi pacienţii cirotici, rămâne neschimbat şi cu largă aplicare.

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NOUTĂŢI

IDENTIFICAREA RAPIDĂ ŞI SIMPLĂ A RISCULUI DE MORTALITATE ÎN INSUFICIENŢA CARDIACĂ

Numeroase scoruri au fost imaginate pentru predicţia riscului de deces în insuficienţa cardiacă. În studiul EURO HEART SURVEY, care a inclus 10701 subiecţi cu insuficienţă cardiacă (IC) recrutaţi din 115 centre europene, un procent important de pacienţi, 1404 (13%) au decedat în primele 12 săptămâni de supraveghere. Într-o analiză univariabilă, vârsta, sexul feminin, un QRS lărgit pe electrocardiogramă, cât şi existenţa unei hiponatremii, hipokaliemii, insuficienţe renale, anemii, a unei insuficienţe mitrale severe, sau a unei insuficienţe ventriculare stângi severe au însoţit un prognostic rezervat. Tratamentul cu inhibitori ai enzimei de conversie (IEC), betablocante, derivaţi nitraţi, sau hipolipemiante au ameliorat prognosticul. Într-o analiză multivariabilă au rămas ca factori predictivi (odds ratio – OR < 1 fiind protectiv): vârsta (OR 1,5), insuficienţa ventriculară stângă severă (OR 1,8), creşterea cretininemiei (OR 1,2), natremiei (OR 0,9), hemoglobinei (OR 0,9). Pe plan terapeutic warfarina s-a dovedit a fi protectoare (OR 0,5), antiagregantele plachetare (OR 0,6), spre deosebire de heparină (OR 1,7) şi medicaţia inotropă (OR 5,5). Alte medicamente, precum IEC (OR 0,5), betablocante (OR 0,7), statine (OR 0,6) sau inhibitori calcici (OR 0,7) au ameliorat toate prognosticul. Este uşor de clasificat subiecţii în cohorte utilizând un scor simplu de risc permiţându-se astfel predicţia mortalităţii pe termen scurt. Acesta ar cuprinde: vârsta, nivelul de afectare al ventriculului stâng, tratamentul cu betablocante, IEC, nivelul creatininemiei. Se pare că este relativ uşor de a identifica rapid şi simplu pacienţii cu risc în cursul unei spitalizări pentru IC (Velavan P et al. Predictors of short term mortality in heart failure — Insights from the Euro Heart Failure survey. *Int J Cardiol* 2010; 138: 63–69).

Al.D. Ciochină

POST COITAL ALLERGY – A CASE REPORT OF POST ORGASMIC ILLNESS SYNDROME

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POST COITAL ALLERGY-A CASE REPORT OF POST ORGASMIC ILLNESS SYNDROME (Abstract) The post-orgasmic illness syndrome (POIS) is a rare, but debilitating cluster of symptoms, occurring after ejaculation. The clinical case presented is a male patient, his complains started after ejaculation, four years ago, with flu-like symptoms, feverishness and sweating, brain fog, muscle pain and heavy legs, followed by concentration and attention difficulties and irritation. This status last for about 5 to 7 days, disappear spontaneously after 3 days and reappear after the next ejaculation. This postcoital illness was worsening during the years. The clinical assessment and differentiation of the diagnosis was difficult and consisted in several clinical criteria, a functional test and allergy valuation. The combination of symptoms and pathophysiological mechanisms pointed to other sex related allergies, such as seminal plasma protein allergy. **Keywords***:* SEMINAL PLASMA PROTEIN ALLERGY, POST ORGASMIC ILLNESS, SEXUAL DISTRESS.

The only universal in human sexuality is variability itself (1). During the last 5 years we have diagnosed and treated three different cases of sex allergy: latex allergy, seminal plasma allergy and, the most recent, post orgasmic illness syndrome (POIS). Sex and intimate contacts can represent a risk factor for allergic reactions, because they may favor direct contact with sensitizing substances(1).

Pathogenesis of POIS might include type 2 immune allergic reactions, but it does not necessary involve atopy (2). The mean serum total IgE in the non-atopic males was 27 kU/L (range 6-78 kU/L), indicating that in these men this immunological marker was normal (2).

Pathophysiology of POIS shows an immunological process which explains the systemic reaction of the body and not only a local genital reaction, since only the immune system is capable of inducing very rapid and serious physical and mental symptoms (3). The support for an immunological cause of these flu-like complaints was found in reports on cytokines inducing a flu-like state (4, 5). There are two types of POIS, a primary type in which POIS is manifest from the first ejaculations in puberty or adolescence and a secondary type, in which POIS starts later in life (2, 6).

CASE REPORT

We present the case of a 34-year-old male patient, with a very demanding job, married young, heterosexual, with two healthy children. He had no intimate relationships with other women after getting

married.

The patient had two appointments at our clinic during the last 4 years, the first in 2014 and the second in 2017. He initially presented for a "strong dysphoric status after intercourse", during the last months before his visit, "aggravating each time, from abdominal pain and nausea to generalized sickness", to which obnubilation, confusion and severe migraine were added.

The particularity of the case is that the patient had no obvious local or generalized allergic reaction, which can delay and even obscure the diagnosis. Previously he had consulted other doctors, urologists and internists, but the allergist was his last chance to have this mysterious disease diagnosed and treated.

Chief complaint: generalized post coital/post orgasmic illness over the last 4 years.

Past medical history: no history of allergy, high blood pressure (145/90 mm Hg), endoscopically diagnosed gastric ulcer, history of recurrent low urinary tract infections. The patient was asymptomatic at both consultations.

Family history: one of his children was diagnosed with atopic dermatitis. No family history of allergies or diabetes.

Current medication: occasionally, sedatives; he was using latex free condoms, with no influence on symptoms.

Self-observation and self-intervention: He was hiding the truth from his wife, refusing to deal with the problem as a couple. *He noticed that when he interrupted intercourse before reaching orgasm, symptoms did not appear*.

Physical exam*:* absence of physical signs or symptoms, no local or generalized eruption or swelling, normal blood pressure and pulse.

Lab tests showed a total IgE = 12 UI/L; seminal-fluid specific IgE = 45kUI/L (normal range < 0.35 kUI/L); CIC (Circulating Immune Complexes) = 45; serum complement = 570 UI; (++)presence of cryoglobulins

Skin prick test with his own semen resulted in a 14 mm wheal and erythema (+1), while patch test with his own semen was refused, as well as diamine oxidase (DAO) determination and the skin biopsy for mastocytosis.

Functional Diagnosis 1. A non-specific clinical test of POIS - advise the patient to stop masturbating or intercourse just before the first genital sensations of an impending ejaculation occur while having a full erection (2-17). 2. Difficult but clarifying: this is not easy to perform as one asks the patient to stop his sexual activity while having an increasing pleasure in this activity. POIS symptoms will not become manifest after this non-specific clinical diagnostic test.

Clinical diagnosis The five criteria of POIS are as follows: 1: One or more of the following symptoms: sensation of a flu-like state, extreme fatigue or exhaustion, muscle weakness, feverishness or sweating, mood disturbances and/or irritability, memory difficulties, concentration problems, incoherent speech, congested or runny watery nose, itchy eyes; 2: All symptoms occur immediately (e.g., seconds), soon (e.g., minutes), or within a few hours after ejaculation that is initiated by coitus, and/or masturbation, and/or spontaneously (e.g., during sleep); 3: Symptoms occur always or nearly always in more than 90% of ejaculation events; 4: Most of these symptoms last for about 2 to 7 days; 5: The symptoms disappear spontaneously.

Allergy diagnosis Skin prick test with autologous semen: the purpose was to objectify the skin reaction after inoculation of the semen using a protocolized intracutaneous (IC) skin-prick test. It was performed with the male's own semen (autologous

semen) and compared with a placebo skin reaction with IC saline 0.9% (6). The patient masturbated at home to produce semen. In hospital the harvested semen sample was defrosted and diluted with saline 0.9% to a concentration of 1:40,000. In addition, 0.05 mL of each dilution was IC injected at the volar side of the left forearm. The skin reaction to autologous semen and placebo were interpreted at 15 minutes after IC injections and found to be positive when the diameter of the wheal was > 5 mm with local erythema (6). The grading system of the skin reactions was as follows: I. wheal and erythema < 5 mm = negative; II. wheal 5-10 mm and erythema of 11-20 mm = 1+; III. wheal erythema of 21-30 mm = 2+; IV. erythema of 31-40 mm = 3+; and V. wheal > 15 mm or erythema of > 40 mm = 4+ (2, 6).

Positive Diagnosis Based on the functional (positive non ejaculatory test), allergy (+1 level) and clinical diagnosis (all 5 criteria were met), we made the following positive diagnosis, as being the most likely: 1. Post orgasmic illness syndrome type II (late onset) 2. Seminal Plasma Protein Allergy (SPPA) (autoimmune)

Differential diagnosis that can explain at least partially the clusters of symptoms included: 1.Seminal Plasma Protein Allergy (SPPA); 2. Systemic Mastocytosis; 3. Drug/Substance abuse; 4. Autoimmune disease with involvement of sexual activity; Unknown syndrome linked to sexual activity (other Rare forms of Post-coital Sickness).

Treatment consisted in desensitization with autologous semen. Since the patient score was +1 (wheal and erythema +14 mm), the treatment was postponed until the patient should accept a patch test and diamono oxidase (DAO) determination. All treatments should be performed in hospital and attentively surveyed by the allergist and emergency care intervention, if needed. Desensitization is made with extremely diluted autologous semen (1/40,000), by gradually higher concentrations of autologous semen. Titration are to be performed according to local skin reactions post inoculation, aiming at a wheal and flare response of 3+. This score has to be maintained for a period of at least 2 years.

DISCUSSION

Clinical data from urology and andrology reported the post-orgasmic disease status at the beginning of 2000, especially in males and after ejaculation. There are only 50 cases around the world. From the three different cases of sexual allergy, latex allergy, seminal plasma allergy and post -orgasmic disease, the latter most challenged, because there is no objective local allergic reaction and all the symptoms are subjectively presented by the patient, not obvious to the physician. The only support is the patient's observation of the inventory and timing of clinical symptoms.

In this case, we benefited from a very patient and self-aware patient who self-intervening if he had given consistent help because he performed the functional test, stopped sexual intercourse before the first genital sensations of an imminent ejaculation, complete erection. The test was positive and gave us the certainty that the allergic reaction is due to autoimmune allergies to its own seminal fluid.

The first differential diagnosis was a co-existing one, because of the high specific IgE to his own seminal plasma protein, a range of 0-0.35kUI/L being the normal values accepted for absence of SSPA. Thus, the patient has a high degree of autoimmunity to his own seminal plasma protein. We can affirm that POIS include SSPA.

The second differential diagnosis was invalidated, because of the absence of ma-

jor and/or minor criteria for mastocytosis. There were no obvious rashes or hematological changes in the number or morphology of mast cells. Also, there are no consistent data supporting the third differential diagnosis, as there was no history or clinical evidence of drug or other substance use, although clinical status was suggestive of intoxication. It is also important to establish any influence on males with POIS, of the 25-OH D vitamin deficiency, if we consider the epidemic character and the implications of this vitamin (7). Is it the semen or the seminal fluid? The question is which part of the ejaculate contains the antigen (Ag) that triggers the immunological reaction? Waldinger (3, 4, 5) reported the occurrence of POIS before and after sterilization in three men. This phenomenon means that the Ag is most likely not bound to the spermatozoa but associated with the seminal fluid. After sterilization, spermatozoa are no longer released into the genital system, but seminal fluid continues to be produced, for example by the prostate and/or the seminal vesicles.

CONCLUSIONS

We have presented an allergy case with a certain immunotherapy status, that is not associated with elevated serum total IgE or urticaria but may be associated with an autoimmune reaction in the plasma protein in the fluid seed.

Indirect clinical evidence suggests that the Ag triggering the POIS systemic reaction is not bound to spermatozoa, but to seminal the fluid produced by prostate tissue.

The crucial benefit of the case is to point out the importance of self-observation and self-intervention of the patient with POIS, for there is no possible diagnosis of this rare disease, without the contribution of the patient, since all symptoms are invalidating and restricting the visit to a doctor and the possibility of an onsite immediate allergology evaluation.

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THE EVOLUTION OF CLINICO-BIOLOGICAL PROFILE OF PATIENTS UNDERGOING PROPHYLAXIS FOR POST-ERCP PANCREATITIS - A PROSPECTIVE STUDY

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THE EVOLUTION OF CLINICO-BIOLOGICAL PROFILE OF PATIENTS UNDERGOING PROPHYLAXIS FOR POST-ERCP PANCREATITIS-A PROSPECTIVE STUDY (Abstract): In most cases, endoscopic retrograde cholangiopancreatography (ERCP) is a safe procedure. However, acute pancreatitis is the most common complication with an average incidence of 4-5.5% and a mortality of 0.5%. **Aim:** The present study aims to evaluate the incidence and the risk factors of post-ERCP pancreatitis (PEP). **Material and methods:** Patients who underwent first-time ERCP for choledocholithiasis were included in a prospective, single center trial. **Results:** The study group consisted of 98 patients (53 women - 54.08% and 45 males - 45.91%) with an average age of 63.56 ± 14.09 years old, without statistically significant differences between genders. From the 98 patients enrolled in the study, 8 patients (8.16%) developed acute pancreatitis manifested clinically after the interventional procedure (6 patients with mild - 75% and 2 with moderate forms). No severe cases have been recorded and in all the cases the evolution was rapidly favorable. Risk factor analysis demonstrated that 77.6% of the patients enrolled in the study had at least one risk factor for PEP. All patients who developed PEP (8 patients) were females; no cases of post-ERCP pancreatitis have been reported in patients under the age of 60; among the patients who developed PEP, 4 of them had normal serum bilirubin and the other 4 had hyperbilirubinemia; 66.7% of patients with excess contrast were diagnosed with PEP. **Conclusions:** The incidence of acute post-ERCP pancreatitis in our study was similar to the one reported in the literature. No severe pancreatitis was recorded, and the progression of the cases with pancreatic complications was rapidly favorable. Out of the risk factors associated with the occurrence of acute post-ERCP pancreatitis, only female sex and excess contrast substance correlated with PEP in the patients of our study. **Keywords:** ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY (ERCP), POST-ERCP PANCREATITIS (PEP), NON-STEROIDAL ANTI-INFLAMMATORY DRUGS.

Endoscopic retrograde cholangiopancreatography (ERCP) originally appeared as a diagnostic method in gallstone pathology. The subsequent diagnosis and thera-

peutic utility of ERCP has been enough demonstrated for a variety of conditions, including endoscopic treatment of biliary stones, diagnosis and management of cholangiocarcinoma and pancreatic neoplasms, and postoperative management of perioperative biliary complications (1, 2).

In most cases, this is a safe procedure, without post-procedural complications. However, there are situations where these may occur and one of the most common complications is acute pancreatitis (PEP), the average incidence is estimated at 4-5.5%, with wide variations in different studies, between 0.4% and 40%, depending on the presence of risk factors, and the reported mortality is about 0.5% (3, 4).

Despite the increasingly performing equipment used in ERCP and the wider experience of endoscopists, the incidence of PEP has remained relatively constant (5).

In this context, it is understandable the permanent interest in the study of physiopathological mechanisms and risk factors, in order to identify methods of preventing acute pancreatitis. (6)

Various therapeutic regimens have been studied to prevent this complication and, currently, European and American guidelines recommend non-steroidal anti-inflammatory drugs - NSAIDs (diclofenac, indomethacin) in various doses and modes of administration (i.v., intrarectal, etc.) (7-9). Pharmacological prophylaxis can be routinely administered or in selected cases, where risk factors for the development of pancreatic complications are identified (10, 11).

Specialty literature reports a number of risk factors involved in the occurrence of acute post-ERCP pancreatitis: those associated with patients- young age, normal serum bilirubin, acute pancreatitis post-ERCP in the past, female gender, sphincter Oddi dysfunction, and those associated with the procedure-difficult cannulation, excess injection of the contrast substance into the pancreatic duct, exaggerated use of the guide wire (5, 6).

Based on these premises, we initiated a study to evaluate the post-ERCP progression of a group of patients who received pharmacological prophylaxis with indomethacin, regardless the presence of risk factors. Patients were monitored to assess the evolution from the perspective of acute pancreatitis as a complication of interventional endoscopy.

MATERIAL AND METHODS

The prospective study was conducted in a tertiary gastroenterology center between January-July 2018. All 108 patients were over 18 years old, with biliary stones, and were proposed for inclusion in the study, due to the indication of performing an ERCP.

Exclusion criteria were the presence of acute pancreatitis or other acute inflammatory diseases at the moment of admission, contraindications for NSAIDs, recent episodes of upper gastrointestinal bleeding (less than one month), pregnancy and patient refusal.

After the initial evaluation, 98 patients were enrolled in the study that met the inclusion criteria and signed informed consent.

RESEARCH PROTOCOL

After signing the informed consent, each patient included in the study was evaluated before the intervention, to identify the risk factors through anamnesis, clinical examination, laboratory tests (complete blood count, pancreatic serum and urinary enzymes, parameters for the evaluation of inflammatory bowel disease, cholestasis

and hepatic cytolysis, renal imaging) and imaging: ultrasound and cholangial-MRI) according to the protocols in force for biliary pathology.

ERCP was performed following the standard protocol, after evaluation and anesthesia physician's opinion. Pharyngeal anesthesia was administered with 2% lidocaine spray and sedation with midazolam, ketamine and propofol. The equipment and materials used for the ERCP included a TJF 160 series (Olympus, Tokyo, Japan), standard sphincterotomes - Olympus and Boston Scientific (Marlborough, MA, United States), Visiglide hydrophilic guide wires (Olympus), and catheter extraction balloon (Boston Scientific and Wilson-Cook Medical Inc.-Bloomington, IN, United States) and / or Dormia basket from Olympus. Contrast agent was water-soluble iopamidol.

All patients were continuously monitored during the intervention, assisted by the anesthesiologist, and parameters related to the procedure (number of cannulations, amount of contrast substance, length of procedure) were recorded in the database.

All patients received 100 mg of indomethacin intrarectal, immediately after ERCP, for the prophylaxis of post-ERCP acute pancreatitis, according to the European guidelines, and administered Ringer's lactate solution (1.5 mL/kg/hr.) during the procedure and 8 hours post ERCP. After the procedure, at 6 and 24 hours, as well as in case of algic post-ERCP symptomatology, the clinical parameters (presence of abdominal pain, vomiting, fever, transit disorders) and some of the parameters (hemoleucogram, C-reactive protein, pancreatic enzymes) were evaluated. In addition, all adverse effects related to the procedure or medication administered, duration of hospitalization was recorded.

The diagnosis of PEP was set if the patient reported post-procedural abdominal pain or increased preexisting pain while pancreatic enzymes increased by at least three times the normal. Pancreatic amylase elevation of at least three times the normal value in the absence of symptomatology was classified as asymptomatic increased amylase.

Monitoring was discontinued 24 hours after ERCP if no special events were reported or after the event had been resolved if it occurred within the first 24 hours post-ERCP. In case of a complicated evolution, the patients were re-evaluated 30 days after discharge.

Data obtained from patient evaluation at all stages of monitoring (pre and post procedural) were set in a database.

The data were presented as raw values, percentages, and mean ± standard deviation. For data comparison *t-Student* test for continuous data and *Chi Square* (χ^2) or *Fisher's* exact tests were used for qualitative variables when appropriate. Results were considered significant when $p < 0.05$. Statistical analysis was conducted using *SPSS 20.0* software version.

The study protocol and the patient consent form were approved by the Ethics committee of "Grigore T. Popa" University of Medicine and Pharmacy Iasi and "Sf. Spiridon" County Clinical Emergency Hospital, Iasi, Romania.

RESULTS

The study group consisted of 98 patients (53 women-54.08% and 45 males-45.91%) with an average age of 63.56 ± 14.09 years old without statistically significant differences between genders.

Study of clinical and biological data at study inclusion

Among the clinical data recorded in the initial evaluation, the most common symp-

tom was the pain in the right hypochondrium, identified in 67% of cases, followed by jaundice (53% of patients).

Related to the biological data recorded in the initial assessment of patients, cholestasis and hepatic cytolysis were the main changes recorded, both identified in 83.7% of patients.

Study of the clinical and biological parameters evolution at 6 hours and 24 hours post ERCP

The PEP diagnosis was recorded based on the clinical examination associated with biological samples (pancreatic enzymes and parameters of inflammatory syndrome-leukocytes, C-reactive protein) monitored at 6 and 24 hours post-ERCP.

From the 98 patients enrolled in the study, 8 patients (8.16%) developed acute pancreatitis manifested clinically after the interventional procedure. It should be noted that in all cases the onset was early, less than 6 hours after the intervention, with no cases of late onset. From the patients who developed acute pancreatitis after ERCP, 6 patients were mild (75%) and 2 moderate forms. There were no severe pancreatitis and all cases had a favorable progression. No adverse effects related to study medication (digestive bleeding, rectal irritation, or allergy) have been reported.

The comparative study of pancreatic enzymes and C-reactive protein at the two monitoring periods (6 and 24 hours post-ERCP) is synthetically represented in first table. At 6 hours post ERCP, amylase increases of more than 3 times the normal value in 18.4% of patients, while in 24 hours only 8.2% of patients had values compatible with the diagnosis of asymptomatic hyperamylasemia.

TABLE I

Dynamics of clinical and biological factors at 6 and 24 hours

	At 6 hrs.	At 24 hrs.
Amylasemia		
< 3 x N	81.6%	91.8%
> 3 x N	18.4%	8.2%
Lipase		
< 3 x N	69.4%	91.8%
> 3 x N	30.6%	8.2%
CRP		
normal	49.0%	49.0%
minor increase	20.4%	20.4%
moderate increase	26.5%	22.4%
important increase	4.1%	8.2%
Clinical-biological status at 6 hours		
Absence of PEP criteria	91.8%	91.8%
PEP	8.2%	8.2%

Lipase values had a similar dynamics to amylase (tab. I), with significant improvement in 24 hours after the intervention. At 6 hours post ERCP, lipase values were

found to be 3 times higher than the upper limit of normal in 30.6% of patients, and this percentage decreased to 8.2% at 24 hours.

Also, the inflammatory status assessed by C-reactive protein determination has undergone significant variations during the follow-up: 51% of patients experienced C-reactive protein increase, more than half of them with moderate/marked elevated values. It should be noted that the inflammatory status rate was maintained in the same proportions at the 6 and 24 hours respectively.

Study of risk factors for PEP development

Another objective of the research was to determine whether there is a correlation between the occurrence of acute pancreatitis and the presence of risk factors associated with the development of PEP in the study group.

77.6% of patients enrolled in the study had at least one risk factor for PEP. We studied the presence of the risk factors recognized in the literature and we identified: female sex (45% of cases), age under 60 years (36.7%), normal bilirubin (20.4%), excess of contrast substance (12.2%) and acute pancreatitis post-ERCP in the past (4.1%) (fig. 1).

We assessed the risk of pancreatitis in the presence and absence of risk factors (fig. 2).

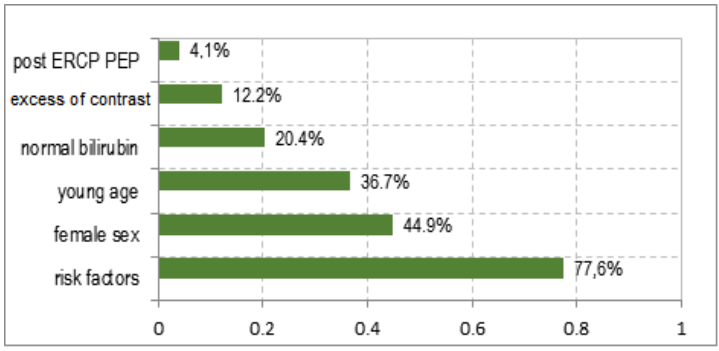

Fig. 1. The distribution of risk factors in the studied group

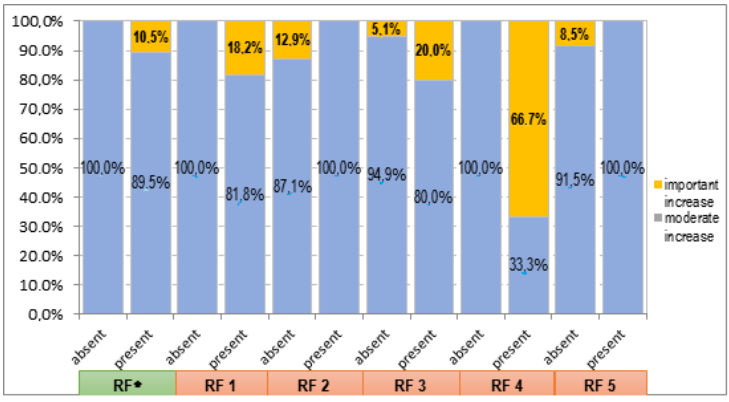

Fig. 2. The relationship between the presence of risk factors and the occurrence of PEP.

Five distinct risk factors (RF), ranging from RF 1 to RF 5, have been studied and have the following meaning:

- RF 1: female gender,
- RF 2: young age,
- RF 3: normal bilirubin,
- RF 4: Excessive contrast substance,
- RF 5: History of PEP

All patients who developed PEP were females. As a result, for 18.2% of the patients who developed the complication, female gender was a risk factor. The value is statistically significant, especially since no male patient presented the complication.

Since no cases of post-ERCP pancreatitis have been reported in patients under the age of 60, we cannot claim that young age has been a risk factor for the patients in our study group.

Also, the normal serum level of bilirubin before endoscopic intervention was not related to the risk of developing the complication. Among the patients who developed acute pancreatitis, 4 of them had normal serum bilirubin and the other 4 had hyperbilirubinemia. As a result, this did not significantly correlate with the occurrence of acute pancreatitis.

Excessive contrast substance administration correlated with the occurrence of acute early pancreatitis (in the first 6 hours): 66.7% of patients with excess contrast were diagnosed with acute pancreatitis, while none of the patients without excess of contrast substance developed the complication. Acute pancreatitis did not statistically correlate with the presence of other episodes of pancreatitis (RF5) in the past. None of the patients with a history of pancreatitis developed acute pancreatitis, whereas among those without a history of pancreatitis, 8.5% of the patients presented the complication. OR risk analysis has shown that the only significant risk factor for pancreatitis is the use of an increased amount of contrast (tab. II).

TABLE II

***Chi-square* test values to compare the presence of PEP depending on the presence of risk factors**

Clinical status	Chi-square	p	OR
RF pres. / abs.	1.261	0.261	-
RF 1 pres. / abs.	5.345	**0.021**	-
RF 2 pres. / abs.	2.529	0.112	-
RF 3 pres. / abs.	2.348	0.125	**4.625**
RF 4 pres. / abs.	31.215	**0.000**	-
RF 5 pres. / abs.	0.185	0.667	-

DISCUSSION

Numerous studies have been reported in the literature, including meta-analyzes, that have assessed the incidence of post-ERCP acute pancreatitis, as well as possible risk factors associated with the occurrence of this complication. The average incidence is estimated at 4-5.5%, with wide variations in different studies, between 0.4% and 40%, depending on the presence / absence

of risk factors (3, 4). In our study, the incidence of PEP was 8.16%. The results are similar to those reported in other studies: Cheng *et al*. reported an incidence of 2-9% in the general population and 15% in high-risk patients (12).

The patients in our group were not selected based on the presence of risk factors and they all received prophylaxis with indomethacin, according to international guidelines. These aspects may explain differences compared to other studies (13, 14).

It should be emphasized that the clinical and biological evolution of the cases that were diagnosed with PEP and asymptomatic hyperamylasemia was favorable, with the improvement of the symptomatology and biological parameters in all patients, with no severe forms or progression to worsening or death. These favorable results may have been obtained because all patients received NSAIDs prophylaxis regardless of the presence or absence of risk factors. According to the literature, prophylaxis with indomethacin is effective, reducing the incidence of acute pancreatitis and improving the clinical and biological evolution of post-ERCP cases of pancreatitis (13, 15, 16).

Another objective was to study whether the factors recognized in the literature as associated with the occurrence of acute post-ERCP pancreatitis (female sex, age under 60 years, normal serum bilirubin, excess contrast substance and personal history of pancreatitis) are associated in our group with the occurrence of acute pancreatitis (17). Risk factor analysis demonstrated that although all of the factors mentioned were present in varying proportions in the patients from the study group, only female gender and excess contrast were correlated with the occurrence of pancreatitis, OR risk assessment demonstrating that the latter influenced the most the risk of PEP.

These results, discordant compared to other published studies, can be explained by the fact that the number of patients included in the study (and implicitly those who developed PEP) is small.

CONCLUSIONS

The incidence of acute post-ERCP pancreatitis in our study was similar to the one reported in the literature. No severe pancreatitis was recorded, and the progression of cases with pancreatic complications was rapidly favorable. Out of the risk factors associated with the occurrence of acute post-ERCP pancreatitis, in the patients in our study, only female sex and excess contrast substance correlated with PEP.

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THE IMPORTANCE OF ARTERIAL STIFFNESS ASSESSMENT IN PATIENTS WITH GRADE 1 HYPERTENSION

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THE IMPORTANCE OF ARTERIAL STIFFNESS ASSESSMENT IN PATIENTS WITH GRADE 1 HYPERTENSION (Abstract): Assessing the arterial stiffness (AS) in hypertensive patients brings complimentary information for the cardiovascular (CV) risk stratification, but current data is rather limited in mild hypertensive patients. The purpose of the study was to evaluate the association between CV risk factors and AS markers in patients with grade 1 hypertension. **Material and methods:** The study was prospective, conducted over a 14-month period and included 58 patients diagnosed with grade 1 hypertension. Patients were extensively analyzed for the presence of CV risk factors and for the determination of AS parameters – pulse wave velocity (PWV) and aortic augmentation index (AixAo). **Results:** Mean age was 63.98 ± 12.24 years, 38% males. PWV was directly associated with age ($r = 0.27$; $p = 0.03$), total cholesterol ($r = 0.33$; $p < 0.01$), LDL-cholesterol ($r = 0.25$; $p = 0.05$), SCORE risk ($r = 0.25$; $p = 0.05$) as well as with the presence of stable angina ($r = 0.31$, $p = 0.02$). AixAo was associated with age ($r = 0.35$; $p = 0.01$), systolic BP ($r = 0.51$; $p < 0.01$) and diastolic BP ($r = 0.37$; $p < 0.01$). Moreover, AixAo was positively associated with total cholesterol ($r = 0.46$; $p < 0.01$) and LDL-cholesterol ($r = 0.36$; $p = 0.01$). **Conclusions:** In patients with grade 1 hypertension, arterial stiffness markers are directly associated with age, lipid parameters, BP and SCORE risk. Therefore, it is essential to apply early specific therapeutic measures to control the CV risk factors in this group of patients. **Keywords:** ARTERIAL STIFFNESS, GRADE 1 HYPERTENSION, ENDOTHELIUM DISFUNCTION, SUBCLINICAL ATHERO-SCLEROSIS, BLOOD PRESSURE.

Arterial stiffness (AS) represents the opposite phenomenon to arterial compliance, defined by the resistance to dilation (diameter variation) determined by a force (contraction of the heart) developed on an elastic body (vascular wall). With aging, but also with the occurrence and maintenance of various conditions (e.g., arterial hypertension, stress) morphological changes occur in the arterial wall, the degradation of the middLe tunic being the first change associated with chronic increase in

AS (1-4). This change is microscopically characterized by the breakdown of the elastin and collagen fibers in the structure of the middle tunic and determines, from an ordered arrangement of the elastin blades, the appearance of fragmented, thinner structures of the elastin and a higher resistance of the collagen resulting in a calcification of the middle tunic. Macroscopically, thickening of the arterial wall appears mainly through hypertrophy, but also through the hyperplasia of smooth muscle cells, altering at last the parietal viscoelastic properties (1-5). These changes determine an increased in peripheral arterial resistance and a decrease in vascular compliance (6-8).

The increased stiffness of the arterial wall and the high rate of propagation of the pulse wave velocity (PWV) places the patient in high group of risk. The degree of AS increases with aging as well as with a number of conditions, which are associated with increased CV risk, such as high blood pressure (BP), diabetes, hypercholesterolemia and chronic renal failure (9-12).

AS has an independent predictive value of all-cause mortality and cardiovascular morbidity, of fatal and non-fatal coronary events and stroke in essential hypertension, diabetes, in patients with chronic end-stage renal disease, in elderly patients and in general to the whole population (9-13). The contribution of AS in increasing the CV risk is explained by a series of mechanisms that converge to promote ventricular hypertrophy, aggravate myocardial ischemia, ischemic stroke or renal impairment (5, 12, 14, 15). Thus, based on the AS parameters measurements we can identify the hypertensive patients at increased risk of cardiac and cerebrovascular events and who can benefit from an earlier and more aggressive cardiovascular treatment. Nowadays, this evidence led to the inclusion of the PWV measurement as a mandatory step in the evaluation of subclinical organ damage in grade 1 hypertensive subjects (16).

The aims of the current study are: - assessment of AS in patients with grade 1 hypertension; - identifying the main CV risk factors associated with increased AS in this class of patients; - the importance of AS analysis as a parameter of increased CV risk in grade 1 hypertension.

MATERIAL AND METHODS

The study was prospective, conducted over a 14-month period and included 58 patients diagnosed with grade 1 hypertension. These patients were extensively analyzed for the presence of CV risk factors as well as for the determination of AS parameters.

The exclusion criteria were the refusal of patients to participate in the study; patients with sinus rhythm disorders at the time of examination; patients who failed to record the parameters of AS; the presence of a cancer in terminal stage; major CV complications (myocardial infarction, stroke, cardiac or peripheral revascularization).

The following parameters and CV risk factors were assessed: age, gender, height (cm) and weight (kg) used to calculate the body mass index (BMI), smoker status and alcohol consumption, as well as hemodynamic parameters: systolic BP – SBP, diastolic BP – DBP and heart rate. All patients have been diagnosed with grade 1 hypertension according to the current guidelines (16). From the patient' personal history we were interested in heart failure, chronic ischemic heart disease, dilated cardiomyopathy, peripheral vascular disease, chronic kidney disease, stroke, atrial fibrillation or

diabetes.

In this study, we focused mainly on the following laboratory parameters: total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, urea, creatinine with determination of glomerular filtration rate (GFR). Moreover, we calculated the CV risk charts Framingham, SCORE and Q-RISK for a better stratification of the CV risk.

Using the Arteriograph® device, several parameters of AS were determined non-invasively but the focus was on PWV and the augmentation index of the aorta (AixAo).

Statistical analysis was performed in SPSS 20.0. The existence of a relationship between variables was evaluated using Pearson correlation coefficient. Descriptive data is presented as mean ± standard deviation. A p value <0.05 was considered statistically significant.

RESULTS

The patients mean age was 63.98 ± 12.24 years, and 38% were males. The main characteristics of the study group is highlighted in table I.

Regarding the medication of the patients, most of them use between 1 and 3 drugs and within the gender representation, women (22) were using more drugs compared to men (13). It is worth mentioning that 12% of patients did not consume any antihypertensive drugs (5 women compared to 2 men). Among the antihypertensive drugs, the most common were beta-blockers and diuretics, both being used by 43%, respectively 34% of participants. The frequency prescribed order was followed by angiotensin conversion enzyme inhibitors and calcium channel blockers. Angiotensin-II-receptor antagonists were used only in 12% of cases.

TABLE I

The main characteristics of the study group

Group profile	Results
Age (years)	63.98 ± 12.24
SBP (mmHg)	150.96 ± 5.96
DBP (mmHg)	85.22 ± 6.83
Years with hypertension	6.28 ± 5.57
BMI (kg/m^2)	28.73 ± 4.84
Framingham score (%)	11.30 ± 7.17
SCORE risk (%)	3.70 ± 2.52
Q-RISC (%)	20.72 ± 11,67
Total cholesterol (mg/dL)	193.46 ± 36.46
LDL-cholesterol (mg/dL)	111.73 ± 34.36
HDL-cholesterol (mg/dL)	54.75±12.39
Triglycerides (mg/dL)	141.74 ± 89.16
Urea (mg/dL)	35.31 ± 12.14
GFR (ml/min/1,73m^2)	79.26 ± 27.22
Glucose (mg/dL)	105.39 ± 31.68
PWV (m/s)	8.75 ± 1.34
AixAo (%)	31.40 ± 14.17

BP-blood pressure, BMI-body mass index, PWV- pulse wave velocity, AixAo-augmentation index of aorta

Among the study group, 51.72% of patients had stable angina, 18.97% had dilatative cardiomyopathy and 43.10% were diagnosed with chronic heart failure. More detailed information can be found in table II.

TABLE II

The presence of other CVD comorbidities in the study group

CVD	All patients	Males	Females	p value
Stroke	5.17%	0.00%	100.00%	0.23
Stable angina	51.72%	40.00%	60.00%	0.47
Chronic venous insufficiency	25.86%	33.33%	66.67%	0.45
Chronic kidney disease	1.72%	0.00%	100.00%	0.08
Diabetes	25.86%	46.67%	53.33%	0.30
Myocardial infarction	5.17%	33.33%	66.67%	0.68
Chronic heart failure	43.10%	8.00%	92.00%	**<0.001**

CVD- cardiovascular diseases

Regarding PWV and the age of the patients, we detected the presence of a statistically significant positive correlation with r = 0.27 and p = 0.03, which shows a trend of increasing PWV value with age (tab. III). A simple linear regression equation was applied to observe the degree of PWV variation with age. As a result, we obtained F = 7.82, p <0.05, R^2 = 0.0787 and therefore a change of 0.25 m/s of PWV increase with one unit in age in our study group (fig. 1).

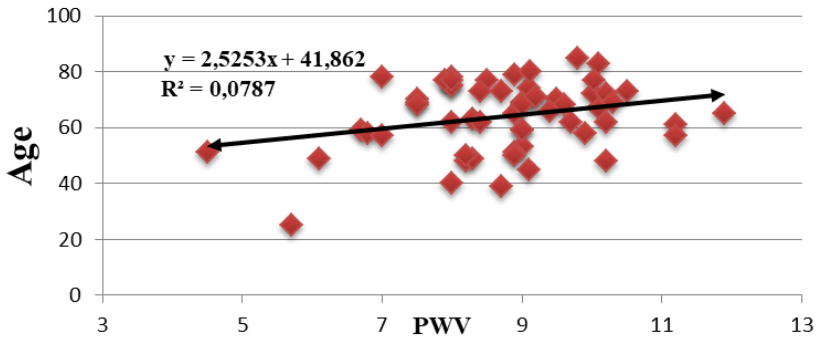

Fig.1. Positive correlation between PWV and age

As for AixAo and age we observed the presence of a positive correlation that is statistically significant with a r = 0.35 and p = 0.01 which demonstrates a tendency of increasing AixAo with growing age (tab. III). The simple linear regression equation pointed out F= 7.40, p <0.05, R^2 = 0.116 and therefore an increase directly proportional of AixAo with age (fig. 2).

Studying the relationship between PWV and total cholesterol values, a positive correlation with statistical significance was detected (r = 0.33; p < 0.01) (tab. III). A simple linear regression equation was applied to analyze the degree of cholesterol variation with PWV which indicated a p

<0.05, R^2 = 0.115 and therefore a change in PWV with 0, 1 m/s may causes a directly proportional change in total cholesterol by 0.90 mg/dL (fig. 3).

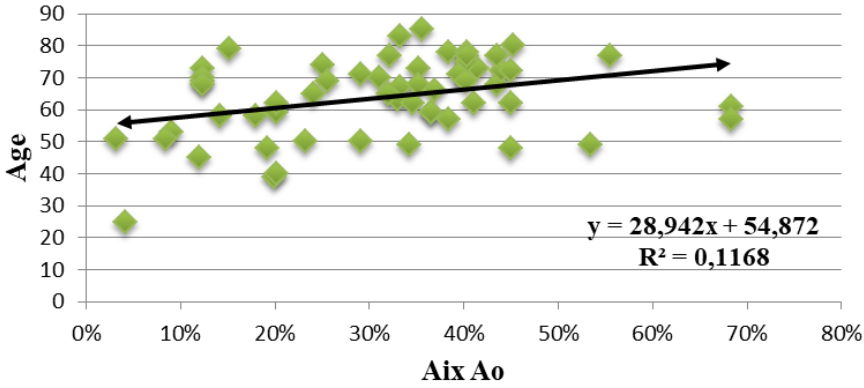

Fig. 2. Positive correlation between AixAo and age

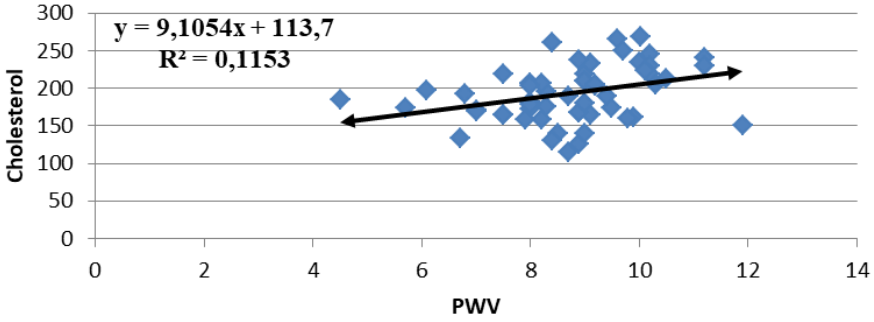

Fig. 3. The positive correlation between PWV and total cholesterol

Analysing the correlation between AixAo and total cholesterol, a positive and statistically significant correlation was detected (r = 0.46, p < 0.01). For AS parameters and LDL-cholesterol analysis, the PWV result was significantly positive (r = 0.25, p = 0.05) while for AixAo the result had increased significance (r = 0.36 and p = 0.01) (tab. III).

By analyzing the relationship between AS parameters and CV risk charts (Framingham, SCORE and Q-RISK) we obtained a positive and statistically significant correlation between PWV and SCORE risk chart (r = 0.25, p = 0.05) which may be translated into an increase in PWV values directly proportional to the increase of SCORE risk (tab. III).

By analyzing the AS markers with the associated CV pathologies, some correlations with statistical significance were obtained, especially in the case of PWV with stable angina (r = 0.031, p = 0.02).

Notably, a strong positive correlation

was observed between AixAo and SBP ($r = 0.51$, $p < 0.01$) as well as with DBP ($r = 0.37$, $p < 0.01$), but not for PWV and BP parameters (tab. III).

TABLE III
Relationship between CV risk factors and AS parameters

	PWV (m/s)		Aix Ao (%)	
	r	p value	r	p value
Age (years)	**0.27**	**0.03**	**0.35**	**0.01**
Total cholesterol (mg/dL)	**0.33**	**<0.01**	**0.46**	**<0.01**
LDL-cholesterol (mg/dL)	**0.25**	**0.05**	**0.36**	**0.01**
SCORE risk chart	**0.25**	**0.05**	0.01	0.96
Stable angina	**0.31**	**0.02**	0.29	0.11
SBP (mmHg)	0.22	0.09	**0.51**	**<0.01**
DBP (mmHg)	0.18	0.16	**0.37**	**<0.01**

DISCUSSION

In this study we aimed to analyze the impact, at an early stage, of the AS parameters on the CV status considering only patients diagnosed with grade 1 hypertension. Lately, a special interest on the structure and function of the arterial wall is increasing mainly due to the association between increased AS and CV morbidity and mortality, but also because AS has become an independent predictive marker for CV risk (9-12,17). However, current data is still limited regarding the AS in patients with small degree of hypertension, our study bringing new information in the comprehensive evaluation of these patients. Thus, we analyzed the AS markers with a series of clinical and paraclinical parameters in order to observe their importance in the evaluation of the hypertensive patients.

It is known that the process of arteriosclerosis is accentuated with aging and is even more accelerated in the presence of several physiological factors such as hypertension. In the present study, PWV is indeed significantly and directly proportional with the age of the patients. Mattace-Raso F. *et al*. confirmed that an increase of PWV is directly proportional with an increase of age on a number of 16,867 patients from 8 European countries (18). Another longitudinal study by AlGhatrif M. *et al*. (the Baltimore study) confirmed this finding on 423 women and 354 men between ages of 21 and 94 years with a more increased PWV values in men (19).

In our study, AixAo presents statistically significant correlations with the age of the patients, result that is accordance with the literature. In the ACCT (Anglo-Cardiff Collaborative Trial) study, the authors found the same increase of AixAo being directly proportional to age (20). In a meta-analysis performed by Baksi A. *et al*. including 13,770 subjects aged 4 to 91 years and aiming for the mechanism of blood pressure changes with aging and indirectly modifying AixAo, the result was a significant change in blood pressure as the aging process is progressing (21). Another study confirmed an increase of AixAo up to the age of 60, but also an increase of BP parallel to the increase in age (22).

Within the study group, the characteri-

zation of the patients was also realized from the perspective of the biochemical parameters. A significant correlation between increased PWV values and total cholesterol was obtained. Moreover, positive correlations between PWV, AixAo and elevated LDL-cholesterol levels were highlighted. Some literature data confirms the existence of this association between these parameters (23). However, other studies did not reveal a statistically significant association between PWV and cholesterol fractions or total cholesterol (24). As well, on a group of participants with ages between 65 and 84 years, the association of plasma cholesterol with the AS of large vessels is denied (25). We found no study that was focused on patients with hypertension debut as was our population.

Regarding the association of the AS markers with the CV pathologies, a significant positive correlation was obtained between the increased PWV values and the presence of stable angina. A Sutton-Tyrrell analysis of 2,248 participants in the Health ABC study revealed the association of increased PWV with 341 coronary heart disease, 94 stroke, and 181 cases of congestive heart failure (26). Another study counting 2000 patients aged over 50 showed a close relationship between PWV and CV mortality independent of previous CV events, age and diabetes (10). Laurent S. *et al*. showed that PWV is a predictor of major CV events in patients with high BP (27).

Moreover, we identified a directly proportional and statistically significant relationship between PWV value and the SCORE risk, result that was also identified on a group of asymptomatic persons (28). Similar, PWV is statistically significant in predicting the CV risk according to SCORE scale more that the Framingham scale explained by the underestimation of the real high CV risk (29).

Statistically significant associations could be observed in the case of AixAo and SBP and DBP. It can be stated that SBP determined noninvasively at the central level represents a better indicator of the process of arteriosclerosis, a fact confirmed by other studies (30) that support the need to determine central blood pressure and arterial stiffness as tests for cardiovascular risk assessment. Moreover, the direct correlation between AixAo and SBP is also confirmed by other longitudinal studies (31), our data having the particularity that investigated only grade 1 hypertensive subjects.

CONCLUSIONS

In patients with grade 1 hypertension, AS parameters are directly associated with age, total cholesterol and LDL-cholesterol. AixAo is directly associated with systolic and diastolic BP while PWV has also positive correlation with SCORE risk chart. Therefore, it is essential to apply early specific therapeutic measures to control the CV risk factors in this group of patients.

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NEWS

VITAMIN D AND THE THERAPEUTIC EFFECT IN NONALCOHOLIC STEATOHEPATITIS

Nonalcoholic steatohepatitis (NASH) is a rapidly progressive component of nonalcoholic fatty liver disease (NAFLD) that can lead to advanced fibrosis, cirrhosis and hepatocellular carcinoma (HCC). This disease is a socio-economic global burden due to the diagnostic methods and lack of effective treatment. NASH represents the main cause of hepatic transplant in United States of America (USA). Vitamin D (Vit D) is a fat-soluble vitamin obtained through diet or synthesized in the skin from 7-dehydrocholesterol. It plays a major role in the pathophysiology of autoimmune diseases. Low seric levels of Vit D correlate with progression of NAFLD. The main target of this study was to show that dietary supplementation with VitD leads to the decrease of histological components in NAFLD / NASH and can improve hepatic function. This was a prospective-observational study that explores the impact of low-fat control diet (LFD) or high-fat/high-sugar Surwit diet (HFSD) and vitamin D3 association in inflammation of steatosis and liver fibrosis. The study was conducted on mice for a 16-week period. The quantity of vitamin D administrated was 500 UI per kg which represents half of the amount recommended by the National Research Council. The results showed a that preventive administration of high dose of Vit D on HFSD mice has counteract gain and adipose tissue expansion, improving liver steatosis, and led to reduced overall NASH activity with a non-significant liver fibrosis remission. In recent years, the treatment for NASH is of great scientific interest, and the information from this study can be extrapolated to the general population, as a way of reducing steatosis and inflammation as well as liver fibrosis, because NAFLD is a major public health problem with an endemic manifestation (Jahn D, Dorbath D, Kircher S, *et al*. Beneficial effects of vitamin D treatment in an obese mouse model of non-alcoholic steatohepatitis. *Nutrients* 2019; 11 (77), doi: 10.3390/nu11010077).

EVALUĂRI ASUPRA REZULTATELOR OBŢINUTE ÎN TERAPIA PERITONITELOR ACUTE. EXPERIENŢA PE 317 CAZURI CLINICE ÎN SPITALUL JUDEŢEAN SUCEAVA

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ASSESSMENT OF ACUTE PERITONITIS TREATMENT RESULTS. SUCEAVA COUNTY HOSPITAL 317 CASES EXPERIENCE (Abstract): The aim of this study was to identify prognostic factors for morbidity, mortality and long term outcome and to analyze the different management modalities for acute diffuse peritonitis. **Material and method**: A clinical retrospective study was carried out on a series of 317 cases with acute peritonitis; admitted in the General Surgery Department, Hospital of Suceava, between 2006-2009. **Results**: There were 212 men (67%) and 105 women (33%) aged between 1 and 89 years. Preoperative concomitant disease was present in 31% of patients: cardiac disease was most common. The most common etiology was acute appendicitis in children 74 of cases (27.6%). Overall, 44 patients died during hospitalization. The cause of death was sepsis and multiple organ failure in 30 patients; malignancy in 8 patients and cardiac failure in 6 patients. Source control at the initial operation was possible in 282 of the patients (89%), of those 28 patients (9%) needed re-intervention. In 32 patients (10%), source control was not possible at the initial operation. **Conclusion**: This study emphasis the importance of prompt surgical treatment and shows the impact of adapting antibiotic treatment. **Key words**: PERITONITIS, MORTALITY, SURGERY, ANTIBIOTHERAPY

INFLUENCE OF COMORBIDITIES ON QOL IN PATIENTS ON CHRONIC RENAL REPLACEMENT THERAPY

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INFLUENCE OF COMORBIDITIES ON QOL IN PATIENTS ON CHRONIC RENAL REPLACEMENT THERAPY (Abstract): Little is known about the quality of life (QoL) and survival in patients on chronic dialysis therapy in Romania. **Aim**: To investigate the impact of comorbidities on QoL in patients with end-stage renal disease (ESRD). **Material and methods**: The 254 patients aged 22-88 years old included in the study were divided into two group as follows: 243 hemodialysis (HD) patients and 11 peritoneal dialysis (PD) patients. QoL was measured by RAND 36-Item Health Survey 1.0 (SF-36). Scores for all dimensions are expressed on a 0-100 scale. Higher scores indicate better health status. Information on comorbid conditions and medical history were obtained from patients' medical and dialysis records. We focused on four chronic diseases: diabetes mellitus (DM), chronic liver disease (CLD), uremic anemia (UA) and oncologic diseases (OD). **Results**: Eighty-two percent of ESRD patients included in this study had at least one comorbidity. Uremic anemia was the most common secondary diagnosis in both groups (HD 63.1%, PD 54.5%) followed by CLD (HD 24.2%, PD 9.1%), DM (HD 14.3%, PD 18.2%) and OD (HD 4.1%, PD 9.1%). DM had a strong negative impact on the physical domain of QoL (p=0.05). **Conclusions**: Comorbidities seem to influence QoL in ESRD patients. **Keywords**: DIALYSIS THERAPY, QUALITY OF LIFE, DIABETES MELLITUS.

Health-related quality of life (HRQoL) is identified as a vital health outcome for studies evaluating the quality of healthcare, assessing the influence of illness and analyzing cost-effectiveness (1, 2). Various studies have shown that socioeconomic status (3), dialysis program, ethnic group, sex, mobility, comorbidities (e.g., diabetes), malnutrition, depression (4) and unsuccessful previous renal transplant affect the QoL of dialysis patients (5). DM is a major cause of ESRD and is recognized as an important comorbidity among ESRD patients; but the situation gets worse because DM has its own complications which affect the QoL of an individual. In Romania, inadequate dialysis, high depression rate, malnutrition and anemia (3) all affect the QoL of HD patients. Chronic disease have a strong impact on physical health, financial status, social life, and ability to perform activities of daily living, but no quantitative evaluation of any of these has been done in Romania. Therefore, the aim of this study was to investigate the impact of comorbidities on QoL of patients with ESRD, especially with respect to DM in HD patients.

MATERIAL AND METHODS

This prospective cross-sectional observational study was performed in in October 2015 in a single dialysis unit, B. Braun Avitum Botosani, Romania. It included a total of 254 hemodynamically stable patients divided into two groups: 243 (95%) patients on HD therapy and 11 (5%) patients on PD therapy. Inclusion criteria were: 1) on regular HD for more than three months; 2) age > 18 years; 3) no hospitalization or acute illness in the preceding 3 months; 4) no psychiatric disorders (such as mental retardation or dementia). Informed consent was obtained from all participants before enrolment in the study. All patients filled out the SF-36 questionnaire.

QoL was measured using the RAND 36-Item Short Form Health Survey (version 1.0) which assesses eight health concept: 1) Physical Functioning (PF); 2) Role limitation due to Physical Functioning (RPF); 3) Bodily Pain (BP); 4) General Health Perception (GH); 5) Vitality (VT); 6) Social Functioning (SF); 7) Role Limitation due to Emotional Functioning (RLE); 8) Mental Health. Raw score of each dimension are transformed into a score ranging from 0-100. Higher scores indicate better health. Information on comorbid conditions and medical history were obtained from each patient medical and dialysis records.

Statistical analysis was made with *SPSS 16.0* and a p value < 0.05 was considered significant. Baseline characteristics of the study group, analyzed using descriptive statistics, are presented as mean ± standard deviation (SD), as appropriate. We used the t-test and Mann Whitney non-parametric test (U) to compare the variables between both groups. Pearson's correlation is used to assess the magnitude and direction of association between comorbidities and SF-36 questionnaire. Kruskal-Wallis test was used for the comparative study of SF-36 scores between patients with different degrees of secondary affections.

RESULTS

Mean age of the study group sample was 57.79 ± 14.30 years, without significant group differences (p = 0.248). In HD group we identified a significantly inverse correlation between age and SF-36 scale scores (tab. I). Sex distribution was relatively balanced (55.1% males, 48.9% females). Females scored significantly lower for all SF-36 scales except for VT, MH and SF (tab. II, fig. 1). Mean dialysis duration was 64.78 ± 47.72 months and had no significant impact on QoL score (tab. III).

TABLE I

Pearson's correlation between age and SF-36 scale scores

AGE		PF	RPF	REP	VT	MH	SF	BP	GH	QoL
HD	Pearson's correlation	-.520**	-.406**	-.281**	-.440**	-.235**	-.439**	-.480**	-.361**	-.502**
	P	.000	.000	.000	.000	.000	.000	.000	.000	.000
	N	243	243	243	243	243	243	243	243	243
PD	Pearson's correlation	-.144	-.193	-.121	.072	.041	.288	.262	.203	.097
	P	.673	.570	.724	.834	.905	.391	.437	.550	.777
	N	11	11	11	11	11	11	11	11	11

HD- hemodialysis group; PD- peritoneal dialysis group; PF- physical functioning. RPF- role limitation due to physical functioning; REP- role limitation due to emotional problems. VT- vitality; MH mental health; SF-social functioning; BP-bodily pain; GH- general perception health.

TABLE II

Mann Whitney comparisons of SF-36 scores between sexes in HD group

SCALE	U	p	SCALE	U	p
PF	5782.00	0.005	MH	7118.00	0.715
RPF	6033.00	0.010	SF	6306.00	0.061
REP	6151.50	0.022	BP	5063.50	0.000
VT	7175.00	0.796	GH	6073.50	0.022
QoL	5763.50	0.004			

HD- hemodialysis group; PF- physical functioning; RPF- role limitation due to physical functioning. REP- role limitation due to emotional problems; VT- vitality; MH mental health. SF-social functioning; BP-bodily pain; GH- general perception health.

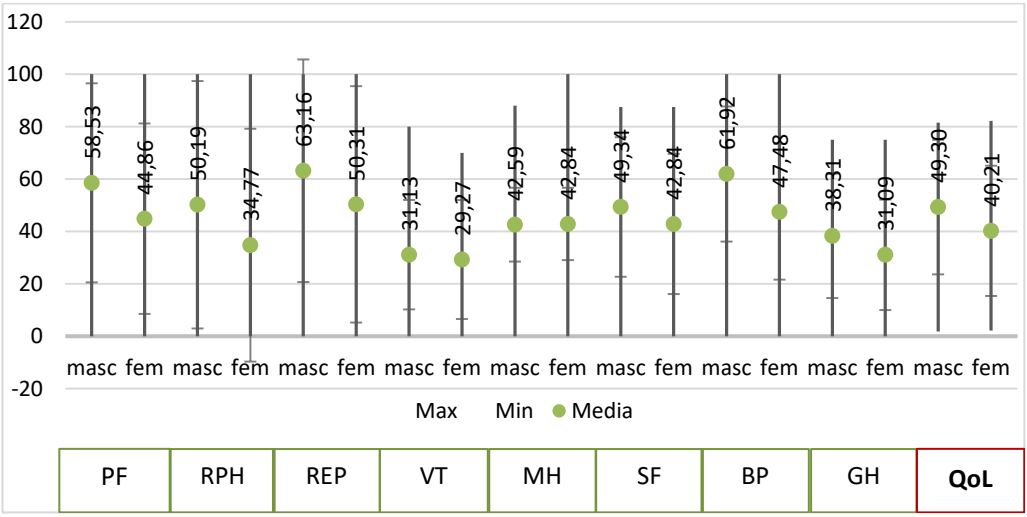

Fig. 1. Mean comparative scores of SF 36 scale between sexes in HD group (see tab. II)

TABLE III

Pearson's correlation between dialysis duration and SF-36 scale scores

Dialysis duration		PF	RPH	REP	VT	MH	SF	BP	GH	QOL
HD	Pearson's correlation	.018	.050	.056	.033	.027	.020	-.018	-.001	.029
	p	.775	.438	.388	.615	.678	.762	.779	.990	.659
	N	243	243	243	243	243	243	243	243	243
PD	Pearson's correlation	.026	.274	.665	.253	-.236	.296	-.483	-.522	.021
	p	.956	.553	.103	.585	.610	.519	.272	.230	.965
	N	11	11	11	11	11	11	11	11	11

HD- hemodialysis group; PD- peritoneal dialysis group. PF- physical functioning; RPF- role limitation due to physical functioning. REP- role limitation due to emotional problems; VT- vitality; MH mental health. SF-social functioning; BP-bodily pain; GH- general perception health.

Eighty-two percent of our study cases had at least one comorbidity besides the main diagnosis of ESRD (fig. 2). UA was the most common secondary diagnosis in both groups (HD 63.1% *vs.* PD 54.5%) followed by CLD (HD 24.2% *vs.* PD 9.1%), DM (HD 14.3% *vs.* PD 18.2%) and OD (HD 4.1% *vs.* PD 9.1%) (fig. 2). We investigated the impact of each comorbidity on SF-36 scales. We focused first on DM. In the majority diabetic HD patients (HD-DM+) the SF-36 scale scores were below 50 (PF, RPH, VT, MH, SF, GH general QoL score) (fig. 3). The most affected domain was PF which showed a statistically significant difference between non-diabetic and diabetic HD patients (54.37± 37.30 and 40.85± 38.75, respectively, p=0.05) (tab. IV). In PD patients diagnosed with DM there were non-systematic fluctuations of SF-36 scores compared to those without this additional diagnosis (fig. 4). REP remained almost constant, the most affected by the presence of this disease being the RPF score. In HD patients with CLD the only influenced scale was GH (p=0.04) (tab. IV). UA and OP showed no significant influence on QoL dimensions in both groups (tabs. IV, V).

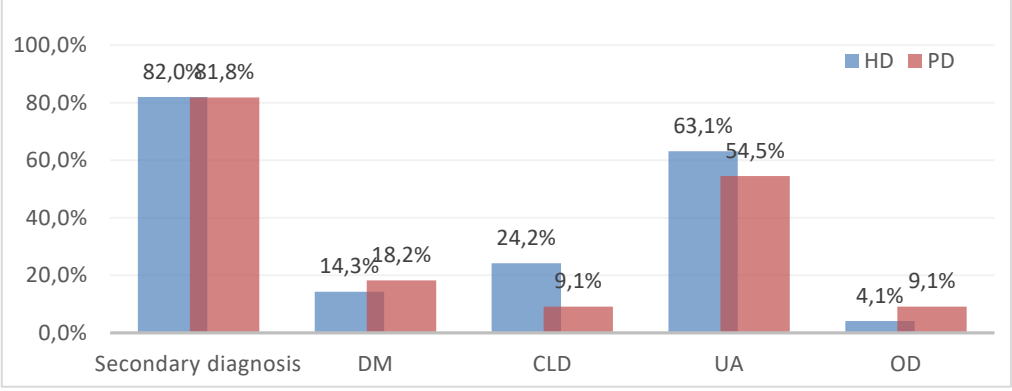

Fig. 2. Comorbidities - Comparative data between HD and PD groups

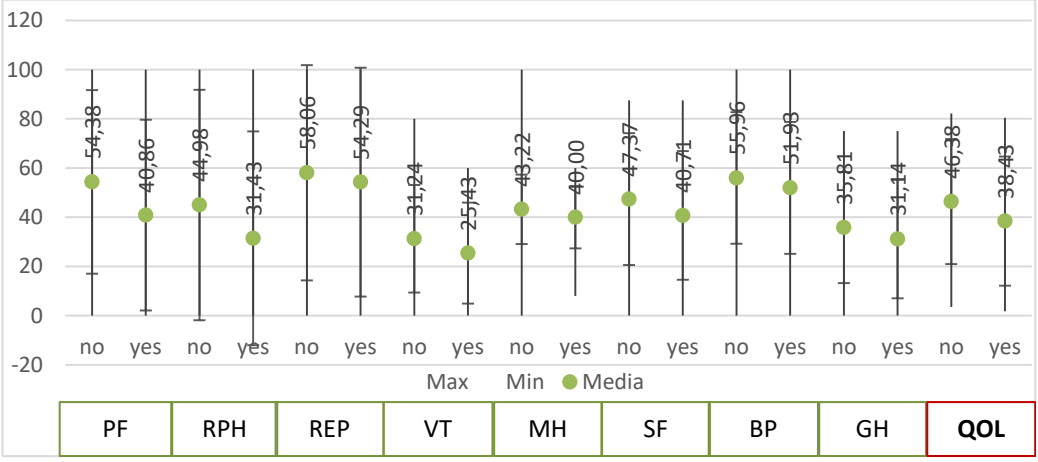

Fig. 3. SF-36 scales -Comparative mean data between diabetic and nondiabetic HD patients (see tab. IV)

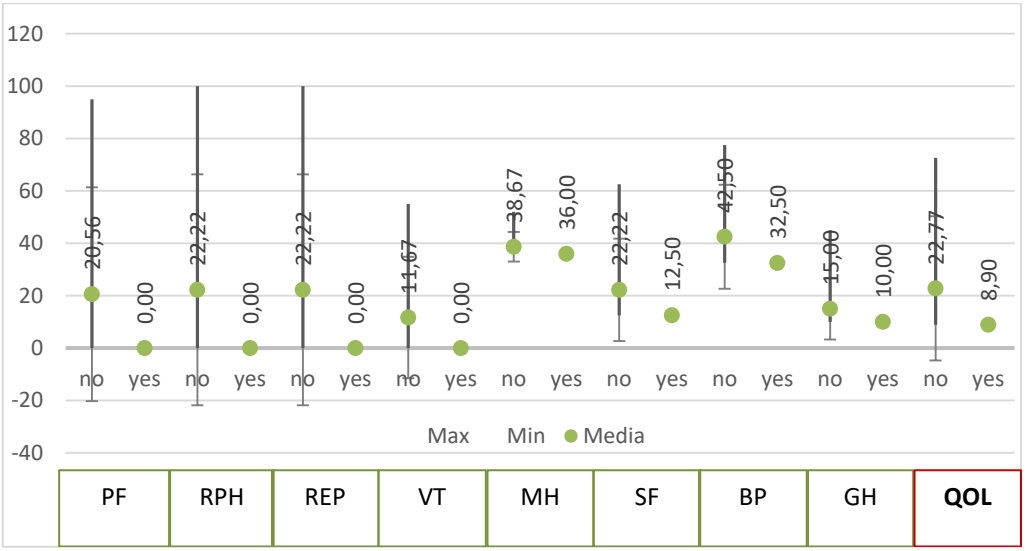

Fig. 4. SF-36 items - Comparative mean data between diabetic and non-diabetic PD patients (see tab. V)

TABLE IV

Comparison of SF-36 scale scores between HD patients with different comorbidities using Mann-Whitney test

SF 36 ITEMS	HD-DM- *vs...* HD-DM+	HD-CLD- *vs...* HD-CLD+	HD-UA- *vs...* HD-UA+	HD-OD- *vs...* HD-OD +
	p	p	p	p
PF	**0.051**	0.605	0.133	0.490
RPF	0.108	0.651	0.387	0.306
REP	0.644	0.380	0.677	0.635
VT	0.117	0.282	0.323	0.980
MH	0.323	0.257	0.171	0.470
SF	0.160	0.818	0.322	0.652
BP	0.489	0.291	0.733	0.417
GH	0.205	**0.043**	0.089	0.861
QoL	0.075	0.411	0.131	0.687

HD-DM-= non-diabetic HD patients, HD-DM+ = diabetic HD patients.
HD-CLD- = HD patients without chronic liver disease; HD-CLD+ = HD patients with chronic liver disease.
HD-UA-= HD patients without uremic anemia; HD-UA+ = HD patients with uremic anemia.
HD-OD-= HD patients without oncologic disease; HD-OD+-= HD patients with oncologic disease.

TABLE V
Comparative mean data of SF-36 scores between PD patients with different comorbidities

SF 36 Scores	PD-DM-	PD-DM+	PD-CLD-	PD-CLD+	PD-UA-	PD-UA+	PD-OD-	PD-OD +
PF	65.56	75.00	68.00	60.00	67.00	67.50	68.00	60.00
RPF	52.78	37.50	50.00	50.00	55.00	45.83	45.00	100.00
REP	66.68	66.70	66.68	66.70	73.36	61.12	63.35	100.00
VT	63.33	57.50	61.50	70.00	64.00	60.83	63.50	50.00
MH	73.78	76.00	75.20	64.00	71.20	76.67	76.40	52.00
SF	77.78	75.00	76.25	87.50	77.50	77.08	78.75	62.00
BP	75.00	66.25	73.00	75.50	86.50	62.50	73.00	77.50
GH	58.89	67.50	60.50	60.00	63.00	58.33	62.00	45.00
QoL	66.72	65.18	66.39	66.96	69.70	63.73	66.25	68.38

PD-DM-= non-diabetic PD patients, PD-DM+ = diabetic PD patients; PD-CLD- = PD patients without chronic liver disease; PD-CLD+ = PD patients with chronic liver disease; PD-UA-= PD patients without uremic anemia; PD-UA+ = PD patients with uremic anemia; PD-OD-= PD patients without oncologic disease; PD-OD+= PD patients with oncologic disease.

DISCUSSION

Chronic diseases like diabetes mellitus, liver disease or oncologic diseases affect various aspects of human life (6). Long-term treatment, financial support and special care are required to deal with such diseases. In ESRD, the main goal of chronic dialysis therapy is to improve QoL, which is usually measured in terms of physical, psychological and social wellbeing. In this study we have evaluated HRQoL among chronic dialysis patients with different types of comorbidities by SF-36 questionnaire. Although HRQoL among patients undergoing chronic dialysis in our country was studied previously (7), there are no data regarding the influence of comorbid conditions on QoL in these group of patients. Our results show that the more chronic diseases the patient had, the more likely he/she was to have poor SF-36 scores. Yang *et al.* (8), in 2010, studied HRQoL in Singapore and revealed that low Charlson comorbidity index was significantly associated with better HRQoL in Asian patients with renal failure. Despite the fact that in our study age was not an exclusion criterion, significantly better scores on SF-36 scales in younger patients were not recorded. Dialysis duration followed a similar trend, and no significant correlation with SF-36 scales was found. On the other hand, males showed significantly higher scores for all SF-36 dimensions. Regarding the comorbid condition, it is known that diabetes is one of the leading cause of ESRD worldwide. We found that HRQoL and both its physical and mental health components were lower in diabetic HD patients compared to the non-diabetic HD group. Notably, the diabetic HD patients reported the lowest level of perceived physical functioning. The various diabetes complication, such as cardiac problems, peripheral vascular diseases and foot amputation, all contribute to impaired physical

abilities and consequently result in low QoL (9,10). In a recent study conducted in Iran (11), diabetic foot infection and catheter infection accounted for 11% and 14% deaths, respectively. With progression of dialysis therapy, the features of cardiac and vascular diseases accelerated in diabetics and it has been reported that the frequency of diabetic foot became 2-5 times higher in dialysis patients in contrast to pre-dialysis period (9,10). Martínez-Castelao *et al.* (12) also found that diabetic patients starting dialysis in Spain have lower HRQoL in contrast to non-diabetics. Diabetic dialysis patients are characterized by reduced self-rated physical health, high prevalence of diabetic complications, and sometimes a relatively good mental health.

Dialysis modality has been identified as a major risk factor of infection with hepatitis B virus (HBV) and hepatitis C virus (HCV), with significantly higher seroconversion rates in HD compared with PD patients (13, 14, 15). In our study, 24% of the HD patients were found to have HBV or HCV infection and only 9% in the PD group. When evaluating the impact of CLD on SF-36 scale scores, a significantly lower score was found only on general health perception. The cross-sectional study by Rostami *et al.* (16) which included 4101 patients from 103 dialysis units, showed that not only general health and physical activity were preserved but also health perception was better among viral hepatitis patients on HD. On the other hand, in another study performed in Turkey (17) which included only 165 HD patients of which 83 were anti-HCV antibody positive revealed that HCV infection impairs QoL, especially in mental aspects. It is important to mention that in our study the QoL of viral hepatitis patients on IFN (interferon) treatment was not determined. It is known that IFN has several adverse effects that can impair QoL during the treatment (18). Akyuz *et al.* (19) performed a study after IFN treatment to evaluate real QoL and revealed that general health and physical activity which were poor, and anxiety were higher in the IFN treated patients as compared with untreated patients. These contradictory results may be explained by the fact that HRQoL in HD patients with chronic hepatitis was evaluated by different questionnaires: Nottingham Health Profile which is a measure of distress in six domains (sleep, energy, pain, mobility, emotional reaction and social isolation) and SF-36, a general questionnaire with eight domains for measuring function and well-being.

CONCLUSIONS

In this study we have evaluated HRQoL among patients on renal replacement therapy with different types of comorbidities using SF-36 questionnaire. We found that lower HRQoL indicated more underlying comorbidities. Diabetes mellitus had a negative impact on physical functioning domain of QoL, possibly in the context of underlying vascular disease under the detrimental effect of uncontrolled glucose level. Chronic liver disease was found to affect only general health perception. Identifying these factors will inform the timely implementation of interventions to improve the quality of life of these patients. Given the small sample size, the data obtained by us in the peritoneal dialysis group are limited, and in order to assess QoL among this group of patients a multicentric study must be conducted.

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STUDY ON PREVALENCE OF ASSOCIATION BETWEEN METABOLIC SYNDROME COMPONENTS

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STUDY ON PREVALENCE OF ASSOCIATION BETWEEN METABOLIC SYNDROME COMPONENTS (Abstract)**:** In Europe, approximately 25% of population are estimated to have metabolic syndrome (MS), with similar prevalence values in Latin America. In general, the prevalence of the metabolic syndrome increases with age with nearly 40% of people aged 60 and older meeting the diagnostic criteria. **Material and methods**: The purpose of this study is to evaluate the prevalence of associations between metabolic syndrome components to find a pattern applicable to patients with this pathology. The study was conducted on a total of 1,528 patients over 18 months, from 01.06.2017 to 31.12.2018, within a general practitioner (GP) medical office, being an epidemiological case-control study. **Results**: Studying the distribution of *Harmonized I* diagnostic criteria in the research group, we obtained the following data: 15.7% of subjects were included into MS with three components (MS3); 6.9% of the participants fall into the MS with four components (MS4); 2.87% of the patients included in the study group all five components of the metabolic syndrome (MS5). Out of a total of 203 women diagnosed with MS, we identified the presence of five-component MS in 10.83% of MS patients, while 31.03% were diagnosed with MS4. **Conclusions**: Early diagnosis of MS by the family doctor may reduce the cardiometabolic risk, the costs it generates, and could increases patients' quality of life. **Keywords**: METABOLIC SYNDROME, OBESITY, CARDIOMETABOLIC RISK.

In Europe, approximately 25% of people are estimated to have metabolic syndrome (MS), with similar prevalence values in Latin America (1). It is also considered an emerging epidemic in developing East Asian countries, including China, Japan, and Korea (2, 3). The prevalence of metabolic syndrome in East Asia may range from 8-13% in men and between 2-18% in women, depending on the population and definitions used. This is believed to be due to the "westernization" seen in recent years in the Asian continent, pertaining mainly to a diet high in carbohydrates and fats and a less active, sedentary lifestyle (4, 5, 6).

In general, the prevalence of the metabolic syndrome increases with age with nearly 40% of people aged 60 and older meeting the diagnostic criteria. Due to the increase in pediatric obesity and diabetes mellitus, the metabolic syndrome is becoming more prevalent in younger population thus it is no longer considered a disease of the elderly (7, 8).

Currently, there are two ways to control cardiometabolic risk factors included in MS: lifestyle management (weight loss by fighting sedentary disease and adopting an antiatherogenic and hypocaloric diet) and drug therapy (9, 10). Although drug therapy aims to control cardiometabolic risk factors according to medical guidelines adopted, achieving therapeutic targets is difficult to obtain. Under these conditions, the introduction of new diagnostic and therapeutic methods based on the etiopathogenesis of MS could improve the quality of life of MS patients and would lead, in the long run, to an increase in average life expectancy (11, 12).

The aim of this study is to evaluate the prevalence of associations between metabolic syndrome components to find a pattern applicable to patients with this pathology.

MATERIAL AND METHODS

The study was conducted on a total of 1,528 patients over 18 months, from 01.06.2017 to 31.12.2018, within a general practitioner (GP) medical office, being an epidemiological case-control study. For the inclusion of patients in metabolic syndrome, the *Harmonized I* diagnostic criteria were applied.

According to *Harmonized I* , MS is defined by the presence of at least three of the five components of the metabolic syndrome: central obesity; triglycerides ≥ 150 mg/dL (1.7 mmol/L) or specific treatment; HDL - cholesterol < 40 mg/dL (1.03 mmol/L) in men and < 50 mg/dL (1.29 mmol/L) in women or specific treatment; systolic blood pressure ≥ 130 mmHg and / or diastolic blood pressure ≥ 85 mmHg or specific treatment; Glycemia *à jeune* ≥ 100 mg/dL (5.6 mmol/L) or diabetes mellitus previously diagnosed. Abdominal circumference (AC) was not included as a mandatory diagnostic criterion.

The components of the metabolic syndrome research used the clinical and laboratory data obtained by clinical examination and laboratory determinations.

The inclusion criteria in the study were: age over 18 years, voluntary participation in the study and MS diagnosed based on the Harmonized criteria I. Exclusion criteria from the study were: age under 18 years, denial to participate in research and women during pregnancy or lactation.

The study protocol was applied according to the recommendations of the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy Iasi, a biding with the ethic norms of the European Community with regard to the investigations carried out on the patients.

Statistical analysis. For statistical processing of the studied parameters we used the programs *Microsoft Office Excel Medcalc Statistical Software 97-2003, SPSS version 16* (Statistical Package for Social Sciences) and the application - 14.3.3. The t-Student test was used to compare the continuous variables in the studied population and comparing the category variables we performed with the Chi square test. In order to compare the proportions calculated in the study we determined the z - test. Were mentioned both the 95% confidence intervals (CI) and the statistical signifi-

cance thresholds (p). P value less than 0.05 was considered statistically significant.

To determine the MS score we applied the diagnostic criteria and we calculated the MS score for each subject: 0 - the absence of the criterion, 2 - the presence of the criterion. The MS score had values in the range of [6-10].

Depending on the number of existing diagnostic criteria, patients who presented three criteria (MS score = 6) were diagnosed with MS with three components (MS3), those who presented four criteria (MS score = 8) were included in MS with four components (MS4), and those who presented all five diagnostic criteria (MS = 10) were classified as MS5 with five components (MS5). MS calculated average score according to the *Harmonized I* criteria was 3.16.

RESULTS

Since our study investigated the prevalence of metabolic syndrome in the population of a general practitioner (GP) medical office in Iasi, Romania, we preferred the use of the *Harmonized I* definition for MS because it includes criteria adapted to a unitary approach of MS, thus allowing to study an increased number of patients diagnosed and who are following correct treatment according to Medical Practice Guidelines. We obtained a 25.39% metabolic syndrome prevalence, in line with reported data for our geographical area.

Studying the distribution of *Harmonized I* diagnostic criteria in the research group we obtained the following data: 15.7% (95% CI: 13.83-17.87) of subjects were included into MS with three components (MS3); 6.9% (95% CI: 5, 55 - 8.23) of the participants fall into the MS with four components (MS4); 2.87% (95% CI: 2.09-3.86) of the patients included in the study group all five components of the metabolic syndrome (MS5) (tab. I).

TABLE I

Distribution of subjects according to the *Harmonized I* criteria

Gender	Classification of subjects according to *Harmonized I*				Total
	Non-MS	MS3	MS4	MS5	
F	610	118	63	22	813 (53%)
M	530	123	41	21	715 (47%)
TOTAL	1,140	241	104	43	1,528

Analyzing the data from the previous table, we calculated that out of 185 male (12.1%) patients diagnosed with MS, 11.35% were classified as MS5, 22.16% in MS4 and 66.49% were diagnosed with MS3.

Regarding female patients, out of a total of 203 (13.3%) women diagnosed with MS, we identified the presence of five-component MS in 10.83% of MS patients, while 31.03% were diagnosed with MS4. A percentage of 58.14% of females which were enrolled in MS had three components of metabolic syndrome (MS3) (fig.1). Also, for each association of MS components diagnosed according to the Harmonised I criteria, we calculated the prevalence within MS (tab. II).

Analyzing the MS components in the 388 (25.4%) patients diagnosed with MS according to the *Harmonized I* criteria, we found that among all components central obesity is the best represented (80.72%, 95% CI: 76.44-84.52), followed by hypertension (78.92%, 95% CI: 74.53-82.87), hypertriglyceridemia (69.15%, 95% CI: 64.30-73.71), hypo-HDL-cholesterolemia (61.23%, 95% CI: 58.76-68.54) and hyperglycemia (56.81%, 95% CI: 51.73-61.79).

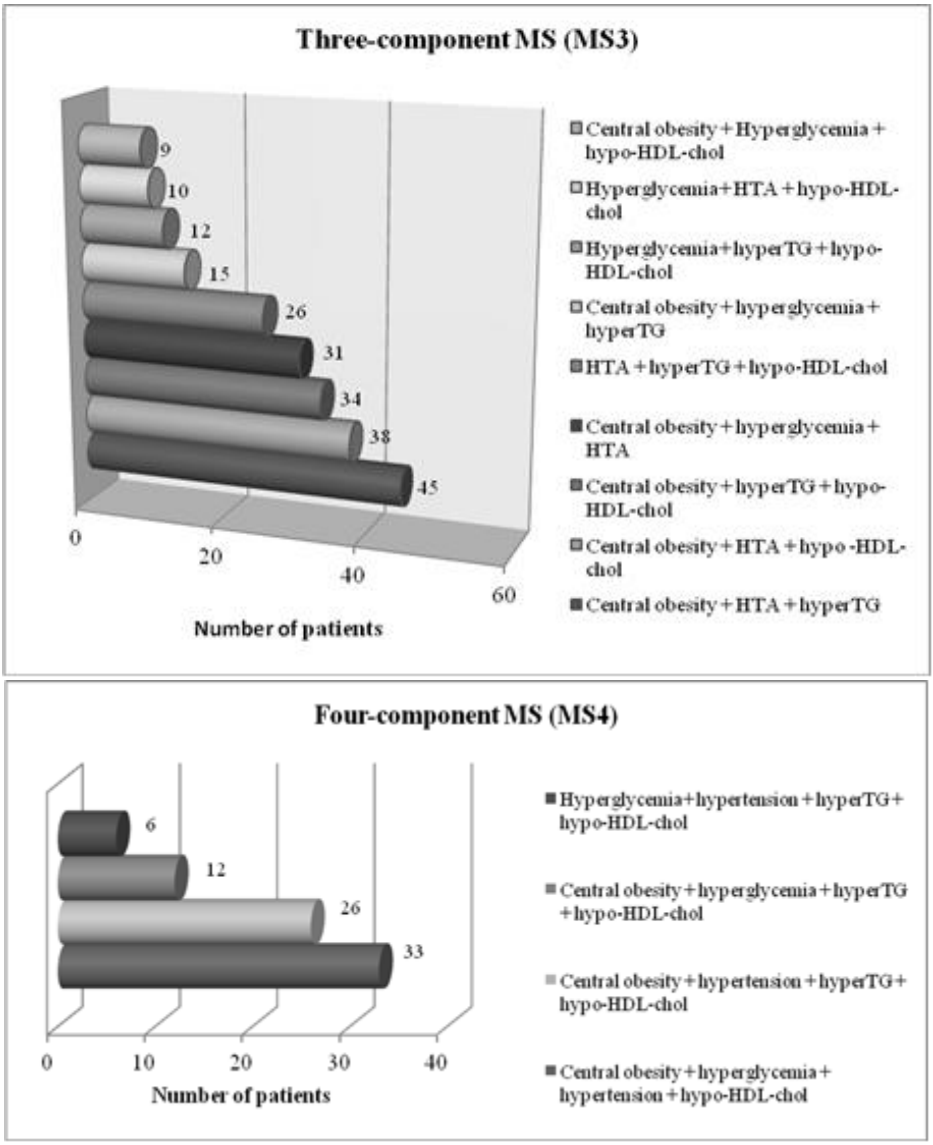

Fig. 1. Associations within the metabolic syndrome
*The chart does not include the 43 patients with MS with 5 components

TABLE II

Prevalence of associations between MS components (according to the *Harmonized I* criteria)

	Number of patients	The percentage in MS patients (95% CI)	The prevalence in the study population (95 % CI)
Three-component MS (MS3)	240	61.95% (54.38–70.29)	15.75% (13.83-17.87)
Central obesity + hypertension + hypertriglyceridemia	45	11.54% (8.44–15.48)	2.94% (2.14-3.93)
Central obesity + hypertension + hypo -HDL-cholesterolemia	38	9.79% (6.91–13.40)	2.48% (1.75-3.40)
Central obesity + hypertriglyceridemia + hypo-HDL-cholesterolemia	34	8.74% (6.05–12.21)	2.22% (1.53-3.10)
Central obesity + hyperglycemia + hypertension	31	7.96% (5.41–11.31)	2.02% (1.37-2.87)
Hypertension + hypertriglyceridemia + hypo-HDL-cholesterolemia	26	6.68% (4.36 –9.79)	1.69% (1.11-2.49)
Central obesity + hyperglycemia + hypertriglyceridemia	15	3.85% (2.15-6.36)	0.98% (0.54-1.16)
Hyperglycemia+ hypertriglyceridemia + hypo-HDL-cholesterolemia	12	3.08% (1.59-5.38)	0.78% (0.40-1.37)
Hyperglycemia+ hypertension + hypo-HDL-cholesterolemia	10	2.57% (1.23–4.72)	0.65% (0.31-1.20)
Central obesity + Hyperglycemia hypo-HDL-cholesterolemia	9	2.31% (1.05–4.39)	0.58% (0.26-1.11)
Four-component MS (MS4)	105	26.74% (21.84-32.39)	6.79% (5.55-8.23)
Central obesity + hyperglycemia + hypertension + hypo-HDL-cholesterolemia	33	8.22% (5.62-11.16)	2.09% (1.43%- 2.95)
Central obesity + hypertension + hypertriglyceridemia + hypo-HDL-cholesterolemia	26	6.42% (4.15- 9.48)	1.63% (1.05-2.41)
Central obesity + hyperglycemia + hypertriglyceridemia + hypo-HDL-cholesterolemia	12	3.08% (1.59-5.38)	0.78% (0.40-1.37)
Hyperglycemia+ hypertension + hypertriglyceridemia + hypo-HDL-cholesterolemia.	6	1.54% (0.56- 3.35)	0.39% (0.14-0.85)
Five-component MS (MS5) Central obesity + Hyperglycemia + hypertension + hypertriglyceridemia + hypo-HDL-cholesterolemia	43	11.31% (8.22-15.18)	2.87% (2.09-3.86)
TOTAL (MS3 + MS4 + MS5)	388	100%	25.42% (22.96- 28.08)

DISCUSSION

The diagnosis of MS involves highlighting its components. Different European and international commissions have proposed various diagnostic criteria, mainly determined by the specializations and concerns of the members of the respective groups.

The MS is a complex pathophysiologic state that originates primarily from an imbalance of calorie intake and energy expenditure but also affected by genetic/epigenetic make up of individual, predominance of sedentary lifestyle over physical activity, and other factors like quality and composition of food and composition of gut microbiota (13).

MS is also defined as the coexistence of abdominal obesity, atherogenic dyslipidemia, high blood pressure and insulin resistance. In a study, Grundy consider that obesity, especially abdominal obesity, is an essential component of metabolic syndrome (14). Abdominal obesity is a most common component of MS in our study, the data being in accordance with those found in the literature.

The condition is progressive, starting with values of risk factors at the boundary line of normal and possibly progressing to categorically pathological values. The MS has been associated with a variety of individual variables, including demographics, lifestyle, clinical measures and physical performance (15).

Civil societies, outside of the strict medical community, need to help disseminate the awareness of the huge impact of MS on the overall health in the global community.

Literature data show that MS is about three times more common than diabetes and the global prevalence can be estimated to be about one quarter of the world population. In other words, over a billion people in the world are now affected with SM (14). In our study it is a less frequent component, the data not being in accordance with those found in specialized literature.

Patient motivation leading to improved lifestyle adherence is a key factor in achieving reduction in metabolic syndrome components (16).

CONCLUSIONS

Applying the MS score along with the implementation of an electronic diagnostic record contributes to rapid, efficient and standardized information processing and to accurate diagnosis of MS in primary care. In this study, most of the patients were diagnosed with three-component metabolic syndrome, followed by patients with four and five component metabolic syndrome. We found that among all components, central obesity is most commonly represented, followed by hypertension, hypertriglyceridemia, hypo-HDL-cholesterolemia and hyperglycemia. Early diagnosis of MS by the family doctor may reduce the cardiometabolic risk, the costs it generates, and could increases patients' quality of life.

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PRIMARY CUTANEOUS PERIVASCULAR EPITHELIOID CELL TUMOR (PEComa). CASE REPORT AND REVIEW OF THE LITERATURE

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PRIMARY CUTANEOUS PERIVASCULAR EPITHELIOID CELL TUMOR (PEComa). CASE REPORT AND REVIEW OF THE LITERATURE (Abstract): PEComas represent a family of uncommon mesenchymal tumors that can develop on the skin and also can affect internal organs with a distinct immunophenotype that typically shows both myogenic and melanocytic differentiation. We present the case of a 49 years old female patient, from urban area, who was hospitalized in Dermatology Clinic Craiova for the presence of a 2 cm diameter tumor, round shaped, reddish-pink, with smooth surface, located on the internal site of the right thigh. The diagnosis of primary cutaneous PEComa was confirmed histopathologicaly and immunohistochemically and from the clinical point of view it represents a challenge for the dermatologist. **Keywords:** PEComa, PERIVASCULAR EPITHELIOID CELL TUMOR, DERMOSCOPY.

PEComas (perivascular epithelioid cell tumors) are mesenchymal tumors that can develop on the skin and also can affect internal organs. Those tumors have histological and immunohistochemical common features. PEComas are tumors expressing both melanocytic and myogenic markers with non-specific clinical features (1-3).

CASE REPORT

We present the case of a 49 years old female patient, from urban areas, was hospitalized in Dermatology Clinic Craiova for the presence of a 2 cm diameter tumor, round shaped, reddish-pink, with smooth surface, located on the internal site of the right thigh (fig. 1).

Fig. 1. Clinical image

Past medical history: appendectomy at six years old. She first noticed the tumor about a year ago. The evolution was to slowly increase in size. The patient did not follow any local or general treatment. At clinical examination we found a patient with BMI = 22.3 kg/m^2, Photo type II, without any pathological changes.

During hospitalization, the following investigations were performed:

Dermoscopic examination revealed a pale pink tumor, with the absence of structural elements (fig. 2).

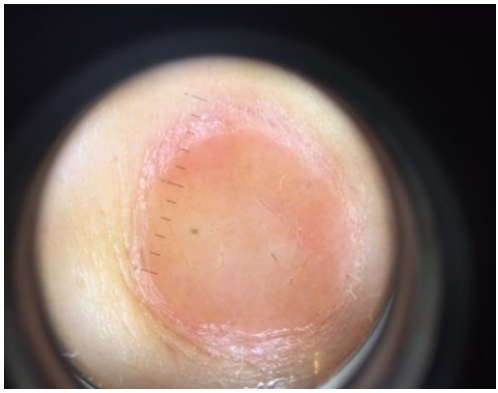

Fig. 2. Dermoscopic features: pale pink tumor, with the absence of structural elements

Under local anesthesia with lignocaine 1%, tumor excision was performed, with oncological safety margins, suture and dressing. The tumor was sent for microscopic examination.

Histopathology revealed atrophic epidermis, dermis and hypodermis cell proliferation with clear cytoplasm, some bi- and multinucleate in a fine collagenous stroma (fig. 3). Immunohistochemical examination is recommended for establishing the origin of the tumor.

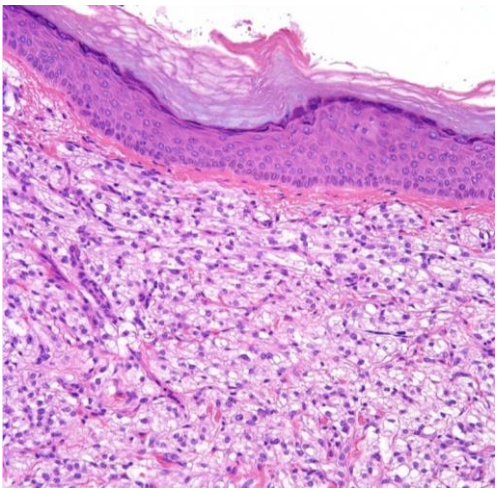

Fig. 3. Histopathological aspects of PEComa

Atrophic epidermis, dermis and hypodermis cell proliferation with clear cytoplasm, some bi- and multinucleate in a fine collagenous stroma

Immunohistochemical examination revealed tumor proliferation composed from epithelioid cells with abundant pale eosinophilic cytoplasm, and slightly hypertrophic, round-oval shaped nucleus, some with visible nucleoli; minimum tumor stroma with numerous thin-walled vessels. HMB45 (human melanoma black), CD44, CD10 were positive in tumor proliferation. S100, Melan A, cytokeratin AE1 / AE3, CK8 / 18, p63, SMA (smooth muscle actin specific), ER (estrogen receptor), chromogranin, synaptophysin were negative in tumor cells. CD34 was positive in numerous intratumorally vessels. Ki67 (proliferation factor): 3%. Histopathological diagnosis is PEComa (perivascular epithelioid cell tumor).

We also performed abdominal ultrasound for our patient, which revealed in the liver the presence of multiple hyperecho-

genic images with hemangiomatous aspect with maximum size of one cm; uterus increased in size; without fluid in the abdominal cavity; without lymphadenopathy. It is recommended to perform thorax, abdomen and pelvis CT. In our case no pathological changes were found.

Based on the medical history, physical examination and investigations, we established the diagnosis of Primary cutaneous perivascular epithelioid cell tumor (PEComa). Differential diagnosis, for our case, was made with dermatofibroma, achromic melanoma and cutaneous metastases. Treatment and management consisted of surgical excision of the tumor. The patient will be assessed every 6 months in the first year after surgery and annually on the long term.

DISCUSSION

In 1996 Zamboni introduces the term PEComa which contain a group of tumors formed from perivascular epithelioid cells located in different organs and tissues, including the skin (4). Tumor origin is explained by three hypotheses: origin from cells with common features with undifferentiated cells developed from neural crest; myoblast origin, with a molecular mutation triggering melanogenesis; pericytes origin (vascular cells).

Primary cutaneous PEComas are rare tumors, usually located on the lower limbs but cases have been described on the upper limbs and thorax. They are usually found in adult women. In a review of literature published in 2016, 34 cases of primary cutaneous perivascular epithelioid tumor were described (5, 6). The evolution of cutaneous PEComas is usually benign, but cases with malignant evolution were described. Folpe has described criteria for malignant PEComas (2005), which include: tumor diameter more than 5 cm; infiltrative tumor growth with high nuclear grade; the presence of necrosis; mitotic activity; tumor with aggressive clinical evolution. The tumor is considered benign when no malignant criteria are present. When there are two or more criteria present, the tumor is considered malignant. If one criteria is present, the risk of malignancy is uncertain (5, 7).

PEComas diagnosis is certified with histopathology and immunohistochemistry examination. In terms of histopathology are well defined dermal tumors composed of epithelioid cells with rich, clear or slightly granular cytoplasm, with central nucleus. The cells are arranged perivascular (4, 8, 9, 10).

Immunohistochemically is characteristic that tumors are positive for melanocytic markers: HMB 45 = human melanoma black (the most sensitive and is expressed by all PEComas published), Melan A, and MITF (microphthalmia transcription factor) and smooth muscle markers: desmin and SMA. Primary cutaneous PEComas are negative for epithelial markers (4, 9, 10, 11).

Charli-Joseph Y *et al.* on a clinicopathological and molecular reappraisal analyzed the clinicopathological features of 8 primary cutaneous PEComas. They were evaluated for DNA copy number changes and for initiating mutations common in melanocytic neoplasms. Primary cutaneous PEComas were assessed using immunohistochemistry, DNA sequencing and comparative genomic hybridization. There were no chromosomal copy number changes or initiating mutations were identified. Primary cutaneous PEComas are a diagnostic challenge, so it was propose that MIT fac-

tor, NKIC3, SMA, desmin, bcl-1, cathepsin K, and 4E-binding protein 1 to be used when evaluating a possible primary cutaneous PEComa (12). Stuart reported 5 additional cases of this rare entity. All showed classic histologic features and expression of either HMB-45 and/or Melan-A/MART-1 (13).

PEComas differential diagnosis can be made with dermatofibroma - the clinical aspect is of brownish tumor, often located on the legs and is slowly growing; cutaneous metastasis - the incidence is 0.7 to 5% of all cancers internal; achromic melanoma represents 10-12% of de novo melanomas; *Xhantomas tuberosus* - dysmetabolic yellowish painless tumors, associated with hyperlipidemia (6, 14, 15, 16). So far, the curative treatment of primary skin PEComas is represented by surgical excision (6).

CONCLUSIONS

The diagnosis of primary cutaneous PEComas is confirmed histopathologicaly and immunohistochemically and from the clinical point of view represents a challenge for the dermatologist.

Given the possibility of malignant evolution, patients will be follow-up continuously after the surgical cure of the tumor.

Our case had a benign evolution, without the presence of malignant signs at 6 months after the tumor excision.

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NEWS

A REVIEW OF PATIENT AND PROVIDER SATISFACTION WITH TELEMEDICINE

According to the Office of the National Coordinator for Health Information Technology, telemedicine (TM) can be defined as "the use of electronic information and telecommunications to support and promote long-distance clinical health care, patient and professional health-related education, public health and health objective measures including clinical outcomes, cost (both direct and indirect), and availability. In addition, patients and providers need to be at least as satisfied with their telehealth experience as they are with in-person visits. If this is not achieved, they both will refuse to use TM and as a result, adoption of the new technology will languish. The use of TM will expand only if patients and providers are at least as satisfied with it as they are with in-person visits. Since deviations from expected care can result in reduced satisfaction regardless of the quality of the visit or objective medical outcomes, it is important to understand and to help form those expectations when possible. Patients consistently report 95-100% satisfaction rate with TM when compared with in-person appointments. They tend to cite the convenience of decreased travel times and costs as the main drivers for satisfaction with TM. Providers tend to be satisfied with TM if they have input into its development, there is administrative support, the technology is reliable and easy to use, and if there is adequate reimbursement for its use. Satisfaction with TM is necessary for adoption of this new technology. To improve satisfaction, it is important to consider factors that drive it both for patients and for providers. (Nguyen Mary, Waller Morgan, Pandya Aarti, Portnoy Jay. *Current Allergy and Asthma Reports* 2020; 20(11): 72)

CARDIAC PACING IN ACUTE RIGHT VENTRICULAR MYOCARDIAL INFARCTION

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CARDIAC PACING IN ACUTE RIGHT VENTRICULAR MYOCARDIAL INFARCTION (Abstract): Isolated acute right ventricular myocardial infarction is rarely seen, being associated in 30-50% of cases with acute inferior myocardial infarction, providing an increased mortality rate. Due to coronary anatomy, an acute occlusion of the culprit artery at different levels may produce conduction disturbances that require temporary or permanent cardiac pacing. Furthermore, adding biventricular dysfunction it may generate cardiogenic shock. **Material and methods:** Our study analyzes the conduction disturbances requiring cardiac pacing in patients with acute right ventricular myocardial infarction admitted in "Prof. Dr. George I. M. Georgescu" Cardiovascular Diseases Institute, Iași, between January 2013 and January 2018. **Results:** 41 patients from 183 with acute right ventricular myocardial infarction presented significant conduction disturbances, 33 requiring cardiac pacing. 48.48% of them had acute occlusion of proximal right coronary artery, having significant statistical association (p=0.0414), requiring cardiac pacing and conduction disturbances (p<0.001). 15.15% of patients presented cardiogenic shock having in most cases acute occlusion of proximal right coronary artery. **Discussion:** Patients with acute right ventricular infarction present in their evolution conduction disturbances related to the occlusion of the proximal right coronary artery in most cases, associating right ventricle dysfunction, our study results being in concordance with literature data. **Conclusions:** Our study, even being an observational one, shows that the presence of a right ventricular myocardial infarction must alert the medical teams in order to consider a possible temporary cardiac pacing to provide a safe transportation to PCI center and a permanent pacemaker after myocardial revascularization.
Keywords: CARDIAC PACING, RIGHT VENTRICULAR MYOCARDIAL INFARCTION.

Acute right ventricular myocardial infarction is associated in 30-50% of cases with acute inferior myocardial infarction (1), meanwhile isolated right ventricular infarction is rarely seen (2).

Being still an underdiagnosed pathology, the association between acute inferior myocardial infarction and right ventricular infarction has a higher mortality comparing with the cases without right ventricle in-

volvement (25-30% versus 6%) (3).

The responsible coronary artery for right ventricular infarction (*"culprit lesion")* is in majority of cases the right coronary artery or the left circumflex artery, depending on the coronary dominance (4).

Studies that include patients with inferior and right ventricle myocardial infarction revealed a high incidence of sustained ventricular tachycardias (16%), with a high rate of conduction disturbances, including complete atrioventricular block (17%) and severe bradycardia (9%), cardiac pacing being necessary in 18% of cases (5).

The anatomical explanation is that the right coronary artery irrigates not only the sinoatrial node and a part of the atria (6), but also the atrioventricular node, therefore an acute occlusion of the upper segment will block the normal impulse pathway to the ventricles. The rapidly revascularization of the artery may avoid a pacemaker implantation (7, 8).

A metanalysis that analyzed the right ventricular myocardial infarction involvement revealed a higher incidence of ventricular tachyarrhythmias and conduction abnormalities in patients with acute inferior and right ventricular myocardial infarction, also with an increased rate of cardiogenic shock and death (9).

Another study showed a higher percentage of cardiogenic shock in the right ventricle involvement and inferior myocardial infarction compared with single inferior myocardial infarction (32% *vs.* 5%) (10), which may be explained by a low biventricular output that is related to a lower heart rate (significant bradycardia or bradyarrhythmia) associated with extensive infarcted area and with proximal occlusion of right coronary artery (11).

Echocardiographic parameters such as tricuspid annular plane systolic excursion (TAPSE) is used to evaluate the right ventricular function, with a cut-off value of 17mm (12), being an easy and feasible measurement parameter, but it mostly measures the longitudinal ventricular contraction. Doppler tissue measurements and right ventricle fraction area change are needed, as well as, for more accurate diagnosis echo procedure, the right ventricle longitudinal strain (13).

MATERIAL AND METHODS

Our study analyzes the incidence of conduction disturbances that require cardiac pacing in patients with right ventricular infarction, corroborating with coronary angiography, cardiac performance parameters and mortality.

We included patients admitted in the Surveillance and Advanced Treatment Unit for Critically Cardiac Patients from "Prof. Dr. George I. M. Georgescu" Cardiovascular Diseases Institute, Iași, between January 2013 and January 2018.

The including criteria consisted in patients with acute right ventricular myocardial infarction and we excepted from analysis acute coronary syndromes other than acute ST- elevation right ventricular myocardial infarction and patients with history of surgical myocardial revascularization. We continued to select the patients that were temporary and/or permanent cardiac electrical stimulated, analyzing the survival rate, the coronary arteries involvement, cardiac performance, TAPSE and the need of surgical revascularization.

Statistical Analysis

Data analysis was performed using *SPSS version 25* software (IBM, NY). Quantitative variables were reported as

mean values ± standard deviation, meanwhile qualitative as case numbers and percent (n %). The qualitative variables association was measured using *Chi-square Pearson* or Yates tests. The comparison of quantitative variables was evaluated based on the *Mann-Whitney* U Test results. Survival analysis was calculated using *Kaplan-Meier* evaluation. For each time moment death rate probability was calculated. Signification value level (p - value) calculated in the performed tests was considered for $p < 0.05$.

RESULTS

A total of 183 patients with acute inferior and right ventricular myocardial infarction were included, 72.13% male.

The mean age of patients was 64.8±11.6 years with significant values (p = 0.000005) smaller in men (62.5±11.5) comparing with women (70.9±9.4). 22.4% (41 cases) from them presented significant conduction disturbances, 37 patients (20.2%) having complete heart block and 4 patients (2.18%) with high degree atrioventricular block.

A group of 33 patients (18.03%) required cardiac pacing, having a mean age of 70.21±10.3, with minimum values of 46 years and maximum of 86 years (fig. 1). Mean hospitalization period was 9.18±4.5 days and 63.63% of them were men (21/33 cases).

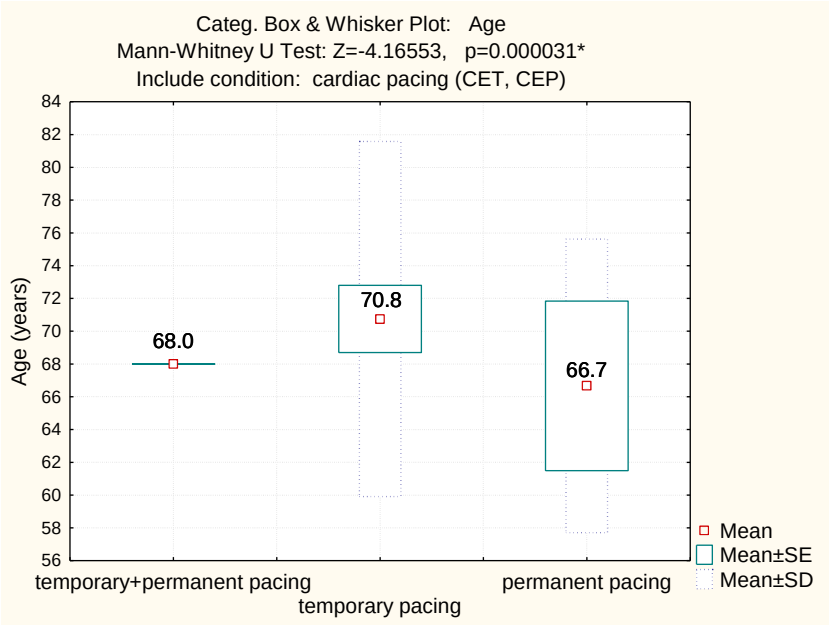

Fig. 1. Mean age value depending on cardiac pacing type

30 temporary pacing procedures were performed, 29 for complete or high degree atrioventricular blocks and 1 for ventricular tachycardia (with antiarrhythmic treatment being necessary), and from them 2 were permanently stimulated. Furthermore, 5 patients required permanent pacing, 3 of them without previous temporary support.

In the cardiac paced group, the most frequent comorbidities were arterial hyper-

tension (51.51%), diabetes mellitus (33.33%, insulin dependent (9.09%) and under oral antidiabetic drugs (18.18%)), and chronic kidney disease (36.36%). However, it was not found a significant association between comorbidities and required cardiac pacing (tab. I).

16 patients from those requiring cardiac pacing (48.48%) had acute proximal right coronary artery occlusion, meanwhile 1 patient (3.03%) presented a 99% stenosis at the same site.

7 patients (21.21%) were diagnosed with acute occlusion of the right coronary artery in the second segment of the vessel and two other patients (6.06%) were diagnosed with 95% and 99% stenosis of the same segment. Ultimately, the distal involvement of the right coronary artery was seen in 4 patients (12.12%).

Acute occlusion of the proximal right coronary artery presented a significant association (p=0.0414) with requiring cardiac pacing (tab. II), being also demonstrated a significant association with conduction disturbances (p<0.001).

TABLE I

Univariate and multivariate logistic regression analysis for cardiac stimulation required depending on the presence of comorbidities

Comorbidities *vs.* required cardiac pacing	Univariate logistic regression				Multivariate logistic regression			
	Odd ratio	C.I. 95%		*p*-value	Odd ratio	C.I. 95%		*p*-value
		Lower	Upper			Lower	Upper	
Arterial hypertension	1.541	0.896	1.993	0.6346	1.102	1.031	1.982	0.2263
Diabetes mellitus	1.116	0.943	1.541	0.3402	1.001	0.943	1.514	0.4571
Chronic kidney disease	1.087	0.174	1.274	0.2163	0.847	0.776	1.771	0.3402
(*) Marked effects are significant at p<0.05								

TABLE II

The Evaluation of association between Acute occlusion of the proximal right coronary artery and Conduction disturbances that required cardiac pacing

Requiring cardiac pacing	Acute occlusion of the proximal right coronary artery		Test Statistic (t) ‡	*p*-value*
	Yes (n=61)	No (n=122)		
Temporary pacing / Permanent pacing, n (%)	16 (26.23%)	17 (13.93%)	4.1590	0.0414*
Temporary pacing, n (%)	16 (26.23%)	14 (11.48%)	6.4588	0.0132*
Permanent pacing, n (%)	2 (3.28%)	3 (2.46%)	0.1028	0.7591
	Conduction disturbances			
	Yes (n=41)	No (n=142)		
Temporary pacing / Permanent pacing, n (%)	28 (68.29%)	5 (3.52%)	78.205	<0.001*
Temporary pacing, n (%)	26 (63.41%)	4 (2.82%)	80.872	<0.001*
Permanent pacing, n (%)	3(7.32%)	2(1.41%)	4.1793	0.4092*
(‡) Yates Chi-square *(*) Marked effects are significant at p<0.05*				

The coronary angiography also revealed the implication of the left anterior descending artery in 24.24% cases (8 patients) and the left circumflex artery in 21.21% cases (7 patients), in addition to the right coronary artery lesions. Tricoronary involvement was seen in 15.15% cases, left main stenosis in 6.06% and the need of cardiac bypass procedure in 9.09% of the total number of patients requiring cardiac pacing.

Left ventricle ejection fraction varied between 15% and 56%, with a mean value of 38.92%±11.25 (mean± standards). Also, mean TAPSE value was 12.42±3.21 mm.

TABLE III

Lifetable for the evaluation of the post-therapy survival rate in the study groups

Lower Limit For Time (days)	Cumulative Proportion Surviving (%)	
	Temporary pacing	Permanent pacing
1.0	100	100
2.6	92.72	100
4.3	92.72	100
6.0	88.41	100
7.6	88.41	100
9.3	**82.52**	100
11.0	82.52	100
12.7	82.52	100
14.3	82.52	100
16.0	82.52	100

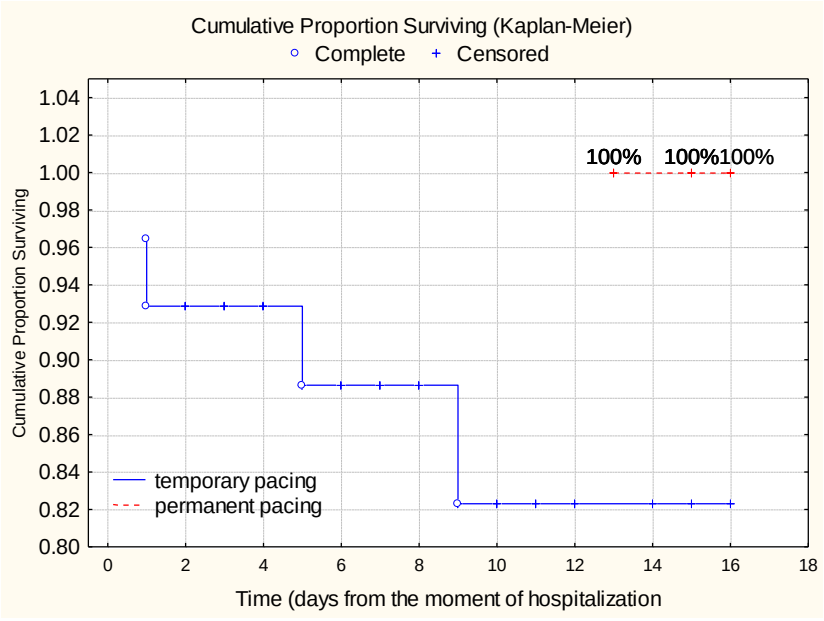

Fig. 2. The Kaplan-Meier survival curves. Survival rate estimation (days) from the admission time depending on the required cardiac pacing

15.15% of cases presented with cardiogenic shock (15/33 cases), 4 of them with acute proximal occlusion of the right coronary artery and the fifth case with acute occlusion of the circumflex artery and 90% stenosis of the right coronary artery in the second segment.

The survival rate in cardiogenic shock was 100%, PCI procedures with drug eluting stents being performed. In this group of 5 patients the mean value of left ventricle ejection fraction was 31.872%±12.8, with a mean TAPSE value of 11±2.82 mm.

The survival rate in temporary paced patients was 82.52%, at 16 days of admission, meanwhile in permanent pacing 100% (tab. III, fig. 2).

7 patients did not undergo stenting procedure due to the fact that 3 of them refused the intervention, 1 case was unsuccessful because of the inadequate coronary anatomy, 1 with cardiac surgery indication, 1 with unsignificant atherosclerotic lesion (40%) and atrial fibrillation and 1 with severe systolic disfunction and cardiac arrest at admission with no response on cardiopulmonary resuscitation.

DISCUSSION

Patients with acute inferior and acute right ventricular myocardial infarction present in their evolution a high incidence of conduction disturbances1 that may be correlated with the occlusion of the culprit artery which in most cases is the right coronary artery.

In our study the highest incidence of conduction delays in patients with acute right ventricular myocardial infarction was seen in cases with proximal occlusion of the right coronary artery, in concordance with literature data (14).

Also, cardiogenic shock was seen in 15.15% of the patients that needed cardiac pacing, associated in most cases with acute proximal occlusion of the right coronary artery, with a mean TAPSE value of 11±2.82 mm, comparing with patients without cardiogenic shock, where TAPSE was 12.42±3.21 mm.

The main consequence of the association of acute right ventricular infarction with acute inferior myocardial infarction is the wider necrotic field process of the myocardial tissue, thereby the necessity of sustaining drugs and filling improvement for maintaining an appropriate cardiac pump function increase.

We believe that the presence of the acute right ventricular myocardial infarction must alert the medical team to be prepared for an eventual transcutaneous or transvenous electrical cardiac pacing, in order to provide a safe transportation to the nearest PCI center and to predict a potential need for a permanent pacemaker.

CONCLUSIONS

Our study has limitations and strengths, being an observational study on patients with acute inferior and right ventricular myocardial infarction, without having a comparator.

In our opinion, acute right ventricular myocardial infarction associated to acute inferior myocardial infarction brings a high rate of conduction disturbances and biventricular disfunction, which may generate the appearance of cardiogenic shock.

Its rapid diagnosis in prehospital phase may improve patients short- and long-term outcomes, but further studies are yet to come.

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THE RISKS OF HEPATOCELLULAR CARCINOMA AND VARICEAL BLEEDING AFTER HCV ERADICATION WITH DIRECT-ACTING ANTIVIRAL THERAPY: A PROPENSITY SCORE ANALYSIS

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THE RISKS OF HEPATOCELLULAR CARCINOMA AND VARICEAL BLEEDING AFTER HCV ERADICATION WITH DIRECT-ACTING ANTIVIRAL THERAPY: A PROPENSITY SCORE ANALYSIS (Abstract): The combination paritaprevir/ritonavir, ombitasvir, and dasabuvir (PrOD), which achieves a high sustained virologic response (SVR) rate and few side effects, has dramatically changed the outcome of hepatitis C virus (HCV) infection. The risk of hepatocellular carcinoma (HCC) and variceal bleeding in patients treated with interferon (IFN)-free direct-acting antivirals (DAAs) is unknown. The aim of this prospective study was to evaluate the rate of HCC and variceal bleeding and identify prognostic factors for decompensation/complications in HCV-1b-infected patients with compensated liver cirrhosis following treatment with PrOD with or without ribavirin. **Material and methods**: A total of 483 HCV patients received PrOD treatment. Patients with a history of HCC were excluded from the study. Prior antiviral therapy was identified in 59% of patients. Patient data were collected at baseline, at the end of treatment (EoT), at 3 months after EOT (SVR) and during an 18-month surveillance period. Patients were divided into two age groups: <60 years (233 patients) and ≥60 years (250 patients). **Results**: The median observation period was 540 days. During this period, HCC developed in 18 patients (3.7%) and variceal bleeding developed in 9 patients (1.9%). A Propensity score matching analysis found no significant difference in the incidence of HCC (p= 0.078) or variceal bleeding (p=0.426) between the two groups. The mean serum AFP levels decreased in the young group (baseline=19.44 ng/dL, SVR=4.57 ng/dL) and the elderly group (baseline=4.52 ng/dL, SVR=10.69 ng/dL). Edge of decompensation and previous esophageal varices were significantly associated with the risk of variceal bleeding. **Conclusions:** The risk of HCC (5.3%) and variceal bleeding (2%) development in elderly patients (≥60years) following viral eradication by IFN-free DAA therapy may follow a specific pattern. **Keywords:** DIRECT ACTING ANTIVIRALS, HEPATITIS C VIRUS, HEPATOCELLULAR CARCINOMA, VARICEAL BLEEDING.

The treatment of hepatitis C virus (HCV) with direct-acting antivirals (DAAs) has significantly changed the approach to patient selection. Interferon (IFN)-free regimens offer an important benefit for patients who are difficult to treat such as liver cirrhosis (1). DAAs have been found to be safe and effective in all patients, including those with hypoalbuminemia or thrombocytopenia, with the exception of patients with decompensated cirrhosis (2, 3). For the 1b genotype, which represents approximately 75% of all infections, an IFN-free therapeutic regimen achieved a sustained virologic response (SVR) in more than 95% of cases considered difficult to treat (elderly patients and those with compensated or decompensated liver cirrhosis, renal failure, or obesity) (4, 5). Based on data reported by clinical trials, SVR correlates with a decrease in mortality, a survival rate in formerly HCV-infected patients comparable to that of the general population, amelioration of portal hypertension, and reduced risks of hepatic disease decompensation and hepatocellular carcinoma (HCC) (6). Among cirrhotic patients with HCV, an annual incidence of HCC of up to 7% was reported (7). Some studies found that IFN- and DAA-based antiviral treatments reduced the risk of HCC (7-9), whereas others found no effect (9). The risk of variceal bleeding after DAA treatment remains unknown.

The aim of this study was to evaluate the risk of HCC and variceal bleeding and identify prognostic factors for decompensation/complications in HCV-1b-infected patients with compensated liver cirrhosis following treatment with paritaprevir /ritonavir, ombitasvir, and dasabuvir (PrOD) with or without ribavirin.

MATERIAL AND METHODS

Patients selection

Between December 1, 2015 and July 31, 2016, patients eligible for the National Insurance House reimbursement programme on PrOD treatment were prospectively enrolled via a regional antiviral therapy register. Patients with compensated HCV cirrhosis (Child-Pugh class A), genotype 1a or 1b, and severe liver fibrosis (METAVIR score 4) were eligible. Patients previously treated with IFN and ribavirin who recorded a lack of virologic response, virologic breakthrough or relapses were considered for PrOD treatment. Patients with decompensated HCV cirrhosis (defined as the presence of ascites, jaundice, variceal bleeding, hepatic encephalopathy; Child-Pugh class B or C) or the presence of HCC or dysplastic liver nodules were excluded. Only patients who achieved SVR were included in this study. We further defined a subgroup of patients who, at the time of therapy initiation, had characteristics that indicated potential decompensation-edge of decompensation group - Child-Pugh class A, score 6 and/or esophageal varices.

Treatment

Patients received the PrOD regimen, with or without ribavirin, for 12 weeks (in the case of genotype 1b HCV cirrhotic patients). The PrOD regimen administered daily included 25 mg ombitasvir, 150 mg paritaprevir, and 100 mg ritonavir in combination with 500 mg dasabuvir. Ribavirin doses of up to 1200 mg daily, adjusted according to hemoglobin level, were added.

Patients evaluation

Patients were evaluated at baseline, at the end of treatment (EoT), at SVR (12 weeks after the completion of treatment) and every 12 weeks until the end of the

follow-up (18 months post-SVR). Data collected prospectively included demographics, medical history, blood tests (full blood count, INR, ALT, AST, bilirubin, creatinine, AFP and HCV RNA quantitative test) and abdominal ultrasonography. The METAVIR score was evaluated using FibroMax™ or liver biopsy. FibroMax™ is a combination of five non-invasive diagnostic tests including FibroTest, ActiTest, SteatoTest, NashTest and AshTest. This method is used to diagnose hepatic fibrosis, viral necro-inflammatory activity, hepatic steatosis, severe alcoholic steatohepatitis (in alcoholic patients) and non-alcoholic steatohepatitis (in overweight, diabetic, or Insulin-resistant patients) (10). The FibroMax™ score is calculated using an algorithm based on a patients' sex, age, weight, height, and specific blood biomarkers (ALT, AST, GGT, haptoglobin, blood glucose, lipids, apolipoprotein A1, alpha-1-macroglobulin) (11).

Liver enzymes and quantitative viral load for HCV were re-evaluated at EoT and SVR (12). SVR was defined as undetectable HCV RNA at 12 weeks after the completion of treatment (11).

Patients found to have high AFP levels (≥20 ng/mL) underwent computed tomography (CT) and/or magnetic resonance imaging (MRI). Patients without suspicion of HCC according to the Liver Imaging Reporting and Data System (LI-RADS) criteria, or evidence of dysplastic nodules requiring surveillance, received antiviral treatment. During follow-up, HCC diagnosis required either a positive ultrasound with confirmation on a second dynamic imaging modality CT or MRI or a diagnostic biopsy (13). Patients who developed variceal bleeding were treated according to the current clinical guidelines (14). The diagnosis of variceal bleeding was confirmed by upper digestive endoscopy, performed in all patients in the first 12 hours from the bleeding event.

Patients were stratified into two groups based on age (<60 years and ≥60 years).

Outcomes

HCC occurrence and variceal bleeding were considered events of interest in both study groups.

Statistical analysis

Continuous variables were evaluated by descriptive statistics and described as the mean (±SD) or median.

Categorical values were expressed as percentages. Student's t-test was performed to compare the mean of continuous variables.

A propensity analysis was performed to match the patients one-to-one by age group (<60 years and ≥60 years (15, 16). Variables entered in the propensity model included BMI, serum albumin, platelet counts, leukocyte counts, AST, ALT, AFP and GGT levels, gender, edge of decompensation status, esophageal varices, comorbidities, T2DM and arterial hypertension.

The risks of HCC and variceal bleeding were analyzed using Cox multivariate analysis in the subgroup of patients matched by propensity score.

Statistical analysis was performed using *SPSS 20.0* software (SPSS Inc., Chicago, IL, USA). A p-value ≤0.05 was considered statistically significant.

This prospective study was performed according to the Declaration of Helsinki and international guidelines for observational studies. Written inform consent was obtained from each patient before starting the study. The study was approved by the National Ethics Committee of Medicines and Medical Devices (17).

RESULTS

Patient characteristics

A total number of 483 consecutive patients who completed the PrOD regimen were evaluated. The baseline characteristics of these patients are described in tab. I. All patients were diagnosed with severe liver fibrosis (F4 METAVIR score), and in 59.0% of cases, patients were previously treated with IFN. One hundred ninety-nine of cases (41.2%) were identified to be on the edge of decompensation. None of the patients were lost during the follow-up.

TABLE I

Baseline Characteristics of HCV Patients (n = 483)

Variable		Parameter
Gender	Male (n = 220)	45.5%
	Female (n = 263)	54.5%
Age (yrs.) (mean ± SD)		59.18 ± 8.30
Weight (kg) (mean ± SD)		76.79 ± 14.94
Height (cm) (mean ± SD)		165.96 ± 0.19
BMI (kg/m^2) (mean ± SD)		27.77 ± 4.19
Edge of Decompensation	Yes (n = 199)	41.2 %
	No (n = 284)	58.8 %
Esophageal Varices	Yes (n=135)	28.0%
	No (n=348)	72.0%
Comorbidities	Yes (n=215)	44.5%
	No (n=268)	55.5%

Patient characteristics by age group (<60 years and ≥60 years) are found in tab. II. There were no statistically significant differences between the two groups except for gender, comorbidities and arterial hypertension.

TABLE II

Clinical Characteristics of Young (<60 years) *vs.* Elderly (≥60years) Groups in 483 Unmatched Cases

Parameter	Young Group (<60 years old) n = 233	Elderly Group (≥60 years old) n = 250	p-value
Gender (Male/Female)	128/105	92/158	<0.001
BMI (kg/m^2)	28.30 (16.80-41.40)	27.12 (19.50-40.63)	0.367
Albumin (g/dL)	4.06 (3.10-5.25)	3.91 (3.04-4.97)	0.248
Platelet Count (x 10^3/mm^3)	142 (34-466)	128 (40-362)	0.44
RNA VHC (log/UI/mL)	5.71 (1.26-6.86)	5.64 (1.06-7.18)	0.473
Leukocyte (x 10^3/mm^3)	6030 (2280-10990)	5270 (1880-12140)	0.571
AST (IU/L)	94 (29-381)	86 (31-342)	0.776
ALT (IU/L)	97 (23-412)	86 (24-382)	0.074
AFP (ng/mL)	9.72 (1.57-131.20)	8.33 (1.13-53.37)	0.483
GGT (IU/L)	92 (17-455)	62 (12-279)	0.379

Parameter	Young Group (<60 years old) n = 233	Elderly Group (≥60 years old) n = 250	p-value
International Normalized Ratio (INR)	1.16 (0.90-4.07)	1.12 (0.92-7.79)	0.307
Edge of Decompensation (Yes/No)	36.6% / 61.4%	43.6% / 56.4%	0.262
Esophageal varices (Yes/No)	25.7% / 74.3%	30.0% / 70.0%	0.298
Comorbidities (Yes/No)	18.01% / 30.23%	26.50% /25.26%	0.002
T2DM (Yes/No)	6.21% / 42.03%	9.73% / 42.03%	0.082
Arterial Hypertension (Yes/No)	8.49% / 39.75%	17.60% / 34.16%	<0.001

The propensity score analysis found 461 matched cases (16 patient variables), where 230 were young patients and 231 were elderly patients (tab. III).

TABLE III

Clinical Characteristics of Young *vs.* Elderly Patient Groups in 461 Matched Cases

Parameter	Young Group (< 60 years old) N=230	Elderly Group (≥ 60 years old) N=231	p-Value
Gender (Male/Female)	126/104	92/139	0.002
BMI (kg/m^2)	28.10 (16.80-41.40)	27.05 (19.50-40.63)	0.32
Albumin (g/dL)	4.04 (3.10-5.25)	3.92 (3.04-4.97)	0.249
Platelet Count (x 10^3/mm^3)	141.5 (34-466)	134 (45-362)	0.472
RNA VHC (log/UI/mL)	5.72 (1.26-6.86)	5.66 (1.06-7.15)	0.489
Leukocyte (x 10^3/mm^3)	5990 (2280-10990)	5330 (2610-12140)	0.616
AST (IU/L)	94 (29-381)	81(31-342)	0.776
ALT (IU/L)	97 (23-412)	84(24-382)	0.096
AFP (ng/mL)	9.67 (1.57-131.20)	7.69 (1.13-53-37)	0.505
GGT (IU/L)	91 (17-455)	63(12-279)	0.373
International Normalized Ratio (INR)	1.16 (0.90-4.07)	1.12 (0.92-7.79)	0.187
Edge of Decompensation (Yes/No)	19.52 %/ 30.37 %	21.04 % / 29.07 %	0.57
Esophageal Varices (Yes/No)	13.02 % / 36.88%	14.10 % / 36.01%	0.675
Comorbidities (Yes/No)	18.66 % / 31.24%	23.64 % / 26.46%	0.038
T2DM (Yes/No)	6.51 % / 43.39 %	7.81 % / 42.30 %	0.506
Arterial Hypertension (Yes/No)	8.46 % / 41.43 %	14.32 % / 35.80 %	0.004

There was no significant intergroup difference in any of the analyzed parameters except gender and concomitant diseases. Ninety young patients and 109 elderly patients were on the edge of decompensation (p = 0.262).

HCC and variceal bleeding incidence

During the observation period, HCC developed in five out of 233 (2.14%) patients in the <60-year-old group and 13 out of 250 (5.2%) patients in the ≥60-year-old group (p=0.077). An analysis of the effect of gender revealed no significant differences between the ≥60-year-oldgroup (p=0.39) and the <60-year-old group (p=0.45). The *male : female ratio* of HCC incidence was 2.5 : 1. The distribution of

events of interest (HCC and variceal bleeding) in different study subgroups are presented in tab. IV and V.

The incidence of HCC was significantly elevated in patients with high levels of AFP (> 7 ng/mL). Mean serum AFP levels decreased in the < 60-year-old group from baseline (19.44 ng/mL) to SVR (4.57 ng/mL). The corresponding mean serum AFP levels found in the ≥60-year-old group were moderate (baseline 14.52 ng/mL, SVR 10.69 ng/mL).

TABLE IV

Annual risk rate of HCC post SVR

Age	N (%)	Edge of decompensation	Compensated	Previous IFN	No previous IFN
All (N; %)	483 (100%)	199 (41.20%)	284 (58.59%)	285 (59%)	198 (40.99%)
HCC (N; %)	18 (3.72%)	9 (4.52%)	9 (3.16%)	10 (3.05%)	8 (4.04%)
Annual Risk Rate (ARR; 95% CI)	0.41 (0.145; 1.207)	1.15 (0.687; 1.929)	0.89 (0.568; 1.411)	0.39 (0.166; 0.927)	1.91 (1.435; 2.546)
< 60 years old	233 (48,24%)	90 (18.63%)	143 (29.60%)	143 (29.60%)	90 (18.63%)
HCC	5 (2.14%)	1 (1.11%)	4 (2.79%)	4 (2.79%)	1 (1.1%)
Annual Risk Rate (ARR; 95% CI)	0.59 (0.281; 1.238)	0.51 (0.088; 2.967)	1.31 (0.839; 2.065)	0.98 (0.475; 2.019)	1.03 (0.348; 3.056)
≥60 years old	250 (51,76%)	109 (22.56%)	141 (29.19%)	142 (29.39%)	108 (22.36%)
HCC	13 (5.2%)	8 (7.33%)	5 (3.54%)	6 (4.22%)	7 (6.48%)
Annual Risk Rate (ARR; 95% CI)	1.40 (1.023; 1.940)	1.37 (0.830; 2.264)	0.72 (0.368; 1.430)	0.14 (0.022; 0.929)	2.22 (1.766; 2.800)

TABLE V

Annual risk rate of variceal bleeding post SVR

Age	N (%)	Edge of decompensation	Compensated	With esophageal varices	Without esophageal varices
All (N; %)	483 (100%)	199 (41.20%)	284 (58.79%)	135 (27.95%)	348 (72.04%)
Variceal Bleeding (N; %)	9 (1.86%)	9 (4.52%)	0	9 (6.66%)	0
Annual Risk Rate (ARR; 95% CI)	0.71 (0.148; 3.453)	0.56 (0.349; 0.907)		2.65 (0.746; 9.476)	0.52 (0.285; 0.970)
<60 years old	233 (48.24%)	90 (18.63%)	143 (29.60%)	60 (12.42%)	173 (35.81%)
Variceal Bleeding (N; %)	4 (1.71%)	4 (4.44%)	0	4 (6.66%)	0
Annual Risk Rate (ARR; 95% CI)	0.81 (0.327; 2.045)	3.00 (0.525; 17.149)	0.33 (0.088; 1.256)	1.50 (0.520; 4.323)	0.50 (0.106; 2.355)
≥60 years old	250 (51.75%)	104 (21.53%)	141 (29.19%)	75 (15.52%)	175 (36.23%)
Variceal Bleeding (N; %)	5 (2.0%)	5 (4.8%)	0	5 (6.66%)	0
Annual Risk Rate (ARR; 95% CI)	1.14 (0.590; 2.226)	0.11 (0.007; 1.177)	0.64 (0.435; 0.950)	0.33 (0.027; 4.186)	0.50 (0.296; 0.844)

Multivariate analysis revealed that probability of HCC development at 540 days was higher in the ≥ 60-year-old group (5.4%) than in the <60-year-oldgroup (1.7%) (Figure 1).

Propensity scores matching analysis found an increased probability of HCC development in the ≥60-year-old group (5.3%) compared to the <60-year-oldgroup (1.8%), with no significant differences observed between the groups (p=0.078) (fig. 2).

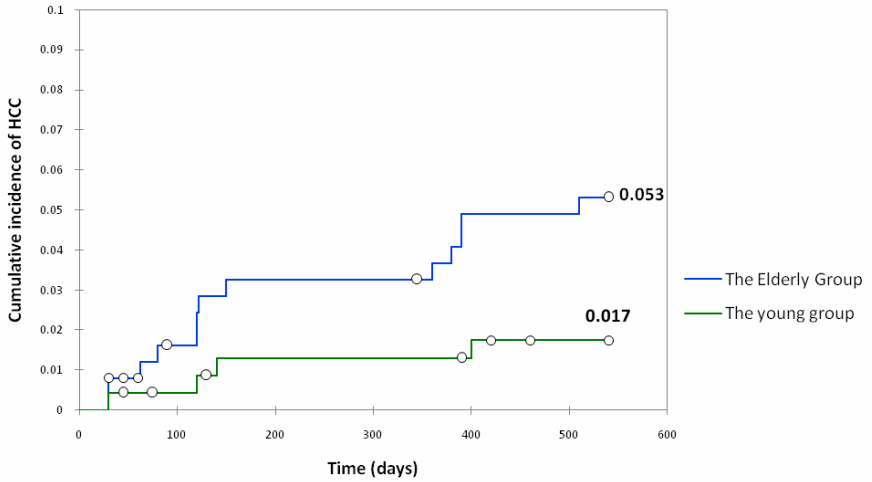

Fig. 1. Cumulative HCC Development-Before Propensity Score Matching

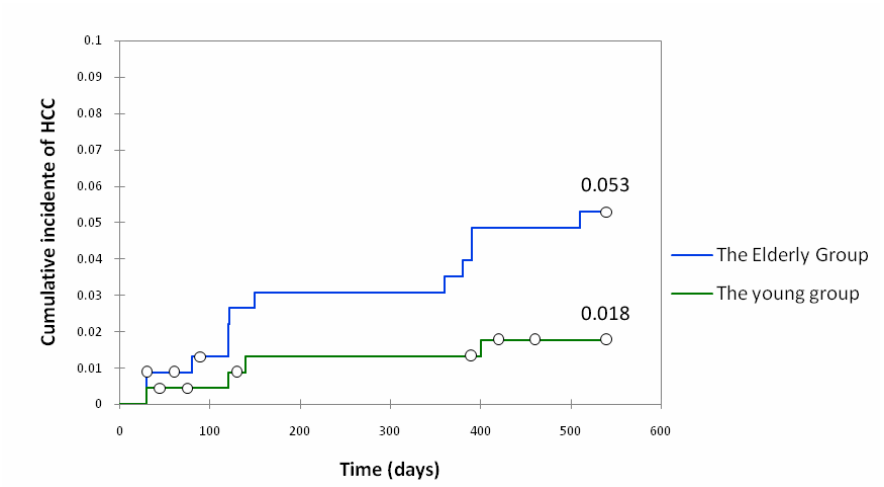

Fig. 2. Cumulative HCC Development in Propensity Score Matched Patients

Variceal bleeding was recorded in nine (1.9%) of all cases. None of the patients without previous described esophageal varices has bled. Esophageal varices at

baseline were found in 25.7% of <60-year-old patients and 30.0% of ≥60-year-old patients. There was no difference regarding the variceal bleeding rate between elderly group and the young group (2.0% *versus* 1.7%, p=0.818). Evolution of variceal bleeding was favorable under standard treatment and no mortality was recorded.

The edge of decompensation had significant influence on HCC development in elderly group. Eight patients (14.50%) in the elderly group were at the edge of decompensation and developed HCC *vs.* 1 patient (1.8%) in the young group (p=0.011).

DISCUSSION

Our study included a homogeneous large cohort of HCV genotype 1b liver cirrhosis patients, followed-up for 18 months in tertiary academic centers, aiming to evaluate the incidence and the risk factors of HCC and variceal bleeding development. Hepatocellular carcinoma (HCC) remains the most important life-threatening complication of HCV cirrhosis. IFN significantly reduced the risk of HCC in this category of patients; however, usage was limited by contraindications (depression, immune diseases). The development of new DAAs surpassed these limitations imposed by IFN usage. The risk of HCC associated with DAA therapy remains unclear. Several studies found that DAAs increased the risk of HCC recurrence (19, 20). These studies investigated HCC recurrence and not incidence, had a limited follow-up period and were underpowered. An extensive retrospective study included more than 3,000 HCV cirrhotic patients treated with either IFN or DAAs or a combination. During a 6.1-year follow-up period, DAA treatments reduced the risk of HCC up to 71%, with no difference between IFN and DAAs (9).

The most important risk factors associated with HCC in HCV cirrhotic patients were age, gender, excessive alcohol consumption, comorbidities (diabetes, obesity, renal diseases, lymphoma, HIV coinfection) (21).

Our study investigated the risk of HCC development in young (<60 years) and elderly (≥60 years) patient groups. During the 18-month follow-up period only 3.7% of cases developed HCC. The *male : female ratio* of HCC incidence was 2.5:1; however, the difference was not statistically significant, likely due to the relatively small sample size.

To overcome the bias due to differences in the distribution of covariates among young and elderly patients treated with PrOD, we created one-to-one matches using propensity score analysis. The model was used to obtain a one-to-one match according to the nearest neighbor matching method, resulting in a sample size of 230 patients per cohort.

The propensity score matching analysis showed an increased probability of HCC development in the elderly patients (5.3%) compared to the young patients (1.8%). The lack of statistical significance may be due to the higher number of females in the elderly patient group. Alpha-fetoprotein is considered an important risk factor for HCC in cirrhotic patients irrespective of cause (22). Long-term follow-up studies have found that approximately 1-8% of patients with cirrhosis develop HCC per year, 2% in HBV-infected cirrhotic patients and 3-8% in HCV-infected cirrhotic patients (23). We found a significant correlation between AFP values and the incidence of HCC (64% of HCC cases were noted in patients with a baseline AFP of more than 7

ng/mL). Edge of decompensation had a significant influence on HCC development.

The second most common cause of death in cirrhotic patients is gastrointestinal bleeding produced by esophageal varices effraction (24).

IFN prevented or delayed the development of de novo esophageal varices; however, its efficacy against gastrointestinal bleeding was not established (25). There was a lack of information regarding effectiveness of DAAs in prevention of variceal bleeding.

In Romania between 2015-2016, a single oral antiviral medication was available for the treatment of HCV, and National Insurance House reimbursement conditions were limited to patients with compensated liver cirrhosis (Child-Pugh class A) (26). The limiting condition for PrOD treatment was related to the demonstration of F4 fibrosis by FibroMax™ or liver biopsy. The majority of patients were evaluated by FibroMax™, and we included only patients with a value of over 0.75, considered a cut-off for an F4 METAVIR score. Patients who were at the limit of F4 on the FibroMax™ examination usually had hepatic stiffness values evaluated with Fibroscan in the clear liver cirrhosis spectrum. Using the FibroMax™ method permitted the inclusion of a population of patients with cirrhosis, sometimes at the edge of decompensation. In this case, the mean value of Fibroscan is above the limit that the Baveno VI (13) consensus considers indicative of clinically significant portal hypertension.

Furthermore, some patients already had esophageal varices (included in the Baveno II stage), some had platelet counts indicating portal hypertension or albumin values indicating a deficiency in liver albumin synthesis. The cohort of patients approved for PrOD treatment due to compensated status as defined by Child-Pugh scores allowed the admission of a wide spectrum of stages of liver cirrhosis into the study. Considering the grading of patients after FibroMax™ and Fibroscan, and adopting the proposed Baveno staging (13), we found that we treated compensated liver cirrhosis patients in a lower proportion than those at the limit of edge of decompensation.

In cirrhotic patients without varices, the rate of developing them was estimated at 5-8% per year (13, 27). Patients with small varices develop large varices at an annual rate of 8% as well (27). The risk of first variceal bleeding is higher in patients with larger varices, with red-signs and decompensated liver cirrhosis, and range between 6-15% per year (28). In our study the annual rate of the first variceal bleeding in patients who already had esophageal varices was 2.65% per year, with no significant difference between the elderly and the young group (p=0.918). No patient without previous described varices had any bleeding. The lower percentage of variceal bleeding after SVR than reported data from literature and the fact that none of patients without esophageal varices developed bleeding could suggest a tendency of improving portal hypertension after SVR and the risk of bleeding which, however, is not eliminated.

Our study has some strengths such as large number of patients, homogeneous groups of cirrhotic genotypes 1b cases, a high proportion at edge of decompensation and excellent adherence. However, the main limitation is due to short period of follow-up.

CONCLUSIONS

The risk of HCC (5.3%) and the vari-

ceal bleeding (2.0%) in elderly patients following viral eradication by IFN-free DAA therapy may follow a specific pattern, comprised of factors like the edge of decompensation, AFP levels, age, esophageal varices.

However, further larger cohort studies with longer follow-up are needed to characterize this pattern.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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NEWS

COVID-19: EVEN THROUGH DEATH WE ARE NO LONGER EQUAL?

A recent research paper entitled *"Racial disparities in COVID-19 mortality are driven by unequal infection risks"* made by a group of researchers led by *Jon Zelner*, after analyzing COVID-19 incidence and mortality data in the state of Michigan, signals a surprising paradigm shift: the unequal distribution of the risks of infection between different race- ethnic groups. The black population was the most affected group of all, with an age-standardized incidence of 1,626/ 100,000 and a mortality rate of 244/100,000. Using Hierarchical models of Bayesian regression for the data analysis and the posterior predictive controls for the results validations, dramatic disparities were found, the age-standardized incidence being 5.5 (95% CrI= 5.4, 5.6) and the mortality 6.7 (95% CrI= 6.4, 7.1) times higher for the Blacks, compared with the Whites. Zelner J, Trangucci R, Naraharisetti R, *et al.* Racial disparities in COVID-19 mortality are driven by unequal infection risks. *Clin Infect Dis* 2020. doi: 10.1093/cid/ciaa1723).

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CATARACT SURGERY IN PATIENTS WITH HISTORY OF UVEITIS - MEDIUM TERM FUNCTIONAL OUTCOMES

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CATARACT SURGERY IN PATIENTS WITH HISTORY OF UVEITIS- MEDIUM TERM FUNCTIONAL OUTCOMES (Abstract). **Aim**: Evaluating the functional outcomes in patients with history of uveitis who previously underwent cataract surgery during 2009-2019, analysis of the best postoperative visual acuity and IOP after first day, one month, 3 months and last postoperative. **Material and methods:** Comparative/retrospective study which included cataract surgery patients with a history of uveitis evaluated between January 2009 and December 2019 in Iaşi, Department of Ophthalmology, "Sf. Spiridon" County Clinical Emergency Hospital. The study included a number of 102 eyes divided into two groups: 59 eyes from 51 patients (25 women and 26 men) the cataract and uveitis group (study group) and 43 cataract eyes (control group). All patients had cataract surgery performed by phacoemulsification and IOL-PC implantation, while the pre- and postoperative evaluation was performed according to a standardized protocol. The database was built in the *EXCEL* program and was statistically processed with the *SPSS 18.0* program. Postoperative follow-up was performed at 1 day, 1 month, 3 months and last postoperative follow up (six month). **Results:** The average age of patients of the 2 groups was 65 years. In all cases, preoperative topical steroid anti-inflammatory treatment was administered for 1 week and postoperatively, for 6 weeks. General anti-inflammatory steroids (CSR) were administered preoperatively and postoperatively in 9 patients with intermediate or posterior uveitis for 8 weeks. In two cases a capsular tension ring was used to stabilize the bag. One case required ECCE + placement of IOL in the sulcus. Only one case required IOL explantation due to severe inflammation. Among the postoperative complications, the inflammatory glaucoma and recurrent uveitis predominated. **Conclusions:** Cataract surgery with IOL implantation resulted in improved postoperative visual acuity in patients with cataract and uveitis, which was maintained at the last follow-up. In the immediate postoperative period, both groups had similar functional results. **Keywords:** CATARACT SURGERY, UVEITIS, PHACOEMULSIFICATION, CYSTOID MACULAR EDEMA, INTRAOCULAR IMPLANT.

Cataract is a major cause of vision loss in patients with uveitis, up to 40% of cases being mainly due to cataracts. Frequent complication of uveitis occurs on the one

hand due to inflammation but also due to chronic treatment with corticosteroids (CSR). The incidence of cataracts in uveitis varies from 57% in pars planitis to 78% in Fuchs' heterochromic iridocyclitis. Cataract surgery in uveitic eyes is difficult and can have many unexpected intraoperative complications. This represents a challenge for the surgeon due to the particular existing situations, namely: small pupils, posterior iris synechiae, the presence of pupillary membranes, fibrous anterior capsule (1, 2). Until the advent of corticosteroids in the early 1960s, eye inflammation was often impossible to be controlled, and articles discussing the results of cataract extraction in swollen eyes reported a high incidence of severe complications (3). In many cases, complications resulted in marked reduction of vision or even loss of the eye. Amit K Reddy *et al.* in a study that included 3,013 eyes from 2,019 patients, found a low incidence (2%) of persistent postoperative uveitis, a 29% incidence of postoperative macular edema and no case of severe granulomatous uveitis. In general, postoperative uveitis is due to the presence of inflammation at the time of surgery, cortical debris after cataract surgery and intraoperative manipulation of the iris (retractors, stretching, sphincterotomies (4). More recent publications have reported a considerable decrease in the incidence of intraoperative and postoperative complications during cataract extraction in patients with uveitis. On the one hand, this is due to a very good control of preoperative inflammation and on the other hand, to the evolution of microsurgical techniques in recent years (4, 5, 6).

MATERIALS AND METHODS

It is a retrospective study that included cataract patients with a history of uveitis evaluated between January 2009 and December 2019 in the Department of Ophthalmology, from "Sf. Spiridon" County Clinical Emergency Hospital, Iasi. The study included a number of 102 eyes divided into two groups: 59 eyes from 51 patients (25 women and 26 men) the cataract and uveitis group (study group) and 43 cataract eyes (control group). Inclusion criteria for the study group were patients with uveitis who underwent cataract surgery. Presence of uveitis had to be confirmed prior surgery. There had to be at least three months with no inflammation in the absence of treatment before cataract surgery. Exclusion criteria: absence of associated uveitis; eyes with active inflammation or under corticosteroid treatment. Patients scheduled for cataract surgery had their surgery planned in advance. The surgery was performed under topical or parabulbar anesthesia. All patients underwent cataract surgery by phacoemulsification and IOL-PC implantation, the pre- and postoperative evaluation was performed according to a standardized protocol. In all cases, in the uveitis group, topical steroid and non-steroidal anti-inflammatory drugs (NSAIDs) were administered preoperative for 1 week and postoperative for 6 weeks; in some selected cases systemic anti-inflammatory corticosteroids were administered. Postoperative follow-up was performed at 1 day, 1 month, 3 months and last control after cataract surgery. The visual acuity was measured with the Snellen optotype, and the IOP was measured by Goldmann applanation tonometry (GAT); slit lamp evaluation of the anterior segment and posterior segment with the Volk 78D lens were also performed; OCT examinations were performed at Oftaprof Clinic

using Zeiss Cirrus HD-OCT to detect cystoid macular edema (CME).

Ethical Approval

During the study, the principles of the Helsinki Declaration were observed. Patients have signed an informed consent, which was attached to the observation sheets.

Statistical Analysis

The database was created in the *EXCEL* program and was statistically processed with the *SPSS 18.0* software.

RESULTS

102 eyes were divided into two groups: the study group (59 eyes from 51 patients) with cataract and uveitis and the control group (43 eyes) with cataract only.

Distribution of patients by study groups. Statistical analysis by age groups highlights the following aspects :

The mean age of patients with cataracts and uveitis (group I) was significantly lower than that of patients with cataracts alone (group II) (57.31 *vs.* 70.12 years).

- the highest frequency of patients with cataract and uveitis is found in the 60-69 age group (25.4%) and 70-79 years (41.9%)

- the incidence peak is noted in the 60-69 years (25.4%) in patients with cataract;

The average age of patients was 57.31 ± 16.30 years in those with uveitis and 70.11 +/- 11.37 in the control group, average of the groups being 65 years (fig. 1). In all cases, in the uveitis group, topical steroid anti-inflammatory and non-steroidal anti-inflammatory drugs (NSAID) were administered preoperative for 1 week and postoperative for 6 weeks, respectively. Systemic anti-inflammatory corticosteroids were administered preoperative and postoperative in 9 patients with intermediate or posterior uveitis for 8 weeks. In most cases the IOL was positioned in the capsular bag. In two cases capsular tension rings were used to stabilize the bag. One case required extracapsular extraction + placement of IOL in the sulcus. Phaco-trabeculectomy combined surgery was performed in 2 eyes. Only one case required IOL explantation due to severe inflammation. The maneuvers performed in case of intraoperative complications were: synechiolysis (in 17 eyes, 50.8%), tension ring implantation (2 eyes), small pupil requiring iris retractors, intracameral phenylephrine (mezatone) (14 eyes) and anterior vitrectomy (2 eyes) (fig. 2, tab. I). Posterior segment complications included: recurrent uveitis (23 eyes, 39%) at 3 months, inflammatory glaucoma (6 eyes, 11.9%) at 1 year, cystoid macular edema (4 eyes, 11.9%) at 6 months and opacification of the posterior capsule (4 eyes, 10.2%) at one year (fig. 3, tab. II).

In the first study group most uveitis was idiopathic (80%), followed by ankylosing spondylitis in proportion of 7.5% and other etiologies in smaller and equal proportions (sarcoidosis, TB, juvenile idiopathic arthritis, Fuchs' heterochromic iridocyclitis, etc.) (fig. 4).

In the first group (cataracts and uveitis) visual acuity varied preoperatively from 0.005 to 0.50; while at the last follow up the visual acuity ranged from 0.004 to 1.0. The mean level increased significantly at 1 day postoperatively by 145% ($p = 0.001$) and still by approximately 34% at 1 month postoperatively ($p = 0.001$). Patients gained on average 3 lines of visual acuity (fig. 5.) The mean intraocular pressure was 15.97 +/- 4.6 mmHg (SD) preoperatively, on the first postoperative day it was 16.8 +/- 5.02 mmHg (SD), and 16.12 +/- 3.89 mmHg (SD) at one month. IOP decreases at 1

month postoperatively by 4% (p = 0.520) and after 3 months the decrease in intraocular pressure from one moment to another was by about 8% (p = 0.146). The IOP changes were not statistically significant (fig. 6). In patients treated with topical steroids, the mean level of postoperative visual acuity was slightly lower (0.37 *vs.* 0.45; p = 0.477), while postoperative IOP was slightly higher (15.58 *vs.* 14.43; p = 0.526). The correlation between postoperative visual acuity and the number of anti-inflammatory drugs was direct, reduced in intensity (r = +0.196; p = 0.138), but statistically insignificant. Severity of postoperative inflammation obviously leads to the use of a higher number of anti-inflammatory drugs (postoperative visual acuity and the period of administration of anti-inflammatory drugs were apparently parametrically independent r = +0.094; p = 0.477).

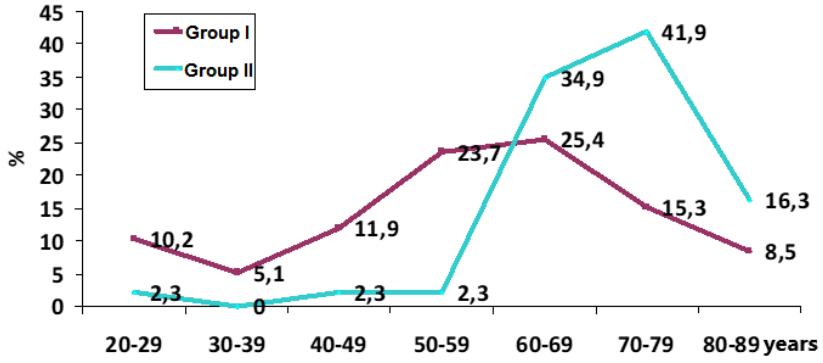


Fig. 1. Patients distribution by age group.
First study group (cataracts and uveitis) *vs.* second study group (only cataracts)

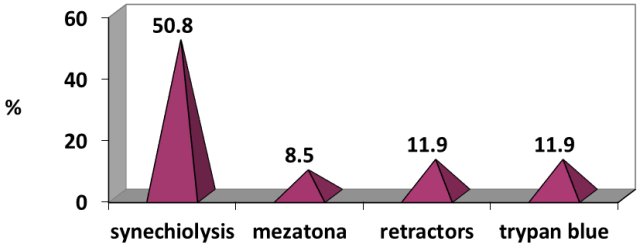


Fig. 2. Maneuvers in case of intraoperative complications

TABLE I

Maneuvers in case of intraoperative complications

Maneuvers in case of intraoperative complications	Synechiolysis	Tension ring implantation	Small pupil Iris retractors mezatona	Trypan blue
Eyes numbers	17	2	14	14

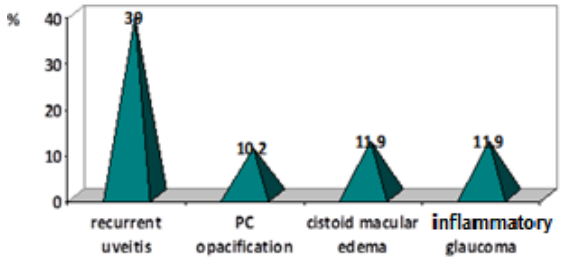


Fig. 3. Postoperative complications in the study group.

TABLE II

Postoperative complications in the study group.
CME- cystoid macular edema, PCO - posterior capsule opacification

Postoperative complications	Inflammatory Glaucoma	CME	PCO	Recurrent uveitis
Eyes numbers	6	4	4	23
Interval	1 year	6 months	1 year	3 months

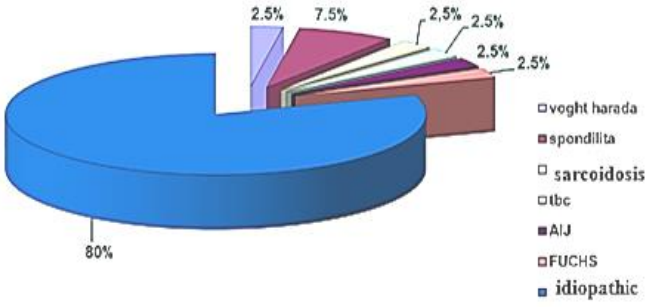


Fig. 4. Distribution of cases according to etiology of uveitis.
JIA- Juvenile Idiopathic Arthritis, TB - Pulmonary Tuberculosis

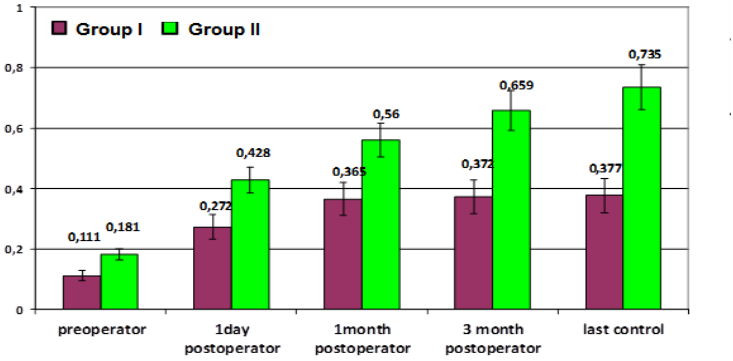


Fig. 5. Evolution of the mean level of visual acuity on study groups
The last control period (six month)

In the cataract control group, visual acuity varied preoperatively from 0.004 to 0.50; whereas at the last follow up, this ranged from 0.10 to 1.0, being significantly higher than that recorded in patients with uveitis (p = 0.001). The average level of visual acuity increased significantly at 1 day postoperatively by 136% (p = 0.001), an approximately extra 31% at 1 month postoperatively (p = 0.001), while after 3 months the visual acuity increased from one follow-up to another by 11-18% (p <0.001). Patients gained on average 5 lines of visual acuity (fig. 6). There is a statistically significant difference in final postoperative visual acuity between the two groups (p = <0.001).

The mean intraocular pressure was 15.88 +/- 3.68 mmHg (SD) preoperatively, the first postoperative day was 16.44 +/- 3.49 mmHg (SD), and at 1 month it was 15.05 +/- 3.49 mmHg (SD) (p <0.039) (fig. 6).

In all cases, in the uveitis group, topical steroid and non-steroidal anti-inflammatory drugs (NSAIDs) were administered preoperative for 1 week and postoperative for 6 weeks, in some selected cases systemic anti-inflammatory corticosteroids were administered.

There was no correlation in the uveitis cases between visual acuity and inflammation-free time interval (fig. 7).

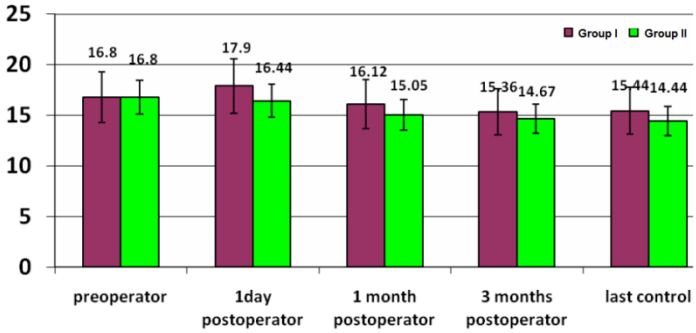


Fig. 6. Evolution of the mean level of intraocular pressure on study groups

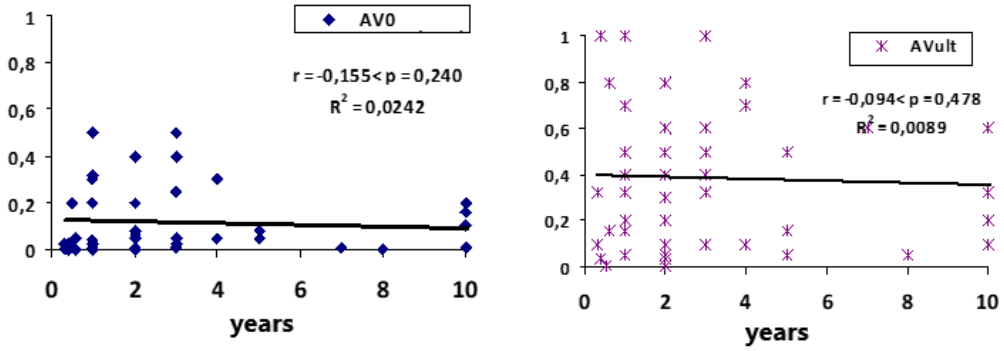


Fig. 7. Visual acuity and inflammation-free time interval correlation

There was no statistical significance between IOP and inflammation-free time interval (fig. 8).

In patients with synechiolysis, visual acuity significantly increased from 0.14 to 0.33 (p = 0.05), and intraocular pressure dropped significantly from 18.2 to 14.9 mmHg (p = 0.05). In patients with recurrent uveitis, visual acuity increased significantly from 0.09 to 0.33 (p = 0.05), and intraocular pressure dropped slightly from 17.3 to 16.65 mmHg (p = 0.623) (fig. 9).

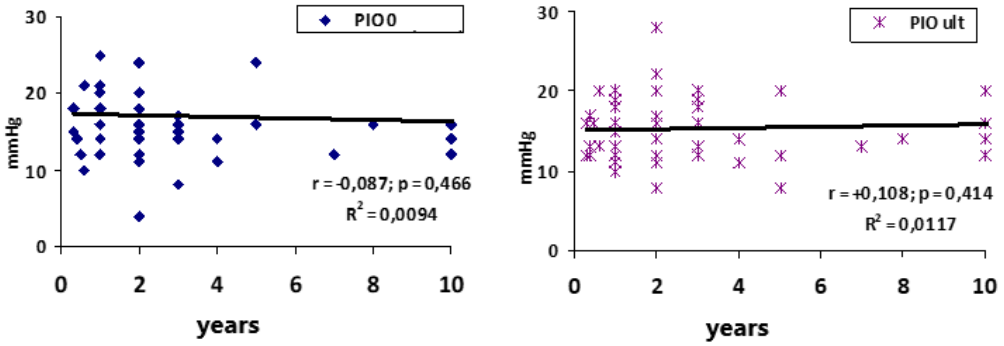


Fig. 8. IOP and inflammation-free time interval correlation

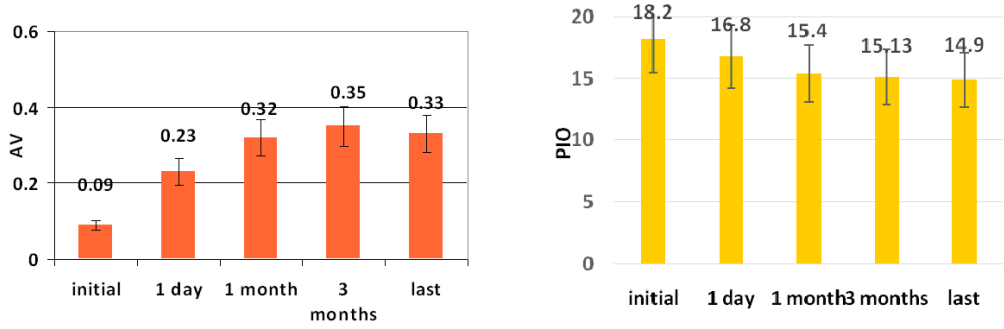
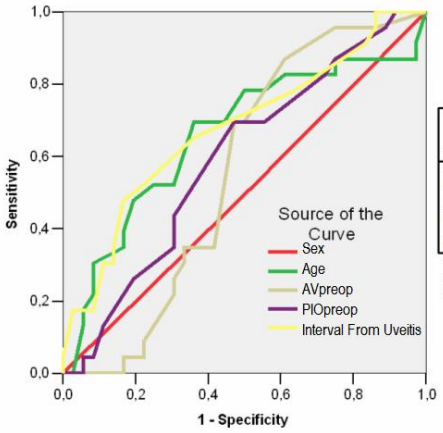


Fig. 9. Functional results of patients with recurrent uveitis

Logistic regression (multivariate analysis) provides a useful means to highlight the relationship between a presumed dependent variable and one or more explanatory variables called “predictors” which can be categorical or continuous. Risk is mathematically modeled as an equation as a collection of predictor variables. Linear regression models showed that visual acuity was significantly determined, in general, by gender, age, preoperative visual acuity - model 3 (y = 0.347 - 0.095 sex + 0.001 age + 0.924 AV0; p = 0.029), but model 5 also highlights the influence of the preoperative IOP and the interval from subsidence of inflammation as parameters that influence the visual acuity result at the last evaluation (y = 0.492 - 0.081 sex +0.001 age +0.914 AV0 - 0.07 PIO0 - 0.002 interval; p = 0.016). Of the preoperative factors, only gender is a good predictor of synechiolysis (AUC = 0.644; 95% CI: 0.501-0.786). Of

the preoperative factors, age (AUC = 0.659; 95% CI: 0.508-0.809) and the interval from extinction of the uveitis (AUC = 0.678; 95% CI: 0.534-0.821) seem to be good predictors of recurrent uveitis determinism (fig. 10).

Multivariate analysis revealed that a greater number of anti-inflammatory drugs administered for a significantly longer period can change the postoperative visual acuity (y = 0.709 + 0.646 AIN number - 0.274 Per Adm; p = 0.042) and postoperative IOP (y = 19.42 + 0.431 AIN number - 0.786 Per Adm; p = 0.008).



Area Under the Curve

Test Result Variable(s	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Sex	,497	,078	,969	,345	,649
Age	,659	,077	,041	,508	,809
AVpreop	,556	,075	,470	,409	,703
PIOpreop	,594	,075	,228	,447	,740
Interval From Uveitis	,678	,073	,022	,534	,821

The test result variable(s): Sex, Varsta, AVpreop, PIOpreop, IntervalDelaUveita has at least between the positive actual state group and the negative actual state group. Statistics may b biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Fig. 10. ROC curve - preoperative parameters which influence the evolution of recurrent uveitis

DISCUSSION

Cataracts are a common complication of uveitis, which occurs on one hand due to inflammation but also due to chronic treatment with corticosteroids. Cataract surgery in patients with uveitis is a serious challenge for the anterior segment surgeon. Today, phacoemulsification with intraocular lens implantation (IOL) in the capsular bag is the care standard for most patients with uveitis. However, despite the remarkable advances in surgical techniques and IOL materials, certain specific considerations should be made in terms of patient selection, preoperative preparation, as well as preoperative and postoperative management for long-term successful outcomes (7, 8). Studies show that there is a number of peculiarities regarding surgery of patients with uveitis, which determine the reduction of inflammation in the postoperative period: minimum number of maneuvers during surgery, short duration of surgery, small instead of large incisions, phacoemulsification with IOL implantation instead of extracapsular extraction of the lens and so on.

Preoperative management depends on the type and etiology of the uveitis, uveitis activity, the absence or presence of preoperative CME - there is no consensus of experts, several protocols being available (5, 6, 9). Thus, in non-granulomatous anterior uveitis Prednisolone acetate 1% eyedrops are administered 4 times/day 3-7 days preoperatively and in anterior uveitis associated with IJA (idiopathic juvenile

arthritis), severe granulomatous, intermediate or posterior uveitis, prednisone is recommended with dosage of 1mg/ kg bw/day, 3 days preoperatively. Also, in all cases, nonsteroidal anti-inflammatory drugs (NSAID) eyedrops 4 times/day 3-7 days before surgery, are proved to be more efficient in preventing the development of cystoid macular edema.

Postoperatively corticosteroid and antibiotic eyedrops are administered, NSAID eyedrops are continued 4 times/day for 6-8 weeks, up to 6 months depending on the case; in severe forms it is continued, tapering the treatment with Prednisone up to a minimum dose of 10 mg/day and combination of cycloplegic-mydriatic eyedrops (tropicamide 1% and phenylephrine 5%) 4times/day 5-7 days (6, 8, 9, 10, 11). Studies show an incidence of only 4% of CME in patients receiving oral and postoperative corticosteroids versus 27% in those without treatment (10, 12). Idan Hecht *et al.* exemplified in a study of 13,368 patients with a mean age of 73 years, who underwent cataract surgery, treated only with corticosteroids, only with NSAIDs or combination of the two, that postoperative treatment with steroids among patients undergoing treatment for uncomplicated cataract surgery was associated with lower rates of opacification of the posterior capsules compared with NSAID-only treatment (10).

The presence of inflammatory glaucoma should be established before surgery and anti-glaucoma therapy (avoiding prostaglandin derivatives) should be initiated. In the case of herpetic uveitis, prophylactic antiviral therapy with acyclovir or valaciclovir should be given at least 1 week before surgery to prevent recurrent viral infection (5, 8). Functional outcomes may be influenced by immediate and late postoperative complications: CME with an increased incidence after ECCE (33-66%) *vs.* Phaco (12-59%); severe anterior segment inflammation (posterior synechia, pupillary membranes); postoperative IOP increase - anti-glaucoma medication + corticosteroids; Posterior Capsule Opacification (PCO) (23-48% at 1 year) - the most common with variable incidence in different studies - requiring Nd: Yag capsulotomy - are prevented through postoperative inflammation control, well-centered circular capsulorhexis, square edge design of the IOL, meticulous removal of OVDs, etc. (5, 8, 10, 11, 13).

In our study, similarly to other literature studies, postoperative visual acuity increased significantly and was maintained at the last follow up. Sonia Mehta and colleagues in a review of literature using a database of 89 articles found that patients with a history of uveitis but no inflammation before surgery had a visual acuity of 20/40 or better in 69% cases especially if cataract surgery was performed through phacoemulsification (2). However, the presence of active uveitis at the time of phacoemulsification was associated with poor visual results (2, 13). In our study, the most frequent postoperative complication was recurrent uveitis (39%), which occurred on average at 3 months, 6 months and the latest at 2 years postoperatively. Most often this was due to intraoperative maneuvers at the pupillary level (stretching, synechiolysis, etc.).

The incidence of CME was 11% in cases with complicated surgery, intermediate or posterior uveitis and prior history of CME. Marie-Lyne Belair and co-workers evaluated the incidence of cystoid macular edema in two groups of patients who underwent cataract surgery, one that associates uveitis (41 eyes) *vs.* the one without

associated uveitis (52 eyes), similar to our study finding an 8% incidence of edema in those with uveitis and its absence in those without. In those treated with preoperative and postoperative corticosteroids the risk of edema was very low (14). In general, PCO occurred at 8 months - 2 years postoperatively, with an incidence of 10%. In a retrospective study of 3,013 eyes Amit K. Reddy evaluated the incidence of persistent anterior uveitis after cataract surgery, which was found in 2% while cystoid macular edema appeared in 29.4% of cases. Regardless of race (Caucasian or African American), uveitis persisted at one year postoperatively, despite moxifloxacin and topical corticosteroid therapy, periocular and intravitreal dexamethasone implant placement. A small percentage showed opacification of the posterior capsule. Visual acuity increased postoperatively in the study group similar to the control group, in accordance with low intraoperative and postoperative complications, as well as prompt treatment with pre- and postoperative anti-inflammatory depending on the cause of ocular inflammation (4). In a study on 111,641 eyes, of which 1,173 with associated uveitis, which underwent cataract surgery, Colin J Chu *et al.* found poorer visual acuity in the uveitis group postoperatively on the first day and at 12-24 weeks and increased intraocular pressure at the same intervals as opposed to the group without uveitis, with an increased frequency of anti-glaucoma procedures (7). In our study in the immediate postoperative period (one day) both groups had almost similar functional results; after one month the functional results in the control group are much better, with statistically significant differences between groups. The presence of a high percentage of recurrent postoperative uveitis (39%) in the study group would be an explanation of the weaker functional outcomes in this group compared to the control group. Preoperative inflammation control and the correct treatment of pre-operative CME can lead to good functional results after surgery. The reached data are comparable to those in the literature. Currently there are no studies comparing the level of visual acuity and intraocular pressure in patients with inflammation *vs.* non-inflammation who have undergone the same type of surgery. Thus, cataract surgery in eyes with uveitis can result in normal or near-normal visual acuity in most cases, at least in the short term, although it is not so common with regard to age-related (non-uveitic) cataracts. Further research is needed on a significant number of patients, in order to increase the level of confidence on which we can make decisions regarding the surgical treatment of cataract in uveitic eyes, and in particular to develop evidence that is specific to the individual clinical entities that make up this heterogeneous group of disease.

The limits of the study consist in the retrospective nature, the small number of enrolled cases and, most importantly, lack of uniformity of the evaluation period from surgery to the last follow-up. The absence of visual acuity results and intraocular pressure at longer intervals postoperatively also represents a serious limitation, and the results cannot be extrapolated to the general population.

CONCLUSIONS

Cataract surgery with artificial lens implantation resulted in improved postoperative visual acuity in patients with cataract and uveitis, which was maintained at the last follow up. In the immediate postopera-

tive period, both groups had similar functional results. Preoperative and postoperative inflammation control was a key factor in achieving relevant functional results. There is a statistically significant difference in final postoperative visual acuity between the two groups. Cataract surgery in uveitis patients requires careful evaluation of the diagnosis to determine the cause of uveitis, adequate patient selection, zero tolerance to preoperative inflammation, vigilant use of drugs to control preoperative inflammation, meticulous surgery, correct decision for implantation of IOL, control of postoperative inflammation and early detection and aggressive management of complications.

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ASSOCIATION BETWEEN DIABETES MELLITUS AND SARS-CoV-2 INFECTION - A RETROSPECTIVE STUDY IN THE PULMONOLOGY CLINIC OF TÂRGU MUREȘ

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ASSOCIATION BETWEEN DIABETES MELLITUS AND SARS-COV2 INFECTION - A RETROSPECTIVE STUDY IN THE PULMONOLOGY CLINIC OF TÂRGU MUREȘ (Abstract): The aim of our study was to establish the relationship between diabetes mellitus (DM) and SARS-CoV-2 infection, given that DM patients have an increased risk of developing severe infections/complications. **Material and methods:** The study included 108 (33.4%) patients with known or unknown DM or prediabetes PD (fasting blood glucose 100-126 mg%) from a group of 323 COVID-19 patients. Age distribution in the first group with DM/PD: 15.7% ≤ 40 years old, 27.7% 41-60 years old, 52.7% 61-80 years old, 3.7% > 80 years old. **Results**: We noted a higher percentage in the age group 61-80 and > 80 years old in nondiabetic patients. 92 (85.16%) from the first group and 174 (80.9%) in the second group also associated obesity/overweight. DM/PD were not previously known in 33.4%. 40% required either introduction of insulin or increased doses, due to marked imbalances. Severity of COVID-19 was higher in the DM: 17.6% *vs.* 53.4% mild forms, 37% *vs.* 27.9% moderate pneumonias, 45.3% *vs.* 18.6% severe forms (CT: multiple bilateral "ground glass" infiltrates or consolidation; respiratory failure) with extrapulmonary complications. Fatality was 12% in the first group and 7.4% in the nondiabetic group. All patients associated increased inflammatory markers (CRP, LDH, ferritin) and lymphopenia. **Conclusions:** DM was an important risk factor for high severity and fatality of infection even in middle-aged people while SARS-CoV-2 infection determined an imbalanced DM and clinical *de novo* DM/PD. Obesity was a major risk factor for SARS-CoV-2 in both groups (with or without DM). The recommendation of close monitoring after discharge is part of a personalized management to prevent further unfavorable evolution and post COVID-19 complications.
Keywords: DIABETES MELLITUS, PREDIABETES, SARS-CoV-2 INFECTION.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the novel coronavirus identified in December 2019 in Wuhan, China, causes the coronavirus

disease (COVID-19), that spread rapidly worldwide and had been declared a pandemic by the World Health Organization (WHO) in March 2020 (1, 2).

SARS-CoV-2 is part of the *Coronaviridae* family and is a positive-stranded RNA virus, surrounded by a protein-decorated lipid bilayer containing a single-stranded RNA genome. The main entry receptors for SARS-CoV2 in human cells are angiotensin-converting enzyme 2 (ACE2) and dipeptidyl peptidase-4 (DPP4), highly expressed in the vascular endothelium, pericytes, lung alveolar epithelial cells (type II pneumocytes), cardiac cells, macrophages, pancreatic islets and enterocytes of the small intestine (1, 3, 4, 5).

Greater risk factors in developing severe COVID-19 with ICU admission or death include advanced age, male sex, cardiovascular diseases (CVD), obesity, type 1 diabetes mellitus (T1DM) or type 2 DM (T2DM), renal chronic diseases, tumors, leukemia, lymphoma or myeloma, immunocompromised states after organ transplants, immunotherapy, chemotherapy, smoking, pregnancy, sickle cell disease, splenectomy, Down syndrome (6).

DM is a complex clinical syndrome, characterized by chronic hyperglycemia due to defects in insulin production, action or both. Besides the classical complications: a) acute (hypoglycemia, ketoacidosis, ketonic coma) and b). chronic (atherosclerosis with increased risk of coronary heart disease, stroke, peripheral arteriopathy, hypertension, retinopathy, nephropathy, neuropathy), DM is associated with a higher susceptibility in developing bacterial, parasitic or viral infections (7, 8, 9). These complications can shorten survival (in 2016 WHO cited 1.6 million deaths directly linked to DM and other 2.2 million that were caused by hyperglycemia) (10) .

The presence of DM and the individual condition of hyperglycemia seem to be directly involved with COVID-19 severity and increased mortality, highlighting the importance of shared disease pathophysiology for therapeutic approaches (11).

MATERIAL AND METHODS

We analyzed the particularities of the association between diabetes mellitus (DM) and SARS-CoV-2 infection in patients hospitalized in the Pulmonology Clinic of Târgu Mureș, Romania.

The study included 323 patients with SARS-CoV-2 infection confirmed in all cases by real time Reverse Transcriptase Polymerase Chain Reaction (RT PCR). The patients were included in the study as they were admitted in the Pulmonology Clinic of Târgu Mureș, Romania, during November - December 2020.

Inclusion criteria for the group 1: patients with SARS-COV2 infection and DM or prediabetes PD (previously documented or at first diagnostic) (108 patients). Prediabetes was defined as the situation in that the patients had fasting blood glucose between 100-126 mg%.

Inclusion criteria for the group 2 (control): patients with SARS-CoV-2 infection without DM or PD (as medical history or during the hospitalization in the Pulmonology Clinic Targu Mureș caused by SARS-CoV-2 infection) (215 patients).

The information recorded from each case file were individual data (gender, age, body mass index BMI, symptoms, medical history), laboratory tests: glucose levels, hemoleucogram, inflammatory markers: C reactive protein (CRP), lactate dehydrogenase (LDH), fibrinogen, ferritin; severity of COVID-19 pneumonia, treatment, as

well as evolution and outcome.

The diagnosis of COVID-19 was based on clinical evaluation and specific investigation RT-PCR and thoracic imaging – Computed tomography CT with intravenous contrast or chest X-Ray.

The aim of our study was to analyze the association between DM/PD and SARS-COV2 infection, the severity of associated diseases, comorbidities and fatality rate in patients hospitalized in the Pulmonology Clinic of Târgu Mureș, Romania. We made some comparisons between the group of patients with DM or PD and the group of patients without DM and PD.

We reviewed some national and international data considering the SARS-CoV-2 infection and association with DM and other comorbidities.

RESULTS

In our study, from 323 patients hospitalized with COVID-19 pneumonia (randomly chosen), 108 patients 33.4% (group 1) were found with DM or PD, indicating that DM and PD are major risks factors for the SARS-CoV-2 infection. We made comparisons with the group 2 (215 patients without DM or PD). The group 1 included 55 men and 53 women, and the male/female ratio was 1.03. The group 2 has a similar male/female ratio 1.05 (109/106).

We noted 3 patients with Type1 DM and in the rest of the group 83 had T2DM and 22 PD. A high number - 37 patients (34.2%) were not known with a previous history of DM or PD. Fasting blood sugar was taken several times for each patient in the morning and 4 time during the day ("glycemic profile") in medium/severe forms and in unbalanced DM).

The age distribution in the first group ranged in all age groups: from 35 to 40-year-old 17 patients (15.7%), 41-60-year-old 30 patients (27.7%), 61–80-year-old 57 patients (52.7%), to > 80-year-old 4 patients (3.7%). A higher prevalence of DM and PD was noticed in the years old Ung/middle age in our study group, 43.4% were between 35-60 years-old, comparing with 35% found in the literature (12). The mean age in our first group was 62-year-old. One possible explanation is that SARS-CoV2 infection in Romania affected more the middle-aged people (the mean age in Romania was 48-year-old in November 2020) (13). Other explanation could be the fragility of diabetic patients against SARS-CoV-2 infection in middle-aged people.

The age allocation in the second group was: 35 - 40-year-old 19 patients (8.83%), 41–60-year-old 42 patients (19.5%), 61–80-year-old 133 patients (61.9%), > 80 years 21 patients (9.76%). We note a higher percentage in patients from the age group 61-80 and > 80-year-old in nondiabetic patients. 92 patients (85.16%) from the DM/PD group also associated obesity or overweight (17 morbid obesity, 36 degree II of obesity, 24 degree I of obesity, 15 overweight) especially in women (tabs. I, IV). At the same time the second group without DM/PD met also high levels of obesity and overweight emphasizing that obesity is a major risk factor for SARS-CoV-2 infection (tab. III)

There were no differences between the prevalence of obesity and overweight in the subgroup with DM and PD, but both have high values. This aspect indicating on one hand the high-risk factor that obesity offers for DM and PD and on the other hand the strong necessity for population weight control. We discovered multiple associated other diseases in both study groups (tab. I).

TABLE I

Obesity and overweight in the DM/PD group

	DM patients (86)	PD patients (22)	Total
Obesity	40 - 46.5%	7 - 31.8%	47/108 - 43.5%
Overweight	37 - 43%	8 - 36,4%	45/108 - 41.66
Total	77/86 - 89.5%	15/22 - 68.2%	92/108 - 85.16%

TABLE II

Comorbidities in the DM/PD group

	DM patients (86)	PD patients (22)	Total group 1 (108 patients)
Coronary heart disease CHD	21	19	40 - 37.0%
Hypertension	27	24	51 - 47.2%
COPD	11	8	19 - 17.6%
Asthma	5	7	12 - 11.1%
Previous renal diseases	7	2	9 - 8.3%

TABLE III

Comparison between comorbidities repartition in the 2 groups of study

	Group 1 DM and PD (108 patients)	Group 2 without DM/PD (215 patients)
Obesity	47 - 43.5%	99 - 46%
Overweight	45 - 41.66%	75 - 34.8%
Coronary heart disease CHD	40 - 37.0%	69 -32%
Hypertension	51 - 47.2%	96 - 44,6%
Chronic Obstructive Pulmonary Disease	19 - 17.6%	33 - 15.3%
Asthma	12 - 11.1%	22 - 10.2%
Previous renal diseases	9 - 8.3%	12 -5.5%
Tumors or immunodepresive treatment	9 - 8.3%	11 - 5.1%

Coronary heart disease (CHD) and hypertension (HTN) were very frequent 37%, respectively 47.2% in the first group (tabs. II, III). This association could at least partially contribute to the higher percentage of DM/PD in SARS-CoV-2 infection, together with some specific susceptibility to SARS-CoV-2 of the patients with DM. In the second group CHD was discover in 32% and HTN 44% of patients.

If we compare case distribution based on gender and nutrition dysfunction, we can observe a higher prevalence of obesity and overweight in women in both groups (tab. IV).

TABLE IV

Association of DM and PD and obesity/overweight to SARS -CoV-2 patients

	Males with DM or PD (55)	Females with DM or PD (53)	Males in group 2 (109)	Females in group 2 (106)
Morbid Obesity	10	7	11	8
Grade II Obesity	16	20	12	20
Grade I Obesity	11	13	19	29
Overweight	6	9	36	39
Total	43 - 78.1%	49 - 92.4%	78 - 71.5%	96 - 90.5%
	92 - 85.16%		174 - 80.9%	

Regarding the severity of COVID-19 cases in the DM/PD group: 19 patients (17.6%) had mild forms, 40 (37%) had moderate pneumonias, 49 (45.3%) were severe cases (CT with multiple bilateral infiltrates in "ground glass" or consolidation, respiratory failure, long evolution ICU admission or death). 21 patients needed ICU admission. There were 13 deaths (12%) in the first group and 16 (7.4%).

Severity of COVID-19 was also extremely high in the nondiabetic patients but lower than first group with DM: 60 patients (27.9%) had mild forms, 115 (53.4%) had moderate pneumonias, 40 (18.6%) were severe cases.

Because patients with extreme inflammation, the so-called "cytokine storm" were considered at great risk for severe COVID-19 disease, including bigger pancreatic inflammation and possible unbalance of the glycemic values (14), we performed a periodically determination of some biomarkers to assess the hyperinflammation (e.g., ferritin, high-sensitivity C-reactive protein, lactate dehydrogenase, hemogram showing lymphopenia). At the same time, analyzing the importance of pro-inflammatory markers, helped us to identify the patients that needed immunosuppression (corticoids or selective cytokine blockade).

Increased inflammatory markers and lymphopenia were noticed in high percentages in our DM study group: severe cases were associated in 49 cases (100%) with positive increased biomarkers, moderate forms 35/40 (87.5%) and mild 6/19 (31%). Newly diagnosed DM and PD cases also had modified biomarkers, but with milder forms of SARS-CoV-2 pneumonia. Inflammatory markers were increased also in the second group emphasizing the severity of hospitalized cases.

Treatment included antidiabetic medication in group 1 (under multidisciplinary team adjustments). 44 patients (40%) required either the introduction of insulin or increased doses due to strong imbalances and daily monitoring of glycemic profile.

In all patients we indicated antiviral drugs upon severity and national guidelines (initially lopinavir / ritonavir, then remdesivir), corticoids (when hyperinflammation was obviously overexpressed and in severe cases), as well as hydroxychloroquine (first period of the pandemic) or tocilizumab, anticoagulants, oxygen therapy (in respiratory failure), antitussive, diarrhea drugs, IV rehydration and vitamins and a sustained psychological support.

We continued the specific medication for cardiovascular comorbidities (antihypertensive and lipid-lowering regimen (including ACE inhibitors and angiotensin 2 receptor-blockers) considered an indication by the European Society of Cardiology (ESC) in all these patients (15). After discharge, all patients were forwarded to ambulatory care units for a cautiously metabolic and cardiac monitoring and adjustment of specific treatment with oral antidiabetics or insulin, according to the evolution after COVID-19 recovery.

DISCUSSION

The high prevalence of DM and PD (33.4%) found in our group of patients with SARS-CoV-2 and the real difficulties for metabolic control achievement, represent addictional data that DM and hyperglycemia are major risk conditions for SARS-CoV-2 infection and for a severe evolution of the disease in hospitalized patients, needing special recommendations (8). Literature data offers several explanations for sustaining this particular group of patients with DM or PD.

Part of the renin-angiotensin-aldosterone system (RAAS), the ACE2 receptor is known to be the primary entry for SARS-COV2, expressed in many human cells and tissues, including the pancreatic islets (1, 16, 17). Studies showed an association between ACE2 and glucose regulation(18), moreover the infection with SARS-COV may be the cause of hyperglycemia in patients without pre-existing DM(19), by direct damage of the pancreatic -cells, which can persist several years after recovery from the infection(19). Two case series made in Seattle and Washington, USA, found that 58%, respectively 33% of the critically ill patients with COVID-19 that were admitted to the ICU had DM as an underlying comorbidity (20, 21). This evidence assumes that RAAS and the ACE2 receptor may have an implication in the connection between COVID-19 and DM.

At the same time the very high incidence of DM/PD met in our study is in concordance with the well-known data from the literature that infectious diseases are more prevalent among patients with DM than on those without(3, 8, 11). One important cause may implicate the hyperglycemic environment that favors immunological abnormalities, such as impaired cellular innate immunity function (low chemotaxis, phagocytosis) of monocytes/macrophages, low T-cells immune response and/or disturbances in humoral innate immunity (low complement factor 4, decreased cytokine response after stimulation) (3, 7), leading to micro- and macrovascular complications of DM (14, 22).

Pathophysiology of SARS-CoV-2 infection led to an elevated inflammatory response among blood mediators such as lipopolysaccharide (22, 23), inflammatory cytokines, toxic metabolites, modulation of natural killer cells with IFNy production (24), that in the end increase the vascular and/or interstitial permeability for pro-inflammatory products (25). Furthermore, the infection with SARS-CoV-2 leads to reactive oxygen species (ROS) production, causing acute lung damage with acute respiratory distress syndrome and lung fibrosis (25). Viral activation of RAAS via ACE2 and together with ROS production, cause insulin resistance (24), hyperglycemia and vascular endothelial damage (25), ending with cardiovascular events, disseminated intravascular coagulation (DIC) and thromboembolism (26).

Elevation of inflammatory markers such as ferritin, D-dimer, fibrinogen and IL-6 (27), contribute to a higher risk for patients with DM for hypercoagulability, sepsis progression and ICU admission (14, 28). Wang *et al.* in a retrospective multicenter study from China regarding fasting blood glucose at admission found out that higher levels (≥ 7.0 mmol/L (≥ 126 mg/dL) were independent predictors of COVID-19-related mortality for patients that did not have DM (29).

In our study, an important part of the group 37 patients 34.2% (17 with DM and 20 PD) presented *de novo* DM or persistent fasting hyperglycemia during SARS-CoV-2 pneumonia.

This observation highlights the importance of daily close monitoring glucose levels and to treat hyperglycemia on patients with progression to severe states of COVID-19. In our group of patients corticoid treatment could be, in addition to infection with SARS-CoV-2, another factor that unbalanced the DM and brought in the light the persistent hyperglycemia. But even after the use of very small doses of corticoids or after corticotherapy exclusion, glucose levels remained high, difficult to control. Thus, we reintroduced corticoster-

oids for their benefits (anti-edematous/anti-inflammatory effects - including the pancreas) and we increased the insulin doses (by subcutaneous or intravenous administration) and recommended a strict diet and adequate fluid intake. The permanent endocrinological consultation and the close monitoring of blood glucose (daily glucose profile - 5 samples) led slowly to improvement.

In a retrospective study performed in Sydney, Australia, eight critically ill patients with COVID-19 and T2DM, three being newly diagnosed, required extremely high doses of insulin (mean peak insulin requirement of 201 units per day) and had a delayed control of the glucose level at the time of peak inflammatory response (30), strongly supporting our study results.

We found that obesity is a major risk factor for the severe evolution of SARS-CoV-2 pneumonia both in DM and non-DM patients.

It is described that patients with COVID-19 and associated overweight or obesity, had worse outcomes than those without, because of the high-grade inflammation and of the compromised immune system (31, 32). Glucagon-like peptide 1 (GLP1) and its analogues are used in some patients with T2DM and cardiovascular comorbidities, with lowered rates of cardiac events (33) and may be a good therapeutic approach for patients with obesity, because of the weight-reducing properties (34), but not recommended in critical situations (e.g., severe cases of COVID-19) because of the slow target dose achievements and side effects such as nausea or vomiting (35).

Several retrospective cohort studies achieved in the USA, China and France analyzed the clinical outcomes regarding patients with COVID-19 and DM. The main features that have been measured were age (mean age), male/female ratio, glycemic status and glycosylated hemoglobin proportion (HbA1c), comorbidities, risk factors for mortality and primary outcomes. The number of patients included ranged from 153 in the China study to 1,317 in the French study. The mean age was 69.8±13.0 in France, 56.2–72.0 in China and 66.7±14.2 in the USA. Besides DM, most commonly discovered comorbidities found were hypertension, coronary heart disease, heart failure, chronic kidney disease and chronic obstructive lung disease. Primarily, added mortality risk factors were older age, male sex, micro and macrovascular complications, higher BMI and insulin administration before admission. In France, the death rate was 29% out of 1,317 patients in day 7 after admission, in China was 20% from a total of 153 patients, 11% being non-DM patients and 33% in the USA (1,276 patients) (36, 37, 38). The Center of Disease Control (CDC), based on a meta-analysis, declared that the risk of a fatal outcome from COVID-19 is 50% higher in patients with DM than in those who do not have DM (39).

In our study group a higher prevalence of DM/PD was noticed in the middle age (43.4% between 35–60-year-old) comparing with 35% found in the literature (12). The mean age in our first group was 62 years-old with a range between 37- and 85-year-old. We note a higher percentage in patients from the age group 61-80 and > 80-year-old in nondiabetic patients (71.66%). An explanation could be the fragility of diabetic patients against SARS-CoV-2 infection even in middle-aged people.

A huge percentage of patients associated obesity or overweight, 92% (bigger in women), 37% had coronary heart disease, 47.2% arterial hypertension, 17.6% COPD,

11.1% asthma and 8.3% renal disease. Cardiovascular comorbidities were in a mild lower frequency in the group 2, without DM. Obesity, COPD and asthma had comparable frequency in the two groups.

Severity of COVID was higher in the DM/PD group compared to the second group without DM: 17.6% *vs.* 53.4% mild forms, 37% *vs.* 27.9% moderate pneumonias, 45.3% *vs.* 18.6% severe forms. At the same time in group with DM/PD we found a surplus of severe forms compared to what has been found in the literature (20% severe forms) (13, 39).

In our DM/PD group of study the fatality (12%) was 4.8 times higher than the COVID-19-related mortality over the country (2.48%) (data from December 2020) (13). The fatality in the group without DM/PD (7.4%) was lower than in the DM/PD group but higher than the national value. Our observation reinforces the fact that DM is a severity factor for SARS-COV2 pneumonia. On the other hand, the high values of severe forms and high fatality in our groups could be explained by the fact that we studied the patients that were precisely hospitalized for severe evolution and our data did not include the general population infected with COVID-19.

After the COVID-19 recovery period, patients with DM or PD will have a strong indication for vaccination. There are not known medical contraindications for vaccination in patients that contracted COVID-19 and had underlying DM. According to CDC, patients with DM are included in the group 1c, as well as other medical conditions that can increase the risk of serious, life-threatening complications from COVID-19. Vaccination of DM patients will take place after 1a group - healthcare professional and long-term care facility residents and 1b group - people aged ≥ 75 years and frontline essential workers (39).

We recommended a close monitoring of patient in post COVID period (by clinical exam, thoracic ultrasound/thoracic scan upon severity, spirometry / bodyplethismography, pulsoxymetry and endocrinological consult) after discharge like a personalized management to prevent further unfavorable evolution and post COVID complications (pulmonary fibrosis, decompensation of the DM) (40).

CONCLUSIONS

DM and PD were important risk factors for an increased severity of SARS-CoV-2 infection and high fatality. SARS-CoV-2 infection determined DM imbalance and clinical *de novo* DM or highlighted PD. In the course of the COVID-19 pandemic, awareness should be raised among patients with underlying DM. Proper management and treatment enhancement (including insulin administration) are essential for a good control of glycemic levels. We found in our study group multiple comorbidities: obesity, overweight, hypertension, cardiac ischemic diseases and COPD and asthma that contributed to a severe evolution.

Obesity was a major risk factor for SARS-CoV-2 in both groups (with or without DM). The recommendation of close monitoring after discharge is part of a personalized management to prevent further unfavorable evolution and post COVID-19 complications. All patients with DM and PD have to follow a customized monitoring in the ambulatory care units for the entire post COVID-19 recovery period, for treatment adjustments.

STUDY LIMITATION. We appreciated the patients with SARS-CoV-2 association with DM/PD that were precisely hospitalized for severe evolution and our data

did not include the general population infected with COVID-19. Because of the high contagiosity of the SARS-CoV-2 infection, it was not possible during the hospitalization an exhaustive analysis of the DM complication.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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DRY EYE SYNDROME IN PATIENTS WITH CARDIOVASCULAR PATHOLOGY

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DRY EYE SYNDROME IN PATIENTS WITH CARDIOVASCULAR PATHOLOGY (Abstract): The present study aims to open a new perspective in addressing patients suffering from the dry eye syndrome through the analysis of biometric markers that explain the long-term evolution of these patients, especially those with cardiovascular dysfunctions. **Material and methods:** In this study were included 200 patients from Comanesti County Hospital who were examined between 2016 and 2018. The cases were structured in 4 groups of 50 patients each, with or without cardiovascular disease, with or without dry eye syndrome. **Results:** Gender distribution showed a slightly higher proportion of women in all study groups. Dyslipidemia was identified in 70% of patients and hypertension in 66% of them. Other frequent pathologies identified in the study group were angina pectoris which was discovered in 28% of patients and diabetes mellitus which was found in 23.3% of them. Heart failure was found in 39% of all patients suffering from dry eye syndrome and it was significantly more frequent in women (57.5% *vs.* 26.7%). **Conclusions:** The heart failure was significantly more common in women and in those aged over 60 years for whom the estimated risk was more than 2.5 times higher in the whole group and about 2 times higher in patients suffering from the dry eye syndrome. Central corneal thickness, corneal resistance, corneal hysteresis are good predictors of the dry eye syndrome with/without cardiovascular damage. **Keywords:** DRY EYE DISEASE, CORNEAL THICKNESS, CARDIOVASCULAR DISEASE, HYPERTENSION.

The purpose of study was to elucidate the correlation of pathophysiological mechanisms and predisposing factors involved in the occurrence of the dry eye syndrome and/or cardiovascular diseases of symptomatic patients, all this for a better life`s quality of patients.

The dry eye syndrome occurs in adults, more commonly after the age of 50-60 years, with females predominating in 90% of cases. The tear secretion is characterized by a significant decrease in aqueous secretion which is also associated with the alteration of the mucin layer of the tear film; the mucin, easily coming into contact with the lipids of the superficial layer, precipitates and stagnates due to the reduction of the aqueous phase. Due to the decrease in aqueous flow on the one hand and the antibacterial lysozyme and lactoferrin on the other, the possibility of developing local infections is high (1-4).

The term *"cardiovascular disease"* (CVD) is attributed to a multitude of dis-

eases affecting the heart and blood vessels, including ischemic heart disease (IHD), cerebrovascular disease, hypertension (high blood pressure) or peripheral arterial disease (PAD). Other cardiovascular diseases are myocarditis (a heart condition caused by rheumatic fever) and congenital heart diseases. The cardiovascular diseases are also associated with heart attacks (myocardial infarction), angina and strokes (5).

MATERIAL AND METHODS

Study lots

The study group consisted of 200 patients selected from Comanesti County Hospital between 2016 and 2018. The study group was divided into four lots according to the presence/absence of the dry eye syndrome and / or cardiovascular comorbidities:

Lot I - 50 patients suffering from the dry eye syndrome with various associated cardiovascular comorbidities;

Lot II - 50 patients suffering from the dry eye syndrome but without associated cardiovascular comorbidities;

Lot III - 50 patients suffering from a cardiovascular disease without suffering from the dry eye syndrome;

Lot IV - 50 apparently healthy patients, chosen as witness group, not suffering from the dry eye syndrome or cardiovascular diseases.

The inclusion criteria were:

- age over 18;
- preoperative diagnosis of the dry eye syndrome;
- significant cardiovascular pathology (grade 3-4 hypertension with high/very high additional risk, NYHA class III heart failure, etc.);
- biochemical parameters within normal limits, allowing ophthalmic surgery.

Exclusion criteria:

- association of neoplasms;
- the refusal to participate in the study or the presence of a psychiatric disorder that does not allow informed consent to be understood;
- other pathologies of the eye (acute/chronic infections) that may affect the biochemistry of the tear film.

The study followed cardiovascular risk factors that cannot be influenced: gender, age, family history (genetic predisposition) and cardiovascular risk factors that can be influenced: obesity, high cholesterol, stress, hypertension, smoking, diabetes, sedentary lifestyle.

The study was approved by the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy from Iasi and is part of a Ph.D. study. Informed consent was obtained from each patient.

Statistical analysis was carried out using *SPSS 20.0* software. For all data, p value <0.05 was considered statistically significant.

RESULTS

The gender distribution showed a slightly higher proportion of women in all study groups, especially in patients from group I (suffering from the dry eye syndrome and a cardiovascular disease) ($p=0.160$) (fig. 1).

The age of the patients ranged from 29 to 85 years, with a group mean age of 61.05 ± 10.30 years and was slightly higher in patients suffering from the dry eye syndrome and a cardiovascular disease (group I: 64.76 years; $p=0.118$). Data are presented in first table.

The weight status did not differ significantly between the study groups. However, it should be noted that the mean BMI level

was slightly higher in patients suffering from cardiovascular diseases without the dry eye syndrome (31.24 kg/m^2; p=0.662) (fig. 2).

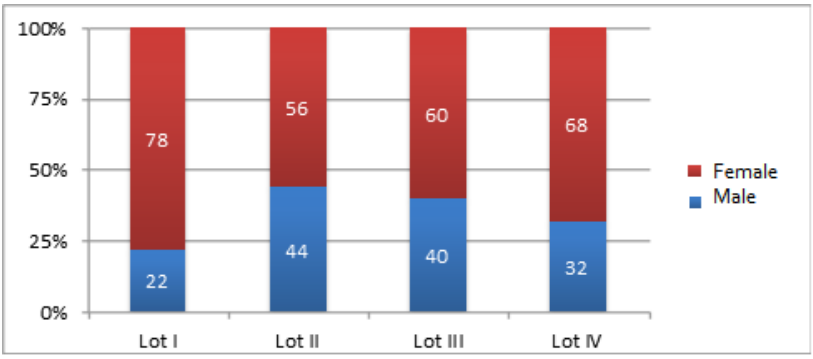

Fig. 1. The structure of lots by gender

TABLE I

Descriptive statistical indicators of age (years) by study groups

Lot	N	Average	Deviation Std.	Error Std.	Confidence range		Min	Max	p
					- 95% CI	+95% CI			
Lot I	50	64.76	8.09	1.21	62.33	67.19	45	85	
Lot II	50	60.63	11.25	1.66	57.29	63.97	34	80	
Lot III	50	60.76	11.63	2.82	54.78	66.75	29	76	0.118
Lot IV	50	58.08	9.94	1.44	55.20	60.97	40	82	
TOTAL	200	61.05	10.30	0.82	59.42	62.68	29	85	

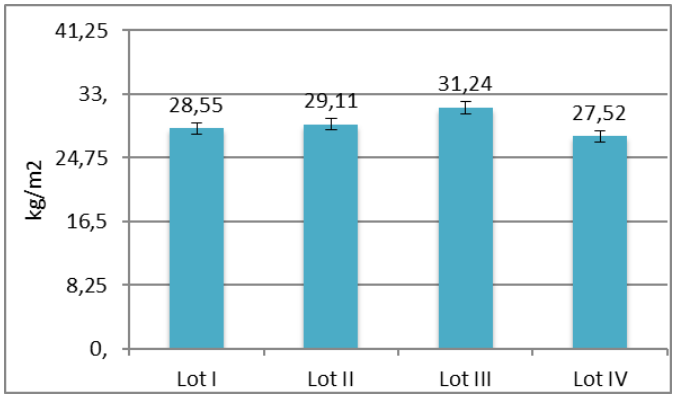

Fig. 2. The comparative weight status by study groups

The heart failure was present in personal pathological history in 34.5% of patients, significantly more frequently in women (60.9% *vs.* 20.6%). The relative risk was about 3 times higher (p=0.001; RR=2.96; 95% CI:1.84-4.75) for these patients. According to NYHA classification on the studied case reports, stage III heart failure was observed in 9.9% of men and stage IV in only 2.3% of them, while in women predominated stage II (29%) and III (23.2%). Heart failure was significantly more common in those aged over 60 years (45.9% *vs.* 20.9%). The relative risk was 2.57 times higher in these cases (p=0.022; RR=2.57; 95% CI: 1.39-4.75). Stages II (19.3%) and III (22%) of heart failure are more common in geriatric age groups according to NYHA staging (tab. II, fig. 3).

Heart failure was found in 39% of all patients suffering from dry eye syndrome and it was significantly more frequent in women (57.5% *vs.* 26.7%). The relative risk was about 2 times higher in this situation (p=0.049; RR=1.95; 95% CI: 1.33-4.15) but with equal weighting by age group (29%) (tab. II, fig. 3).

The angina pectoris was stable in 28% of patients, having no significant percentage differences between gender: 29.9% men and 19% women (p=0.446; RR=1.57; 95% CI: 0.63-3.95) or age groups: 31.5% in patients under 60 years and 25.3% in those over 60 years (p=0.515; RR=1.24; 95% CI: 0.74-2.08).

Hypertension was present in 66% of patients, with no significant percentage differences between gender: 71.4% of women and 64.8% of men (p=0.751; RR=1.10; 95% CI: 0.81-1.48) or age groups (p=0.686; RR=1.07; 95% CI: 0.85-1.35).

TABLE II

The distribution of heart failure cases by gender and age group

IM	Men		Women		< 60 years		≥ 60 years		Total	
	n	%	n	%	n	%	n	%	n	%
The whole group										
N/A	104	79.4%	27	39.1%	72	79.1%	59	54.1%	131	65.5%
NYHA II	11	8.4%	20	29.0%	10	11.0%	21	19.3%	31	15.5%
NYHA III	13	9.9%	16	23.2%	5	5.5%	24	22.0%	29	14.5%
NYHA IV	3	2.3%	6	8.7%	4	4.4%	5	4.6%	9	4.5%
The dry eye syndrome										
N/A	44	73.3%	17	42.5%	25	61.0%	36	61.0%	61	61.0%
NYHA II	5	8.3%	10	22.0%	5	12.2%	10	16.9%	15	15.0%
NYHA III	10	16.7%	10	25.0%	8	19.5%	12	20.3%	20	20.0%
NYHA IV	1	1.7%	3	7.5%	3	7.3%	1	1.7%	4	4.0%

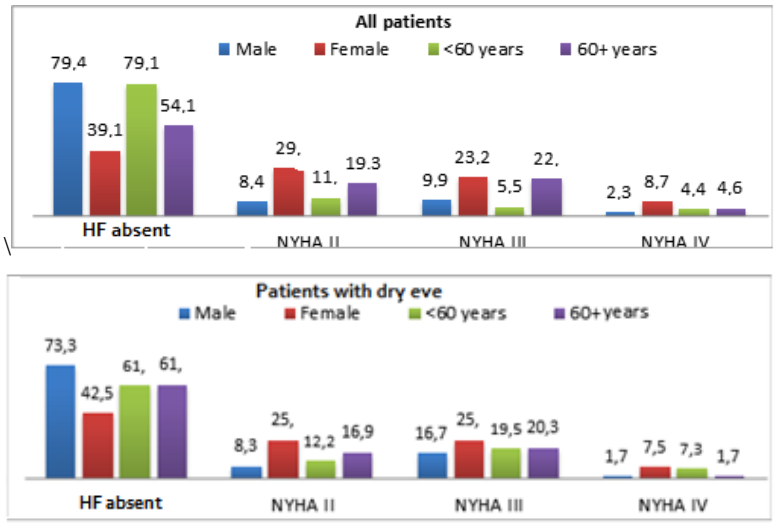

Fig. 3. The distribution of heart failure cases by gender and age group

Diabetes mellitus was identified in 23.3% of patients. The gender distribution was 33.3% in women and 21.7% in men, inducing a slightly higher relative risk in females (p=0.373; RR=1.54; 95% CI: 0.77-3.06). Diabetes was identified in 27% of patients under 60 and 19.7% of those over 60, but the percentage differences were not statistically significant (p=0.388; RR=1.37; 95% CI: 0.76-2.47).

Dyslipidemia was present preoperatively in 70% of patients and the gender distribution was 76.2% in women and 69% in men, with statistically insignificant percentage differences between genders (p=0.681; RR=1.10; 95% CI: 0.85-1.44). The percentage distribution of cases with dyslipidemia according to the age group was 81.1% in patients under 60 years of age compared to 59.2% of those over 60 years of age, with a slightly higher relative risk at younger ages (p=0.006; RR=1.37; 95% CI: 1.10-1.70).

Biometric evaluation

Central corneal thickness (CCG) ranged from 481 to 634 µm, with no significant differences in average levels compared by study groups (tab. III).

The lowest average intraocular pressure (IOP) level was recorded in patients suffering from the dry eye syndrome with various cardiovascular comorbidities (group I) and the highest was recorded in patients suffering from the dry eye syndrome without cardiovascular comorbidities (group II) (16.13±2.94 mmHg *vs.* 18.31±4.19 mmHg; p=0.004) (tab. III).

Individual corneal hysteresis (HC) values exceeded 10.8±1.5 mmHg in 7.7% of patients. The lowest average level was found in patients suffering from dry eye syndrome without any cardiovascular comorbidities associated (group II) (9.39±1.56 mmHg; p=0.001) (tab. III).

The corneal resistance factor (CRF) ranged from 8 to 18 mmHg and recorded a significantly lower average level in the groups suffering from the dry eye syndrome compared to the highest average level recorded in the group with cardiovascular diseases only (group I and II *vs.* group III) (10.97±1.68 mmHg; 10.45±2.09 mmHg *vs.* 12.15±2.53 mmHg; p=0.011) (tab. III).

TABLE III

Descriptive statistical indicators of biometric markers comparison by study groups

Lot	N	Aver-age	Deviation Std.	Error Std.	Confidence range		Min	Max	p
					- 95% CI	+95% CI			
Central corneal thickness (µm)									
Lot I	50	558.67	26.64	3.97	550.66	566.67	495	599	0.561
Lot II	50	553.42	42.56	6.34	540.64	566.21	453	681	
Lot III	50	566.35	25.70	6.23	553.14	579.57	495	599	
Lot IV	50	556.94	27.79	4.01	548.87	565.01	481	634	
Intraocular pressure (mmHg)									
Lot I	50	16.13	2.94	0.44	15.25	17.02	11	24	**0.004**
Lot II	50	18.31	4.19	0.62	17.05	19.57	12	30	
Lot III	50	17.76	2.51	0.61	16.47	19.06	12	22	
Lot IV	50	16.46	2.06	0.30	15.86	17.06	12	20	
Corneal hysteresis (mmHg)									
Lot I	50	10.12	1.87	0.28	9.56	10.68	6	16	**0.001**
Lot II	50	9.39	1.56	0.23	8.92	9.86	5	13	
Lot III	50	10.15	1.65	0.40	9.31	11.00	6	14	
Lot IV	50	11.09	1.74	0.25	10.59	11.60	9	17	
Corneal resistance (mmHg)									
Lot I	50	10.97	1.68	0.25	10.46	11.47	8	15	**0.011**
Lot II	50	10.45	2.09	0.31	9.82	11.08	5	15	
Lot III	50	12.15	2.53	0.61	10.85	13.46	9	20	
Lot IV	50	11.48	1.99	0.29	10.91	12.06	8	18	

TABLE IV

Multivariate analysis. Dependent variable of the dry eye syndrome.

Model	R	R square	Adjusted R square	Err. Std. of estimation	P F Change
1. Age	0.236	0.056	0.050	1.176	0.003
2. Age. CCG	0.237	0.056	0.044	1.180	0.760
3. Age. CCG. FRC	0.275	0.076	0.057	1.171	0.077
4. Age. CCG. FRC. HC	0.342	0.117	0.093	1.149	0.009
5. Age. CCG. FRC. HC. PIO	0.409	0.167	0.128	1.127	0.006

The multivariate linear analysis confirms the following:

- The linear regression model 4 (p=0.009) highlights as good predictors in the determinism of the dry eye syndrome and/or cardiovascular damage: age, central corneal thickness, corneal resistance and corneal hysteresis;
- The linear regression model number 5 (p=0.006) highlights, in addition to

age, central corneal thickness, corneal resistance and corneal hysteresis as good predictors in the determinism of the dry eye syndrome and/or cardiovascular damage and intraocular pressure.

DISCUSSION

Dry eye disease is more frequent in women than men and has an increased prevalence with age (3). The prevalence of dry eye syndrome varies from approximately 5 to 50% in population-based studies. Approximately 1 out of 7 individuals with ages between 65 to 84 years old reports frequently symptoms of dry eye (4).

Most cardiovascular diseases can be prevented by measures that address behavioral risk factors: smoking, unhealthy diet, sedentary lifestyles, obesity, alcohol in unhealthy doses. A low standard of living, a disadvantaged social background and educational deficiencies dramatically reduce the adherence to these prophylactic measures. The ageing of the population should also be mentioned, as age is a non-influential risk factor for cardiovascular disease (5).

Recent studies of dry eye disease classification and epidemiology suggest that a patient with dry eye may have multiple etiologies (6-9). In our study, dyslipidemia was identified in 70% of patients and hypertension in 66% of them. Other frequent pathologies identified in the study group were angina pectoris which was discovered in 28% of patients and diabetes mellitus which was found in 23.3% of them. These results are similar with a study conducted in 2018 that identified hypertension, thyroid disease, type 2 diabetes, sleep apnea, depression, and cardiovascular diseases associated with dry eye syndrome (6).

Type 2 diabetes represents a common risk factor for ocular diseases (10). A study conducted by Chew *et al.* showed that diabetes was found in more than 24% of those with newly diagnosed and prevalent dry eye disease (11). These results are similar with the ones found in our study.

In our study, across all comorbidities evaluated, hypertension was the most commonly observed cardiovascular comorbidity and was found in 66% with or without dry eye disease. These findings are similar with the ones from a study conducted by Dana *et al.*, who found that hypertension was the most frequent comorbidity in both groups with or without dry eye disease (6).

A study conducted by Butovich *et al.* showed that the relationship between hypercholesterolemia and dry eye disease can be explained as the high levels of cholesterol in the meibomian lipid will lead to increased viscosity and plugging of the meibomiam orifice (12). In our study. dyslipidemia was identified in 70% of patients.

Numerous epidemiological studies noted that dry eye prevalence increases in women and men every five years after the age of 50, with higher prevalence in women compared to men (13-19). Age and female sex have been found to represent the most important risk factors for dry eye. These findings are supported by the reduced tear production in women through the sixth decade of life (20-22). These results are similar with the ones found in our study where the gender distribution showed a slightly higher proportion of women in all study groups, with a mean age of 61.05 years old.

In our study of all patients suffering from the dry eye syndrome, 39% of them had a history of heart failure, significantly

more frequently in women (57.5% *vs.* 26.7%), for whom the relative risk was about 2 times higher. These results are similar with the ones found in a study conducted by Shokr *et al*. in 2021, which showed that patients with positive diagnosis of dry eye disease present a higher risk for developing cardiovascular diseases (23).

A study conducted by Borrego-Sanz *et al.* showed that dry eye disease associated with Sjögren's syndrome can influence corneal biomechanical properties (24). In our study. central corneal thickness, corneal resistance and corneal hysteresis turned out to be important predictor factors for dry eye disease.

CONCLUSIONS

There were no significant percentage differences in sex. age and weight status characteristics in relation to the association of the dry eye syndrome with/without cardiovascular impairment.

Heart failure was significantly more common in women (60.9%) over the age of 60 (45.9%), with an estimated risk more than 2.5 times higher in the whole group and about 2 times higher in patients suffering from the dry eye syndrome.

Central corneal thickness, corneal resistance and corneal hysteresis are good predictors of the dry eye syndrome with/without cardiovascular damage.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding the scientific research.

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NEWS

BELATACEPT - A GOOD OPTION AS AN IMMUNOSUPPRESSIVE DRUG IN LIVER TRANSPLANT

Judicious use of immunosuppression is imperative after liver transplantation to achieve an adequate level of immunosuppression while also balancing dose-dependent adverse effects associated with these medications. Authors describe the successful use of belatacept-based immunosuppression in a liver transplant recipient who experienced treatment related adverse effects to first- and second-line immunosuppressive agents. Six months post-initiation belatacept-based immunosuppression, the patient had stabilization of his chronic kidney disease, improvement in lung function, and remains without evidence of rejection. Belatacept is a novel, first in class, fusion protein that blocks signal 2 of T cell activation. However, before routine use of belatacept in liver transplant recipients can be recommended, further safety and efficacy data are needed. (Lang K, Kane C, Van Wagner BL. Belatacept as salvage maintenance immunosuppression in a liver transplant recipient. *Transplantation Reports* 2020; 5: 100070).

CHRONIC OBSTRUCTIVE PULMONARY DISEASE - OBSTRUCTIVE SLEEP APNEA OVERLAP SYNDROME CHOOSING THE RIGHT POSITIVE PRESSURE THERAPY

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CHRONIC OBSTRUCTIVE PULMONARY DISEASE - OBSTRUCTIVE SLEEP APNEA OVERLAP SYNDROME CHOOSING THE RIGHT POSITIVE PRESSURE THERAPY **(**Abstract): Patients with chronic obstructive pulmonary disease (COPD) - obstructive sleep apnea (OSA) overlap syndrome have worse outcomes than patients with either COPD or OSA alone. Positive pressure therapy (continuous positive airway pressure - CPAP or non-invasive ventilation - NIV) is still a matter of debate. The **aim** of the study is to identify clinical (symptoms, changes in physical examination) and paraclinical (spirometry, arterial blood gases and respiratory events during sleep) parameters that could orientate the physician towards choosing a certain type of positive pressure therapy in patients with COPD-OSA overlap syndrome. **Material and method**: 84 patients with overlap syndrome were included. A very thorough anamnesis, physical examination, blood samples, spirometry and ventilatory polygraphy was performed in all patients. **Results:** Most patients had severe sleep apnea with an average of 38 events per hour of sleep, and moderate COPD - stage II GOLD. The positive therapy used was mainly CPAP (57%), and the preferred mask was oronasal. **Conclusions:** Patients with overlap syndrome appear to prefer CPAP as positive therapy treatment and oronasal mask as interface. However, we could not establish any correlation between any clinical, functional or ventilatory polygraphy parameters that could explain the choice of one or another type of positive pressure devices. **Keywords:** COPD, OSA, OVERLAP SYNDROME, OBSTRUCTIVE SYNDROME, DAYTIME SLEEPINESS.

Obstructive sleep apnea (OSA) is a common comorbidity in patients with chronic obstructive pulmonary disease (COPD) (1). The term "overlap syndrome" refers to the association of the two conditions in the same patient - COPD-OSA

overlap syndrome (OVS). These patients have in general a worse prognosis compared with those with either OSA, either COPD due to the significant desaturation during the night with longer periods of hypoxemia and/or hypercapnia.

The triad that clinically characterizes obstructive sleep apnea is snoring, sleep apnea, and excessive daytime sleepiness in a typically male patient, obese, active, or former smoker (2, 3, 4, 5). Cardiovascular complications are present in both those with OSA and COPD (6, 7, 8). In patients with OVS, however, arterial hypertension, arrhythmias, pulmonary hypertension, ischemic heart disease occur more often and earlier in the course of the disease. Positive pressure therapy, at least in patients with OSA, appears to reduce the costs associated with healthcare and mortality. The choice of continuous positive pressure (CPAP) versus non-invasive ventilation (BiPAP) in patients with OVS is still the subject of much debate (7, 8, 9, 10, 11, 12). While some authors suggest that CPAP is as effective as bilevel positive airway pressure (BiPAP) in patients with OVS (7-9) there are some who recommend adjusting the therapy according to the severity of the lung involvement, practically the severity of COPD (10, 11, 13). The main reason for prescribing long term non-invasive ventilation in patients with OSA and/or COPD is persistent hypercapnia ($PaCO_2$ > 45 mmHg). The main question is however: what causes CO_2 elevation in patients with OVS? Is it only the COPD or has obstructive sleep apnea a certain contribution, and the use of CPAP improves ventilation by reducing $PaCO_2$ such as in patients with hypoventilation obesity syndrome? (12, 13). The question is even more interesting as obstructive sleep apnea seems to be more common among patients with mild to moderate COPD (FEV1 > 50%). The more severe the COPD, the lower the likelihood of developing OSA. In other words, the patients that are more prone to develop hypercapnia are most unlikely to have obstructive sleep apnea. CPAP, in addition to improving survival in patients with OVS, is also effective in reducing COPD exacerbations (14-19).

Therefore, **the aim of the present study** is to identify clinical (symptoms, changes in physical examination) and paraclinical (spirometry, arterial blood gases, respiratory events during sleep) parameters that could orientate the physician towards choosing a certain type of positive pressure therapy in patients with COPD-OSA overlap syndrome.

MATERIAL AND METHODS

It is a prospective, observational study performed in the "Leon Daniello" Clinical Hospital of Pneumology in Cluj Napoca between 2019 and 2020.

Study population: were included 84 patients hospitalized for sleep study and with newly diagnosed COPD (diagnosed made according to GOLD guideline 2019) (1). Initial evaluation included a very thorough anamnesis and physical examination performed by the same physician. All patients completed the Epworth Sleepiness Scale (ESS). Blood samples, arterial blood gases, spirometry and cardio-respiratory polygraphy was performed in all patients. The polygraphy was performed using the Nox Medical polygraph, Model 8000. The data were interpreted using the NOXTURNAL software version 4.4.2.14926/2016. All results were manually validated by the same doctor with competence in sleep medicine. The validation and interpretation

were performed according to the recommendations of the American Sleep Society 2007. If the apnea-hypopnea index (AHI) was > 15 events / hour of sleep, patients were offered CPAP. To ensure a better titration quality, the patient slept one night only with the positive pressure device without the polygraph. Titration was performed depending on the result of the polygraphy and the presence or absence of hypercapnia with CPAP (DV 54 Auto adjust CPAP, ResMed) or BiPAP S or S / T (DV 57AutoBilevel, ResMed). The initial pressures for auto CPAP were the positive end-of-expiratory pressure (EPAP) minimum of 4 cmH_2O, and maximum depending on the patient's tolerance up to 20 cmH_2O. If the pressures needed by the patient were too high or not tolerated by the patient, or if hypercapnia was present then CPAP was replaced with BiPAP. For BiPAP: EPAP was at least 6 cmH_2O and IPAP varied, being at least 10 cmH_2O. Masks used were FlexiFit407 and F&P Simplus (Fisher and Paykel), nasal or oronasal mask depending on the patient's preference or patient characteristics (mouth breathing, nasal obstruction).

Statistical analysis

Data was analyzed using *IBM SPSS STATISTICS 25.0 for Windows*. The normal distribution of continuous variables was tested using the Kolmogorov-Smirnov test. The median [25th percentile (Q1) and 75th percentile (Q3)] was calculated for the quantitative variables with normal distribution. In the case of non-normally distributed quantitative variables, they were expressed using mean ± standard deviation. Qualitative variables were expressed in numbers / frequencies and percentages. To evaluate the association between two quantitative variables, the Pearson correlation coefficient was calculated if the variables were linear and the Spearmen's coefficient, otherwise.

RESULTS

84 patients with COPD - OSA overlap syndrome were included in the study. The clinical characteristics and paraclinical parameters (arterial blood gases, spirometry, cardio-respiratory polygraphy values) are summarized in table I A and I B.

TABLE I A

Patients demographic characteristics

Parameter	Frequency
Gender	
Male	71 (82.6%)
Female	15 (17.4%)
Environment	
City	55 (64%)
Countryside	29 (33.7%)
Smoking status	
1. never smoker	23 (26.7%)
2. active smoker	22 (25.6%)
3. former smoker	39 (45.3%)
Night shifts	39 (45.3%)
Comorbidities	
1. Obesity	77 (89.5%)
2. Arterial hypertension	80,2 (69%)
3. Endocrine diseases	16 (18.6%)
4. Dyslipidemia	64 (74.4%)
5. Gastro-esophageal reflux	15 (17.4%)
6. Stroke	3 (3.5%)
7. Ischemic heart disease	36 (41.9%)
8. Cardiac failure	31 (36%)
9. Cor pulmonale	30 (34.9%)
10. Type II diabetes	37 (43%)
Inhaler treatment	
1. Long-acting beta 2 bronchodilators	31 (36%)
2. Short acting bronchodilator as needed	1 (1.3%)
3. Bronchodilator and inhaled corticosteroids	54 (62.8%)

TABLE IB
Patients clinical characteristics

Parameters	Median 25th percentile; 75th percentile (mean ± SD)
Age (years old)	61 (51; 66)
Height (m)	1.7 (1.66; 1.75)
Weight (kg)	110 (100; 122)
BMI (kg/m²)	37.37 (33.25; 41.8)
EPWORTH score	9.5 (6; 16)
Pack-a-year index	21.25 (0; 35.5)
Neck diameter	46.04 ± 3.73
Abdominal circumference	128.66 ± 14.33
Abdominal-waist index	1.08 ± 0.09
AHI	38.2 (22.4; 63.9)
ODI	43.85 (22.4; 72.4)
Min O_2 saturation	73.5 (61; 81)
Average O_2 saturation	89 (85; 92)
HR average	67 (61; 75)
HR max	103.5 (89; 116)
HR min	48 (37; 53)
FVC (L) **FVC (%)**	2.69 ± 0.86 72.75 ± 21.44
FEV1 (L) **FEV (%)**	2.03 ± 0.84 67.05 ± 24.81
FEV1 / FVC	66.2 (63.54; 68.98)
MEF 50 (L) **MEF 50 (%)**	1.74 (0.9; 2.8) 45 (26.95; 71.8)
HCO_3	27.3 (25.5; 29.8)

The patients were symptomatic with an Epworth score of 9.5 (6; 16) out of 24. After obesity, the most common comorbidity was arterial hypertension. As for COPD treatment most, patients had a combination of inhaled corticosteroids and a bronchodilator (either long acting muscarinic or beta2 agonist) (tab. IA). In the studied population, sleep apnea syndrome was severed with the predominance of apnea (21.91 ± 17.34 events per hour of sleep, while hypopnea were 19.1 (9.6; 29.3) Desaturation index was 43.83/hour of sleep with significant desaturation during the night (tab. IB). COPD was moderate (FEV1 = 67.05 ± 24.8% of predicted) and arterial blood gases were characterized by mild hypoxemia 69.36 ± 11.39 mmHg and mild hypercapnia 42.5 mmHg (39; 46.3). The lower the oxygen saturation measured by ABG was, the

lower the FVC measured in liters (L) and percentage. Regarding the symptoms reported by patients with OVS, the predominance of snoring is observed from afar, which is found in 90.7% of patients, followed by daytime sleepiness (74.4%) - Epworth score of 9.5 and morning headache (72.1%). Nocturnal dyspnea (70% of patients), nycturia (61% of patients) and insomnia (67%) were also very frequent among patients with OVS. Positive pressure therapy of patients included in the study was mainly CPAP (57%), with a mean pressure of 8 cmH_2O (tab. II). Patients who needed non-invasive ventilation (BiPAP) were ventilated in Bi-PAP S mode with a mean IPAP of 18 cmH_2O and a mean EPAP of 8 cmH_2O. The most used interface was the oronasal mask. EPAP was higher the more severe the obstructive sleep apnea.

TABLE II

Treatment with positive pressure of patients with overlap syndrome

Parameter	Frequency	Mean ± SD
Device **BiPAP** **CPAP**	 36 (43%) 48 (57%)	
IPAP (cm H_2O)		18 (16; 20)
EPAP (cm H_2O)		8 (6; 10)
Interface **Nasal mask** **Oronasal mask**	 28 (30.2%) 56 (62.8%)	
Associated oxygen therapy	26 (30.2%)	

DISCUSSION

84 patients with COPD-OSA overlap syndrome who presented to the pulmonology department for sleep studies were evaluated. Most patients had severe sleep apnea with an average of 38 events per hour of sleep, and moderate COPD - stage II GOLD (FEV1 = 67.2% of predicted value). The positive therapy used was mainly CPAP (57%), and the preferred mask was oronasal. Our results are like those reported in the literature: CPAP appears to be the most used therapy in the treatment of patients with OVS (2, 3, 14-17). The data are however scarce, and the official recommendations sometimes contradictory. It is still debatable if CPAP or BIPAP should be prescribed for OSV. Which patients? In which conditions? One of the main reasons for prescribing non-invasive ventilation in OVS patients is persistent hypercapnia $PaCO_2 > 45$ mmHg, due mainly to type II respiratory failure a common complication of COPD patients. There are patients with OSA that could present with hypercapnia that does not correct with CPAP. Therefore, even though McNichols (8) proposed as therapeutic algorithm the use of CPAP if OSA has the predominant features and BiPAP if the COPD it's the predominant diseases, we still don't know in what measure the presence of OSA contributes to the presence of hypercapnia.

Secondly there are few particularities

that may explain patient's preference for CPAP in the present study: 1) **The population** - the present study was performed on patients who presented for sleep apnea on their own initiative and the diagnosis of COPD was made afterwards, in the clinic. This situation is quite different from patients with known COPD that would come for evaluation or with exacerbation and it would be proposed to perform ventilatory polygraphy to diagnose obstructive sleep apnea. From the beginning we have patients in whom the clinical picture is dominated by sleep apnea, so, according to the existing data we will be more prone to prescribe CPAP. Since they came on their own initiative we can suspect a greater interest in their own health, unlike COPD patients who come with exacerbation, an indirect sign of the fact that they neglect the treatment, therefor their disease. Moreover, we observed in our patients a high Epworth score (9.5 out of 24) which means that the patients were very symptomatic, and sleepiness affects greatly life quality. As reported in several studies the symptomatic patients are more likely to follow the treatment that those without symptoms (8, 9, 10, 11). We must consider, nevertheless the low sensibility of Epworth in evaluating daytime sleepiness. It has a sensibility (Se) of 27-72% and a specificity of 50-76% and a diagnostic accuracy of up to 59% for mild form obstructive sleep apnea (AHI = 5-15/ hour of sleep) and a Se = 36%, Sp = 77% for moderate and severe SASO (AHI > 15 / hour of sleep) (4). An ideal situation would be to have a comparative group of patients with OVS where COPD is the predominant disease. According to existing data, patients with obstructive sleep apnea prefer the nasal mask. It has less contact with the face compared to the oronasal face and brings an additional benefit to the patient by minimizing side effects, especially pressure lesions. There are also fewer losses (7, 8, 11, 13, 14). However, they are more difficult to tolerate, especially when the ventilation pressures are high. This may be one of the reasons why our patients preferred the oronasal mask. Educational, supportive, and behavioral interventions are very important, helping patients to accept the need for regular use of therapy (13).

2) **The patients must pay for their therapy**. In our country positive pressure therapy is not reimbursed by the National Insurance House in sleep apnea or COPD patients. Given the purchase costs, which are often prohibitive for patients, most people resort to renting the device. The cost of rent, depending on the distribution company and the characteristic of the device varies between 180-250 Ron (Romanian money) - for a CPAP, and for a BIPAP depending on the characteristics and ventilation modes offered varies between 250-350 (S, S / T), 600 - 1000 those with hybrid modes (AVAPS, AVAPS-AE, iVAPS). There should also be considered mask cost, which should normally be replaced annually. The high costs of therapy are one of the main reasons why patients either refuse the device or are not compliant to the therapy. There are companies that, under certain conditions, offer free mask (usually nasal). Despite that, however patients still prefer oro-nasal mask (25, 26). We must take this in consideration when analysis the present study results, and we consider it to be one of studies strengths despite its limitations: 1. it's performed in a single center, on a small sample; 2. the OSA diagnosis was done using ventilatory polygraphy and not nocturnal polysomnography and some patients might have been missed. Oxygen saturation, assessed by ABG, was positively correlated with age and FVC measured

in liters and percentages predicted ($r = 0.324$, $p < 0.012$). The interest of this observation lies in the fact that in older patients with OVS only positive pressure therapy may not be sufficient, additional oxygen may also be needed.

CONCLUSIONS

The positive pressure therapy used in patients with OVS seems to be CPAP and the preferred mask the oronasal mask. However, it is not possible to establish a correlation between any clinical, functional or ventilatory polygraphy parameter that could explain the choice of one or another of the devices.

Particular reason for CPAP preferences in the present study might be the studied population (patients that come for sleep studies) and the non-reimbursed therapy cost in our country.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding the scientific research.

Nicoleta Ștefania Motoc and Claudia Toma had equal contribution to this study.

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NEWS

BISULFITE PYROSEQUENCING AS A FIRST LINE DIAGNOSTIC TOOL OR SILVER-RUSSELL SYNDROME

Silver-Russell syndrome (SRS) is a pre- or post-natal growth retardation disorder caused by (epi)genetic alterations. The molecular basis and clinical value of sequential epigenetic analysis in pediatric patients with SRS were evaluated. 28 patients who met ≥ 3 Netchine-Harbison clinical scoring system (NH-CSS) criteria for SRS were enrolled. Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) was performed as the first diagnostic step and then bisulfite pyrosequencing (BP). BP for 11p15 detected methylation defects in the 11 patients for whom a diagnosis was obtained using MS-MLPA and in three patients (10.7%) who did not show abnormal results on MS-MLPA. In contrast to MS-MLPA, BP analysis provides quantitative values for methylation abnormalities and increases the diagnostic yield, particularly in cases with mild and marginal methylation abnormalities. Therefore, BP should be considered in patients for whom a diagnosis of SRS is highly suspected, even if they showed negative results in MS-MLPA. (Soo YK, *et al*. Clinical Application of Sequential Epigenetic Analysis for Diagnosis of Silver-Russell Syndrome. *Ann Lab Med* 2021; 41(4): 401-408).

ADIPOKINE PROFILE AND METABOLIC SYNDROME IN PATIENTS WITH SIMILAR ADIPOSE TISSUE MASS AND DISTRIBUTION

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ADIPOKINE PROFILE AND METABOLIC SYNDROME IN PATIENTS WITH SIMILAR ADIPOSE TISSUE MASS AND DISTRIBUTION (Abstract): In order to establish more efficient ways to prevent metabolic syndrome (MetS) in clinical settings, we need to thoroughly understand the physiopathology behind this cluster of risk factors for cardiometabolic diseases. The main point of focus concerning MetS still embraces the metabolic and secretory products of the abdominal adipose tissue. Our study aims to clearly define an adipokine profile for patients with MetS and assess possible correlations between obesity parameters, biochemical markers and adipokines. **Material and methods**: This is a cross-sectional study, elaborated over a period of two years, which involved 104 patients divided into 2 groups: with MetS and without MetS. Patients were considered as having MetS if they presented WC > 88 cm (women) / 104 cm (men) and more than one criterion of the following: glucose ≥ 100 mg/dL, HDL < 40 mg/dL (men) / < 50 mg/dL (women), TG ≥ 150 mg/dL, SBP/DBP ≥ 130/85 mmHg. Dual-Energy X-ray Absorptiometry (DEXA) was performed to assess adipose tissue distribution. All patients underwent clinical and paraclinical evaluation, including the measurements of insulin, adiponectin and leptin. **Results**: Leptin levels present a strong and positive correlation with almost all obesity parameters evaluated, with or without MetS. The strongest correlation is observed for trunk fat percentage (without MetS: r=0.648, p<0.001; with MetS: r=0. 723, p<0.001). In the group of patients without MetS adiponectin level is not associated with fat percentages, whereas in the group of patients with MetS adiponectin reports a weak and positive correlation between total fat, arms fat and legs fat percentages. Adiponectin is negatively correlated with HOMA-IR and insulin in both groups, suggesting that insulin resistance is linked to the change in adiponectin levels. Patients that present MetS also report an association between HDL and adiponectin (r=0.280, p=0.02). **Conclusions**: Patients with MetS that did not follow prior treatment for any chronic disease and are characterized by similar adipose tissue percentage and distribution, report the following: lower adiponectin levels than patients without MetS, but still a positive association with HDL; no modification in leptin levels compared to patients without MetS; negative correlation between adiponectin and insulin resistance; strong association between leptin and adipose tissue mass. **Keywords:** ADIPOKINES, ADIPOSE TISSUE, OBESITY, METABOLIC SYNDROME.

Adipose tissue is an active and complex endocrine organ, with important implications in glucose and lipid metabolism. The development of metabolic syndrome (MetS) focuses mainly on high abdominal fat, considered the most pathogenic depot that contributes to metabolic abnormalities (1, 2). The pathways that describe MetS are not yet clearly explained in the literature and debates still arise when the role, consequences and regulation of the secretory function of the adipose tissue is being discussed.

Adipokines are a class of cytokine mediators secreted by adipose cells. Adiponectin and leptin are two of the most studied adipokines, presenting opposite effects (3). Adiponectin has anti-atherogenic, anti-inflammatory and anti-diabetic properties (4, 5), while leptin promotes inflammation immune response and high cardiovascular risk (6). Thus far, the specific values of these adipokines vary among study populations with MetS. A possible bias might be the medication participants are taking for chronic diseases in studies or maybe the adiponectin or leptin paradox already present.

Therefore, a correct interpretation of the values for adiponectin, leptin and their ratio are very important in order to thoroughly stratify the pathologies the patient is at risk for (dyslipidemia, diabetes mellitus, hypertension). The first step of our study was to assess an adipokine profile in patients with MetS, but with no prior treatment, no known chronic disease and no antecedent acute atherosclerotic pathology. The next step was to identify and compare possible correlations between fat mass, MetS biochemical parameters and adipokine levels, alongside insulin and insulin-resistance, in patients with or without MetS.

MATERIAL AND METHODS

Our study was conducted over a period of 2 years and included participants that did not present any known chronic disease and had no antecedent acute atherosclerotic pathology. The study was approved by the University Ethics Committee, number 1/27.07.2020 and all participants signed an informed consent before entering the study. Exclusion criteria included pregnant women and patients that refused to sign the informed consent. All investigations were performed by trained personnel, in the same clinic and laboratory, using the same tools and analyzers, during the entire study.

The population included in the study was divided into two groups: one group of patients that presented MetS (35 participants) and one group without MetS (69 participants). The status of MetS was defined as present if patients had waist circumference (WC) > 88 cm (women) / 104 cm (men) and met at least 2 of the following criteria: glucose ≥ 100 mg/dL, HDL < 40 mg/dL (men) / < 50 mg/dL (women), TG ≥ 150 mg/dL, SBP/DBP ≥ 130/85 mmHg (7).

Clinical and
anthropometric measurements

Prior to clinical evaluation, patients did not engage in physical exercise at least 30 minutes before and did not consume caffeine or alcohol. Anthropometric parameters were measured: height (tape meter stadiometer), weight (Tanita BC730), waist and hip circumference (flexible tape) at the umbilical and greater trochanter level, respectively. Blood pressure was measured twice with a valid automated device and a mean value was considered.

Adiposity measurements

In order to accurately appreciate adiposity percentages and fat mass values, along-

side lean mass and bone mineral content, Dual-Energy X-ray Absorptiometry (DEXA) was used, as a gold standard for this measurement. We report values expressed as kg of adipose tissue per whole body/specific areas of the body or percentages of adipose tissue calculated from total body weight or a specific area weight (left and right arm, left and right leg, trunk).

Biochemical parameters

Patients included in the study were evaluated during morning time, blood samples being collected after 12 hours overnight fast. Serum samples were obtained after centrifugation at 4°C and 4,500 x g, for 10 minutes. To avoid preanalytical errors, samples were transported to the laboratory within a 2-hr. frame, with a special isothermal bag for biological samples. There was an exception for insulin, which required sample centrifugation in maximum 15 minutes after collection. Serum for insulin, adiponectin and leptin was stored in separate tubes at -20°C.

Insulin levels were measured using an enzyme-labeled chemiluminescent immunometric assay kit, Immulite 2000 Insulin, provided by Siemens (catalog number L2KIN2). Quantitative determination of the adiponectin and leptin levels was performed using enzyme-linked immunosorbent assay (ELISA) kits supplied by Biovendor-Laboratorni medicina a.s., Brno, Czech Republic: Adiponectin Human ELISA Competitive kit, CE-IVD, limit of detection 26 ng/mL, catalog number RD195023100 and Leptin Human ELISA, CE-IVD, limit of detection 0.2 ng/mL, catalog number RD191001100.

To calculate the homeostasis model assessment for insulin resistance (HOMA-IR) we applied the formula reported in the literature (8):

HOMA-IR = [insulin ($\mu U/mL$) *x* glucose *(mg/dL)*] / 405.

Markers for the metabolic syndrome were determined using the spectrophotometric method applied to each specific kit. HDL-chol was measured using the direct method (elimination/peroxidase), TG using the glycerol phosphate oxidase method and glucose using the glucose-6-phosphate dehydrogenase method.

We estimated the visceral adiposity per total body with the previous validated equations (9):

$$\text{Men: eVAI} = \left(\frac{\text{AC}}{39.68 + (1.88 \times \text{BMI})}\right) \times \left(\frac{\text{TG}}{1.03}\right) \times \left(\frac{1.31}{\text{HDL} - \text{chol}}\right)$$

$$\text{Women: eVAI} = \left(\frac{\text{AC}}{36.58 + (1.89 \times \text{BMI})}\right) \times \left(\frac{\text{TG}}{0.81}\right) \times \left(\frac{1.52}{\text{HDL} - \text{chol}}\right)$$

Normal values are 1 for both men and women, if they are non-obese and present normal adipose tissue distribution.

Statistical analysis

The database of all 104 patients was elaborated in *Microsoft Excel 2003 (Microsoft Corporation*, Redmond, WA, USA) and the statistics of the data was performed with *SPSS version 23.0* (IBM Corporation, Armonk, NY, USA). There were no missing data in our database. For each continuous variable we reported the mean ± standard deviation (SD), minimum value and maximum value. Categorical values were expressed as frequencies (percentages). One-way *ANOVA* and chi-square tests were applied for comparison of parameters between the group with MetS and the group without. Statistical power was calculated and presented as eta squared (η^2) for each

variable. In order to assess the presence/absence of correlations between variables and their strength, Pearson correlation was performed. A p value < 0.05 was considered statistically significant.

RESULTS

Characteristics of the study population divided in the 2 groups (with MetS and without MetS) are presented in first table. Our groups did not differ concerning age, gender, BMI or fat mass parameters, suggesting a good homogeneity and thus preserving possible bias at a minimum level. Regardless of no significant differences in percentages of adiposity, eVAI is significantly higher in patients with MetS, because of the variation of HDL, TG and WC.

Our study further investigated correlations between obesity parameters and adipokine levels, including their ratio and insulin values, alongside insulin resistance (tab. II).

TABLE I
Baseline characteristics of the study population

	Patients without MetS (n=35)			Patients with MetS (n=69)				
	Mean±SD	Min value	Max value	Mean±SD	Min value	Max value	p	η^2
Age (years)	58.29±7.54	45	75	60.13±8.48	39	75	0.28	0.01
Gender							0.16	-
Females	82.9% (29)	-	-	69.6% (48)	-	-		
Males	17.1% (6)	-	-	30.4% (21)	-	-		
WC (cm)	102.75±11.62	89	138	110.34±12.22	90	160	*0.003*	0.08
HC (cm)	111.86±9.33	95	132	115.43±10.87	100	155	0.10	0.03
WHR	0.92±0.06	0.79	1.08	0.96±0.06	0.81	1.08	*0.003*	0.08
BMI (kg/m^2)	30.61±5.08	22.75	44.40	32.38±5.42	21.91	53.31	0.11	0.02
FM Total (%)	40.17±6.50	18.9	49.40	40.07±6.32	26.10	53.20	0.94	<0.01
Trunk Fat (%)	39.29±6.93	17.10	50.40	40.82±6.13	28.30	60.20	0.25	0.01
Arms Fat (%)	43.91±8.26	18.89	54.98	42.80±8.97	23.99	59.20	0.54	<0.01
Legs Fat (%)	42.21±7.87	20.16	55.18	40.10±8.05	22.44	55.46	0.20	0.02
eVAI	3.36±1.47	1.38	8	7.88±5.42	1.42	33.66	*<0.001*	0.19
SBP (mmHg)	131.34±23.44	107	222	141.14±17.60	94	198	*0.01*	0.05
DBP (mmHg)	82.17±12.92	65	130	87.85±11.13	53	119	*0.02*	0.05
Adiponectin(*μg/mL)*	15.74±13.03	4.89	25.65	13.03±4.83	4.53	28.53	*0.01*	0.06
Leptin (ng/mL)	24.21±15.02	2.60	47.60	24.80±14.93	3.80	58.20	0.851	<0.01
LAR	0.53±0.41	0.06	1.81	0.61±0.40	0.08	2.27	0.317	<0.01
Insulin (*μU/mL)*	12.28±7.41	2.13	32.20	20.83±12.07	5.80	52	*<0.001*	0.13
HOMA-IR	2.98±1.94	0.49	8.53	6.48±5.08	1.48	27.25	*<0.001*	0.13
Glucose (mg/dL)	96.54±15.53	76	157	120.58±40.13	85	336	*0.001*	0.10
TG (mg/dL)	97.63±27.73	53	149	183.91±84.76	49	488	*<0.001*	0.25
HDL (mg/dL)	59.03±12.29	34	82	49.39±13.23	20	85	*0.001*	0.11

TABLE II

Correlations between adiposity parameters and hormones related to obesity

	Group		Adiponectin	Leptin	LAR	Insulin	HOMA-IR
BMI (kg/m^2)	without MetS	r	-0.22	0.510*	0.538*	0.338*	0.238
		p	0.195	*0.002*	*0.001*	*0.047*	0.168
	with MetS	r	-0.157	0.412*	0.436*	0.436*	0.387*
		p	0.199	*<0.001*	*<0.001*	*<0.001*	*0.001*
WC (cm)	without MetS	r	-0.208	0.404*	0.410*	0.322	0.253
		p	0.229	*0.016*	*0.014*	0.059	0.142
	with MetS	r	-0.181	0.167	0.271*	0.498*	0.501*
		p	0.137	0.171	*0.024*	*<0.001*	*<0.001*
HC (cm)	without MetS	r	0.012	0.470*	0.344*	0.152	0.120
		p	0.947	*0.004*	*0.043*	0.385	0.492
	with MetS	r	-0.108	0.465*	0.485*	0.378*	0.340*
		p	0.376	*<0.001*	*<0.001*	*0.001*	*0.004*
WHR	without MetS	r	-0.346*	0.101	0.248	0.326	0.247
		p	*0.042*	0.565	0.151	0.056	0.153
	with MetS	r	-0.158	-0.401*	-0.241*	0.300*	0.358*
		p	0.194	*0.001*	*0.046*	*0.012*	*0.003*
FM Total (%)	without MetS	r	0.063	0.673*	0.572*	0.218	0.200
		p	0.717	*<0.001*	*<0.001*	0.208	0.250
	with MetS	r	0.310*	0.760*	0.583*	-0.024	-0.115
		p	*0.010*	*<0.001*	*<0.001*	0.842	0.346
Trunk Fat (%)	without MetS	r	-0.056	0.648*	0.593*	0.284	0.216
		p	0.751	*<0.001*	*<0.001*	0.098	0.212
	with MetS	r	0.174	0.723*	0.605*	0.120	0.020
		p	0.153	*<0.001*	*<0.001*	0.328	0.873
Arms Fat (%)	without MetS	r	0.078	0.670*	0.539*	0.277	0.272
		p	0.655	*<0.001*	*0.001*	0.108	0.114
	with MetS	r	0.315*	0.736*	0.571*	-0.102	-0.164
		p	*0.008*	*<0.001*	*<0.001*	0.405	0.178
Legs Fat (%)	without MetS	r	0.211	0.535*	0.403*	0.044	0.093
		p	0.223	*0.001*	*0.016*	0.802	0.595
	with MetS	r	0.443*	0.659*	0.430*	-0.207	-0.279*
		p	*<0.001*	*<0.001*	*<0.001*	0.088	*0.020*
eVAI	without MetS	r	0.040	0.280	0.258	0.251	0.246
		p	0.818	0.103	0.135	0.146	0.155
	with MetS	r	-0.121	-0.067	0.018	0.095	0.180
		p	0.321	0.584	0.881	0.438	0.139

*WC=waist circumference, HC=hip circumference, WHR=waist-hip-ratio, FM total=fat mass total, eVAI=estimated visceral adipose index, LAR=leptin-adiponectin-ratio.

Leptin levels present a strong and positive correlation with almost all obesity parameters evaluated, with or without MetS. A stronger association is reported in

the MetS group concerning leptin and fat mass (total, trunk, arms, legs), suggesting that in patients with MetS adipose tissue is associated with a higher stimulation of leptin secretion. Moderate to strong correlations are observed between LAR levels and all obesity parameters. These values do not vary significantly between groups.

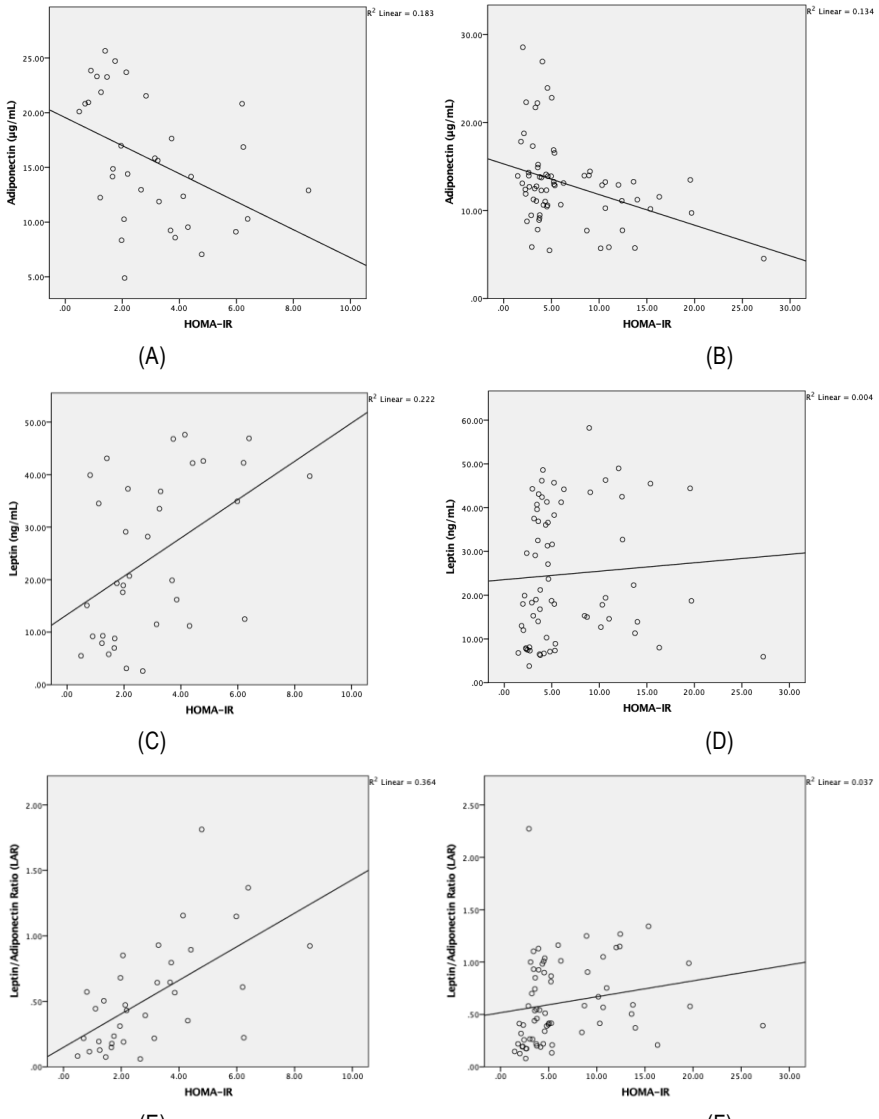

Fig. 1. Correlations between Adiponectin and HOMA-IR in (A) group without MetS (r=-0.428, p=0.01) and (B) group with MetS (r=-0.366, p=0.002); Correlations between Leptin and HOMA-IR in (C) group without MetS (r=0.471, p=0.004) and (D) group with MetS (r=0.066, p=0.592); Correlations between LAR and HOMA-IR in (E) group without MetS (r=0.604 p=<0.001) and (F) group with MetS (r=0.191, p=0.116).

In patients with MetS, insulin has higher values once the BMI is higher, but this does not seem to be associated with fat mass percentages of the whole body or of different areas of the body. At the same time, our results report moderate and positive correlations with fat mass expressed in kg and not percentages: trunk fat (kg) (r=0.369, p=0.029), left arm fat (kg) (r=0.504, p=0.002), right arm fat (kg) (r=0.496, p=0.002). In the other group, insulin is also associated only with fat mass expressed in kg: total fat (kg) (r=0.332, p=0.005), right arm fat (kg) (r=0.300, p=0.012), trunk fat (kg) (r=0.419, p<0.001).

In the group of patients without MetS adiponectin level is not associated with fat percentages in different areas of the body or total fat percentage, whereas in the group of patients with MetS adiponectin reports a weak and positive correlation between fat mass total, arms fat and legs fat percentages. Trunk fat percentage is not associated with adiponectin levels (p>0.05). Therefore, our study further analyzed the relationship between adipokines, and HOMA-IR. Adiponectin is negatively correlated with HOMA-IR and insulin in both groups, suggesting that insulin resistance is linked to the change in adiponectin levels (fig. 1).

Regarding biochemical parameters, glucose presents moderate correlations with HOMA-IR in the MetS group (r=0.563, p < 0.001) and in the group without MetS (r=0.521, p=0.001). Patients that present MetS also report an association between glucose and insulin (r=0.238, p=0.049) and between HDL and adiponectin (r=0.280, p=0.02).

DISCUSSION

Adiponectin levels differ statistically between the 2 groups, suggesting that the value decreases when MetS is present, even if patients present the same percentage of adipose tissue per total body and per different areas of the body. This result is supported by other studies from the literature that report a lower secretion rate of adiponectin in obesity-related diseases, including diabetes mellitus, dyslipidemia, hypertension or atherosclerotic cardiovascular disease (10-12). Recent reviews treat adiponectin as a potential therapeutic target for MetS, discussing drugs that increase plasma adiponectin (PPARγ agonists thiazolidinediones, phytochemicals, L-cys-teine), alongside their safety concerns and limitations (12-14). However, several clinical studies and a more recent meta-analysis report associations between high adiponectin and mortality (15-17). This adiponectin paradox seems to be characterized by adiponectin resistance, due to altered signal transduction and insulin sensitivity, as a consequence of T-cadherin hydrolysis (18-19).

In our cohort, adiponectin level correlates well with insulin resistance in both groups, while with body fat parameters the correlation is not present in the group without MetS. Having a very similar mean concerning the percentages of adipose tissue reported for specific areas of the body and even for the whole-body surface, these results suggest that insulin and insulin resistance are more relevant for adiponectin levels than obesity. Studies support our findings even though the entire mechanism of action of adiponectin is not yet known. When binding to its receptors AdipoR1 and AdipoR2, adiponectin activates adenosine monophosphate dependent kinase (AMPK), PPARγ and probably other signaling pathways that still need to be discovered (12, 20).

Leptin presents strong correlations with adipose tissue in both groups, reflecting an even stronger relationship in patients with MetS. Of all the areas analyzed, trunk area reports the strongest correlation, implying that abdominal adipose tissue plays a key role in leptin secretion.

Our study included the index calculated for visceral adipose tissue (eVAI), which did not correlate with adipokines or insulin. A paper on 5113 participants reports that ethnicity plays a role in establishing the cardiometabolic risk of patients and that in Europeans visceral adipose tissue (measured by ultrasonography) showed stronger positive associations with most of the cardiometabolic risk factors than subcutaneous fat (21). However, another recent study on samples of abdominal adipose tissue obtained from both subcutaneous and visceral fat depots show that adipokine dysregulation is first seen in subcutaneous adipose tissue, rather than in visceral adipose tissue. This conclusion suggests that endocrine dysfunction in the subcutaneous adipose tissue may represent an early risk sign of MetS (22).

Results from our analysis present LAR as a parameter with a direct proportional relationship with fat mass, regardless of the body surface area studied. The correlations are always stronger in patients with MetS, but not as strong as leptin alone. Although LAR levels are higher in patients with MetS, the difference between the 2 groups is not statistically significant, possibly because of the similar leptin values between the 2 groups. Even though adiponectin values differ significantly, leptin presents a stronger correlation and therefore weights more in the ratio between the 2. In the literature, high LARs report a higher risk of developing type 2 diabetes mellitus (23). Also, in a study with a 2.8-year follow-up timeframe, LAR had a better predictive value than adiponectin alone for the regression of MetS, suggesting that LAR may be used as a clinical marker for the management of MetS (24).

HOMA-IR is shown to correlate best with adiponectin in patients with MetS and with LAR in patients without MetS. Leptin and LAR do not correlate with insulin resistance in patients with MetS, but it is important to highlight those only patients with no known metabolic or cardiovascular disease were included in this study. There are some patients with high glucose values, but they are not confirmed with diabetes. Therefore, this can explain the lack of association between LAR and HOMA-IR in patients with MetS.

Several studies reported a positive correlation between adiponectin and HDL (25-28), similar to our findings in the study. Adiponectin is an antiatherogenic adipokine, with an important role in prevention. It has been demonstrated that hypoadiponectinemia is an effective predictor for decreased cholesterol efflux capacity (a measure of HDL quality and quantity) in adults, regardless of the obesity status or degree (29).

CONCLUSIONS

Patients with MetS that did not follow prior treatment for any chronic disease and are characterized by similar adipose tissue percentage and distribution, report the following: lower adiponectin levels than patients without MetS, but still a positive association with HDL; no modification in leptin levels compared to patients without MetS; negative correlation between adiponectin and insulin resistance; strong association between leptin and adipose tissue mass.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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COMPUTER VISION SYNDROME IN MEDICAL STUDENTS AND HEALTHCARE WORKERS

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COMPUTER VISION SYNDROME IN MEDICAL STUDENTS AND HEALTHCARE WORKERS (Abstract). The present study aims to open a new perspective about Computer Vision Syndrome in medical students and healthcare workers. This work aims to analyze whether there are any differences in ophthalmological and musculoskeletal symptoms between occupational groups. **Material and methods:** In this study were included 407 participants from different occupational groups: students, doctors, nurses, pharmacists and university teachers All participants completed a specially designed questionnaire (CVS-F3) via Google Forms**. Results:** The frequency of female participants was higher (68.1% *vs.* 31.9%). The most common ophthalmological symptoms encountered in students were dry eyes (73.3%), focusing difficulties (72%) and eye fatigue (69.3). The most common ophthalmological symptoms encountered in doctors were discomfort at near vision (48.3%) and blurred vision (24.4%), in nurse blurred vision (5.6%), in pharmacists focusing difficulties (5.6%) and in university teachers focusing difficulties (2.8%). The most frequently encountered Musculo-skeletal symptoms, regardless of profession, were neck, shoulder, and back pain. The number of hours spent during the day or at night correlated significantly with age and the most used electronic device was smartphone. **Conclusions:** The ophthalmic symptoms are different depending on the medical category. Instead, the musculoskeletal symptoms most often encountered in all medical categories were neck, shoulder and back pain. **Keywords:** COMPUTER VISION SYNDROME, VIDEO DISPLAY TERMINALS, MEDICAL STUDENTS, DOCTORS, NURSES.

The aim of the present study is to analyze the relationship between Computer Vision Syndrome (CVS) and exposure to various types of gadgets in healthcare workers and medical students. Moreover, this work aims to analyze whether there are differences between occupational groups.

According to the Sixth European Working Conditions Survey (Eurofound, 2017), more than half of European workers use various gadgets such as laptops, computers, tablets or smartphones at work. The economic sectors with the most widespread use of gadgets are financial services, public administration, education and health (1).

Due to the SARS-CoV-2 pandemic, the use of gadgets in healthcare has increased substantially due to online work. Worldwide, online consultations using various electronic devices with digital screens have been promoted to avoid face-to-face contact (2).

Computer Vision Syndrome represents a series of eye, musculoskeletal and behavioral conditions caused by prolonged use of digital screen devices. The first symptoms that appear are headaches, blurred vision, red eyes, neck and back muscle pain, eye fatigue, visual accommodation difficulties and attention disorders (3).

Blehm *et al.* classified CVS into four categories:

• The first category is CVS associated with asthenopia, which includes symptoms of eye fatigue, irritated, red, and dry eyes.

• The second category is CVS associated with changes in the ocular surface and includes dryness, burning sensation and feeling of foreign body in the eyes.

• The third category is CVS associated with visual disturbances and includes symptoms such as blurred vision, diplopia, focusing difficulties and presbyopia.

• The fourth category are extraocular symptoms, mainly shoulder, neck and back pain (4).

MATERIAL AND METHODS

We conducted a cross-sectional study, which included 407 participants, as follows: Students in "Grigore T. Popa" University of Medicine and Pharmacy from Iasi; Doctors; Nurses; Pharmacists and Teaching staff of "Grigore T. Popa" University of Medicine and Pharmacy from Iasi.

The study took place between December 2021 and March 2022. All participants completed a specially designed questionnaire (CVS-F3) via Google Forms. Each participant read and signed the "Informed Consent" certifying the agreement to participate in the study and to consent to the publication of the results, for scientific purposes and under the total protection of anonymity. Prior to patient enrollment, the study protocol was approved by the Research Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi.

The data were systematized and centralized in an *SPSS 18.0* database and were processed with the statistical functions to which they lend themselves. Confidence intervals at the 95% significance threshold were used in the data presentation.

For the descriptive statistical analysis, the ANOVA test was used, which highlighted the following indicators:

• primary indicators: minimum, maximum, frequency:

• average value indicators: simple arithmetic mean, median;

• dispersion indicators: standard deviation, standard error, CI of the mean;

• the Skewness test ($-2 < p < 2$) validates the normality of the series of values, it is used when the examined variable has continuous values.

RESULTS

Of the total subjects (n=407), 71.7% were students at University of Medicine and Pharmacy Grigore T. Popa, Iasi, 4.2% were pharmacists, 20.6% doctors, 3.2% nurses. From the total doctors, 2.5% were also university teachers at "Grigore T. Popa" University of Medicine and Pharmacy from Iasi.

The series of values for age was homogeneous, which suggests that statistical significance tests can be applied: variations in the range of 18-66 years, group average 29.25 years ± 11.36, median 24 years, the result of the Skewness test $p = 1.423$ (tab. I).

The frequency of female participants was higher (68.1% vs 31.9%). Over 68% of the participants had been using electronic devices for over 5 years. Comparing by gender, it is noted that 70.8% of men and

66.8% of women used gadgets for over 5 years (p=0.125).

TABLE I .
Descriptive data regarding age (years)

		Study group
N		407
Mean		29.25
Median		24
Standard deviation		11.36
Variance		38.84
Skewness Test		1.423
Er. standard Skewness		0.121
Minimum		18
Maximum		66
Percentile	25	22
	50	24
	75	31

Of the total subjects (n=407), 292 (71.7%) were students, predominantly female (64.7% vs 35.3%; p=0.019). From the total students, 44 .5% were studying general medicine, 39.1% nursing, 7% dentistry, 4.7% pharmacy, and 44.3% of them being in the years 2-3 of study.

The total number of hours spent on electronic devices did not correlate significantly with age (r= -0.079; p=0.113), but the number of hours spent during the day or at night correlated significantly with age, highlighting the fact that older people use electronics devices more hours during the day (r= 0.110; p=0.026), and younger people use gadgets more hours at night (r= -0.242; p=0.001) (fig.1).

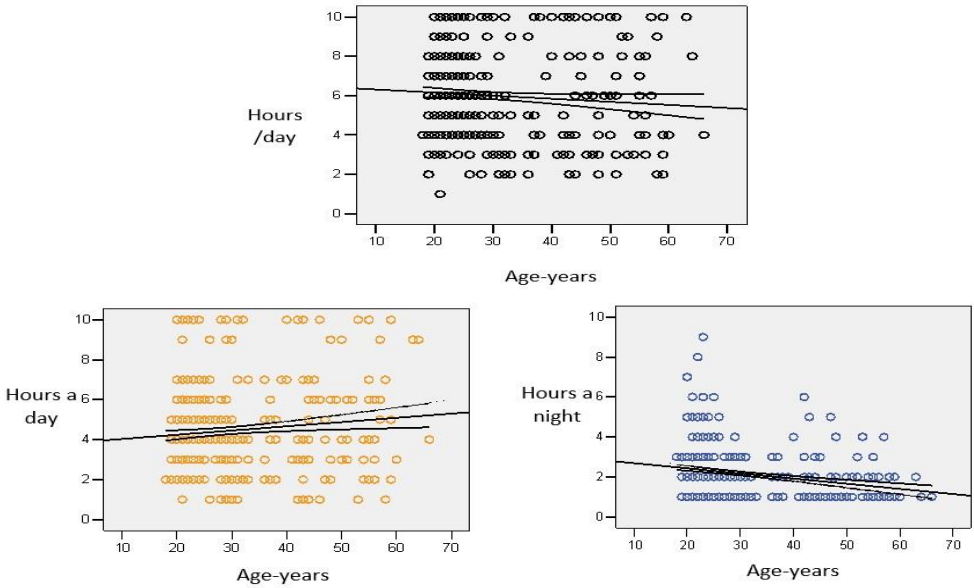

Fig. 1. Correlation of age with time spent on electronic devices

In our study, the most used electronic device was smartphone (83.8%), followed by laptop (78.4%), computer (21.4%) and tablet (17.4%). Regardless of the device, the daily usage time was 5-8 hours. From the total of participant, 88.2% stated that the hours spent using the gadgets are interrupted, and only 11.8% spent continuously

hours using gadgets.

Of the 214 subjects (52.6%) who wear glasses (71%) were female and 102 (47.7%) changed diopters in the last 12 months. Of the total 407 participants, 65.4% of students, 24.3% of doctors, 4.7% of pharmacists and 2.8% of nurses wore glasses. Only 3.9% of students wore contact lenses.

Myopia was the most common refractive error, being found in 37.1% of students, 33.3% of doctors, 35.3% of pharmacists and 38.5% in nurses (fig. 2).

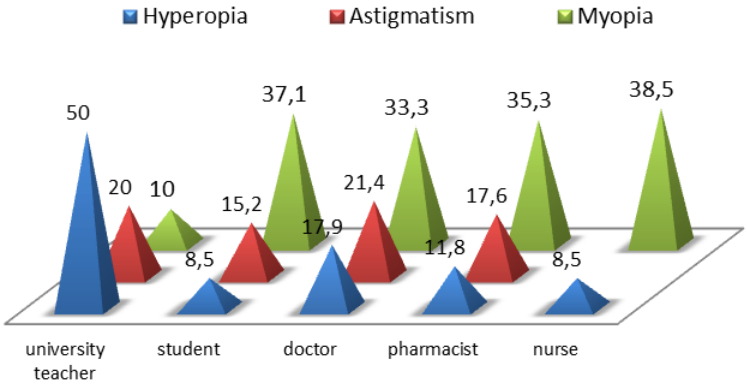

Fig. 2. Frequency of refractive errors according to profession

With the exception of smartphone, where the degree of illumination of the screen was most often between 50-70%, in the other types of gadgets, the degree of illumination of the screen was most often 10-40% (fig.3).

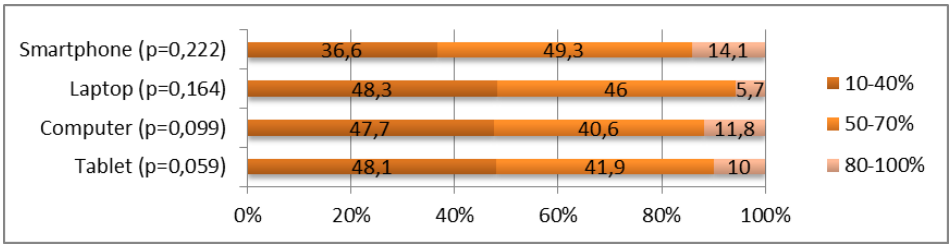

Fig. 3. Frequency of gadgets used according to screen lightning

Of the total study participants, 47.66% declared that they use artificial light at the office while using gadgets, and 52.33% declared that they use natural light, the percentage difference being minor. Of the 407 participants, 20.3% declared that they suffer from dry eyes, and they use artificial tears.

The most common ophthalmological symptoms encountered in students were dry eyes (73.3%), focusing difficulties (72%) and eye fatigue (69.3) (fig.4). The most common ophthalmological symptoms encountered in doctors were discomfort at near vision (48.3%) and blurred vision (24.4%), in nurse blurred vision (5.6%), in pharmacists focusing difficulties (5.6%) and in university teachers focusing difficulties (2.8%) (fig. 4).

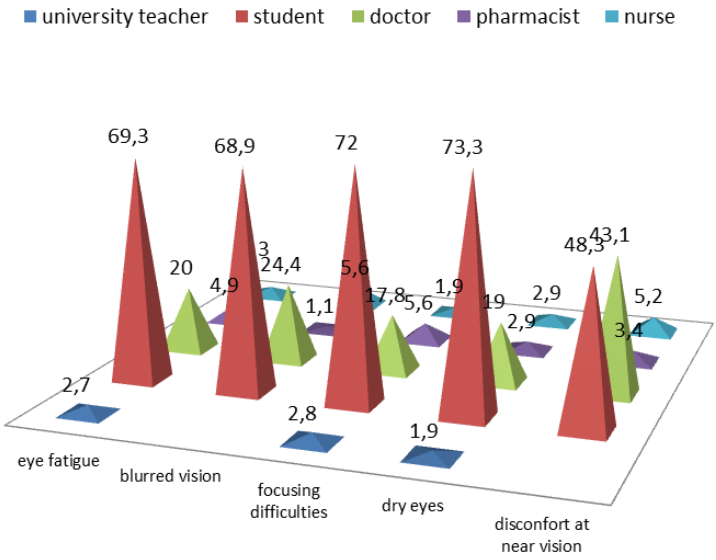

Fig. 4. Frequency of ophthalmological symptoms according to profession

The most frequently encountered musculoskeletal symptoms, regardless of profession, were neck, shoulder, and back pain.

Depending on the profession, there is an awareness of the fact that prolonged use of gadgets can affect health and lifestyle: 80.9% of students, 79.8% of doctors, 84.6% of nurses, 88.2% of pharmacists and 90% of university teachers believe that gadgets affect their health and lifestyle and 83.3% of total participants are willing to reduce the number of hours spent in front of gadgets.

DISCUSSION

Very few published studies have analyzed the effects of exposure to various gadgets on the visual system of healthcare workers. A North American study conducted on 380 radiologists who used computers at work estimated a prevalence of eye strain of 36% (5). Other hospital-based studies either focused on a sample of caregivers and computer operators (6) or did not specify whether their sample consisted of healthcare workers (7,8). However, a review of the literature concluded that the occurrence of CVS should be considered in doctors, nurses and other healthcare workers due to the use of tablets and laptops in hospitals (9).

A study conducted in 2021 on 382 students revealed that 63.5% of participants have been using gadgets for over 4 years (10). This data is similar to the results of our study where over 68% of participants have been using electronic devices for over 5 years. The results of the same study show that the most used gadget is the smartphone 71% of the participants (10), these results being similar to those of the present study in which the most used gadget was also the smartphone, being used by 341 participants.

The results of a study conducted on 100 medical students showed that the most common symptoms associated with pro-

longed use of gadgets were dry eyes, blurred vision, and pain in the neck, shoulders and back (11). The most common symptoms associated with Computer Vison Syndrome reported in another study of 80 medical students were headache in 50% of participants and dry eye in 45% of them (12). These data are similar to our research where the most common symptoms associated with gadget use were dry eyes found in 73.3% of students, blurred vision in 68.9%, and neck, shoulder and back pain were declared by 65.2% of the students.

The same study mentioned earlier showed that only 13% of college students use gadgets during the day, and 87% of them said they use gadgets during the night (11). These data are similar to the results of our study in which the number of hours spent during the day or at night correlated significantly with age, highlighting the fact that older people use electronic devices more hours during the day ($r= 0.110$; $p=0.026$), and younger people use gadgets more hours at night ($r= -0.242$; $p=0.001$).

A 2022 study that analyzed the effects of prolonged gadget use on physical and mental health among healthcare workers found that the longer the time spent working in front of electronic devices with digital screens, the greater the risk of muscle pain, headaches and stress at work. The results of the study showed that it is important to reduce the daily exposure to gadgets for doctors and nurses (13). Another 2022 study found a high prevalence of CVS among healthcare workers (14). In our study, 79.8% of doctors and 84.6% of nurses believe that digital screens affect their health and lifestyle.

Another study conducted in 2022 that evaluated the frequency and determinants of CVS among radiologists in Saudi Arabia showed that the most common symptoms perceived by the participants were headache (72.1%), dry eye (70.7%), eye burning sensation (63.7%) and blurred vision (56.3%) (15). These results are similar to those in the present study where doctors reported that the most common eye symptoms were near vision discomfort (48.3%), blurred vision (24.4%), eye fatigue (20%) and dry eye (19%).

In our study, 65.4% of students, 24.3% of doctors, 4.7% of pharmacists and 2.8% of assistants wore glasses. Only 3.9% of students wore contact lenses. These data are similar to those in the study conducted by Iqbal et al. in which 72% of students wore glasses or contact lenses (16).

Several studies have demonstrated the health disadvantages of CVS, highlighting the fact that prolonged use of gadgets causes visual disturbances, nervousness, irritability and fatigue. This will determine a decrease in the ability to concentrate and work time, even leading to a lack of interest in personal life. Because of these negative effects, studies have suggested that special campaigns in schools, universities and media are particularly important to inform the public about the risks and disadvantages of CVS on health, work and lifestyle (17-23).

In our study, 80.9% of students, 79.8% of doctors, 84.6% of nurses, 88.2% of pharmacists and 90% of university teachers believe that digital screens affect their health and lifestyle. Of the 407 participants, 83.3% are willing to reduce the number of hours spent in front of gadgets. These percentages are higher than those in the study conducted by Gammoh which showed that 69.2% of students believe that gadgets affect their health and quality of life, and only 59.2% are willing to reduce

their time using gadgets (10), the results of our study being therefore more satisfactory.

The most effective way to prevent CVS is to develop health programs that should support a healthy lifestyle (stretching exercises, smoking cessation and adequate sleep time/quality) and to increase workers' awareness of the ergonomics of their workstation (adjustment of chair height, desktop height, keyboard and mouse arrangement, screen contrast and brightness and viewing distance) (24-26).

Several limitations should be considered when interpreting the results from our work. All participants were from different healthcare branches, so the study results may not be generalizable. Moreover, relevant information about video display terminals (VDT) working time was obtained through self-answered questionnaires, which are not always reliable. Further larger-scale studies are needed to address the effect of VDT use on CVS in medical students and healthcare workers and provide guidance regarding good working practices.

CONCLUSIONS

In the present study, the number of hours spent during the day or at night were significantly correlated with age, highlighting the fact that older people use electronic devices more hours during the day, and younger people use gadgets more hours at night.

The most common ophthalmological symptoms encountered in students were dry eyes, focusing difficulties and eye fatigue. The most common ophthalmological symptoms encountered in doctors were discomfort at near vision and blurred vision, in nurse blurred vision, in pharmacists focusing difficulties and in university teachers focusing difficulties, demonstrating that ophthalmic symptoms are different depending on the medical category. Instead, the musculoskeletal symptoms most often encountered in all medical categories were neck, shoulder and back pain.

Depending on the profession, there is an awareness of the fact that the use of electronic devices for a long time affects health and lifestyle, and the vast majority of participants are willing to reduce the number of hours spent in front of gadgets, thus highlighting the fact that regardless of the category in which found, the participants of the present study are aware of the negative effects caused by Computer Vision Syndrome and want to improve their quality of life by using gadgets for a shorter time.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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THE TIME FOR SURGERY IN THYROID NODULES

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THE TIME FOR SURGERY IN THYROID NODULES (Abstract): The aim of the study was to improve the diagnosis and surgical treatment of thyroid nodules. **Material and methods:** During 2012-2022, a serial, retrospective and prospective clinical study was conducted on 124 patients, with clinically unresponsive thyroid nodules, with/without signs of compression on adjacent structures, and/or with suspected ultrasonographic, scintigraphic, cytological malignancy. **Results:** Depending on the hyperplastic (goiter), tumor (adenoma, carcinoma), or autoimmune etiology of nodules, surgical treatment consisted of total and subtotal thyroidectomies, unilateral hemithyroidectomies, nodules enucleation, isthmectomies, combined - unilateral hemithyroid-ectomies and enucleation of the nodule or partial contralateral lobe resections and total thyroidectomy with lymphadenectomy. In 82 patients was performed frozen section for intraoperative diagnosis of malignancy and establishing the definitive volume of thyroidectomies. Complications of the intra- and postoperative period were not determined, except for one patient who developed transient paresis of a recurrent laryngeal nerve due to posttraumatic edema in the early postoperative period, with complete recovery after one month. The relapse of pathology was not identified. **Conclusions:** Once detected, thyroid nodules require clinical and imaging surveillance regardless of nodule size. Surgical treatment is indicated for nodules with clinical and paraclinical signs of malignancy, refractory to conservative treatment, and those associated with compression signs. The time for surgery should be decided in common with endocrinologists, family physicians, and other specialists in every suspicious case. **Keywords:** THYROID NODULES, SURGICAL TREATMENT.

Thyroid nodules are discrete lesions of thyroid gland parenchyma and morphologically can denote the hypertrophic form of autoimmune thyroiditis, cysts, oncocytic, follicular, or colloid adenomas and carcinomas of the thyroid gland (1, 2). Thyroid nodules are frequent occurrences in the general population and may be detected up to 76% by imaging methods. They are found four times more in women than in men, and their prevalence increases with age and body weight (3, 4).

The main clinical concern about thyroid nodules is malignant potential, causing compressive symptoms or/and thyroid dysfunction (5).

Exposure to ionizing radiation and endemic iodine deficiency are the important factors that persist in the Republic of Moldova, and other states of Eastern Europe and influence the thyroid nodule's tumorigenesis. In 1985, the morbidity index for thyroid

gland cancer was 1.35 new cases per 100,000 population annually, and after the Chernobyl accident, it continuously increased, in 2019 reaching 8.3 new cases per 100,000 population (6). In the last 10-15 years there are 8-10 times increase in the incidence of endemic diseases of the thyroid gland (7). Iodine deficiency induces a lack of synthesis of thyroid hormones with nodular hypertrophy of the thyroid gland and consequently an increase in the risk of malignancy. Follicular and undifferentiated thyroid carcinomas are more related to iodine deficiency, meanwhile, papillary thyroid cancer has significantly increased following the Chernobyl disaster (8).

For the last 30 years were published thousands of articles, books, and guidelines from different clinics and international Associations of Thyreology about the diagnosis and treatment of thyroid nodules. Due to the multidisciplinary of the problem regarding endocrinology, nuclear medicine, imaging, surgery, and oncology no unique consensus on the management of thyroid nodules was decided. In this context, every clinician concerned about patients with thyroid nodules should decide on better management for each patient according to guidelines recommendations, the presence of risk factors, and the particular evolution of the disease.

MATERIAL AND METHODS

In the clinic of the Department of Surgery no. 5 of SUMPh "N. Testemitanu", Chisinau, Republic of Moldova, during 2012-2022, a serial, retrospective, and prospective clinical study was conducted on 124 patients, with clinical and paraclinical signs of thyroid nodules. The patients included in the study, according to the inclusion criteria, previously signed informed consent.

In the first stage, all patients were examined clinically and paraclinical through standard investigations - complete blood count test, biochemical blood test, coagulogram, blood type, and Rh-factor, rapid plasma reagin, urinalysis, electrocardiogram, and chest X-rays.

The patients were examined by thyroid ultrasound. The sonographic appearance of thyroid nodules was described according to their site, size, shape, composition, echogenicity, edges, presence of calcifications, and lymphadenopathy, which were systematized into TI-RADS (Thyroid Imaging Reporting and Data System) categories: TI-RADS 1 – normal thyroid gland; TI-RADS 2 – benign nodules; TI-RADS 3 – probably benign nodules; TI-RADS 4 – highly suspicious nodules; TI-RADS 5 - probably malignant nodules. Dopplerography and son elastography have supplemented the informativeness of ultrasound in assessing the type of vascularization and, respectively, the degree of elasticity of the thyroid nodules.

In the assessment of thyroid function and nodule functional autonomy were measured total or free serum concentrations of thyroxine FT_4/T_4 and triiodothyronine FT_3/T_3, as well, were performed Technetium 99m pertechnetate thyroid scan, also were estimated markers of autoimmunity - anti-TPO (anti-thyroperoxidase), anti-TG (anti-thyroglobulin) antibodies and the tumor marker – calcitonin.

The cytological diagnosis of thyroid nodules was obtained in 53 cases by FNAB – Fine Needle Aspiration Biopsy, being reported to the Bethesda system: I – non-diagnostic or unsatisfactory; II – benign; III – atypia of undetermined significance or follicular lesion of undetermined significance; IV – follicular neoplasm or suspi-

cious for a follicular neoplasm; V – suspicious for malignancy; VI – malignant.

In addition, a frozen section was performed intraoperatively in 82 patients, for diagnosis of malignant thyroid nodules with the decision on the volume of thyroidectomies.

Based on the postoperative histological confirmation of thyroid nodules were decided the subsequent tactic and follow-up of patients in the postoperative period and served in the interpretation of pre- and intraoperative clinical and paraclinical results to establish criteria of malignancy.

The results were processed and analyzed using statistical IBM Corporation, SPSS software version 26.0. The mean, absolute and relative values, and the association coefficient were measured. Statistical Pearson's chi-square test and Fisher's exact test were applied. The p-value < 0.05 was accepted as statistically significant.

RESULTS

The obtained data revealed that thyroid nodules were detected predominantly in women 83.9% (n=104), in a ratio of 5:1 compared to men 16.1% (n=20). The age of the patients included in the study ranged from 19 to 71 years, with an average of 47.55 years among women and 42.85 years among men.

The complaints reported by the patients were: discomfort/pain while swallowing (96.0%), globus sensation ("lump in the throat") (85.5%), fatigue (72.6%), the presence of a lump in the anterior cervical region (60.5%), excitability and emotional lability (44.4%), palpitations (34.7%), dyspnea (16.1%), tremors (12.1%), sweating (8.9%), weight loss (4.0 %), dysphonia (3.2%).

All the patients were on evidence at the family physician or/and endocrinologist, from which 13.7% did not follow conservative treatment, due to the rapid increase in the size of the nodular lump and the high suspicion of malignancy, 83.9% did not obtain disease remission, despite the administered treatment, 1.6% had contralateral recurrence after hemithyroidectomy and 0.8% - ipsilateral recurrence after subtotal lobectomy (the last cases had surgeries in other clinics). 75 (60.48%) patients presented with a solitary nodule, and 49 (39.51%) with multinodular goiters. The average duration of the disease was 47.13 months, which means approximately 4 years, with a minimum of one month and a maximum of 10 years.

According to the serum levels of thyroid hormones and TSH, 92.7% of patients were euthyroid, 5.6% - hyperthyroid, and 1.6% - were hypothyroid. Autoimmune etiology (Hashimoto's Thyroiditis) of nodular goiters was confirmed by elevated Anti-TPO values and ranged from 170 IU/ml to 1140 IU/ml. Anti-TG and thyroglobulin levels did not correlate with the detected nodules, being clinically insignificant. In three cases calcitonin values exceeded the reference range (165.0 - 585.0 pg/ml) and were associated with follicular adenoma, oncocytic cell carcinoma, and papillary carcinoma, which excluded affinity to a certain malignant process.

The ultrasound size of thyroid nodules ranged from 0.8 cm to 6.0 cm, with a mean size of 2.41 cm. More affected was the right lobe in 35.5% of cases, compared to bilateral lesions at 29.8%, left lobe – at 29.0%, and isthmus - at 5.6%. By composition, mixed echoic nodules (with solid and fluid components) predominated in 64.5%, followed by solid ones in 30.6% of cases. Most nodules showed isoechogenicity

(41.9%) and hypo echogenicity (37.1%). The shape of the nodules "taller than wide" was detected only in 8.9%, irregular edges in 14.5%, and the presence of calcifications in 15.3%. Laterocervical lymphadenopathy was visualized sonographically in 8.1% but was confirmed intraoperatively only in one case (0.8%). Dopplerography showed mixed vascularization in 42.7% of examined nodules. In sonoelastography, 74.2% of nodules were distinguished by elastic tissue, and 16.9% with stiffness. Table I shows the thyroid nodule size and its correlation with malignancy.

TABLE I

Thyroid nodules size and correlation with malignancy

Nodules size	Frequency	Valid Percent	Malignant nodules frequency	Malignant nodules valid percent
0,8-1,9	56	45.2	9	40.9
2,0-2,9	32	25.8	5	22.7
3,0-3,9	20	16.1	6	27.3
4,0-4,9	11	8.9	2	9.1
5,0-6,0	5	4.0	-	-
Total	124	100.0	22	100.0

On scintigraphy, thyroid nodules were norm functional (warm) in 6.5% of cases, hypofunctional (cold) in 74.2% of cases, and hyper functional (hot) in 8.9% of cases. In 3.2% of cases, the thyroid scan was not informative because the nodules were situated posterior, and in multinodular goiters, some of the ultrasound nodules <1.0 cm were not detected.

FNAB was performed for nodules ≥1.0 cm, both solitary and those dominant in unilateral multinodular goiters. Cytologically tested nodules were included in a greater proportion in Bethesda categories II, III, and IV as shown in table II. It should be noted that, in 6 situations, the patients categorically refused to perform FNAB, due to the risk of complications.

TABLE II

Thyroid nodules cytology and correlation with malignancy

FNAB categories	Frequency	Valid Percent	Malignant nodules frequency	Malignant nodules valid percent
Bethesda I	2	3.77	-	
Bethesda II	17	32.1	4	33.3
Bethesda III	14	26.41	2	16.7
Bethesda IV	11	20.75	3	25.0
Bethesda V	8	15.1	2	16.7
Bethesda VI	1	1.87	1	8.3
Total	53	100.0	12	100.0

Indications for surgical treatment were nodules with suspicion of clinical and paraclinical malignancy, nodules continuously growing despite thyroid suppression treatment, cold nodules with hypervascularization and/or stiffness, nodular or multinodular goiters with signs of compression or with several nodules ≥ 1.0 cm, nodules or nodular goiter associated with hyperthyroidism.

The volume of thyroidectomies was decided according to patient consent and age, international guidelines recommendations, uni- or bilateral thyroid lesions, and intraoperatively results of frozen section. In 16 cases cancer was confirmed by frozen section and we extended for total thyroidectomy. The structure of performed surgeries is unfolded in table III.

Thyroidectomies were performed within the limits of healthy tissues, highlighting and respecting the adjacent anatomical structures, especially the recurrent laryngeal nerve, parathyroid glands, and trachea.

TABLE III
Type of surgery performed in the study

Surgical procedure	Frequency	Valid Percent
Hemithyroidectomy	72	58.1
Subtotal thyroidectomy	5	4.0
Total thyroidectomy	28	22.6
Isthmectomy	3	2.4
Enucleation of nodule	3	2.4
Combined	11	8.9
Reoperation	1	0.8
Thyroidectomy with lymphadenectomy	1	0.8
Total	124	100.0

Postoperatively the histopathology confirmed: Goiter - 43 cases (34.7%), Papillary carcinoma - 8 cases (6.5%), Follicular carcinoma - 10 cases (8.1%), Papillary – follicular (mixed) carcinoma - 3 cases (2.4%), Oncocytic carcinoma - 1 case (0.8%), Follicular adenoma - 56 cases (45.2%), Papillary adenoma - 1 case (0.8%), Oncocytic adenoma - 2 cases (1.6%). Thyroid carcinoma was detected in 22 cases (17.74%).

The postoperative period in all patients was favorable. The average length of stay in the hospital was 6.95 days. One of the early postoperative complications that occurred was temporary dysphonia in a patient after total thyroidectomy, caused by posttraumatic edema, which recovered completely within one month. Other early and late postoperative complications were not recorded. Thyroid hormone replacement therapy was individually corrected by endocrinologists according to serum values. Patients were monitored at an interval of 1, 3, 6, 12, and 24 months. A relapse of the pathology was not detected.

DISCUSSION

Thyroid nodules, according to the literature data, have a low malignancy rate of 5 - 15%, but due to the high prevalence in the general population, they require a conscientious approach. (1,2,4,5).

In the evaluation of thyroid nodules, the main keys are the measurements of serum TSH, ultrasonography of the thyroid gland, and FNAB (1,2,5). American Thyroid Association (ATA), American Association of Clinical Endocrinologists (AACE), American College of Endocrinology (ACE), and Associazione Medici Endocrinology (AME) recommend scintigraphy only in cases of low TSH (9,10). In our study, 92.7% of patients with thyroid nodules were euthyroid with a normal TSH and the use of scintigraphy was important for the detection of "cold" nodules, which were associated with a high risk of malignancy, as well as for determining the functional autonomy of the nodules.

TIRADS ultrasonographic criteria (ACR-TIRADS, EU-TIRADS, K-TIRADS, C-TIRADS) are sometimes not sufficient to establish the malignant potential of nodules because not all thyroid cancers have the same image. In addition to ultrasound, dopplerography and sonoelastography can be performed, which provide high informativeness, as we mentioned in the study results, sometimes equivalent to the cytological examination. In this way, invasive diagnostic procedures in long-term monitoring can be avoided (11).

Another side of the problem in the diagnosis of thyroid nodules is performing FNAB. Although in most studies cytological examination showed promising results, there are two main limitations – indeterminate cytology and inadequate specimen. The sampling of qualitative cytological material, to reduce the false negative rate requires scoring the solid areas of the nodules (12). Bethesda categories III and IV are considered indeterminate due to diagnostic errors and sometimes exceeding the moment of organ-preserving surgery (13). Furthermore, FNAB is recommended as a follow-up tool for benign nodules, but no guideline indicates the deadline for performing a biopsy of benign nodules and a certain landmark when a patient should be subjected to surgery. In a long-term surveillance of nodules and persistence of proliferative stimuli of the thyroid gland multinodularity may appear (14,16). More recent studies describe the states of discomfort, stress, and anxiety associated with repeating FNAB and the risk of complications (hematomas, dissemination of the process) (15).

The intraoperative frozen section is a valuable diagnostic test (12). In our study, it was important in the diagnosis of malignant nodules, especially with indeterminate cytology, in the decision on the volume of thyroidectomies, for solving cases in a single surgical procedure, avoiding reoperation and repeated anesthetic risk.

The diagnosis and treatment of thyroid nodules require close collaboration between different specialists, and it has to be mentioned that any suspicious malignancy case should be subjected to surgery. The increase in time for surgery may worsen the evolution and even survival of patients with low-risk thyroid cancer.

CONCLUSIONS

Thyroid nodules are clinically important due to their malignant potential, the degree of compression on adjacent structures, and the dysfunction of the thyroid gland. Once detected, thyroid nodules require clinical

and imaging surveillance regardless of nodule size.

Surgical treatment is indicated for nodules with clinical and paraclinical signs of malignancy, refractory to conservative treatment, and those associated with compression signs. The time for surgery should be decided in common with endocrinologists, family physicians, and other specialists in every suspicious case.

CONFLICT OF INTEREST AND FUNDING

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NEWS

IS CANNABIS A POTENTIAL TREATMENT FOR OBESITY AND ASSOCIATED PATHOLOGIES?

Few and often under-effective drugs are available to address the increasing prevalence of obesity and type 2 diabetes. Glucagon-like peptide 1 receptor (GLP-1R) agonists improve glycemic control but have suboptimal efficacy against adiposity and insulin resistance. Recently developed peripherally restricted CB1R inhibitors, overcome these safety issues, while drastically lowering body weight in obese animal models. These compounds may also improve specific metabolic systemic and tissue complications, including insulin and leptin resistance, dyslipidemia, nonalcoholic fatty liver disease, inflammation, β-cell loss, and diabetic nephropathy. In particular, GLP-1 secretion is modulated by changes in CB1R signaling, while insulinotropic action of GLP-1R agonism is enhanced in CB1R-deficient mice, suggesting the existence of reciprocal functional interactions between GLP-1R and CB1R. Obesity is a heterogeneous disease that requires pharmacological approaches capable of targeting several pathways. GLP-1R-based unimolecular multiagonists that affect multiple metabolic targets have exceptional preclinical efficacy and are being investigated in more and more clinical trials. Chemical coupling of these newly developed molecules with an increasing number of peripheral CB1R inhibitors may have a major impact on the pharmacotherapy of obesity and diabetes, an interesting prospect given the structural and functional plasticity of CB1R and the emergence of new generations of peripheral “fusioned” CB1R inhibitors (Zizzari P, He R, Falk S, *et al.* CB1 and GLP-1 Receptors Cross Talk Provides New Therapies for Obesity. *Diabetes*, 20121; 70(2): 415-422 / doi.org/10.2337/db20-0162).

ZINC POTENTIATES THE ANTIOXIDANT EFFECT OF DAPAGLIFLOZIN IN RATS WITH EXPERIMENTAL-INDUCED DIABETES

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ZINC POTENTIATES THE ANTIOXIDANT EFFECT OF DAPAGLIFLOZIN IN RATS WITH EXPERIMENTAL-INDUCED DIABETES (Abstract). Diabetes mellitus (DM) is a multifaceted disorder that disrupts the body's overall metabolic equilibrium, giving rise to various organ-related complications such as cardiovascular, neuronal, and renal problems. In this research, we **aimed** to investigate the effects of zinc chloride (Zn), dapagliflozin (DAPA) and their combination on some biochemical parameters evaluating the liver function, lipid metabolism and cellular oxidative processes in rats with streptozotocin-induced diabetes. **Material and methods:** The rats were distributed into five groups (of five animals each): Control: citrate buffer; STZ and fat diet; STZ+Zn and fat diet; STZ+DAPA and fat diet; STZ+DAPA+Zn and fat diet. DM was induced in rats by intraperitoneal administration of a single daily dose of streptozotocin (STZ) of 20 mg/ kg body weight (kbw), for three consecutive days, and fat diet (10 g cholesterol/100 g diet) for 4 weeks. Citrate buffer (0.1 mL/100 g body) was intraperitoneal administered to the control rats. DAPA 1 mg/kbw, and Zn 5 mg/kbw were administered orally as a single daily dose for 4 weeks. **Results:** The treatment with Zn, DAPA and their combination resulted in weight loss and a decrease in blood glucose level, the higher effect being evidenced in STZ+Dapa+Zn group. The use of Zn, DAPA and Zn+DAPA in STZ-induced DM in rats with fat diet led to a decrease in blood values of transaminases, total cholesterol, triglycerides and in malondialdehyde activity, most substantially in the group treated with DAPA and Zn association. **Conclusions:** These outcomes suggest that the supplementation with Zn of DAPA treatment, enhanced the DAPA effect on decreasing the glycemia, restored the liver function, improved the lipid metabolism, and reduced the oxidative stress in STZ-induced DM in rats. **Keywords:** ZINC, STREPTOZOTOCIN, DIABETES, RATS, ANTIOXIDANT.

Type 2 DM is a chronic and widespread disease that arises from a combination of genetics and environmental factors, including sedentary lifestyle and obesity. The disease and its complications pose a significant public health challenge globally affecting both developed and developing countries with high rates of morbidity and mortality.

Unfortunately, the prevalence of type 2 DM is rapidly increasing, particularly in populations undergoing modernization. The disease often remains undiagnosed until serious complications emerge, and many risk factors are associated with it. The existing therapies are often ineffective and expensive, highlighting the need for new, efficient prevention and treatment strategies.

The selective glucose-sodium cotransporter 2 (SGLT2) inhibitors (empagliflozin, dapa-gliflozin, canagliflozin) belong to a new class of drugs approved for use as antihyperglycemic therapy (1). They reduce plasma concentrations of glucose through the renal inhibition of its reabsorption, inducing glucosuria (2). Their actions include: the decreasing glycated hemoglobin, the lowering postprandial blood glucose, reducing body weight and blood pressure (3-5).

In healthy individuals, 90% of the filtered glucose is reabsorbed by the glucose-sodium cotransporter 2 (SGLT-2) in the renal proximal convoluted tubule, and the remaining 10% is reabsorbed by the SGLT-1 transporter with a high affinity, in the right segment of the proximal descending tube (6, 7). In nondiabetic subjects, no glucose appears in the urine until the plasma glucose concentration exceeds 180-200 mg/dl, at which point all excess filtered glucose is eliminated. In people with DM, the threshold at which glucose appears in the urine, as well as the maximal renal tubular reabsorption capacity, are significantly increased, thus contributing to the maintenance of hyperglycemia (8, 9).

SGLT2 is expressed in the proximal tubule and mediates the reabsorption of approximately 90% of the filtered glucose. SGLT2 inhibitors promote renal glucose excretion and therefore modestly reduce high blood glucose levels in patients with type 2 diabetes (10). The ability to lower blood glucose and glycated hemoglobin levels is limited by the amount of filtered glucose and the osmotic diuresis caused by this medication. Although the SGLT2 inhibitors currently on the mark*et al*most completely block tubular glucose reabsorption, the measured inhibition is less than 50%, estimated based on urinary glucose excretion (11,12).

Zinc is a trace element with an essential role for growth, immunity, has antioxidant properties, and participates in the storage and processing of insulin in the body (13). Data from the literature show that the administration of zinc supplements in patients with type 2 DM improves the symptoms of the disease, as a result of the decrease in the level of cholesterol and glycated hemoglobin in the blood (14-16). Normally, zinc is concentrated in the pancreatic beta cells, at the level of the dense nucleus of the insulin-secreting granules, being essential for the formation of insulin crystals, its release, transport, storage and processing in the body (17,18).

The **purpose** of our study was the experimental research on the effects of DAPA and its association with Zn in rats with experimental-induced DM.

MATERIAL AND METHOD

Substances

STZ (molecular weight of 265.221 g/mol, catalogue code: S0130), buffer citrate (0.1 M, pH 4.5, catalogue code: P4809), DAPA (≥ 98% HPLC, molecular weight of 408.87 g/mol, catalogue code: SML2804) and Zn (molecular weight of 136.30 g/mol, catalogue code: 429430) were procured from Sigma-Aldrich, Chemical Co (Steinheim, Germany).

Animals

Healthy, non-genetically engineered white Wistar rats, weight between 150 and 200 g weight) were purchased from the National Medical-Military Institute for Research and Development, Băneasa, Bucharest, Romania through the Biobase of "Grigore T. Popa" University and Medicine and Pharmacy from Iasi and CEMEX (Advanced Research and Development Center for Experimental Medicine), Iasi, Romania.

The rats were housed in individually plexiglass cages and acclimatized for 7 days, in constant laboratory environment with a controlled temperature of 21±2°C, a relative humidity of 50-70% and 12-hours light/12-hours darkness alternative cycle. Standard granulated food and tap water were *ad libitum* offered, the daily intake was measured and the animal`s behavior was carefully monitored. All animals were weighted after one and four weeks in the experiment.

The protocol of the experiment

Rats were randomly assigned into five groups (5 animal per group), as follows: Control: citrate buffer; STZ and fat diet; STZ+Zn and fat diet; STZ+DAPA and fat diet; STZ+DAPA+Zn and fat diet. DM was induced in rats, by intraperitoneal administration of a single daily dose of STZ of 20 mg/ kg body weight (kbw), for three consecutive days, and fat diet (10 g cholesterol/100 g diet) for four weeks. After dissolving in citrate buffer (25 µg), STZ was immediately injected. Citrate buffer (0.1 mL/100 g body) was intraperitoneal administered to the control rats. DAPA 1 mg/kbw and Zn 5 mg/kbw were administered orally as a single daily dose, for four weeks.

Few drops of blood collected from caudal vein were used to measure glycemia in twelve hours fasting rats, using an Accu-Chek Go digital glucometer (Roche, Warsaw, Poland). The animals with levels higher than 200 mg/dL were recognized as having diabetes and included in the investigation.

Laboratory investigations

Before inducing diabetes (moment zero - baseline), a week and four weeks after, 0.3 mL blood were collected for biochemical analysis to assess the: ALT (alanine-aminotransferase), AST (aspartate-aminotransferase), cholesterol, HDL (high density lipoprotein), triglycerides using the VITROS 750 XRC Analyzer (Alphasoft, Bochum, Germany) with specific Johnson & Johnson (Johnson, New Haven, USA) reactors.

The influence of substance tested on the oxidative processes was investigated by evaluation of SOD (superoxide dismutase) and MDA (malondialdehyde) activity. The estimation of SOD activity was carried out by spectrophotometric analysis, using specific kits from the RANSOD kit (catalogue code19160-1KT-F) of RANDOX Laboratories Ltd. (Crumlin, UK). The analysis of SOD activity is based on the inhibition of the reduction of nitroblue tetrazolium, with xanthine-xanthine oxidase, used as a superoxide generator (19). The measurement of MDA blood values was performed by chromatography, using the thiobarbituric acid test as a marker of lipid peroxidation and free radical generation (20). For the HPLC (high performance liquid chromatography) method, a particular kit (catalogue code ABIN772058) bought from Redox, Bucharest, Romania was used.

Ethical aspects of the research

The research methodology was approved by our University Committee for Research and Ethical Issues (Certificate No. 30/14.01.2021) and was carried out in accordance with international standards (21)

about the handling of laboratory animals.

Statistical analysis

The mean ± standard deviation (S.D.) of average values was calculated for each group and the statistical differences among the groups were assessed using SPSS software version 17.0 for Windows and EXCEL application (IBM, New York, United States). The probability (*p*) values below 0.05 were estimated to be significant versus control group.

RESULTS

At the beginning of the experiment, no significant variations in the animals body weight between STZ, STX+Zn, STZ+DAPA, STZ+DAPA+Zn groups, and the group treated with buffer citrate were observed (fig. 1). The administration of STZ and cholesterol was associated with a continuous increase in the rats' body weight over time, which became statistically relevant versus control group (*p<0.05), but also compared to the beginning of the experiment (♦p<0.05), after one month (fig. 1). The use of Zn, DAPA and DAPA+Zn in rats with STZ and fat diet, lowered the body weight; but no significant differences were revealed between groups, as well as compared to buffer citrate group, and to baseline, at both moments of the evaluation (fig. 1).

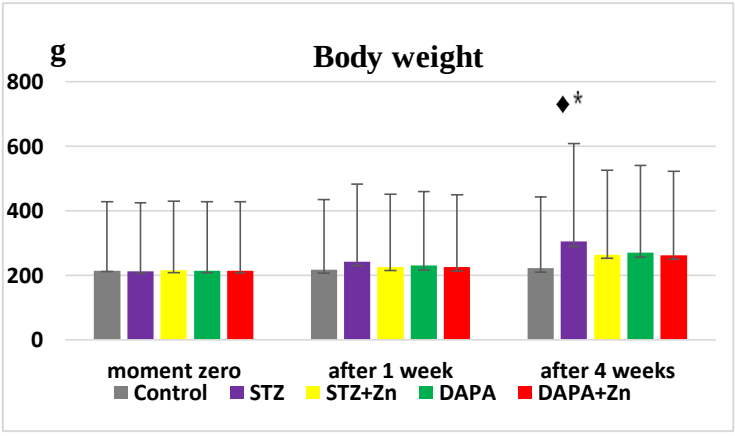

Fig. 1. The influence of DAPA, Zn and their association on the body weight in rats with STZ-induced diabetes. *p<0.05 compared to control; ♦p<0.05 compared to moment zero.

Before the inducing of DM, no modifications in glycemia values between the groups studied were observed (fig. 2). The rats with DM after the administration of STZ injection and fat diet, exhibited a progressive increase in serum sugar levels, with a statistical relevance significant compared to control group, after 7 days (**p<0.01), respectively after 4 weeks (**p<0.01). At both moments of the observation the blood glucose values were considerably higher (♦♦p<0.01) versus baseline (fig. 2).

In the diabetic rats the treatment with Zn, DAPA, or their association, prevent the increase in glycemia, which were still slightly elevated, but without relevance compared to buffer citrate group, after one week. Thereafter, the blood glucose levels were progressive diminished becoming insignificant compared to the control group after one month in the experiment (fig. 2).

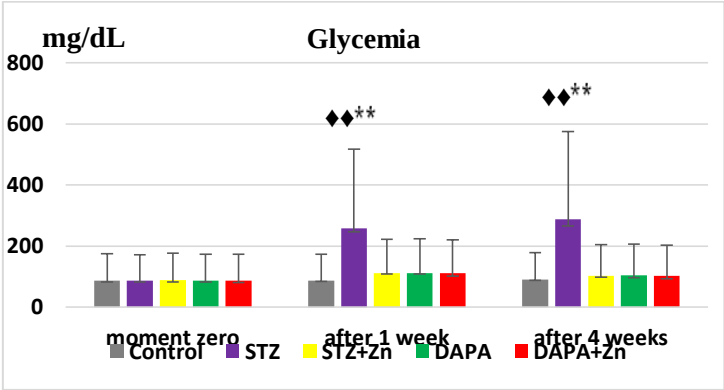

Fig. 2. The effects of DAPA, Zn and their association on the blood glucose levels in rats with STZ-induced diabetes. **p<0.01 compared to control; ♦♦p<0.01 compared to moment zero.

The induced DM with STZ on rats was accompanied by an increase of ALT and AST activity which became relevant after one month, compared to citrate buffer group (*p<0.05), as well as to the moment zero (♦p<0.05) in the experiment (tab. I). The use of Zn, DAPA, respectively DAPA+Zn in animals with STZ-induced diabetes resulted in a considerable decrease in the blood values of these two liver enzymes, their levels being comparable with those of control group at both moments of the investigation (tab. I).

The effects of the substances tested on the decreasing of ALT respectively of AST activity after 7 days, were in the descending comparison as following: STZ+DAPA+Zn > STZ+Zn > STZ+DAPA. Similar ranking was revealed for the blood levels of the transaminases after 4 weeks in the experiment (tab. I).

There were evidenced no important dissimilarities in the blood values of total cholesterol, HDL and triglycerides in the groups studied at moment zero (tab. II). In the STZ group a significant increase in serum total cholesterol levels, after one week, as well after four weeks of monitoring, statistically significant compared to control group (*p<0.05), respectively to the moment zero (♦p<0.05) (tab. II). At the same time an important increasing in triglyceride and a reduction in HDL values were noted after one month in the experiment, statistically relevant versus buffer citrate group, as well as versus the baseline (tab. II).

The treatment with Zn, DAPA and DAPA+Zn was accompanied by a diminution of the cholesterol and triglycerides and increasing of the HDL blood values during the experiment. At both moments of the determination, the intensity of the effects produced by the substances investigated on these parameters of the lipid metabolism, were in descending ordering as following: STZ+ DAPA+Zn > STZ+Zn > STZ+DAPA (tab. II).

No evident variations in the SOD and MDA blood values were shown between STZ, STZ+Zn, STZ+DAPA, STZ+DAPA+Zn and buffer citrate group at the beginning of the experiment (tab. III). In rats with STZ-induced diabetes, was revealed a continuous reduction of SOD, and increase of MD activity, with a statistical significance after four weeks, compared to the control group (*p<0.05), respectively to baseline (♦p<0.05) (tab. III). The treatment with Zn, DAPA and DAPA+Zn alleviated the disturbances of these two enzymes activity

during the interval of monitoring. At both time moments of the evaluation their serum levels were similar to control group, respectively to baseline (tab. III).

TABLE I.

The influence of DAPA, Zn and their association on the ALT and AST activity in rats with STZ-induced diabetes.

***p<0.05 compared to control; ♦p<0.05 compared to moment zero**

	Moment of evaluation	ALT (U/L)	AST (U/L)
Control	moment zero	40.54±4.29	91.64±7.27
	after 1 week	42.72±5.55	90.87±7.43
	after 4 weeks	41.36±4.17	91.25±6.67
STZ	moment zero	40.63±4.83	90.45±6.33
	after 1 week	48.24±4.45	113.39±7.45
	after 4 weeks	**69.33±5.22*♦**	**124.63±7.18*♦**
STZ+Zn	moment zero	41.48±4.67	91.82±6.83
	after 1 week	48.51±5.13	100.36±6.33
	after 4 weeks	45.75±5.05	99.48±6.29
STZ+DAPA	moment zero	41.28±3.83	91.52±5.37
	after 1 week	51.17±4.29	101.36±6.45
	after 4 weeks	48.21±5.67	100.65±6.33
STZ+DAPA+Zn	moment zero	40.92±4.41	91.68±5.29
	after 1 week	46.46±3.83	97.36±5.41
	after 4 weeks	44.21±3.55	95.45±7.17

TABLE II.

The influence of DAPA, Zn and their association on the blood values of cholesterol, HDL-cholesterol and triglycerides in rats with STZ-induced diabetes.

***p<0.05 compared to control; ♦p<0.05 compared to baseline**

	Moment of evaluation	Cholesterol (mg/dL)	HDL (mg/dL)	Triglycerides (mg/dL)
Control	moment zero	65.48±5.67	45.44±2.43	51.82±4.25
	after 1 week	64.33±5.21	46.62±2.17	52.25±4.67
	after 4 weeks	67.26±6.30	45.73±1.55	52.16±5.43
STZ	moment zero	64.82±4.13	45.35±2.21	52.44±5.21
	after 1 week	**78.63±6.45*♦**	35.36±2.67	60.59±5.33
	after 4 weeks	**80.54±6.33*♦**	**34.28±2.05*♦**	**63.78±6.17*♦**
STZ+Zn	moment zero	66.42±4.55	46.14±2.33	51.34±4.83
	after 1 week	74.37±5.05	36.65±1.29	56.26±4.79
	after 4 weeks	75.76±5.83	37.30±1.43	54.26±5.55
STZ+DAPA	moment zero	66.56±5.17	46.52±2.17	51.67±4.37
	after 1 week	76.45±5.33	36.54±2.35	57.48±5.43
	after 4 weeks	75.86±6.21	36.78±2.21	55.42±5.67
STZ+DAPA+Zn	moment zero	65.78±4.55	46.22±1.41	51.24±5.21
	after 1 week	71.43±5.05	41.64±2.55	54.67±5.33
	after 4 weeks	70.36±5.83	43.13±1.67	53.83±4.45

TABLE III.

The influence of DAPA, Zn and their association on the activity of SOD and MDA in rats with STZ-induced diabetes.

***p<0.05 compared to control; ♦p<0.05 compared to moment zero**

	Moment of evaluation	SOD (U/mg protein)	MDA (nmol/mg protein)
Control	moment zero	104.48±6.43	22.54±1.35
	after 1 week	105.63±6.55	23.27±2.11
	after 4 weeks	106.39±7.27	22.65±1.67
STZ	moment zero	105.22±6.43	22.83±1.43
	after 1 week	98.33±4.51	29.48±2.13
	after 4 weeks	**79.85±5.13*♦**	**32.67±1.25*♦**
STZ+Zn	moment zero	105.34±5.83	21.25±1.33
	after 1 week	103.62±6.67	26.71±2.21
	after 4 weeks	104.29±6.21	25.44±1.17
STZ+DAPA	moment zero	104.88±6.41	22.36±1.41
	after 1 week	100.32±6.45	27.34±1.55
	after 4 weeks	101.48±6.37	26.82±1.41
STZ+DAPA+Zn	moment zero	104.64±6.55	21.74±1.52
	after 1 week	104.13±7.21	25.22±1.13
	after 4 weeks	104.29±6.21	23.46±2.17

The effects on the specific markers of oxidative reactions varied in intensity following the exposure to the studied substances, and were observed both at one and four weeks, being in descending classification as following: STZ+DAPA+Zn > STZ+Zn > STZ+DAPA (tab. III).

DISCUSSION

Literature data revealed that SGLT2 inhibitors readily reduce blood pressure and body weight, particularly due to their glycosuric effect. Currently, empagliflozin and canagliflozin have also been shown to improve cardiovascular status in high-risk patients and slow the progression of kidney disease in diabetics (22, 23). Regarding their clinical efficacy, it is important to emphasize the obvious metabolic benefit of SGLT2 inhibitors, when used in place of other antihyperglycemic agents, such as sulfonylureas, or to reduce the daily dose of insulin (24, 25). Very important are their osmotic diuretic and natriuretic effects, which contribute to the reduction of plasma volume and the decrease of systolic and diastolic arterial pressure, which explains the cardiovascular and renal benefits (11, 26). SGLT2 inhibition is also associated with a rapid, dose-dependent decrease in glomerular filtration rate and reduction in albuminuria (27, 28).

It is known that in patients with diabetes, the zinc content of the pancreas is very low, the serum zinc levels are low, as a result of its loss due to excessive urination. Low levels of zinc in the blood plasma prevent the islets of Langerhans from secreting and producing insulin (29). Also, this divalent cation plays an important role in the antioxidant defense in patients with type 2 diabetes, in particular by acting as a cofactor of

superoxide dismutase, by modulating glutathione metabolism and metallothionein expression, by competing with iron and copper, at the cell membrane level and by inhibiting nicotinamide adenine dinucleotide phosphate oxidase. Zinc alleviates oxidative stress in these patients, by reducing chronic hyperglycemia, with the promotion of insulin receptor phosphorylation, as a result of increased glucose transport in cells (30).

Recent outcome trail data indicate that DAPA not only improves hyperglycemia but also slows the progression of DM-associated glomerulosclerosis and liver fibrosis by improving hyperglycemia-induced tissue inflammation and oxidative stress by reducing levels of ROS, MDA and increased antioxidant enzymes, including SOD and glutathione peroxidase (31,32). Other studies highlight that diabetic rat subjected to treatment with Zn experienced weight loss and a reduction in blood sugar levels. The addition of Zn to their diet resulted in a significant decrease in oxidative stress, preserved the pancreatic architecture, and restored both liver and kidney function and structure in rats with STZ-induced diabetes (33, 34).

In our research we evidenced an increase in body weight occurred in all groups in the study throughout the duration of the experiment, both the one measured at seven days and the one measured at four weeks. It was found that in the control group, the weight increase was the lowest, the highest increase being registered in the STZ and fat diet group. Although an increase in body weight versus baseline was noticed in all the other groups, this was the lowest in the STZ+Dapa+Zn group, probably due to the synergistic action of DAPA and Zn. Similar observation could be done about the modification in blood glucose values.

In rats with STZ and fat diet-induced DM, an increase in ALT and AST enzymes activity, in total cholesterol and triglyceride levels and a decrease in HLD-cholesterol blood values were observed in all study groups compared to the control group and baseline. Also, the weight gain caused by the administration of STZ, and high-fat diet was recorded in all study groups, but the most significant decrease was observed in the group treated with DAPA+Zn, probably due to the synergistic action of the two substances.

The treatment with Zn, DAPA and their combination resulted in a significant decrease in blood sugar levels in diabetic rats, in all groups studied. The administration of Zn, DAPA, respectively DAPA+Zn in animals with STZ-induced DM led to a significant improvement in the blood values of liver enzymes ALT and AST, total cholesterol, triglycerides and HDL-cholesterol, most substantially in the DAPA+Zn group.

CONCLUSIONS

In our experimental conditions, we demonstrated that supplementation with Zn of DAPA treatment enhances the DAPA effect, reduced oxidative stress, restored the liver function and improve lipids levels in STZ-induced DM in rats.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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IS THE MANNHEIM INDEX STILL USEFUL IN THE PROGNOSIS OF PATIENTS WITH SECONDARY ACUTE PERITONITIS?

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IS THE MANNHEIM INDEX STILL USEFUL IN THE PROGNOSIS OF PATIENTS WITH SECONDARY ACUTE PERITONITIS? (Abstract): Digestive tract perforations or anastomotic dehiscence are the main causes of secondary acute peritonitis, in 80% of cases rapid surgical intervention combined with antibiotic therapy leads to a favorable prognosis. The Mannheim index represents a simple and inexpensive score used in acute peritonitis, which was originally derived from a study on 1253 patients with peritonitis treated between 1963 and 1979. **Material and methods:** We performed a retrospective study on 185 patients, hospitalized with the diagnosis of acute peritonitis who underwent surgery, in the general surgery clinic of the Emergency Clinical Hospital "Bagdasar-Arseni" Bucharest between 2016 and 2019. The patients were grouped into 4 risk groups according to the Mannheim index as follows: < 12, 12 - 20, 21 - 27, > 27. **Results:** A value of the Mannheim index below 12 was recorded in 75 patients with a mortality of 8%, values between 12 and 20 were in 59 patients with a mortality of 13.55%, values between 21 and 27 were in 40 patients with a mortality of 25% and a Mannheim index value above 27 were in 11 patients with a mortality of 63.63%. **Conclusions:** The Mannheim Index may be a useful predictor of progression, and further analysis may be beneficial to explore this relationship further. **Keywords:** PERITONITIS, DIGESTIVE PERFORATION, PEPTIC ULCER, MANNHEIM INDEX.

Digestive tract perforations or anastomotic dehiscence are the main causes of secondary acute peritonitis, in 80% of cases rapid surgery combined with antibiotic therapy leads to a favorable prognosis (1), however, mortality remains high ranging between 17 and 63% (1-6). Severe forms of peritonitis are accompanied by a systemic inflammatory response whose intensity is difficult to anticipate, so we can use the Mannheim index, which represents a simple and cheap prognostic method (7, 8).

The Mannheim index represents a simple and inexpensive score used in acute

peritonitis, which was originally derived from a study of 1253 patients with peritonitis treated between 1963 and 1979 (7). It was composed by analyzing 17 risk factors among which 8 represented prognostic factors, being widely used in the prediction of mortality from acute peritonitis (9).

MATERIAL AND METHODS

We performed a retrospective study on 185 patients, hospitalized with a diagnosis of acute peritonitis and operated on, in the general surgery clinic of the Emergency Clinical Hospital "Bagdasar-Arseni" Bucharest between 2016 and 2019. The patients were grouped into 3 groups of risk, < 21, between 22 and 29 respectively > 30, and then in 4 risk groups according to the Mannheim index (Table 1) as follows: < 12, 12 - 20, 21 - 27, > 27.

The data obtained from the analysis of the variables recorded from the patients were subjected to statistical analysis through *Microsoft Excel* and *SPSS* applications, using Crosstabulation, Chi-Square, and Symmetric Measures tests.

TABLE I.

Mannheim peritonitis index

Mannheim peritonitis index	
Risk Factor	Score
Age > 50 years	5
Female sex	5
Organ failure	7
Malignancy	4
Origin of sepsis not colonic	4
Diffuse generalized peritonitis	6
Preoperative duration of peritonitis	4
Intraperitoneal exudates	
Clear	0
Cloudy, purulent	6
Fecal	12
Organ failure	
Kidney	
Creatinine level	≥ 177 μmol/L (≥ 2,31 mg/dL)
Urea level	≥ 167 mmol/l (≥467,78 mg/dL)
Oliguria	<20 ml/h
Lung (mmHg)	
PaO_2	< 50
$PaCO_2$	> 50
Shock	Hypodynamic or hyperdynamic
Intestinal obstruction (only if profound)	With paralytic ileus > 24 h, complete mechanical

RESULTS

Of the 185 patients included in the study, we had a total of 115 male patients (62.2%) and 70 female patients (37.8%), aged between 9 and 86 years, with a value average of 51.46 years and a median of 49 years, 48.64% aged over 50 years, most of the patients in the studied group, 60.54%,

coming from the urban environment.

A percentage of 92.43% of patients presented as an emergency, and the most common causes of peritonitis were represented by acute appendicitis with a percentage of 27.6% and perforated ulcer with a percentage of 24.9%, only 16.76% of the causes of peritonitis being of a malignant nature. The onset of symptoms was in most patients more than 72 hours before presentation to the emergency room, the most important symptoms being abdominal pain, nausea, and vomiting. We also mention the fact that at the presentation a percentage of 56.8% of the patients presented a good general condition, 25.4% presented an alteration of the general condition, and 15.7% of the patients at the time of presentation had established toxic-septic shock.

At the time of presentation, organ failure was found in 18 patients, in most cases, 10, being multiple organ failure.

Regarding the intraoperative appearance, in 55.68% of cases, the peritonitis was generalized, the appearance of the intraperitoneal fluid in 52.4% of cases was clear, in 37.3% purulent, in 6.5% fecaloid and 3.8% bilious.

Out of a total of 185 patients, 154 (83.2%) showed a favorable evolution, while 31 died, thus registering a mortality of 16.75%.

The values of the Mannheim index were between 0 and 43 with an average of 15.43 and a median of 15. We decided to group the patients into 3 risk groups, < 21, between 22 and 29 respectively > 30 according to the Mannheim index, in the group with values below 21 were 149 patients of which 13 died, revealing mortality of 8.72%, in the group with values between 22 and 29 we had 27 patients of which 10 died, revealing mortality of 58, 82%, and in the group with values over 29 we had 9 patients, 6 of whom died, revealing mortality of 66.66%. (tab. II). The data were analyzed statistically; thus, Chi-Square tests were performed obtaining a p-value between 0.000000554 and 0.000000087 and Symmetric Measures with a p-value between 0.000000554 and 0.000242805.

TABLE II.

Distribution of patients by Mannheim Index and mortality

		Favorable	Death	Total	Mortality (%)
Mannheim Index	< 21	134	13	149	8.72
	22 - 29	17	10	27	58.82
	>29	3 2	6	9	66.66
Total		154	31	185	16.75

Since the mortality in the group with values between 22 and 29 and in the group with values above 29 was approximately close, we decided to group the patients into 4 categories according to the Mannheim index as follows, < 12, between 12 and 20, between 21 and 27 and >27. Thus, in the group with a value below 12, only 75 patients were registered, out of which 6 died, revealing a mortality rate of 8%, in the group with values between 12 and 20, there were 59 patients, of which 8 died, revealing a mortality rate of 13.55%, in the group with values between 21 and 27 there were 40 patients of which 10 died revealing a mortality rate of 25% and in the group with Mannheim index value over 27 there were 11 patients of which 7 died revealing a

mortality rate of 63.63%. (tab. III) The data were analyzed statistically; thus, Chi-Square tests were performed obtaining a p-value between 0.00001824 and 0.00024998 and Symmetric Measures with a p-value between 0.00001183 and 0.00008787.

TABLE III.
Distribution of patients by Mannheim Index and mortality

		Favorable	Death	Total	Mortality (%)
Mannheim Index	<12	69	6	75	8.00
	12 - 20	51	8	59	13.55
	21 - 27	30	10	40	25.00
	>27	4	7	11	63.63
Total		154	31	185	16.75

DISCUSSION

Mortality in acute peritonitis is between 6 and 40% (2, 10-15), an important risk factor being organ failure (16) in our study, mortality was 16.75%.

In our study, 62.16% of patients were male, compared to other studies in which 70.6% of patients were male (10) but also different from other studies in which the percentage of male patients was between 43 and 52% (11, 13). At the same time, the studies do not record gender as a risk factor in acute peritonitis (10, 11, 13).

In many studies, Mannheim index values are divided into 3 groups: under 21, associated with low mortality, between 21 and 29 and over 29, associated with a mortality rate of up to 100% (12, 17, 18), many authors consider that a Mannheim index greater than 26 is associated with high morbidity and mortality (2, 5, 8, 10, 11, 17, 19-21).

In our study, in the case of Mannheim index values below 21, we had a mortality of 8.72%, the group with a value between 22 and 29 had a mortality of 58.82%, and patients with values above 29 had a mortality of 66.66%. For better statistical relevance in our study, we divided the patients into 4 categories according to the Mannheim index as follows, < 12 associated with a mortality of 8%, between 12 and 20 associated with a mortality of 13.55%, between 21 and 27 associated with a mortality of 25% and >27 associated with a mortality of 63.63%. This fact indicates that this prediction cannot be applied individually in terms of making therapeutic decisions in a single individual, a fact also confirmed by multicenter studies performed on large groups of patients (2, 10).

Contrary to the statements previously mentioned, certain studies support the fact that the Mannheim index can be used as a criterion in choosing the optimal treatment of acute peritonitis (17, 18 ,20), laparoscopic treatment is indicated for a Mannheim index with a value below 29 (10, 17, 22, 23).

The Mannheim index represents a severity score that is easy to use in the clinic, studies that compared it with the APACHE II score, peritonitis index of Altona, and sepsis severity score demonstrated its value, being equal or superior in assessing the prognosis (5, 12, 18, 24, 25).

CONCLUSIONS

The Mannheim index is a useful score in predicting mortality in acute peritonitis.

Based on the results obtained in our study we can conclude that there is a significant association between the Mannheim index and the evolution of patients. The Mannheim index may be a useful predictor of progression, and further analysis may be beneficial to explore this relationship further.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

RESEARCH ETHICS STATEMENT

The research was conducted with the Approval of the Ethics Committee of "Bagdasar-Arseni" Clinical Emergency Hospital.

AUTHORS' CONTRIBUTION

The first and the second authors contributed equally to this paper's achievement.

DISCLAIMER

The data presented in the article are part of a larger study, which is part of a Ph.D. thesis.

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TYPES OF DYSPLASTIC LESIONS AND COMPLIANCE WITH FOLLOW UP IN NORTHEASTERN ROMANIA – A RETROSPECTIVE STUDY

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TYPES OF DYSPLASTIC LESIONS AND COMPLIANCE WITH FOLLOW UP IN NORTHEASTERN ROMANIA - A RETROSPECTIVE STUDY (Abstract): Cervical cancer is a worrisome condition as it correlates with a high mortality rate although being one of the most preventable cancer types. **Material and methods**: The medical data of all patients subjected to an excisional procedure between 2000 and 2020 were retrospectively analyzed. Thus, data regarding age, obstetric history, types of dysplastic lesions in the history, HPV infection status, colposcopic findings, identified dysplastic lesions, and the number of screening tests performed were analyzed. Data entry and analysis were performed using SPSS software version 20. **Results:** The study included 455 patients aged between 19 and 62 years old, mean age of 36 years and a standard deviation of 9.07. The analysis of the initial cytology results revealed that the most frequently identified dysplastic lesion was HSIL (High Grade Squamous Intraepithelial Lesion), closely followed by LSIL (Low-grade squamous intraepithelial lesion). The analysis of HPV DNA test recommendations highlighted the fact that in over 50% of cases this test was not performed. In the cases in which DNA HPV test was performed, strains 16 and 18 were most frequently identified. The colposcopic examination revealed that the lesions located within the transformation zone were the most numerous. Of the described lesions, the acetowhite epithelium was most often identified, either isolated or in combination with punctation, mosaic pattern of vascularization or leukoplakia. Conization and LLETZ (Large Loop Excision of the Transformation Zone) were the two excisional procedures used in 46.4% and 44% of the patients, respectively. Histopathological examination showed: 12.5% of the patients presented high-grade HSIL (CIN3) lesions, 47.5% low-grade CIN 1 lesions, and 7% were diagnosed with carcinoma in situ. In the post-intervention follow-up, a total of 202 patients (44.39%) showed up for the first and only 66 patients (14.5%) returned for the second post-intervention follow-up. **Conclusions**: The types of dysplastic lesions identified by cytology were found to be in percentages similar to those reported in the literature, with the exception of HSIL diagnosis, which was the most frequently identified. This high rate could explain the increased incidence of cervical cancer in our country. Conization and excision procedures represent a valuable method of conservative treatment, preserving at the same time patient's fertility and avoiding a radical intervention. Low compliance with follow-up among patients from the northeastern region of Romania and the increasing migration of the groups at high risk made us use these excisional procedures even in the case of low-grade lesions in an attempt to reduce the incidence of this disease. **Keywords:** CERVICAL DYSPLASIA, MONITORING, LLETZ, CERVICAL CANCER.

Globally, cervical cancer is the most commonly diagnosed cancer in 23 countries. In recent decades, incidence and mortality rates have decreased in most areas of the world. These declines are attributed to factors related either to the increase in the average socioeconomic status of the target population, or to a lower risk of persistent infection with high-risk HPV resulting from improved genital and sexual hygiene, low parity, and a decreasing prevalence of sexually transmitted diseases. Cervical cancer screening programs have greatly improved the prognosis of this disease after their implementation in many countries in Europe, Oceania and North America. In the absence of effective screening, as is the case in Eastern Europe and Central Asia, rapid increases in premature mortality from cervical cancer have been recorded in recent years (1). Cervical cancer is a worrisome condition because it correlates with a high mortality rate, although being one of the most preventable types of cancer (2). Regarding the measures that can be taken in order to decrease the prevalence, incidence, and mortality of cervical cancer, an optimal system of actions should include the long-term follow-up of both the processes and the results of the three basic activities: vaccination, screening and treatment - as outlined in the global strategy of the World Health Organization (WHO) to eliminate cervical cancer as a public health problem. However, these measures have not been implemented fairly and directly in all countries. As of May 2020, less than 30% of low-income countries have implemented national HPV vaccination programs, compared to over 80% in high-income countries (1). Unfortunately, the importance of early diagnosis and prevention of cervical cancer is still underestimated by the general population of Romania; however, as shown by the analyzed data, in the past 10 years young patients (aged 25-40 years) have demonstrated an increased interest in routine gynecological check-ups. So, in this context, we conducted a retrospective study that evaluates the types of cervical lesions detected in northeastern Romania over a 20-year period, compliance with follow-up, as well as the risk of cervical dysplasia recurrence among patients who underwent an excisional procedure.

MATERIAL AND METHODS

To achieve this goal, we retrospectively analyzed (using the electronic archive and specific registers) the medical data of all patients subjected to an excisional procedure for dysplastic lesions of various degrees in the interval 2000-2020. The diagnosis and excisional procedure were carried out in a private clinic in Iasi town, being performed by gynecologists with over 10 years of experience in the field. All included patients gave consent for the use of personal information for health research and subsequent inclusion in the study. The data on appointments, consultations, results, and interventions in a population of 500 patients from the northeastern region of Romania, namely Suceava, Botosani, Iasi, Neamt, Vaslui, and Bacau counties, who presented themselves at the clinic between 2000-2020 were processed. Following the evaluation and application of the inclusion and exclusion criteria, a number of 45 patients were excluded from the study. Thus, data from 455 patients were analyzed, information related to age, obstetric antecedents, types of dysplastic lesions in antecedents, HPV infection status, colposcopic aspects, identified dysplas-

tic lesions, and the number of follow-ups. The inclusion criteria were presence of cervical dysplasia or HPV infection, age 18-65 years, sexually active, non-pregnant, intact uterus, without previous treatment for cervical intraepithelial neoplasia. The exclusion criteria were withdrawal of consent, invasive genital cancer at diagnosis, pregnancy, previous cervical excisional procedures, acute bacterial or mycotic cervical-vaginitis, chronic diseases such as liver cirrhosis, kidney failure, cardiovascular diseases, drug addiction or psychiatric diseases. The main goal of the study was to evaluate the types of dysplastic lesions in the northeastern region of Romania, as well as patients' compliance with follow-up in order to identify a possible cause that could explain the increased incidence of cervical cancer among the population in this geographical region. Data entry and analysis were performed using software *SPSS version 20* using descriptive and inferential statistics. In order to maintain data confidentiality and protect the identity of the patients included in the study, the personal data were anonymized. The presented cytological findings are from cervicovaginal smears collected with a specific cervical brush with the help of which cells were taken from the level of the endo and exocolus and prepared mainly in ThinPrep liquid medium (70% of cases), but also from conventional smears, the staining method being the classic one, Papanicolaou. For reporting the results of cervical cytology, the 2014 Bethesda System was used. HPV detection and genotyping were performed by isolation from the collected cervical samples on ThinPrep medium by the gynecologist, later a polymerase chain reaction (PCR) with colorimetric detection and hybridization being used. All samples were processed in RENAR accredited laboratories. Colposcopic and histological examinations were performed if a diagnosis of atypical squamous cells of undetermined significance (ASCUS) or high-grade atypical squamous cells (ASC-H), low-grade intraepithelial lesion (LSIL), high-grade intraepithelial lesion (HSIL) or carcinoma in situ was made. The colposcopic examination was performed with Video colposcope Full HD DCS 102 PRO by doctors certified in colposcopy and with more than 10 years of experience. During the colposcopic examination performed in the patients included in the study 3 parameters were considered to determine the need for biopsy. Criterion I: colposcopic impression of lesion severity or lesion grade (G) with three degrees of severity: G1: colposcopic appearance of low-grade lesion, G2: colposcopic appearance of high-grade lesion, but without signs that could suggest invasion, G3 : colposcopic appearance of invasive lesion with signs of severity: atypical vessels, ulcerations, congestion, erosions, vegetative lesions; Criterion 2: the position of the inner line of the transformation zone (TZ) or the squamocolumnar junction line (SCJ): Type 1 (TZ1): The SCJ is fully visible and is located fully on the ectocervix; Type 2 (TZ2): The SCJ is fully visible and is located either fully or partially within the endocervical canal; Type 3 (TZ3): The SCJ is invisible being located within the endocervical canal; Criterion 3: atypical size or area of the transformation zone (Q): Q1: a single quadrant covered by the atypical transformation zone, Q2: two quadrants covered by the atypical transformation zone, Q3-Q4: three or four quadrants covered by the atypical zone transformation (3). Resection techniques included conization, Loop Electrosurgical Excision Proce-

dure (LEEP) or Large Loop Excision of Transformational Zone (LLETZ). The specimens were initially examined macroscopically and later the blocks were paraffin embedded and stained using the classic hematoxylin-eosin (HE) staining method.

This study was conducted in compliance with the principles of the Declaration of Helsinki and approved by the Research Ethics Committee of the Iasi "Grigore T. Popa" University of Medicine and Pharmacy, registration number 57/17.03.2021.

RESULTS

The study included 455 patients who underwent excisional procedures for dysplastic lesions of various degrees. The study group was divided into 5 groups depending on the type of dysplastic lesions, but also based on cytology findings according to the Bethesda classification: ASCUS (20.4%), ASCH (14.9%), LSIL (30.3%) , HSIL (32.3%), CIS (2%) (tab. II). The study patients were aged 19 to 62 years, average age 36 years, standard deviation = 9.07. The highest percentage (38%) of patients presenting to the clinic for the assessment of dysplastic lesions were aged 31-40 years, and the lowest rate of presentation to the doctor, of only 9%, was recorded in patients aged 19-24 years. As to seeking medical advice, a first maximum peak was recorded in patients aged 27 and a second in those aged 34-35, subsequently the number of patients falling below 15% after the age of 44 (fig. 1).

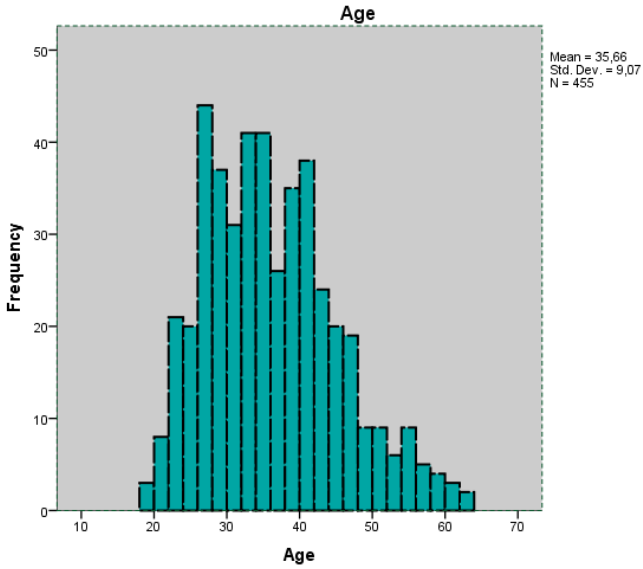

Fig. 1. Age-group frequency

Obstetric history included questions about the number of births and abortions, data that could influence the management of the identified cervical lesions. A maximum of 6 births per patient (with an average of 2 births per patient) with a standard deviation of 1.05, respectively 5 abortions per patient (with an average of 1 abortion per patient) with a standard deviation of 1.07 were recorded (tab. I). The Kruskal

Wallis test was used to compare the abortion and birth rates by maternal age and found that the distribution of abortions does not change with patients' age ($p>0.05$), while obviously the number of births varied by age ($p<0.05$) .

TABLE I.

Obstetrical history of the study patients. Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Number of Births	455	0	6	1.23	1.053
Number of Abortions	455	0	5	0.72	1.079

The analysis of the initial cytodiagnosis revealed that the 455 patients presented the following pathological aspects: the most frequent diagnosis was HSIL, closely followed by LSIL. An initial cytological diagnosis of carcinoma in situ (CIS) was made in 2% of cases. The distribution of these lesions is detailed in table II. Age-distribution according to the type of lesions differed ($p<0.05$), being higher in the case of carcinomas and high-grade lesions and lower in the case of low-grade lesions (fig. 2).

TABLE II.

Distribution of dysplastic lesions

	Frequency	Percent	Valid Percent	Cumulative Percent
ASCH (2)	68	14.9	14.9	14.9
ASCUS (1)	93	20.4	20.4	35.4
CIS (5)	9	2.0	2.00	37.4
HSIL (4)	147	32.3	32.3	69.7
LSIL (3)	138	30.3	30.3	100.0
Total	455	100.0	100.0	

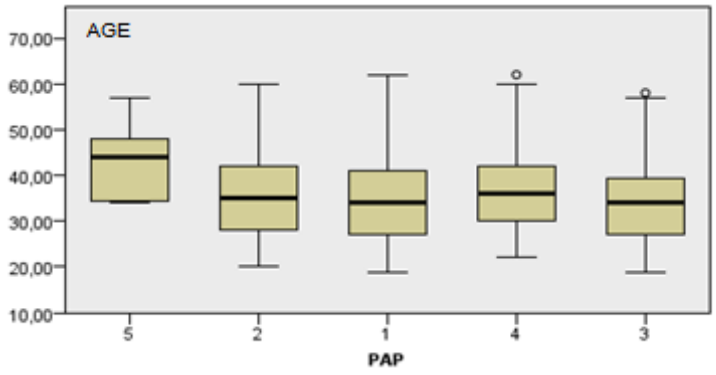

Fig. 2. Age distribution according to the type of dysplastic lesions

The analysis of the data on HPV DNA testing revealed ca over 50% of the study women never had a HPV DNA test, and in the patients in which virus typing was done

the most frequently identified were strains 16 si 18, both as unique strain and in association with other high risk (HR) and low risk (LR) strains.

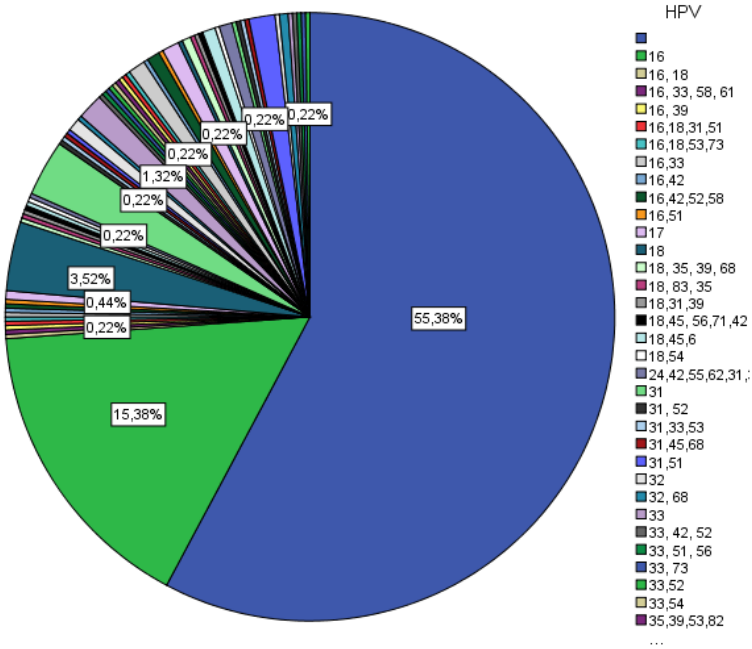

Fig. 3. Distribution of cases according to HPV genotyping results

Distribution of HPV strains in the tested patients revealed that the dominant strain was HPV 16, with most of the test being performed in the 26-30 years age group. The prevalence of HR-HPV and HPV 16 strains was found in women aged 24-41 years, after that the infection rate started to decrease.

All 455 study participants underwent colposcopic examination. Colposcopy results showed that the SCJ was visible in 72.81% of cases (fig. 4). Later, during the colposcopic examination, the 3 parameters used to determine the need for biopsy were analyzed: lesion grade (G), squamocolumnar junction (SCJ) and surface of the atypical transformation zone (Q). The obtained results were: G1/low-grade lesions were identified in over 60% of cases (fig. 4); SCJ was fully visible in 56.4%, partially visible in 30.79%, and could not be identified in 12.81% of cases. The percentage of lesions located solely within the TZ was the highest, thus confirming the potential for missed diagnosis of neoplasias when they develop predominantly at this level.

Of the described lesions, acetowhite epithelium was the most frequently identified, either isolated or in combination with mosaic and punctate vascular patterns or leukoplakia. The mosaic pattern was also seen in 116 examinations and the punctate pattern in 119 examinations. It was found that Mosaic patterns were also seen in 116 examinations and the dotted pattern in 119 examinations. Most colposcopic examinations with pathological findings had lesions limited to 1 or 2 quadrants; in the group with lesions extended to 3 or 4 quadrants, 10 cases with endometrial or vaginal extension were identified.

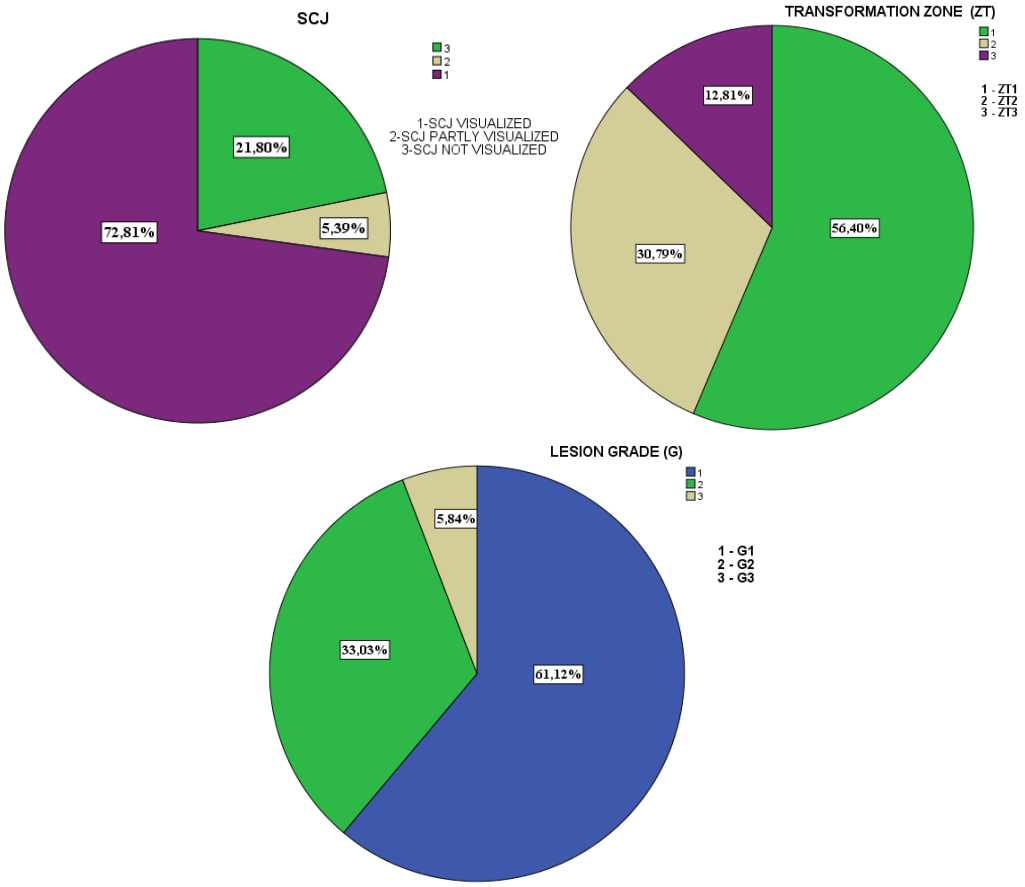

Fig. 4. Squamocolumnar junction (SCJ), Transformation zone, and Lesion grade

Excisional techniques consisted of 2 procedures performed separately or together: conization performed in 46.4% and LLETZ in 44% of the cases, in only 9 cases a colonoscopy with targeted biopsy being performed (tab. III).

TABLE III.
Types of performed excisional procedures

	Frequency	Percentage	Valid Percentage	Cumulative Percentage
	35	7.7	7.7	7.7
BIOPSY	9	2.0	2.0	9.7
CONIZATION	211	46.4	46.4	56.0
LLETZ	200	44.0	44.0	100.0
TOTAL	455	100.0	100.0	

The histopathological findings were: 12.5% of patients had high-grade HSIL (CIN3) lesions (combined or not with lower-grade lesions), 47.5% had a diagnosis of CIN1 (low-grade lesions), and 7% were diagnosed with carcinoma in situ.

Regarding the post-intervention follow-up, a total of 202 patients (44.39%) showed up for the first follow-up. The first follow-up was performed at 1 to 12 months, on average being performed at 3 months with a standard deviation of 2.22. No direct correlation was found between patients' age and post-interventional follow-up, and the low compliance with follow-up did not prove to be related to patients' age, the correlation between the two variables being very weak (R2= 0.01 (fig. 6). However, correlating age with the follow-up rate depending on the performed excisional procedure, most patients showed up for follow-up on average 3 months after the interventional procedure, with LLETZ being the most frequently performed. It is also found that the patients who showed up for the second follow-up were in the age range 26 -45 years old, therefore women of reproductive age. However, a statistically significant correlation between these variables could not be identified (R2=1.18). Thus, we can conclude that the type of performed procedure and compliance to follow-up are not age-dependent (fig. 5).

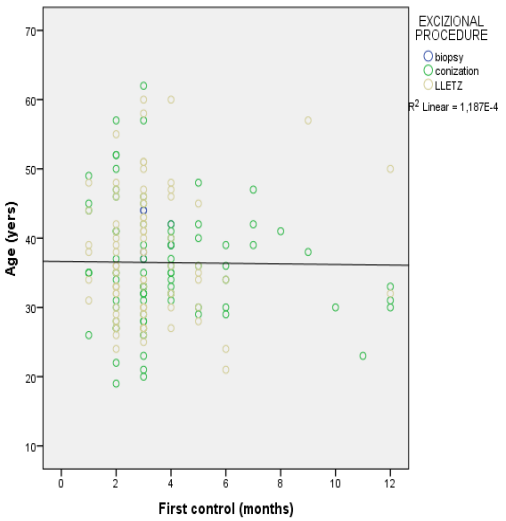

Fig. 5. Correlation between patients' age and compliance with follow-up depending on the excision procedure

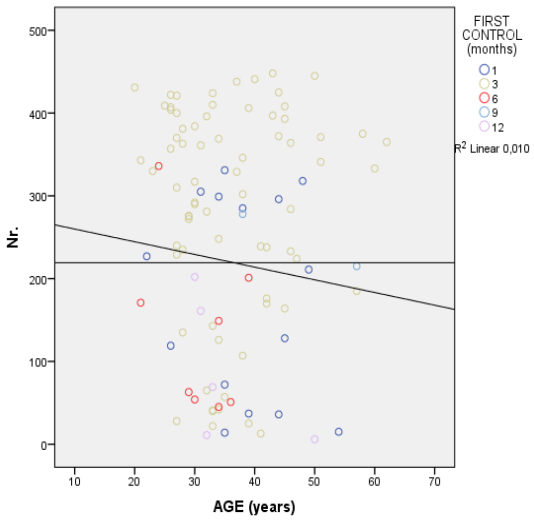

Fig. 6. Correlation between patients' age and time to the first post-interventional follow-up

Of the patients attending the first post-interventional follow-up, only 24 had an HPV test: 12 patients had a positive HPV test result and the remaining 12 negative results, demonstrating a low compliance with performing these investigations. A positive finding is that over 50% of the patients who attended the follow-up had a normal colposcopic appearance of the cervix or had a cytological diagnosis negative for

intraepithelial or malignant lesions; only 6 cases were diagnosed at the cytological re-examination with ASCUS, in 7 cases high-risk lesions were identified (CIN3, HSIL), and in two other patients the colposcopic examination revealed atypical vascularization. As for low-grade lesions, 16 patients who showed up at the first follow-up received a diagnosis of LSIL, CIN1 or CIN2. A single case was diagnosed with CIS 2 months after the first intervention. As for the colposcopies performed in the patients attending the first follow-up, in 15 patients the TZ was visible in different degrees (TZ1, TZ2, TZ3), and on examination the aceto-white epithelium (AWE) had a clear outline in 4 patients or was classified G1 or G2 in 9 patients. In 16 cases a chronic inflammatory process and in other 3 patients an incomplete post-conization scarring were found.

TABLE IV.
Diagnostic findings at the first follow-up

Diagnosis at first follow-up	Number of patients	Percentage
CIS	1	0.49%
HSIL	7	3.46%
LSIL	16	7.92%
ASCUS	6	2.97%
Inflammatory aspects	16	7.92%
NILM	123	60.89%
Colposcopic visualization ZT	15	7.42%
Colposcopic aspect EAA G1/G2	13	6.43%
Colposcopic atypical vascularization	2	0.99%
Other diseases	3	1.48%

Only 66 (14.5%) patients showed up for the second post-interventional follow-up. Cytological examination revealed: 7 patients presented ASCUS features and 4 patients LSIL type lesions, 19 patients had a cytodiagnosis negative for intraepithelial lesions or malignancy (NILM), 14 patients had normal colonoscopic appearance, and 4 patients presented inflammatory aspects (tab. V). Other pathological findings were suggestive of various diagnoses, such as Naboth cysts (1 case), genital mycosis (1 case), TZ ectopy or TZ2 (5 cases), or bacillary flora (1 case). None of the followed-up patients developed CIS or invasive carcinoma. There was only one case with HSIL (followed up at 2- and 4-months post intervention) which at the 7-month follow up was reclassified as LSIL.

Regarding the correlation between age and post-interventional follow-up, no direct correlation was found, low compliance with follow-up being independent of the patients' age. However, the fact that most patients showed up for follow-up 3 months after the interventional procedure, predominantly LLETZ, deserves mention. And so is the fact that the patients who did not miss the follow-up appointments were aged between 26 and 45 years, that is the reproductive years.

TABLE V.
Diagnostic findings at the first follow-up

DIAGNOSIS AT FOLLOW-UP 2	NUMBER OF PATIENTS	PERCENTAGE
ASCUS+LSIL	11	16.6%
Inflammatory aspects	4	6.06%
NILM	19	28.78%
Normal colposcopic findings	14	21.21%
Other diseases	18	27.27%

DISCUSSION

The study population included patients who required an excisional procedure, so the types of dysplastic lesions identified by cytology tests were in different percentages compared to the data in the literature on this topic. Thus, ASCUS-type lesions were identified in 20.4% of our patients compared to 4-5% in the literature. In our study LSIL lesions had an incidence of 30.3%, similar to that of 25-30% reported in the literature on this topic, while for HSIL lesions the incidence in our study was 32.3% versus 8% in the literature. As to carcinomas, the percentages are similar, 2% in our study vs 1% in the literature. These differences can also be explained by the profile of the study population, but at the same time they can raise a question mark regarding the increased incidence of cancer in the northeastern region of our country. The high percentage of severe lesions could be a predictive factor for the high rate of cervical cancer in our country.

Most initial HPV infections are believed to be cleared by the immune system. However, there are people in whom the HPV virus persists in a latent state in cells of the cervix, the latter becoming active through replication mechanisms, leading to dysplasia at a given time in the future. HPV induces pathological changes in the cervix due to the effects of viral proteins expressed by the virus. These changes include basal cell proliferation, nuclear multiplication with hypernucleosis, koilocytosis and abnormal mitoses (4).

The natural history of HPV infection demonstrates that it is often transient in young women, most CIN I and II cases regressing within 2 years of primary infection (5,6). The age range in the population analyzed in our study was 19 - 62 years, average age 36 years, thus most of them socially active young women. We believe that analyzing if patients saw a doctor or general practitioner and the classification of cervical diseases by age group due to future different obstetric implications are an interesting addition. Regarding the first age group in our study, patients under 24 years of age, they accounted for only 9% of all study patients and did not require HPV testing because it is a group at high risk for infection but also for spontaneous remission within 2 years mainly due to individual immunological mechanisms. However, at least those women who are already sexually active should be screened for cervical cancer by cytology.

The age group 25-40 years accounted for 64% of the patients diagnosed with cervical dysplasia included in the study. If to these we add the age group under 24

years we get a total of 73%, meaning that three quarters of the women who require consultations for dysplasia are of childbearing age, the obstetric prognosis being important and essential for this age category. Thus, in addition to preventing cervical cancer, preserving the obstetrical future of these patients has to be another main concern. These observations are in agreement with the data regarding the obstetric history of these patients, the statistical analysis showing that the number of births differs according to age, women aged between 19 and 40 years having more babies than those over 40. So, in women over 40 years, the obstetric prognosis is no longer a priority, the main concern being the prevention of cervical cancer. Also, we found that these women seek medical help less than the younger ones, accounting for only 27% of the examined patients.

The risk of HPV infection in a population increases sharply after the first intercourse. Cohort studies show that after a rapid increase in young women, the incidence of cervical HPV infection decreases with age. On one hand, this decrease is due to fewer sex partners and, on the other hand, to the specific immunity developed by some patients. Of the 203 patients assessed for HPV DNA in our study, 41.38% tested positive for HPV 16, 10.84% tested positive for HPV 18, and 32.02% tested positive for other HPV HR strains than the 2 above mentioned. Thus, the percentage of tests in which high-risk strains were detected exceeded 80%. Comparing the different age groups with positive HPV genotyping an upward trend can be observed, with a peak in high-risk viral DNA detection in patients up to 30 years of age, and a decrease after the age of 40. Fifty-one of the positive cases were up to 30 years old, the result being mainly influenced by the increased rate of infections with high-risk strains at young ages, but also possibly due to an increased level of information of this age group or a particularly favorable socio-economic status. The age-related distribution of HPV is also supported by other studies such as that by Aro *et al.* who evaluated the distribution of HPV types in a cohort of 1,279 women with cervical dysplasia (7) and found that the distribution was distinctly polarized by age. So, in terms of incidence, HPV 16/18 proved to be more common in young women. Another study reported comparable results, HPV16/18-related cervical dysplasia being found in 64%, 58%, and 35% of women aged <30, 30–44, and ≥45, respectively(8). The results are somewhat similar to those obtained in our study.

In our study, excisional interventions were performed in 47.5% of the patients with CIN1 dysplasia, which is low-grade dysplasia, and in 25.3% of the patients with CIN2 dysplasia. This therapeutic approach was based on the following considerations: they were performed when the lesions did not regress spontaneously, in low grade lesions but worrisome for the examiner because they were extended, and also in patients who did not want or did not have the possibility (for various reasons) to comply with the specific follow-up essential to adopt a "wait and see" attitude, as in the case of those from a disadvantaged socio-economic environment. Added to these is the particular group of patients who migrate to other countries for a longer period of time, who do not benefit from medical insurance, and who request a quick solution.

It would have been interesting to study how the patients with cervical anomalies

were followed-up before making the decision to perform excisional interventions, knowing that a high percentage of the dysplastic lesions, especially the low-grade ones, are likely to regress spontaneously. Unfortunately, the vast majority of women included in the study saw a doctor because of a more or less specific symptomatology, rather than for a screening assessment. In these conditions, it is not surprising that the most common therapeutic attitude was conization or LLETZ. It is obvious that from the point of view of specific management, the actions taken, and the approach used in these cases are not in accordance with the international guidelines regarding the management of the cases in which small asymptomatic lesions are discovered.

A discrepancy can be noted between the compliance with follow-up of the patients in the northeastern region of Romania included in the study and that of patients with cervical dysplasia from other countries where a follow-up appointment is scheduled every 6 months in the first 2 years after diagnosis and annually thereafter, and includes pap smear, colposcopy, and colposcopic guided biopsy, of course if clinically indicated. As a rule, HPV testing is performed at the first examination after conization in patients with documented HPV infections. Persistence of HPV infection is defined as HPV identification at the first specific examination after conization (9). In this study, we failed to establish a correlation between the persistence of HPV infection and the degree of cervical lesions, since only 24 patients underwent HPV testing after the excisional procedure.

As to the follow-ups after excisional procedures conducted in the first 3 months after the intervention, it was found that a total of 202 patients showed up for the first post-procedural follow-up, which accounted for over 50% of all examinations. Sixty-six of these patients showed-up for a second follow-up, of which 43 patients in the first 6 months after the intervention. Age-related differences in the low compliance with follow-up were noticed, this aspect not only influencing the visits to a doctor, but also the subsequent follow-up. Although most initial infections in young women are generally eradicated, there are patients in whom the HPV virus persists in the cervical cells in a latent state, the latter becoming active through replication mechanisms leading to dysplasia at a certain time in the future (4). In young women, most CIN 1 and CIN 2 cases regress within 2 years, the treatment and excision being recommended only when CIN2 persists for over 2 years on biopsy according to the guidelines. Regarding the CIN3 lesions, a small percentage will regress, but most of them will progress to cervical cancer. Current recommendations support that CIN3 in young women under 24 years to be managed as is adult women, excisional procedures being recommended (10). Although these new recommendations are medically reasonable, it is important to consider the issue of compliance. Unfortunately, a substantial number of patients is lost to follow-up as shown by our study, where less than half of the patients who underwent excisional procedures showed up for follow-up. Delayed follow-up may result in a delayed initiation of the specific treatment. Data in the literature demonstrate low compliance at 2-year follow-up even after an excisional procedure such as a LEEP (11, 12). Failure to comply with the follow-up protocol was associated with young age, lower education level, unmarried status, inadequate health insurance, better overall health, low grade

cervical lesions, as well as the lack of understanding the severity of their condition (13). Women with a low socioeconomic status and those from minority communities were identified as less likely to accept cervical cytology as a screening method. Furthermore, compliance with follow-up in these cases is much lower after an abnormal result. The absence of monitoring can substantially contribute to an increase in the incidence of invasive cervical carcinoma, this increased incidence already being reported among poor and minority women (14, 15). For all these reasons we believe that delaying additional testing for more than 12 months in patients with abnormal cytology may contribute to a delay in diagnosis and management.

Our study shows that there are no differences regarding patients' compliance with follow-up regardless of disease severity or the performance of an excisional procedure. This lack of compliance with follow-up in the analyzed population may be explained by their low socioeconomic status and increased population exodus. According to the National Institute of Statistics, "Romania continues to be a country of emigration, the phenomenon of emigration being the second main cause of the declining population. The international migration balance of Romania in 2021 was negative" (16). The number of Romanians living abroad is around 5.7 million according to the data of the Ministry of Foreign Affairs, accounting for almost 30% of the country's population (17). This problem explains the sometimes slightly more aggressive therapeutic approach compared to international guidelines. The low compliance with follow-up and the fact that some patients showed up for the first follow-up after the interventional procedure at 6 or 12 months did not allow a relevant correlation of cytodiagnosis in dynamics. Based on the results of the first follow-up after the excisional procedure we can report that 11 patients presented low-grade lesions (ASCUS+LSIL) and HPV HR infection, the group being too small for a statistically significant result. It can be noted that after a longer time interval, over 12 months, no residual high-grade lesions were observed. The literature supports the fact that the persistence of high-risk HPV infection (both HPV16/18 or other HPV HR strains) was correlated with an increased risk of recurrence of CIN 2-3 cervical lesions. Persistence of HPV infection is associated with recurrence, much more than margin status after excisional procedure, being one of the main factors predictable of relapse. This is also supported by Zhang *et al.* (18) who analyzed the data of over 500 patients who underwent conization and observed that positive margins after the excisional cause a 6-fold increase in the risk of recurrence, while the persistence of HPV HR infection cause to a 20-fold increase in the risk of recurrent disease. Considering these results, it is imperative to implement cotesting both for the detection of cervical lesions and for their monitoring (9).

A possible solution could be an organized screening and a much more effective vaccination campaign, which could increase patients' compliance with health services, being able to address this condition before the onset of clinical symptoms and thus minimizing the interventional procedures. In this respect, better ways to inform the population and the mobilization of the target population, the involvement of several health professionals, mobile screening units facilitating access to healthcare services, treatment optimization and fol-

low-up systematization, as well as the establishment of a national registration and tracking register are needed (19). Measures to inform and educate the population about this disease also necessary. Increasing the degree of awareness of this disease severity would increase compliance with follow-up. Primary prevention by vaccination against HPV virus was initiated in 2008 but was eventually abandoned because of parental refusal of vaccines. This opposition is found even in medical circles and even in cancer services. For convincing the parents about the value and safety of vaccination it is still essential that professionals to send a clear message and inspire through the power of example. Secondary prevention through a systematic screening program was initially implemented in 2012 and abandoned subsequently; a new campaign with mobile units was resumed in 2020, the target population being women aged of 25 to 65 years, which is approximately 4.2 million women in Romania. The large sample size is the main strength of the present study, and the most important limitation was the inclusion of a large group of patients who were not tested for HPV. Although being a limitation it reflects the current practice in a "real life" setting. Low compliance with follow-up also reflects a current practice, being a favorable factor for the increased incidence of cervical cancer in our country.

CONCLUSIONS

Although the etiopathogenesis of cervical cancer is strongly associated with HPV infection, not all lesions will undergo malignant transformation as cases of spontaneous remission were demonstrated under adequate monitoring. Cervical cancer is the neoplasia with the most effective preventive methods including vaccination and primary or secondary screening, thus making possible the early detection of precancerous lesions. The types of dysplastic lesions identified by cytological examination are in similar percentages with those reported in the literature, except for the diagnosis of HSIL which is the most frequently identified. This increased rate could be the basis for the increased incidence of cervical cancer in our country. Conization and excisional procedures represent a valuable method of conservative treatment while preserving the patient's fertility and avoiding a radical intervention.

The low compliance with follow-up among the patients in this region of the Romania and the increased population migration are two particular elements that lead to the use of excisional procedures even in the case of low-grade lesions in an attempt to reduce the incidence of cervical cancer. This low compliance to follow-up and the absence of vaccination can justify the fact that Romania ranks first in Europe in terms of the incidence of cervical cancer. The risk of recurrence identified after excisional procedures was low, being correlated with the persistence of HPV infection. Improving the organized screening programs and a more effective vaccination campaign could increase patients' compliance with public health services, being able to deal with this condition before symptoms arise thus minimizing the interventional procedures and progression to cervical cancer.

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ANTIBIOTIC TREATMENT AND BACTERIAL FLORA IN SPUTUM IN CHRONIC PULMONARY DISEASE EXACERBATIONS

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ANTIBIOTIC TREATMENT AND BACTERIAL FLORA IN SPUTUM IN CHRONIC PULMONARY DISEASE EXACERBATIONS. **Background and aims:** A judicious use of antibiotics for Chronic Obstructive Pulmonary Disease (COPD) or asthma frequent exacerbation is a provocation for clinician. In this context, the aim of our study was to identify and describe the commonest bacterial pathogens and the preferred antibiotic for the treatment of the patients with recurrences for COPD and asthma exacerbations because this category of patients has worse health status and increased morbidity. **Materials and methods:** A retrospective study investigated patients with recurrent COPD or bronchial asthma, more than 2 exacerbations/year and at least one early re-admission, admitted in Iasi University Clinical Hospital of Pulmonary Diseases from North-East Romania, from 2014 to 2019. **Results:** Only 33 patients were selected according to inclusion criteria. A small proportion of sputum culture identified the pathogen (20.35%) and most frequent were *Streptococcus pneumoniae* followed by *Pseudomonas aeruginosa* and *Haemophilus influenzae*. The bacteria identified were resistant to aminopenicillins and 1st - 3rd cephalosporins generation. Quinolones (levofloxacin) and colistin were the preferred antibiogram-based therapy. C**onclusions:** COPD and asthma exacerbation can be a start to antibiotic stewardship because these patients are the most exposed to inappropriate antibiotic use, decompensations, increased hospital stay, increased treatment costs. **Keywords**: ANTIBIOTIC TREATMENT, EXACERBATIONS OF COPD, EXACERBATIONS OF ASTHMA, BACTERIA.

The judicious use of antimicrobial chemotherapy is a priority of global interest, an urgent situation to be also considered in countries where the surveillance programs for their administration did not become yet a priority that requires local and national resources to stop antibiotic resistance.

Chronic respiratory diseases, like asthma and Chronic Obstructive Pulmonary Disease (COPD), are one of the main causes of mortality and morbidity worldwide. In 2017, hospital in-patient admission rates for asthma ranged from 0.12% in Italy to 0.19% in Bulgaria and those for COPD and bronchiectasis ranged from 0.62% in Cy-

prus to 4.70% in Romania (1). Our country it is on second place for hospital discharge rates for in-patients with diseases of the respiratory system, in 2019, after Bulgaria (2). In-patient average length of stay for asthma in our country was 7.8 days in 2014 and 7.9 days in 2019 (3).

Both bacterial and viral infections are predominant causes of COPD exacerbations, but viral infections are predominant causes of asthma exacerbations. Bacterial pathogens in COPD causes approximately half of reported cases. The common bacteria are *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa* and *Enterobacteriaceae* and atypical pathogens such as *Chlamydia pneumoniae* (4). According to GOLD recommendations, five to seven days of oral antibiotics should be given to COPD exacerbations that include all three increasing symptoms - sputum purulence, sputum volume and increased dyspnea or evident purulence of sputum. It is recommended to start with an association between amino penicillin and clavulanic acid, tetracyclines (doxycycline) or macrolides (azithromycin), with careful attention to multiple bacterial infections, resistant pathogens, intensive care stay, where identification of the bacteria or cultures are needed (5). According to GINA 2023 recommendations (6), antibiotic treatment is not recommended routinely for asthma exacerbations, unless is evidence of bacterial infections with fever, purulent sputum, radiographic evidence. On the other hand, a recent review of Normansell *et al.*, 2018, showed that the most prescribed antibiotics are macrolides (clarithromycin, azithromycin), and aminopenicillins (7).

A judicious use of antibiotics for COPD or asthma exacerbation is a provocation for clinician. In this context, the aim of our study was to identify the commonest bacterial pathogens and the preferred antibiotic for the treatment of the patients with recurrences for COPD and asthma exacerbations due to bacterial exacerbation of the disease.

MATERIALS AND METHODS

Study Design

A single-center, cross-sectional, retrospective study included recurrent COPD and asthma patients admitted in Iasi University Clinical Hospital of Pulmonary Diseases from North-East Romania from June 1, 2014 to May 31, 2019 (340 pulmonary, thoracic surgery and therapy beds). The study was approved by the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy of Iasi, Romania, and the Ethics Committee of Clinical Hospital of Pulmonary Diseases of Iasi, Romania (approval date 04.06.2020, respectively 22.01.2021).

Study Population and Data Collection

Patients of more than 40 years, with diagnosis of COPD, based on spirometry (with the ratio post-bronchodilator forced expiratory volume in one second (FEV1)/ forced vital capacity (FVC) < 0.70), or of asthma (with a 12%- or 200-mL improvement in FEV1 from the previous value), admitted in the hospital from June 2014 to May 2019, were screened to select patients with frequent hospitalizations.

The inclusion criteria were: 1) either COPD or bronchial asthma with specific diagnosis codes established by International Classification of Diseases 10th Revision (ICD-10): J44.0 – COPD with lower airway infection, J44.1 – COPD with acute, non-specific exacerbation, J44.8 – other specific COPD and J44.9 – non-specific COPD with all-cause rehospitalization; J45 and J46

diagnosis codes of asthma; 2) more than 2 exacerbations that needed hospital admission in the previous year; 3) at least one early re-admission (hospitalization in the first 30 days after previous discharge from hospital); 4) more than 24 hours hospitalization and 5) received at least one antibiotic during hospitalization.

The exclusion criteria were: admitted to the intensive care unit (ICU); pulmonary interstitial disease; cystic fibrosis diagnosis, pulmonary embolism; any type of cancer disease; under anti-tuberculosis treatment; and incomplete data in clinical record file.

Data extraction was from electronic and paper-based medical clinical records. The following data were collected: date of admission and discharge from hospital, length of hospitalization, age, gender, residence, comorbidities, COPD airflow severity based on spirometry, antibiotic use, bacterial pathogen from antibiogram, recommended treatment.

The main outcome was to identify the commonest bacterial pathogens isolated and the type of antibiotic chosen for the treatment.

Data Analysis

Demographic characteristics were summarized as a mean ± standard deviation (SD) for continuous variables and as a percentage of the group for categorical or dichotomous variables. The data were presented as absolute values and percentages for categorical variables

RESULTS

There were included 33 patients with frequent admissions in hospital (951 admissions in 5 years), 29 patients due to COPD exacerbations and 4 patients for asthma exacerbations (tab. I), with age between 44 years and 84 years, average age of 63 ± 9 years. The number of asthmatic patients is small because most of asthma exacerbations are not bacterial and we selected only patients with frequent hospital admissions due to bacterial exacerbation of the disease. The predominant comorbidity was arterial hypertension. Most of these patients 22 (67%) were on long-term oxygen treatment at home. Hospitalization average length was 12 ± 5 days, the longest hospitalization duration being of 47 days due to comorbidities.

TABLE I.

Patient characteristics (n=33)

Age (mean ± SD) (years)	63 ±9
Gender:	
Male	24 (73%)
Women	9 (27%)
Residence:	
Rural	25 (76%)
Urban	8 (24%)
Comorbidities:	
Hypertension	17 (52%)
Tuberculosis sequelae	20 (61%)
Heart failure	9 (27%)
Cor pulmonale	5 (15%)
Diabeties mellitus	5 (15%)
Atrial fibrillation	4 (12%)
COPD GOLD stage:	
GOLD 3	5 (82%)
GOLD 4	24 (18%)

Out of 948 sputum samples, 193 (20.35%) were positive culture sputum samples, 75 (39%) were *Streptococcus pneumoniae* and 98 (51%) were Gram Negative bacteria (fig. 1). There were no MRSA (methicillin resistant *Staphylococcus aureus*) in the sputum of these patients.

In each patient with asthma were identified in the sputum *Streptococcus pneumoniae* (6 samples in total), followed by gram negative bacilli (4 samples in total).

Each bacterium was with resistance at aminopenicillins and cephalosporins and the patients were treated with Ciprofloxacin, Gentamicin or Colistin according to antibiograms, with a medium of 10 days treatment.

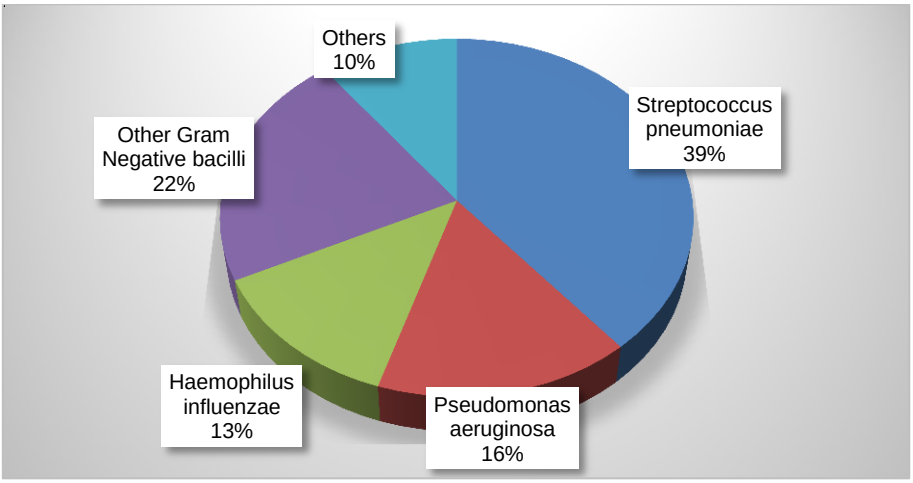

Fig. 1. Type of bacterial flora in positive sputum cultures. Other: *Klebsiella oxytoca, Serratia odorifera, Moraxella catarrhalis, Proteus spp., Escherichia coli.*

In COPD patients most of the time treatment was started with cephalosporins, rarely with aminopenicillins and changed on quinolones (levofloxacin *per os*, ciprofloxacin intravenously) most of the time, or colistin intravenously or associated with other antibiotic in aerosols. Other antibiotic that was recommended in small proportion were clarithromycin, gentamicin, clindamycin, trimethoprim/sulfamethoxazole. The identified bacteria showed resistance to aminopenicillins and frequently to cephalosporins 1st - 3rd generation, rarely sensitive at Ceftamil.

DISCUSSION

Our findings showed that bacteria in patients with frequent exacerbations can be hard to find and in most of the time these are resistant to usual antibiotics. The antibiogram of the sputum is the key point to stat antibiotics if the patient is stable, or to start with antibiotics according to previous bacteria identifications and antibiograms after sputum collection.

According to GOLD recommendations, the route of administration can be intravenous and the therapy days can be longer in COPD exacerbations. The choice of antibiotic should take into consideration also the local hospital/ region antimicrobial resistance map (5).

Chronic pulmonary diseases imply multidisciplinary approach, clear management plan for patient, closely treatment supervision and evaluation, comorbidity control, with intervention on modifiable risk factors like reduce tobacco and alcohol use, control blood pressure, environmental risk factors.

In Europe, the 2022 Eurostat report on respiratory diseases deaths in 2019 found them to be on 3rd place, after cardiovascular diseases deaths and lung cancer diseases

deaths. Romania is on 8th place at lung cancer death and second place on cardiovascular death in European Union. In our country population reporting that they suffering from asthma females were less than 2%, but more than males, for other chronic respiratory diseases than asthma our country reported less than 2% for males, but more than females (5).

World Health Organization found that COPD deaths was on the third place among global death causes in 2020 (6% of total deaths). These patients are fragile patients with frequent exacerbation due to pollution, cigarettes consumption, infections, other airway injuries, leading to frequent exacerbations with or without hospitalizations, disease progression, disease complications like heart failure, pulmonary embolism, frequent antibiotic consumption, negative health status and socio-economic effect (8, 9).

Literature studies identified chronic respiratory diseases exacerbations factors for asthma and COPD like poor adherence to treatment, pneumonia or other infections prior hospitalization, disease severity, oxygen therapy at home, and smoking. Moreover, pollution, climate change, aeroallergens, and pollen season, are other important factors for asthma exacerbations (1, 9).

Most existing studies in the literature analyses the duration of antibiotic therapy in exacerbations of chronic lung diseases such as COPD, asthma, cystic fibrosis, less the type of antibiotic used in these exacerbations, especially as the bacterial etiological agent could not be identified (10-13).

In a recent meta-analysis, authors investigate whether shorter courses of oral antibiotic treatment for asthma and COPD exacerbations are associated with different outcomes when compared with longer courses (14), in our findings most the treatment were a long one 10-14 days and without prolonged tine up to readmission in hospital.

We found that *Streptococcus pneumoniae* was frequent isolated in sputum in accordance with literature, *Hemophilus influenzae also* (4, 15-16). We found that *Pseudomonas aeruginosa* was isolated in increased number, frequent with resistance at usual antibiotic that are recommended in COPD/asthma exacerbation and the choice was Levofloxacin, Gentamicin, Colistin. The frequent isolation of the same bacteria at the same patient raise the possibility of colonization of the airway a long time.

Limitations of the study

Our date base is a small one, the number of patients with frequent chronic respiratory diseases (asthma and COPD) it is a small one, with a large number of hospitalizations.

CONCLUSIONS

COPD and asthma exacerbation can be a start to antibiotic stewardship because these patients are the most exposed to decompensations, increased hospital stay, increased treatment costs. Not all the time exacerbations are provoked by bacteria but it is hard to decide in some cases if it is just viral or other causes and clinicians are tempted to give antibiotics, leading to unnecessary use of antibiotics.

Together with a judicious use of antibiotics, adequate management of the disease, comorbidities, social and financial support of these patients is necessary to prevent frequent exacerbations.

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THE INFLUENCE OF CO_2 PRESSURE IN THE LAPAROSCOPIC OR ENDOSCOPIC APPROACH TO INCISIONAL HERNIAS

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THE INFLUENCE OF CO2 PRESSURE IN THE LAPAROSCOPIC OR ENDOSCOPIC APPROACH TO INCISIONAL HERNIAS (Abstract): Laparoscopic surgery is a transformative approach to surgical procedures, with significant benefits for patient recovery. However, the impact of carbon dioxide (CO2) pressure in laparoscopic incisional hernia surgery has been underexplored. **Materials and methods:** A retrospective analysis of 36 patients undergoing laparoscopic incisional hernia surgery over three years was conducted. Patients were categorized into lower and normal CO2 pressure groups. Post-operative factors, including pain, ileus, transit resumption, and hospital stay, were evaluated. **Results:** Patients in the normal CO2 pressure group reported significantly higher post-operative pain ($p = 0.05$) and a greater incidence of postoperative ileus ($p < 0.01$). Normal pressure patients also exhibited delayed transit resumption ($p < 0.01$). Hospital stay duration did not significantly differ ($p = 0.35$). **Conclusions:** Lower CO2 pressures during laparoscopic incisional hernia surgery are associated with lowered post-operative pain, improved gastrointestinal recovery, and a lower risk of ileus. These findings emphasize the potential benefits of optimizing CO2 pressure management to improve patient comfort and expedite recovery. This study underscores the multifaceted nature of post-operative outcomes, necessitating comprehensive assessments of surgical techniques and patient-specific factors. Enhanced CO2 pressure management holds promise for enhancing patient experiences and healthcare resource allocation. **Keywords:** LAPAROSCOPIC SURGERY, CO_2 PRESSURE, INCISIONAL HERNIAS, POST-OPERATIVE OUTCOMES, PAIN MANAGEMENT.

Surgical techniques have come a long way, and one of the most significant advancements in recent years is laparoscopic surgery or minimally invasive surgery. This approach has revolutionized patient care by making surgeries less painful, reducing hospital stays, and speeding up recoveries (1, 2). But there's a hidden factor that plays a crucial role in this process – the use of carbon dioxide (CO2) during surgery (3). The amount and pressure of CO2 used in the abdomen can have a profound impact on how patients feel after the surgery (4, 5).

The rationale for this study is grounded in the recognition that the ramifications of surgical interventions extend far beyond

the confines of the operating room, recovery playing an important medical but also economical role (6). While laparoscopy offers undeniable advantages, the nuances of CO2 pressure modulation have surfaced as a potential factor affecting post-operative well-being (7). Past research hints at the possibility that higher CO2 pressures, a characteristic of conventional laparoscopy, may not be benign (8), while other studies contradict this idea (9). They could be linked to a spectrum of post-operative issues, such as increased pain, delayed gastrointestinal recovery, and prolonged hospitalization.

The reason we're looking into this is that CO_2 pressure in surgery is a bit like a balancing act. On one hand, it's needed to create the space required for the surgeon to work inside the abdomen. On the other hand, using too much CO_2 pressure might not be good for the patient, and it could cause problems like more pain, slower recovery of the stomach, and a longer hospital stay (10). Some studies have suggested that if we're careful and use less CO_2 pressure, we might be able to avoid these problems and help patients get back to normal more quickly.

Our research is based on the idea that the balance between creating the right surgical environment and ensuring patient comfort is crucial. This study is motivated by the fact that we still have many questions about how CO_2 pressure in laparoscopic surgery affects patients after the incisional hernia operation. We aim to fill this knowledge gap by looking into how CO2 pressure influences things like post-operative pain, how quickly the bowels start working again, and how long patients stay in the hospital. Our goal is to understand how this can improve surgical practices and patient care. These have a significant impact on healthcare providers, hospital management, and, most importantly, the patients.

MATERIALS AND METHODS

The study involved a retrospective analysis of patients who underwent laparoscopic or endoscopic procedures for incisional hernias. These are patients that were treated for eventrations over a three-year period (2018-2021). These patients are part of a lager study comparing the approach of incisional hernias treated using either minimally invasive or classic intervention (11). To investigate the effects CO_2 pressure used in minimally invasive techniques used for treating incisional hernia, we evaluated post-surgery management of pain and recovery.

Thus, we measured the CO_2 pressure used during the intervention. We then defined two groups of patients, ones with lower values, for whom CO_2 pressures between 8 and 11 mmHg were used, and normal values, for whom CO_2 pressures between 12 and 15 mmHg were used. We evaluated pain presence at palpation, immediately after surgery, which was evaluated using a five-points pain scale, one being minimum and five being maximum. Other monitored aspects were: presence of pain after 12 and 24 hours, the need for antalgic medication, ICU post-intervention stay, and intestinal transit presence.

We used descriptive statistics (mean ± standard deviation or counts and percentages, depending on the type of variable). For comparing the two groups of patients we used nonparametric tests (Mann Whitney U for comparing continuous variables, or Chi-square test for nominal variables or Fisher test for 2x2 tables). Relative risk was calcu-

lated to assess the risk of complications, and 95% confidence intervals and p values were also calculated. Statistical significance was considered to be achieved when values o p lower than 0.05 were obtained.

RESULTS

Characteristics of the sample

We evaluated the basic characteristics of the patients included in the study (Table 1), and compared them among the two created groups. The lower values group of patients had a number of 26 cases (72.2%) and the group of patients with normal values of CO2 pressure had 10 cases (27.8%). We provide an analysis of the demographic and clinical characteristics of patients in the study, highlighting the differences or lack thereof between the lower and normal CO_2 pressure groups (tab. I).

TABLE I.

Characteristics of the sample

	Total (36)	Lower pressure (26)	Normal pressure (10)	p-value
Age				
Mean ± SD	50.3±13.7	51.1±12.5	48.4±17.1	0.67
Sex				
Male	19 (52.8%)	15 (57.7%)	4 (40%)	0.46
Female	17 (47.2%)	11 (42.3%)	6 (60%)	
Environment				
Urban	28 (77.8%)	18 (69.2%)	10 (100%)	0.07
Rural	8 (23.2%)	8 (30.8%)	0 (0%)	
Weight				
Mean ± SD	90.4±14.9	91.7±15.6	87±13.0	0.48
Localization				
Supraumbilical	18 (50%)	18 (69.2%)	0 (0%)	<0.01
Umbilical	6 (16.7%)	6 (23.1%)	0 (0%)	
Sub umbilical	10 (27.8%)	0 (0%)	10 (50%)	
Inguinal	2 (5.5%)	2 (7.7%)	0 (0%)	
Size of the defect (cm)				
Mean ± SD	10.03±4.8	10.44±5.7	9.7±4.1	0.95

The mean age of the patients in the study was 50.3 years with a standard deviation of 13.7. There was no statistically significant difference in mean age between patients who underwent laparoscopic surgery with lower CO_2 pressure (51.1±12.5) and those with normal CO_2 pressure (48.4±17.1) groups (p=0.67), indicating that age was not a significant confounding factor in the study. The study included 19 male patients (52.8%) and 17 female patients (47.2%). The proportion of male patients was slightly higher in the lower CO_2 pressure group (57.7%) compared to the normal CO_2 pressure group (40%), but this difference was not statistically significant (p=0.46). In terms of the patient's environmental setting, 77.8% of patients came from urban areas, while 23.2% came from rural areas. Interestingly, all patients in the normal CO_2 pressure group were from urban areas, whereas 30.8% of patients in the lower pressure group came from rural areas. Considering that the pa-

tients come from South-eastern part of Romania, namely Constanta County, this is a normal distribution, as the County has, according to the latest data, approximately 70% of the people living in urban areas. The difference in the distribution of patients from rural and urban settings between the two CO2 pressure groups approached statistical significance (p=0.07). The mean weight of patients in the study was 90.4 kg with a standard deviation of 14.9. There was no statistically significant difference in mean weight between the lower CO_2 pressure group (91.7±15.6) and the normal CO_2 pressure group (87±13.0) (p=0.48), suggesting that weight did not significantly differ between the groups.

Regarding the localization of incisional hernias, it's worth noting that a statistically significant difference was observed between the two CO_2 pressure groups. All patients with supraumbilical hernias were in the lower CO_2 pressure group, while the normal CO2 pressure group had no cases of supraumbilical hernias. This difference was highly significant (p<0.01). The mean size of the hernia defect was 10.03 cm with a standard deviation of 4.8. There was no statistically significant difference in the mean size of the defect between the lower CO_2 pressure group (10.44±5.7) and the normal CO_2 pressure group (9.7±4.1) (p=0.95), indicating that the size of the defect did not significantly vary between the two groups.

We analyzed the outcome of the interventions using three major criteria. The first one is related to actions taken during the intervention (lidocaine infiltrations) and the duration of the procedure. The second criterion was related to the post-surgery pain management and the last criterion was related to post-intervention recuperation.

Characteristics of the intervention

The results are depicted in table II. The mean duration of the surgical intervention in the study was 100.8 minutes with a standard deviation of 30.8. There was no statistically significant difference in the mean duration between patients who underwent laparoscopic surgery with lower CO_2 pressure (98.1±30.8 minutes) and those with normal CO_2 pressure (108±31.2 minutes) groups (p=0.4). This suggests that the duration of the surgical procedure did not significantly differ between the two CO_2 pressure groups. We observed a difference in the use of lidocaine infiltrations during the surgical procedures. A significant difference was observed in the use of lidocaine infiltrations during the surgical procedures. In the lower CO_2 pressure group, 38.9% of patients received lidocaine infiltrations, while all patients (100%) in the normal CO2 pressure group received lidocaine infiltrations. The relative risk was 6.5 (CI 95% 2.63-16.01), indicating a substantial disparity in the use of lidocaine infiltrations between the two CO_2 pressure groups. This difference was highly statistically significant (p<0.01).

TABLE II.

Intervention characteristics

	Total (36)	Lower pressure (26)	Normal pressure (10)	p-value
Duration of the intervention (minutes)				
Mean ± SD	100.8±30.8	98.1±30.8	108±31.2	0.4
Lidocaine infiltrations				
No	22 (61.1%)	22 (84.6%)	0 (0%)	<0.01
Yes	14 (38.9%)	4 (15.4%)	10 (100%)	

The mean pain intensity reported by patients after surgery was 2.03 (on a scale from 1 to 5, 1 being no pain and 5 being unbearable pain), with a standard deviation of 1.05. There was a statistically significant difference in mean pain intensity between patients who underwent laparoscopic surgery with lower CO_2 pressure (1.77±0.8) and those with normal carbon dioxide pressure (2.7±1.4) groups (p=0.05). This suggests that patients in the normal CO_2 pressure group experienced higher pain intensity after surgery compared to those in the lower CO_2 pressure group.

TABLE III.
Pain characteristics

	Total (36)	Lower pressure (26)	Normal pressure (10)	p-value
Pain Intensity after surgery				
Mean ± SD	2.03±1.05	1.77±0.8	2.7±1.4	0.05
Pain at 12 hours				
No	21 (58%)	17 (65.4%)	4 (40%)	0.26
Yes	15 (41.7%)	9 (34.6%)	6 (60%)	
Pain at 24 hours				
No	25 (69.4%	18 (69.2%)	7 (70%)	1
Yes	11 (30.6%)	8 (30.8%)	3 (30%)	
Analgesic post-surgery treatment				
No	22 (61.1%)	18 (69.2%)	4 (40%)	0.14
Yes	14 (38.9%)	8 (30.8%)	6 (60%)	

At twelve hours post-surgery evaluation, a difference was observed in the incidence of pain reported by patients. In the lower carbon dioxide pressure group, 41.7% of patients reported pain, while in the normal carbon dioxide pressure group, 60% of patients reported pain. The relative risk for a patient on whom a $_{CO2}$ pressure between 12 and 15 mmHg was used to experience pain, compared to a patient for whom a CO_2 pressure of no more than 11 mmHg was used was 1.73 (C.I. 95% = 0.83-3.60). The result did not reach statistical significance (p=0.26), indicating that the incidence of pain at 12 hours did not significantly differ between the two CO2 pressure groups.

The incidence of pain reported by patients at 24 hours post-surgery also demonstrated no statistically significant difference between the lower and normal CO_2 pressure groups. In the lower CO_2 pressure group, 30.6% of patients reported pain at 24 hours, while in the normal CO_2 pressure group, 30% of patients reported pain (p=1), with relative risk of 0.97 (C.I. 95% = 0.32-2.95).

The use of analgesic post-surgery treatment revealed a difference between the two CO_2 pressure groups. In the lower CO_2 pressure group, 38.9% of patients received analgesic treatment, while in the normal CO_2 pressure group, 60% of patients received analgesic treatment. Although this difference did not reach statistical significance (p=0.14), it indicates a potential disparity in the need for post-surgery analgesic treatment between the two groups. The relative risk was 1.95 (C.I. 95% = 0.90-4.19).

Post-surgery evolution

A significant difference was observed in

the incidence of postoperative ileus between patients who underwent laparoscopic surgery with lower CO_2 pressure and those with normal CO_2 pressure. In the lower CO_2 pressure group, 7.7% of patients experienced postoperative ileus, while in the normal CO2 pressure group, 60% of patients experienced this complication. Thus, the relative risk was 7.8 (C.I. 95% =1.87-32.41). This indicates a significantly higher risk of having post-surgery ileus in patients within the normal CO_2 pressure group. This difference was highly statistically significant ($p<0.01$).

TABLE IV.

Post-surgery evolution

	Total (36)	Lower pressure (26)	Normal pressure (10)	p-value
Postoperative ileus				
No	28 (77.8%)	24 (92.3%)	4 (40%)	<0.01
Yes	8 (22.2%)	2 (7.7%)	6 (60%)	
Days to transit resuming				
1	12 (33.3%)	10 (38.5%)	2 (20%)	<0.01
2	16 (44.4%)	14 (53.8%)	2 (20%)	
3	8 (22.2%)	2 (7.7%)	6 (60%)	
Days of stay in ICU				
1	12 (33.3%)	8 (30.8%)	4 (40%)	0.65
2	7 (19.4%)	6 (23.1%)	1 (10%)	
3	17 (47.2%)	12 (46.2%)	5 (50%)	
Days of hospitalization				
Mean ± SD	2.72±0.9	2.62±0.85	3±1.15	0.35

The time to resumption of transit (bowel function) following surgery showed a statistically significant difference between the two CO_2 pressure groups. In the lower CO_2 pressure group, 38.5% of patients resumed transit within 1 day, 53.8% within 2 days, and 7.7% within 3 days. In contrast, in the normal CO_2 pressure group, only 20% of patients resumed transit within 1 day, 20% within 2 days, and 60% within 3 days. These differences were highly statistically significant ($p<0.01$), indicating a substantial delay in transit resumption in the normal CO2 pressure group.

There was no statistically significant difference in the number of days patients spent in the Intensive Care Unit (ICU) between the lower and normal CO_2 pressure groups. The distribution of patients spending 1, 2, or 3 days in the ICU was similar between the two groups, and the p-value was 0.65.

The mean length of hospitalization for all patients was 2.72 days, with a standard deviation of 0.9. There was no statistically significant difference in the mean length of hospitalization between patients who underwent laparoscopic surgery with lower CO_2 pressure (2.62±0.85 days) and those with normal CO_2 pressure (3±1.15 days) groups ($p=0.35$). This suggests that the overall length of hospitalization did not significantly differ between the two CO2 pressure groups.

DISCUSSION

This study delves into the critical question of how the management of CO_2 pres-

sure during laparoscopic surgery can impact postoperative outcomes in patients with incisional hernias.

The analysis of demographic and clinical characteristics, including age, sex, environment, weight, hernia localization, and size of the defect, did not show significant differences between the lower CO_2 pressure and normal CO_2 pressure groups. These findings suggest that patients in both groups were generally comparable in terms of these baseline factors, reducing the potential for confounding variables. Also, we observe that there are no disparities between patients, as observed in other studies where, for example environment or sex played important roles in diagnosis capacities (12, 13).

One of the key findings of this study was the significant difference in postoperative pain intensity between the two CO_2 pressure groups. Patients in the normal CO_2 pressure group reported higher postoperative pain intensity compared to those in the lower CO_2 pressure group. This finding raises important questions about the potential role of CO_2 pressure in influencing pain perception following laparoscopic surgery. These findings are in line with previously published papers on the topic (14).

Additionally, the data indicate a substantial difference in the use of lidocaine infiltrations between the two groups. A significantly higher proportion of patients in the normal CO2 pressure group received lidocaine infiltrations as part of their pain management strategy. The increased use of lidocaine in the normal V pressure group may have contributed to the observed differences in pain intensity. This usage of lidocaine has the potential of alleviating the pain immediately after surgery (15), and also to influence the need of post-surgery analgesic medication (16, 17), even an effect on chronic pain being observed (18). When referring to pain management, there is a real endeavor in identifying also non-pharmaceutical methods of reducing it, not only post-surgery (19) but also in other types pain-inducing interventions (20).

Postoperative ileus, a common complication following abdominal surgery, was significantly more prevalent in the normal CO_2 pressure group. Patients in this group experienced a substantially higher incidence of postoperative ileus, which is consistent with previous literature suggesting that higher intra-abdominal pressures, may impact gastrointestinal motility and contribute to ileus (21, 22). Moreover, the time to resumption of bowel function, measured as days to transit resuming, was significantly prolonged in the normal CO_2 pressure group. The delayed transit resumption in this group may be attributed to the impact of higher CO_2 pressures on gastrointestinal function, as well as the increased incidence of postoperative ileus. These findings emphasize the potential clinical importance of CO_2 pressure management in laparoscopic surgery to mitigate postoperative ileus and promote earlier recovery of bowel function. The length of hospitalization and the number of days spent in the Intensive Care Unit (ICU) did not show significant differences between the two CO_2 pressure groups. While both groups had similar lengths of hospital stay and days in the ICU, these parameters may be influenced by various factors beyond CO_2 pressure, such as surgical technique, patient recovery, and hospital protocols (23).

The findings from this study have several clinical implications. The higher postoperative pain intensity, increased inci-

dence of postoperative ileus, and delayed transit resumption observed in the normal CO_2 pressure group underscore the potential benefits of carefully managing CO_2 pressures during laparoscopic surgery. Reduced CO_2 pressure may contribute to improved postoperative pain control and a faster return to normal gastrointestinal function. These findings should encourage further research into the optimization of CO_2 pressure levels to enhance patient outcomes in laparoscopic surgery.

This study has several limitations, mainly the relatively small sample size. Larger prospective studies are needed to confirm and expand upon these findings. Additionally, other variables, such as surgical techniques, perioperative care, and patient factors, may have influenced the outcomes studied and should be considered in future investigations.

CONCLUSIONS

Carbon dioxide pressure is a critical factor in laparoscopic and endoscopic approaches to incisional hernias. Surgeons must carefully consider the balance between optimal visualization, patient comfort, and surgical challenges. The findings suggest that a lower carbon dioxide pressure remains a reliable choice for creating a conducive working space. However, the use of elevated pressures should be approached cautiously due to the potential for increased dissection difficulty and patient discomfort. Similarly, while low pressures might alleviate pain and discomfort, the associated risks of bleeding and compromised visualization cannot be ignored.

This study contributes to a deeper understanding of the impact of carbon dioxide pressure on surgical outcomes and patient experiences in the context of incisional hernia procedures. Further research could explore strategies to mitigate the challenges posed by elevated carbon dioxide pressures or investigate innovative approaches to address issues related to low pressures.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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BREAST FIBROADENOMAS: ULTRASOUND APPEARANCE AND POTENTIAL ASSOCIATION WITH PERIPHERAL VEIN THROMBOSIS

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BREAST FIBROADENOMAS: ULTRASOUND APPEARANCE AND POTENTIAL ASSOCIATION WITH PERIPHERAL VEIN THROMBOSIS (Abstract): The **aim** of this study was to characterize the ultrasound appearance of breast fibroadenomas and explore a potential association with peripheral vein thrombosis. **Materials and methods:** We retrospectively studied between January 2021 and December 2022 the imaging records and reports of 159 women with fibroadenomas who underwent breast ultrasound (US) with an 11-18 MHz linear transducer and 7 of them lower extremity US for suspicion of peripheral vein thrombosis with an 11-18 MHz linear or a 3-6 MHz curved transducer. **Results:** We found a total of 293 fibroadenomas, and the most frequent pattern was: isoechoic (69.62% lesions), homogenous (77.47% lesions), with well-defined (100%), macrolobulated margins (51.88%), oval-shaped (95.22% tumors), with a Solbiati index greater than or equal to 2 (51.19%), without Doppler signal (73.72%). We studied the correlation between the Doppler signal and contour pattern, the number of lobulations, and the Solbiati index using a function in Python, and we found the following results: the dimensions of the fibroadenoma (r = 0.117438) and the number of lobulations (r = 0.282) have a positive correlation, but the Solbiati index (r = -0.012) and contour pattern (r = -0.243945) have a negative correlation with the presence of the Doppler signal. The association of multiple breast fibroadenomas with peripheral vein thrombosis was rare (7.46% cases). We monitored 147 patients with simple fibroadenomas every six months for two years, and none of them had a significant variation in the dimensions. **Conclusions:** The association of multiple breast fibroadenomas with peripheral vein thrombosis was rare, so we considered it to be a random association. In our study, the most frequent pattern of fibroadenomas was isoechoic, homogenous, oval-shaped lesions with macrolobulated margins, a Solbiati index greater than or equal to 2, and no Doppler signal. According to our experience, we offered, for typical fibroadenomas, the option of conservative management. A fine needle biopsy was advised in one case with atypical-appearance lesions at ultrasound.
Keywords: FIBROADENOMAS, VEIN THROMBOSIS, ULTRASOUND.

INTRODUCTION

Fibroadenomas are benign lesions with minimal or no malignant potential (malignant transformation 0.01-0.3%), frequent between ages 15 and 35, with predilection for the upper outer quadrant, and a slightly

variable dimension in time or with the period. There are at least two special types of breast fibroadenomas: complex fibroadenomas (which can grow over time) and giant fibroadenomas (growing quickly to a diameter larger than 5 centimeters) (1). Multiple fibroadenomas account for 20% of breast fibroadenoma cases, and their management presents particular challenges because they may have other associated diseases and it is difficult to follow up on every single lesion (2, 3).The cause of fibroadenomas is not known, but the relationship with ovarian hormones is considered important. Simple fibroadenomas are predominantly encountered in women of reproductive age (the incidence is 7-13% in women from adolescence through the mid-20s) (4). The hormonal fluctuations during the menstrual cycle, puberty, pregnancy, menopause, and oral contraceptives increase the risk of fibroadenomas, may stimulate fibroadenomas' growth, and increase the risk for peripheral vein thrombosis (2, 5). Typically, ultrasound depicts a round or ovoid, homogeneous, hypoechoic, well-circumscribed, or macrolobulated mass; intralesional calcification in (~10% of cases) and a thin echogenic rim (pseudocapsule) may be seen (6). Mammography and MRI contribute to the diagnosis (7, 8). When a lesion has the typical features of a fibroadenoma on ultrasound and there are no clinical issues, it can be safely followed. When lesions enlarge or have atypical imaging findings, an ultrasound-guided core biopsy will give a diagnosis (9).

MATERIALS AND METHODS

We retrospectively studied between January 2021 and December 2022 the imaging records and reports of 159 women with fibroadenomas who underwent B mode and Doppler mode breast US with an 11-18 MHz linear transducer and 7 of them lower extremity US for suspicion of deep vein thrombosis with an 11-18 MHz linear or a 3-6 MHz curved transducer (which provides a deeper field view) using a General Electric Voluson E6 ultrasound machine.

Breast US study protocol included bilateral examination with transversal, longitudinal, radial, and antiradial sections of the breast and evaluation of the axillary, subclavian, supraclavian, and internal mammary regions; the patient is lying down in supine position (with right and left oblique rotation in case of large breasts), with the hands above the head (for better exposure of the breast and evaluation of internal mammary and axillary regions), and with the hands nearby the body (for the examination of subclavian and supraclavian regions). For a better visualization of the pathology, the focal zone of the probe must include the lesion, the power of the ultrasound waves, time gain compensation, and gain carefully adjusted for optimal visualization of the deep structures of the breast, for compensating the rapid attenuation of the waves when high frequencies are used, and for well-differentiating the solid and cystic lesions, respectively. The compression of the lesions with the transducer is useful to determine the hardness of the solid ones and to demonstrate the mobility of the internal echoes of the cysts.

The ultrasound report must include the exact location of the lesions, echogenicity (compared with the fatty tissue), number, dimensions (at least two: the longest dimension and the transversal dimension), structure, compressibility, associated acoustic changes, and Doppler signal characteristics. This study was focused on lesion number,

echogenicity, the longest dimension (L), ratio between the longest (L) and the transversal (T) dimensions (L/T ratio, Solbiati index), structure, presence and location (central and peripheral) of the Doppler signal, and lesion growth over time.

If after the first ultrasound examination the patient had lesions with a typical pattern for fibroadenomas, we indicated follow-up at a 6-month interval, and if the lesions did not grow more than 10%, we considered them to be stable, and the diagnosis of fibroadenoma was made with confidence. Conservative treatment with follow-up every 6 months for 2 years was applied. If, in time, one of the fibroadenomas grows more than 10%, we return to a 6-month follow-up for another two years. If the lesions have an odd feature (anechoic components, micro calcifications, poorly defined margins), the patient was referred to a fine needle biopsy to correlate with morpho-pathology.

Ultrasound examination for lower limb deep vein thrombosis was extended from the common femoral vein to the anterior tibial, posterior tibial, and peroneal veins, with transversal and longitudinal sections and segmental compression every 2 cm. The patient lies down in supine position with the head elevated to help with venous pooling, and the examined leg is externally rotated with the knee slightly bent (for better access to the femoral and popliteal veins) (10, 11).

RESULTS

We studied 159 women, age range17 to 74 years, who had 293 fibroadenomas, among them 67 had multiple fibroadenomas, and we studied the US pattern of the lesions, the associated breast diseases (fibrocystic breast disease, mammary duct ectasia) and peripheral vein thrombosis (which was found in 5 cases).Most of the woman (121 -76.10%) had at diagnosis ages between 26 and 49 years, accordingly to the literature (1)(fig. 1).

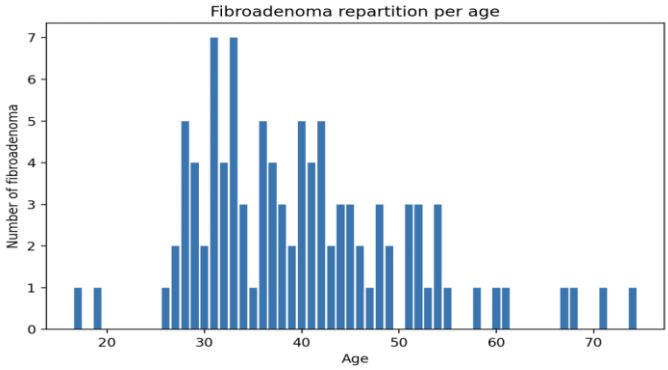

Fig. 1. Number of fibroadenomas - repartition per age

The average number of fibroadenomas was 1.85 per person, and the highest number of lesions was in the case of a 21-year-old woman (7 lesions). Among the 159 patients, we found 92 (57.86%) (age range 27 to 60 years) with a single fibroadenoma and 67 (42.14%) (age range 27 to 50 years) with multiple fibroadenomas, most of them associating fibrocystic breast disease (61.95% and 76.11%, respectively), and 62 (38.99%) presented mammary duct ectasia.

In the case of 7 patients with multiple

fibroadenomas, we also depicted lower extremity edema and referred them to the cardiologist, who found at clinical examination unilateral lower extremity edema, tenderness, erythemas or cyanosis, dilated superficial veins, palpable thrombotic veins, and suspected deep vein thrombosis of the lower extremity confirmed by B mode and Doppler mode US in 5 cases. The association of multiple breast fibroadenomas with peripheral vein thrombosis was rare (7.46% cases), so we considered it to be a random association, results that are similar to the literature data (12) (fig. 2).

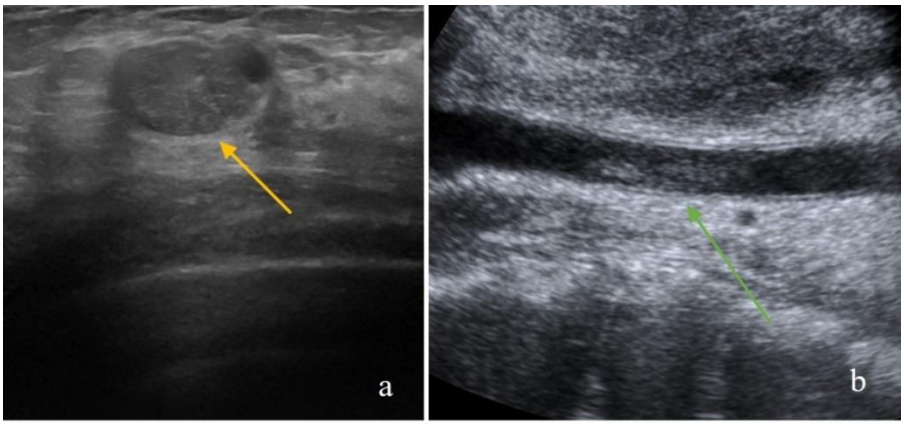

Fig. 2. B mode breast ultrasound- isoechoic, macrolobulated, oval shaped inhomogeneous (yellow arrow) fibroadenoma (a); B mode thigh ultrasound femoral vein thrombosis

The most frequent pattern of fibroadenomas was: isoechoic (204-69.62% lesions), homogenous (227-77.47% lesions), with well-defined (100%), macrolobulated margins (152-51.88%), oval-shaped (279-95.22%) tumors, with a Solbiati index greater than or equal to 2 (150-51.19%), and without Doppler signal (216-73.72%). A small number of tumors were round 14 (4.78%), with Solbiati index smaller than 2 (142-48.46% cases), microlobulated (42-14.33%) or regular (99-33.79%) margins, hipoechoic (89-30.38%), and inhomogenous structure in 66-22.29% of lesions (the most special types of inhomogeneities being macrocalcifications 4.09%, microcalcifications 1.70%, or anechoic inclusions 1.70%). In our study, most of the tumors had the largest diameter smaller than 2 cm (255-87.03%), and only 38 (12.97%) tumors had L diameter between 2 and 7 cm; 77 (26.28%) had Doppler signal present (mostly peripheral, 47-16.04%) (fig. 3). Microlobulated margins (found in 14.33% of the tumors) are considered in the literature a problem for a confident diagnosis of fibroadenoma, but the microlobulated lesions did not grow over time, so in the end, this pattern was also included in benign lesion features (6). No lymphadenopathies were found.

One special case was a 27-year-old female who had four hypoechoic lesions: two lesions were simple fibroadenomas (followed-up at a 6-month interval for 24 months), and two mases had an uncertain appearance (hypoechoic, inhomogeneous, one of them containing an irregular anechoic area, and the other one with microcalcifications) and were referred to a fine needle biopsy, which established the diagnosis of complex fibroadenomas (fig. 4).

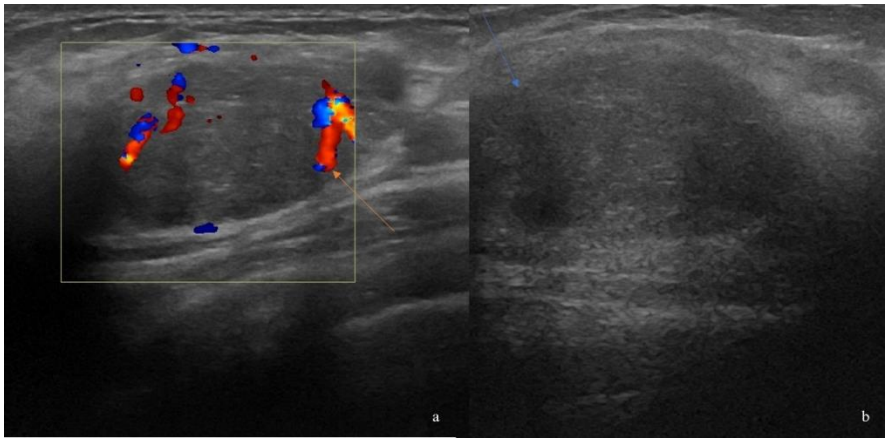

Fig. 3. B and Doppler mode breast ultrasound: isoechoic, well-circumscribed fibroadenoma with central and peripheral Doppler signal (orange arrow) (a); B mode breast ultrasound: isoechoic fibroadenoma, with macrolobulated margins (blue arrow) (b)

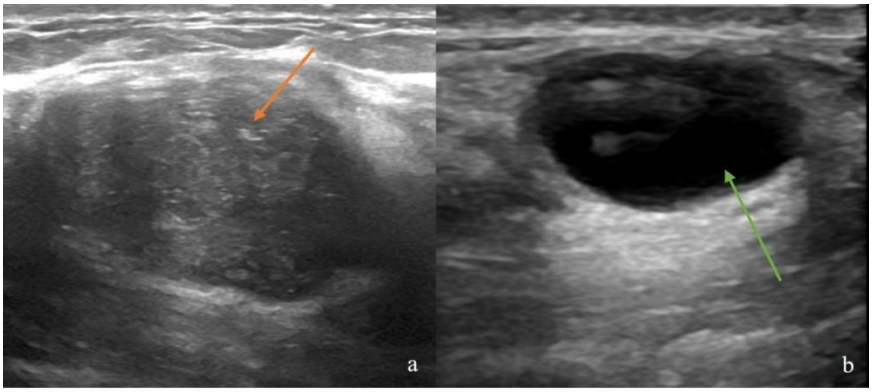

Fig. 4. B and Doppler mode breast ultrasound, BI-RADS 4a lesions: hypoechoic, macrolobulated, oval shaped lesion, with microcalcifications (orange arrow) (a); inhomogeneous hypoechoic, microlobulated, oval shaped lesion, containing an irregular anechoic area (green arrow) (b)

We studied the correlation between the Doppler signal and the following features of the lesions: contour pattern (regular or lobulated margins), number of lobulations, dimensions, and length-to-thickness ratio using a function in Python that has the following definition: correlation is used to summarize the strength and direction of the linear association between two quantitative variables. It is denoted by r and has values between -1 and +1. A positive value for r indicates a positive association, and a negative value for r indicates a negative association. We found the following results: lobulated margins (r = -0.243945), the number of lobulations (r = 0.282), dimensions (r = 0.117438), and length-to-thickness ratio (r = -0.012), and we concluded that the larger the dimensions of the fibroadenoma and the higher the number of lobulations will corre-

late with the presence of the Doppler signal; the length-to-thickness ratio and contour pattern have a negative correlation with the Doppler signal.

To correlate Doppler signal with three variables at once (contour pattern, number of lobulations, and dimensions), we used the algorithm Ordinary Least Squares Regression, and the most interesting of all results was the coefficient (Coef), which suggests a moderately positive correlation between Doppler signal and the number of lobulations (tab. I).

According to all the features we had studied, we concluded that 182 (62.12%) patients had simple fibroadenomas, which had to come for follow-up every 6 months for two years; nine (3.07%) patients with complex fibroadenoma and one (0.34%) patient with a giant fibroadenoma came every 3 months for 6 months, and then at a 6-month interval, and one (0.34%) patient with an uncertain lesion was referred to a fine needle biopsy, which confirmed the diagnosis of complex fibroadenoma (13). The compliance for follow up was good enough: we monitored 158 lesions for at least 2 years; among them most fibroadenomas (116-73.42%) had stationary dimensions, 23 (14.56%) fibroadenomas were increasing in size, 14 (8.86%) fibroadenomas had decreasing sizes, 5 (3.16%) fibroadenomas had variable size over time (increasing and decreasing) (fig. 6).

TABLE I.

Correlation between Doppler signal and contour pattern, number of lobulations and dimensions with Ordinary Least Squares Regression algorithm

Doppler signal	Coef.	Std. errs.	T
Dimensions (cm)	0.048	0.049	0.981
Contour pattern	-0.048	0.233	-0.207
No. of lobulations	0.379	0.167	2.267

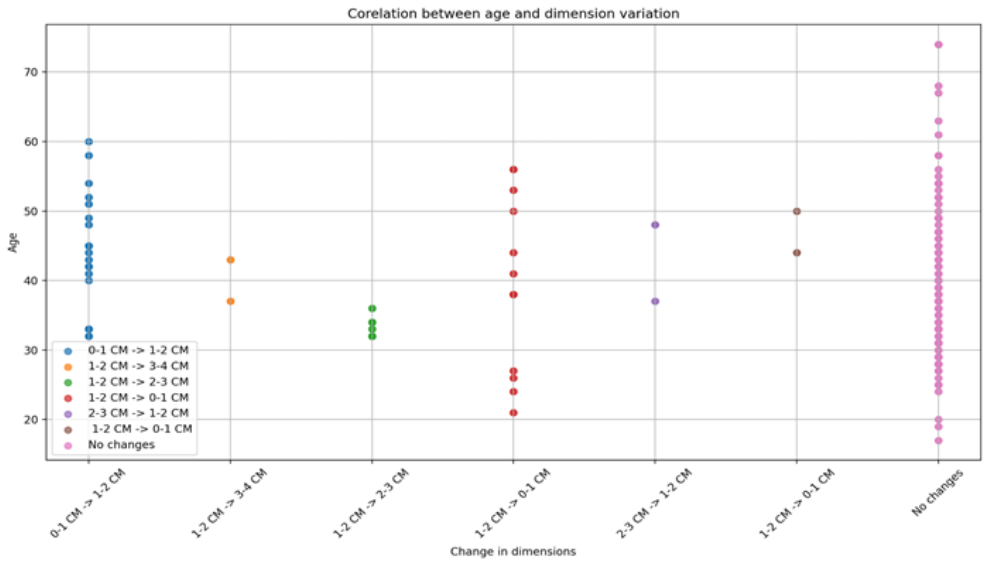

Fig. 6. Correlation between age and dimension variation over time

DISCUSSION

The most frequent pattern of fibroadenomas was isoechoic, homogenous oval-shaped lesions with a Solbiati index greater than or equal to 2, well-defined margins, and no Doppler signal, which was similar to the literature data (4). Other features of the lesions were discordant with the literature data: macrolobulated margins (51.88%) were more frequent than regular margins (33.79%) (1).

The association between multiple breast fibroadenomas and peripheral vein thrombosis was rare (5-7.46% cases), so we considered it to be a random association, despite the fact that both lesions have hyper estrogenism among the etiology (12).

Most of the lesions were homogeneous, slightly inhomogeneous, or contained macrocalcifications; only 10 (3.38%) tumors had microcalcifications or anechoic inclusions, which raised the suspicion of complex fibroadenomas and indicated follow-up at a 3-month interval (nine cases) and a fine needle biopsy (in one case) (4).

Most of the tumors had the largest diameter smaller than 2 cm (87.03%), and the number of tumors with diameters between 2 and 7 cm was fewer (12.97%), according to the literature data (9). The only lesion considered to be a giant fibroadenoma was an isoechoic 7/3 cm tumor with central Doppler signal present in the case of a 29-year-old woman, which was followed up initially at 3 months with insignificant growth (0.5 cm) over this period of time.

We studied the correlation between Doppler signal and contour pattern, number of lobulations, largest dimensions, and length-to-thickness ratio using a function in Python; lobulated margins ($r = -0.243945$) and Solbiati index ($r = -0.012$) have a negative correlation, but the number of lobulations ($r = 0.282$) and lesions' largest dimension ($r = 0.117438$) have a positive correlation with the presence of Doppler signal.

In terms of follow-up, our data were similar to those in the literature (13). We managed to monitor the simple fibroadenomas (according to the compliance of the patients) in 147 cases every 6 months for two years, and none of them had a significant variation of the dimensions in order to raise diagnostic issues; nine with complex fibroadenomas and one with giant fibroadenoma came every 3 months for 6 months, and then at a 6-month interval for two years, and one patient with complex fibroadenomas was referred to a fine needle biopsy.

CONCLUSIONS

The association of multiple breast fibroadenomas with peripheral vein thrombosis was rare, so we considered that a random association.

In our study, the most frequent pattern of fibroadenomas was an isoechoic, homogenous oval-shaped lesion with a Solbiati index less than 2, well-defined macrolobulated margins, and no Doppler signal. A small number of tumors were round, microlobulated, hypoechoic, with inhomogeneous structure due to macrocalcification, microcalcifications, or anechoic inclusions, with a Solbiati index larger or equal to 2, and a Doppler signal present, mostly in the periphery of the lesions. Most of the tumors were smaller than 2 cm; less frequently, we encountered tumors with the largest diameters between 2 and 7 cm.

We found, using a function in Python, that the Doppler signal has a negative correlation with lobulated margins and the L/T ratio and a positive correlation with the

number of lobulations and lesions' largest dimension.

According to our experience when offered the option of conservative management, in our study, most women preferred follow-up and not biopsy. A fine needle biopsy was advised in one case with atypical-appearance lesions at ultrasound.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflicts of interest. This research received no external funding.

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EVOLUTION OF INFLAMMATORY MARKERS IN PATIENTS WITH PROSTHETIC JOINT INFECTION TREATED WITH *STIMULAN*

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EVOLUTION OF INFLAMMATORY MARKERS IN PATIENTS WITH PROSTHETIC JOINT INFECTION TREATED WITH *STIMULAN* (Abstract): Total hip arthroplasty is a commonly performed procedure with a known risk of postoperative infection. Diagnosing an infection after total hip arthroplasty can be challenging, as there are no consistently reliable preoperative tests for infection in patients requiring a revision arthroplasty. This study **aimed** to assess the common investigative methods for diagnosing infection at the site of a previous arthroplasty and the evolution of these biomarkers in patients treated with biocomposite and traditional surgical treatment. **Materials and methods:** We conducted a prospective analysis of pre- and postoperative investigations used to diagnose infection in 44 patients admitted from January 2017 to September 2023 at the "Sf. Apostol Andrei" County Clinical Emergency Hospital of Galati. **Results:** Our study group included 31.81% women and 68.19% men. The most common symptoms experienced by patients were pain and functional incapacity of the lower limb. Only ESR and CRP were elevated in all patients at admission. Their values decreased postoperatively, and determinations at two and six weeks postoperatively showed normalization of ESR, while CRP remained slightly elevated. **Conclusions:** The biological modifications were generally typical when considering the preoperatory moment, with notable rapid improvement in inflammatory status within the first six weeks post-surgery. The novel approach that includes bio composites seems to have major clinical impact on the future management of prosthetic joint infections given the cure rate and the general evolution. **Keywords:** PROSTHETIC JOINT, INFLAMMATORY MARKERS, INFECTION, BIOCOMPOSITE.

After joint replacement surgeries, most patients find relief from preoperative pain and disability. While the surgeries are generally successful and lead to high patient satisfaction, it's important to be aware of the associated risks. Infection is one of the

most concerning complications of this type of operation.

In addition, it can be challenging to confirm a diagnosis of prosthetic joint infection (PJI) after total hip arthroplasty (THA). Patients with this type of infection often do not display typical signs and symptoms, such as fever, chills, or elevated white blood cell count.

In some cases, microorganisms enter the joint during the insertion of the prosthesis (direct inoculation), while in others, bacteria travel to the joint through the bloodstream (hematogenous spread).

Some bacteria, like coagulase-negative staphylococci, have low virulence and can persist due to prosthetic material and biofilm. These infections usually cause a chronic low-grade infection with few systemic symptoms. On the other hand, diseases caused by high-virulence organisms, such as Staphylococcus aureus, can lead to severe conditions like septicemia and bacterial endocarditis (1).

Staphylococci species are the most common causative organisms of joint infections, followed by streptococci, enterococci, and Gram-negative bacteria. Gram-negative bacteria account for about 8% of cases (2).

The erythrocyte sedimentation rate (ESR) and white blood cell (WBC) count are said to have low reliability as indicators of prosthetic joint infection (3). However, these indicators can be influenced by age and medical conditions. An ESR greater than 30 mm/h and a CRP greater than 10 mg/L are considered elevated levels (4). The sensitivity and specificity of ESR and CRP can vary widely in the available literature. If both the ESR and CRP are normal, however, the probability of infection is around 3% (5).

This research is part of a larger study evaluating the efficacy of using an antibiotic-loaded bio composite (*Stimulan*) in addition to conventional treatment. One of the objectives of this original research was to evaluate the inflammatory biologic profile and its evolution in this category of patients. To our knowledge, this is the first research of its kind in our country, with a major clinical impact on the future management of prosthetic joint infections.

MATERIALS AND METHODS

We carried out a retrospective study from January 2017 to September 2023 at the "Sf. Apostol Andrei" County Clinical Emergency Hospital of Galati. Our research involved patients who were hospitalized for hip arthroplasty.

During the specified period, 44 out of 4,244 hip arthroplasty surgeries were included in this study. The study focused on patients with hip arthroplasty-related infections who were treated with the biocomposite *Stimulan* in addition to the classical surgical treatment.

Patients with chronic inflammatory conditions, those with malignancy, or patients who had previously received antibiotic treatment before surgery were excluded based on the criteria.

Data on age, gender, BMI, laboratory results, type of intervention, progression, and post-surgery outcomes were collected.

A patient was considered to have an infected THA if they met any of the following criteria (6):

1. Presence of a sinus tract communicating with the prosthesis

2. Isolation of a pathogen by culture from two separate tissue or fluid samples obtained from the affected prosthetic joint

3. Any four of the following six criteria:

a. Elevated serum erythrocyte sedimentation rate (ESR) or serum C-reactive protein (CRP) concentration

b. Elevated synovial white blood cell (WBC) count.

c. Elevated synovial neutrophil percentage (polymorphonuclear leukocyte % [PMN%])

d. Presence of purulence in the affected joint

e. Isolation of a microorganism in one culture of periprosthetic tissue or fluid

f. Greater than five neutrophils per high-power field in five high-power fields observed from histologic analysis of periprosthetic tissue at 400 times magnification.

In order to be included in this study, all the patients completed an informed consent form. The ethics approval was received in 2022 (no. 33/23.12.2022 issued by the Ministry of National Education-Faculty of Medicine and Pharmacy, "Dunărea de Jos" University of Galati, Romania).

The statistical analysis of the patients data was performed using the *SPSS program version 20.0* and the calculation of the chi square test, as follows: $p<0.05$, the statistical value is moderately significant (S, 95% c); $p<0.01$, the statistical value is very significant (S, 99% confidence); $p<0.001$, the statistical value is highly significant (HS, 99.9% confidence); $p> 0.05$, the statistical value is not significant (NS).

RESULTS

From a total of 4,244 patients admitted for hip arthroplasty, 76 were diagnosed with an infection associated with the surgical intervention, and from those, in 44 cases, *Stimulan* was used complementary to the classical surgical treatment.

Our study group included 31.81% women and 68.19% men. Women were older, had a bigger BMI. In their case, the infection appeared later, and the hospitalization was shorter. DAIR procedure was preferred in most women, while in the case of men, there was an equal percentage DAIR versus two-stage revision. All the characteristics of the study group are presented in the first table.

TABLE I.

Characteristics of the study group

		Total Group (n-44)	Female (n-14)	Male (n-30)	P
Age (mean ± SD) (years)		66.77±12.02	72.02±11.59	63.30±11.57	0.04
Living environment	Urban	18 (40.90%)	6 (42.85%)	12 (40%)	0.65
	Rural	26 (59.10%)	8 (57.15%)	18 (60%)	0.57
BMI		27.79±3.42	30.22±3.37	26.67±2.83	0.0007
Temperature		36.62±0.75	36.32±0.85	36.76±0.84	0.12
Time between the initial surgery and the onset of infection (days)		387 (127.5;720)	429 (150;817.5)	360 (112.5;750)	0.65
Hospitalization days		18.5 (10;46.5)	15.5 (8;48)	21.5 (10;44.25)	0.51
Nasal culture test		5 (11.36%)	0 (0%)	5 (16.66%)	-
Pharyngeal culture test		3 (6.81%)	0 (0%)	3 (10%)	-
Urine culture		2 (4.54%)	0 (0%)	2 (6.66%)	-
DAIR		25 (56.81%)	10 (71.42%)	15 (50%)	0.18
Two-stage revision		20 (45.45%)	4 (28.57%)	15 (50%)	0.38

BMI, Body Mass Index; DAIR, Debridement, antibiotics, and implant retention.

Upon admission, the most common symptoms experienced by patients were pain and functional incapacity of the lower limb. In addition, prolonged standing resulted also in discomfort/pain (fig. 1).

The main aspects of the wound are described in fig. 2.

Before surgery, WBC values varied from 2290/mm^3 to 16600/mm^3, only 40.90% of the patient having elevated levels. Only ESR and CRP were elevated in all patients at admission (fig 3).

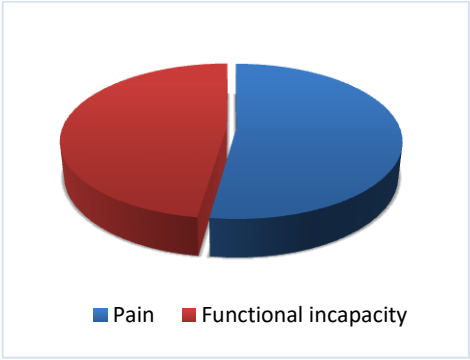

Fig. 1. Symptoms at admission

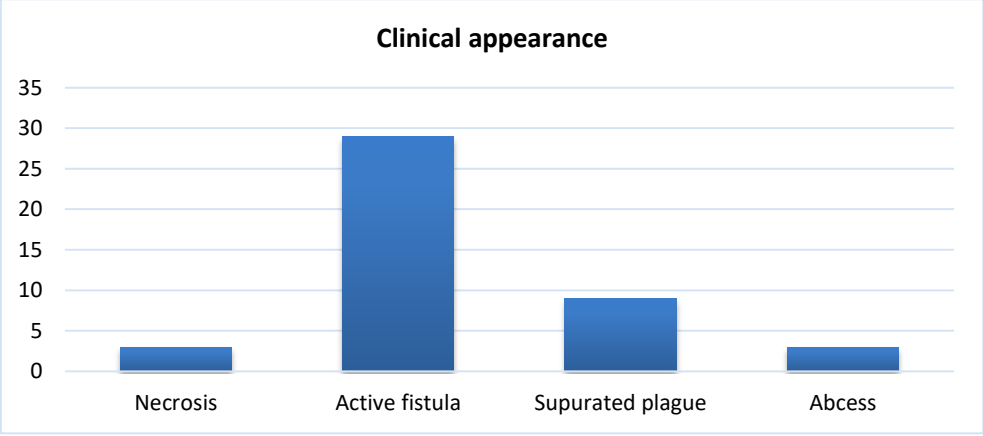

Fig. 2. Aspect of the plague

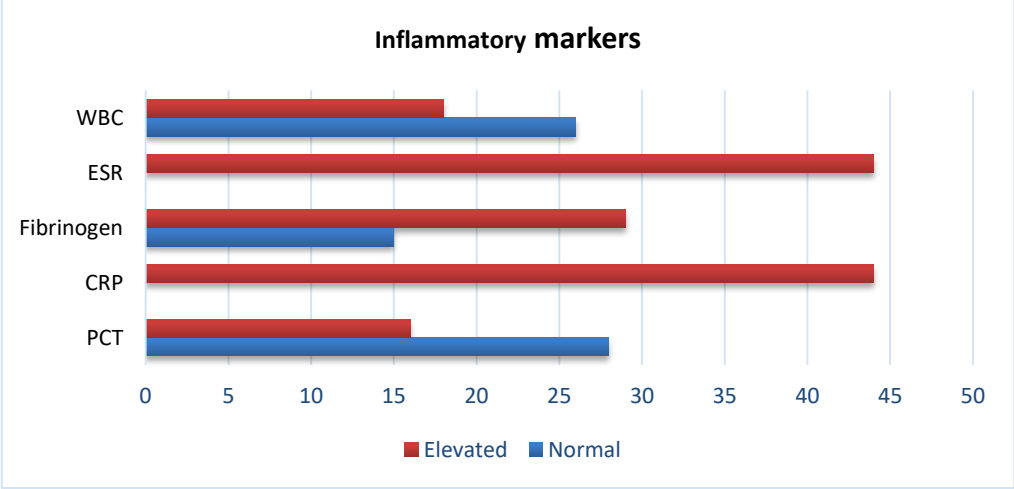

Fig. 3. Inflammatory markers upon admission

The evolution of the laboratory parameters showed a significance difference in the case of the ESR, CRP and hemoglobin (tab. II).

When we analyzed the pathogens involved in the infection, the most frequent were Methicillin-resistant Staphylococcus aureus-MSSA (34%), coagulase-negative staphylococcus-SCN-MRS (20.45%) and Methicillin-susceptible *Staphylococcus* MSS (9.09%). 4 cases had negative antibiograms (fig. 4).

TABLE II.

Evolution of the laboratory parameters

	Before intervention	2 weeks after intervention	6 weeks after intervention	P
WBC	8798 (7297;10230)	7620 (6242.25;9445)	8626 (6732;10532)	0.75
ESR	80 (61.25;120)	21.11 (15;22)	19.5 (15.25;25)	< .00001
CRP	174(147.25;223.25)	11.5 (8.07;29.25)	8.25 (4.31;9.36)	< 0.00001
Fibrinogen	455 (302.25;573)	365.5 (321.25;436.25)	321.75(257.5;385.75)	0.000043
PCT	0.66±2.08	0.17±0.54	0.07±0.05	0.06
Hemoglobin	12.70±2.08	12.36±2.22	11.50±2.17	0.009
Glycemia	104.40±23.30	108.29±31.40	103.20±21.11	0.80
Urea	41.88±16.90	38±15.47	38.29±13.23	0.27
Creatinine	1.12±0.36	1.05±0.36	1.13±0.45	0.84

WBC, white blood cells; ESR, erythrocytes sedimentation rate; CRP, C reactive protein; PCT, procalcitonin.

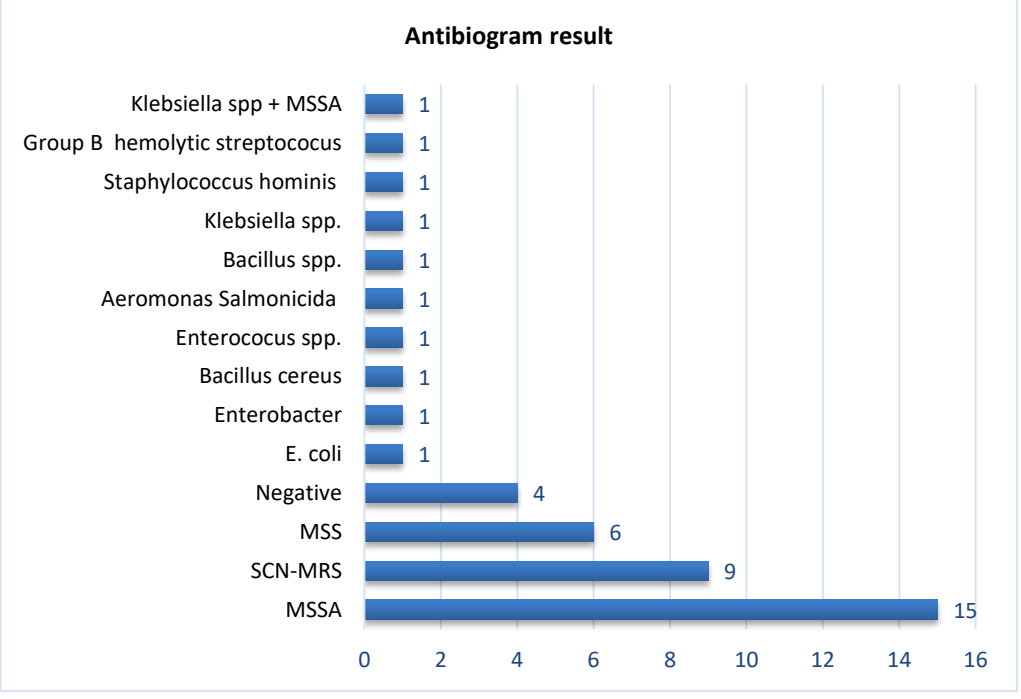

Fig. 4. The pathogens involved in the infection.

DISCUSSION

In recent arthroplasty developments, there has been a focus on gender-specific implants for the hip and knee to account for potential anatomical differences between males and females (7). Specifically, regarding the hip, attention is given to the femoral side as there are differences in the location of the femoral head center and the shape and size of the femoral canal based on gender. Females generally have a lower offset, or a smaller femoral axis compared to male. With aging, females also tend to experience increased femoral medullary width and greater bone loss at the medial calcar and lateral cortex compared to males (8). Currently, available implant designs offer various options in stem length and diameter, neck offset, and neck length but it remains unclear whether gender makes a difference in the outcome of the patents after the hip surgery. Using nongender-specific implants, creates a difference in implant survivorship in the long-term? Are there any differences in implant-related complications?

The Swedish Total Hip Replacement Register and other large registries have demonstrated that the revision rates for hip replacements have been consistent for both men and women over the years. Additionally, the reasons for hip replacement revisions have also been similar between men and women (9).

In addition to the anatomical aspect, recent studies have begun to emphasize the psychological impact, quality of life and impact on daily activities. However, research is very limited (10).

In the present study, there are some differences between the female and male groups that are worth discussing. Women tend to undergo joint arthroplasty at an older age, possibly due to a heightened apprehension of surgery and a tendency to delay the procedure in order to minimize the impact on their caregivers' responsibilities.

BMI was higher in women, and the time interval between the first intervention and presentation to hospital for the symptoms mentioned was also longer. We have no data to confirm whether the time interval to the onset of the infectious complication was longer, or patients delayed presentation to hospital.

After the primary THA intervention, the patients declared they were called for follow-up at 14 days, at which time the sutures were removed. No intermediate follow-ups were performed until the current presentation.

At the time of the current presentation, laboratory tests and pelvis / hip X-ray were performed. In most of the cases, the X-ray did not show any specific modifications.

Detecting PJI continues to be a significant challenge for orthopedic practitioners. Research by Virolainen *et al.* (11) suggests that there is no single laboratory test currently available to accurately diagnose PJI before revision arthroplasty. Common laboratory tests such as ESR, WCC, and CRP, which are typically used as initial diagnostic tools for PJI, are not consistently reliable. Therefore, it is widely acknowledged that there is a pressing need to discover new and dependable indicators of inflammation that can facilitate the prompt and accurate diagnosis of PJI (12).

The C-reactive protein is a substance produced by the liver in response to inflammation, infection, and neoplasm. After surgery, its levels peak two to three days later and return to normal around three weeks post-surgery. Currently, most ex-

perts recommend using both the blood erythrocyte sedimentation rate and C-reactive protein level as markers for evaluating patients with a suspected prosthetic joint infection. When both markers test negative, the likelihood of infection is very low.

Following a standard joint arthroplasty, the erythrocyte sedimentation rate initially increases, peaks at five to seven days postoperatively, and then gradually returns to preoperative levels over three to twelve months.

In our study, both inflammatory markers were elevated preoperatively. Their values decreased postoperatively, and determinations at 2 and six weeks postoperatively showed normalization of ESR, while CRP remained slightly elevated. The results are different from those published in the literature, where ESR often remains slightly elevated until the end of the first year after the procedure (13), this aspect may be due both to the use of the bio composite and to the particularities of each individual patient.

In arthroplasty patients, the time taken for plasma CRP levels to return to normal after surgery has been reported as 2 (14), 3 (15) or 6-8 weeks (16), whereas White *et al.* (17) reported that, at the first follow-up visit after discharge from hospital, the CRP levels had returned to normal, although the exact time-period was not reported.

Plasma fibrinogen is a key coagulation indicator traditionally used to assess venous thrombus embolism and to regulate inflammation associated with several organ infections (18, 19). Previous research has shown that plasma fibrinogen is closely linked to the diagnosis of PJI also, with Xu *et al.* (20) being the first to show that plasma fibrinogen levels are elevated in PJI compared to aseptic failure.

The latest findings indicate that coagulation-related markers show potential for diagnosing PJI. Coagulation-related indicators offer a simple, relatively inexpensive, and noninvasive diagnostic approach compared to other tests. Additionally, coagulation function testing is a standard practice following admission. The primary function of the coagulation system is to facilitate hemostasis, and it has a close connection to infection. This system plays a role in preventing the spread of viruses through the coagulation cascade (21). Synovitis can generate significant amounts of fibrin, and the breakdown of fibrin can lead to heightened indicators with subsequent inflammatory effects (22).

The white blood cell count is a commonly used lab test that can indicate the presence of infection or inflammation. While the WBC count alone cannot definitively diagnose a specific disease, it is a valuable tool in diagnosing many diseases (23). While some studies suggest that the serum WBC count can be useful in diagnosing prosthetic joint infection (PJI), other research has conflicting findings (24, 25).

Toosi *et al.* (26) investigated the effectiveness of using white blood cell count to diagnose periprosthetic joint infection. The study revealed that the sensitivity of WBC count in diagnosing PJI was 55%, and the specificity was 66%. These findings align with the widespread opinion that serum WBC count and differential have limited utility and should not be relied upon when evaluating patients suspected of having PJI.

In our study, white blood cells were elevated in only 40% of the patients, this agrees with the results in the literature. There were no statistically significant differences between the three determinations.

The usefulness of serum procalcitonin in diagnosing periprosthetic joint infection is limited, unlike its effectiveness in detecting systemic bacterial infection. Busch *et al.* (27) conducted a study that revealed serum PCT to have low sensitivity (13%) in diagnosing PJI. Additionally, a recent meta-analysis by Yoon *et al.* (28) concluded that serum PCT plays a minimal role in the evaluation of patients with suspected PJI.

In the current study, only 16 patients showed elevated procalcitonin levels. It's important to mention that there was a consistent decrease in the levels of this marker in the postoperative follow-up assessments. The renal function remained stable, with slight postoperative increases in urea and creatinine, which later normalized.

Despite experiencing significant inflammatory symptoms, preoperative glycemic values remained below 200 mg/dL for diabetic patients and below 140 mg/dL for non-diabetic patients. These levels showed a noticeable decrease in the weeks following the surgery. Similar to other major surgeries, patients with diabetes have a higher risk of postoperative complications. Stress-induced high blood sugar is frequently seen in surgical patients and is typically caused by hormonal responses and inflammatory mediators. Elevated blood sugar levels can be due to medications, stress, poor glucose tolerance, or unmanaged diabetes. However, the patients we studied had well-managed blood sugar levels after surgery.

THA is linked to reduced hemoglobin levels, largely due to blood loss during the operation. These low Hb levels following surgery could potentially hinder the healing of surgical wounds (29-31). In our study, hemoglobin values were maintained at an average of 12.36 g/L, with a slight decrease at 6 weeks postoperatively.

PJI involves a complicated interaction between germs, mostly bacteria and sometimes fungi, and the body's immune response. Even a small number can start a PJI, as disease-causing organisms can stick to the surfaces of joint replacement components and create biofilms. The bacteria usually come from the skin's microbial community and may enter during the implantation surgery. Alternatively, these pathogens can infect the implant after surgery through the bloodstream or direct contact with nearby infected tissues (32).

Staphylococci species are the most frequent causative organisms. In our study, staphylococci account for 66% of PJI cases, confirming the previous studies' data.

The overall data of the patients included in the study seem to follow the evolution of the biological parameters of patients in previous studies, where no additional treatment (biocomposite) to the classical technique was used. The pain management and the quality of healing were important elements in the outcome analysis (33-35).

The advantages are however preserved, given the overall results and the increased cure rate.

The limitations of the study are the small number of patients, although it can be considered a positive aspect given the low infection rate.

CONCLUSSIONS

The impact of periprosthetic joint infection on patients is significant, affecting them physically, socially, and emotionally while also leading to increased healthcare costs, patient suffering, and higher mortality rates. In this study, involving 45 patients who underwent surgical reintervention and received a biocomposite following total hip

arthroplasty, the biological progression was generally typical when considering the preoperatory changes, with notable rapid improvement in inflammatory status within the first six weeks post-surgery.

However, diagnosing PJI is challenging as there is no universally accepted definition, and it can occur anywhere from 4 weeks to 2 years after the initial surgery. Additionally, the current tests used for diagnosis may only be useful in combination. The ideal scenario would be the discovery of a single biomarker with high sensitivity for accurately diagnosing PJI and relate to the evolution during the post-operative period.

CONFLICT OF INTEREST AND FUNDING

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The author Bogdan Mihnea Ciuntu contributed equally to the first and corresponding authors.

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DRUGS REPURPOSING FOR HEART FAILURE - COMPUTATIONAL APPROACHES TARGETING SGLT2

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DRUGS REPURPOSING FOR HEART FAILURE - COMPUTATIONAL APPROACHES TARGETING SGLT2 (Abstract): Sodium-glucose co-transporter 2 (SGLT2) inhibitors are the latest approved treatment for heart failure (HF), demonstrating a significant reduction in cardiovascular outcomes associated with this condition. Based on the increasing prevalence of HF and the urgent demand for safer and more effective therapeutic options, this study employed *in silico* approaches to systematically screen a comprehensive library of conventional drugs for their binding affinity to the SGLT2 protein. **Materials and methods**: More than 70 drugs, belong to several chemical and pharmacological classes, such as proton pump inhibitors, oral hypoglycemic drugs (sulfonylureas, gliptins), statins, nonsteroidal anti-inflammatory drugs, H_1/H_2 antagonists, antineoplastic drugs, alfa-adrenergic agonists, vitamins, antioxidants, were screened for their SGLT2 affinity using molecular docking method. In addition, for the drugs with the best docking score, a pharmacokinetic profile prediction using computational tools was also performed. Canagliflozin, empagliflozin and dapagliflozin were used as SGLT2 reference inhibitors. **Results and discussion:** The docking simulations revealed that the binding of the ligands to SGLT2 involves direct hydrogen bonds and van der Waals interactions with amino acids in the active site, such as H80, G83, L84, F98, E99, V85, V157, K154, L283, V286, V287, Y290, W291 and Q457. The best docking score, much better than SGLT2 reference inhibitors, was obtained for sulfonylureas (gliquidone, glipizide, glibenclamide, glimepiride), statins (atorvastatin, simvastatin), rutin and vitamins (vitamin K and E). **Conclusions**: Based on *in silico* results, in term of affinity for SGLT2 and pharmacokinetic profile prediction, several drugs show theoretical potential for beneficial effects in HF and could be considered for further investigation in advanced studies to validate these findings. **Keywords**: IN SILICO, HEART FAILURE, SGLT2, DOCKING SCORE, PHARMAKOKINETC PREDICTORS.

INTRODUCTION

Heart failure (HF) is the primary cause of mortality worldwide, with an incidence of approximately 10 cases per 1,000 individuals aged 65 and older (1). Among those diagnosed, approximately 50% will died within five years (2). This syndrome currently affects 65 million individuals globally, and more than 15 million in Europe, and estimations for the next dec-

ades indicate a worsening situation (3, 4). It is estimated that in the US by 2030, 8 million patients will be diagnosed with HF (1).

The prevalence of HF in Western countries is estimated to range from 1% to 14%, reflecting variations in demographic factors and epidemiological trends (5, 6). Moreover, due to the aging population and modest improvements in survival after therapeutic interventions, the HF prevalence is expected to rise by up to 46% by 2030 (7). This increase will affect all age groups and will be primarily attributable to the growing prevalence of risk factors such as hypertension, diabetes mellitus, obesity, and sedentary lifestyle. In Romania, approximately 4.7% of individuals aged 35 and older are affected by HF, corresponding to an estimated 560,000 patients (8).

HF is defined as a complex clinical syndrome that arises from any structural or functional injury affecting the two primary phases of the cardiac cycle: the filling phase (diastole), during which the heart receives oxygenated blood from the lungs, and the emptying phase (systole), in which the heart contracts to pump the accumulated blood into the arteries (9).

Clinically, HF is characterized by exercise intolerance, dyspnea, edema, pulmonary hypertension, and pulmonary edema. These symptoms are primarily caused by mechanisms including cardiac hypertrophy, fibrosis, and impaired angiogenesis within the myocardium (3).

The pathophysiology of HF is driven by a complex cascade of events involving inflammatory processes and neurohormonal activation, which together contribute to cardiac remodeling, diastolic dysfunction, and impaired cardiac output. In the context of HF, systemic inflammation, hypoxia, cardiomyocyte dysfunction and apoptosis, mechanical stress, and the release of profibrotic cytokines stimulate the migration and proliferation of cardiac fibroblasts to the damaged myocardium. These fibroblasts differentiate into activated myofibroblasts which develop stress fibers, acquire enhanced contractility, and secrete profibrotic signaling molecules such as transforming growth factor beta (TGF-β), tumor necrosis factor alpha (TNF-α), and angiotensin II (7). These factors, in turn, promote cardiomyocyte hypertrophy and remodeling process, further compromising cardiac function (1).

The first-line treatment of HF therapy includes beta-adrenergic blockers, angiotensin-converting enzyme (ACE) inhibitors, mineralocorticoid receptor antagonists, and, more recently, sodium-glucose co-transporters 2 (SGLT2) inhibitors (2). Originally developed as oral anti-diabetic agents to lower blood glucose by inhibiting SGLT2 in the kidneys, SGLT2 inhibitors unexpectedly demonstrated beneficial effects on cardiovascular outcomes, both in patients with type 2 diabetes mellitus (T2DM) and in those without the condition (10). These favorable outcomes may be attributed to the pleiotropic effects of SGLT2 inhibitors on the cardiovascular system, which include blood pressure management, attenuation of inflammation, protection against cardiac remodeling, weight reduction, and preservation of kidney function (11).

SGLT2 inhibitors target the SGLT2 transporters located in S2 and S3 segments of the renal proximal tubules (and, to a lesser extent, in the small intestine), where they facilitate glucose reabsorption. By inhibiting SGLT2, these agents induce

glucosuria, natriuresis, and osmotic diuresis, leading to reductions in body weight, blood pressure, plasma volume, and both cardiac preload and afterload. Additionally, SGLT2 inhibition activates key nutrient-sensing pathways, particularly the SIRT1/PGC-1α/FGF21 axis, which in turn enhances lipolysis and ketogenesis. These hemodynamic and metabolic effects are central to the cardiovascular benefits attributed to SGLT2 inhibitors. Moreover, SGLT2 inhibitors exert direct effects on the heart by targeting the cardiac sodium-hydrogen exchanger 1 (NHE1), mitigating the detrimental consequences of elevated intracellular Na^+ and Ca^{2+} levels, and thereby alleviating mitochondrial dysfunction (10, 12).

Drug repurposing (or drug repositioning) is an emerging and promising approach in drug discovery focused on identifying novel therapeutic applications for existing, well-studied drugs. This strategy presents a valuable alternative to traditional de novo drug development, especially considering that the success rate for developing a new molecular entity is just 2%, and the number of new drug approvals has been steadily declining since the 1990s. Over the past decade, approximately one-third of drug approvals have resulted from drug repurposing (13).

In this study, a large library of conventional drugs from various chemical and pharmacological classes was screened for their affinity to the SGLT2 protein using *in silico* methods. Additionally, the pharmacokinetic profiles of the top-ranking drugs, were predicted using different descriptors such as the volume of distribution at steady state (VDss), fraction unbound to plasma proteins, blood-brain barrier (BBB) and CNS permeability.

MATERIALS AND METHODS

In silico SGLT2 docking study

Protein preparation

The 3D structure of SGLT2 protein, established using X-ray crystallography analysis, was obtained from RCSB Protein Data Bank. Prior starting the docking studies the protein structure was processed in terms of removing the ligands, the water molecules, cofactors and ions. Using AutoDock4.2.6 the protein's structure was optimized by designating bond angles, bond ordering, and topology and was stored in PDBQT format for docking (14).

Drug library preparation

For virtual screening, in terms of affinity for SGLT2, were used more than 70 drugs belong to several pharmacological classes: proton pump inhibitors, oral hypoglycemic drugs (sulfonylureas, gliptins), statins, nonsteroidal anti-inflammatory drugs, H_1/H_2 antagonists, antineoplastic drugs, alfa-adrenergic agonists, vitamins, antioxidants. The 2D structures of the used drugs were designed in ChemDraw and then converted to PDB coordinate files using Chimera 1.14. The PDB file of each drug contains special keywords such as ROOT, ENDROOT, BRANCH, and ENDBRANCH in order to be recognized by AutoDock4.2.6. The PDB file defines a fixed set of atoms and rotatable atom groups connected to the rigid base moiety. Furthermore, TORSDOF was utilized to estimate the change in free energy associated with the loss of torsional degrees of freedom upon ligand binding. Each structure underwent energy minimization before being converted into the PDBQT file format for docking (15).

Virtual screening and molecular docking

Using AutoDock-GPU software, the prepared drug library was screened against

SGLT2 using AutoDock4.2.6 scoring function, which calculate the protein-ligand binding affinity. This tool facilitates the docking of large molecular libraries and accommodates the modeling of molecules with increasing size and complexity (16). The results, represented as Gibbs free energy values (ΔG), were compared with those of currently approved SGLT2 inhibitors, including canagliflozin, empagliflozin, and dapagliflozin.

The Autogrid program was employed to generate a grid map of 65×60×60 points with a spacing of 0.375 Å between grid points and the grid box center was put on x = 38.809, y = 48.916 and z = 45.968 to calculate the ΔG for each ligand-protein complex. The binding site of SGLT2 ligands was determined through the use of protein visualization tools, including PyMOL, Chimera 1.14, and DS Visualizer. The most promising ligand-protein complexes are those with high negative ΔG value.

For virtual screening, a molecular docking file (DPF) for each ligand was generated. The DPF specifies the grid map files to be used, the ligand molecule to be docked, the docking center, the number of torsions, the docking algorithm, and the number of docking runs. The 3D ligand conformation was generated using the Lamarckian Genetic Algorithm (LGA) with the following settings: 200 runs per docking procedure, a population of 300 individuals, a maximum of 27,000 generations per LGA run, and a maximum of 25,000,000 evaluations. A mutation rate was set to 0.02, and the crossover rate to 0.80 while other docking parameters were set to their default values a. After completing the molecular docking simulations, the best ligands candidates were identified and evaluated based on their docking scores which measured the hydrophilic and lipophilic interactions between the active sites of ligand and SGLT2 protein.

The docking results were clustered using a root mean square deviation (RMSD) of 0.5 Å and analyzed using PyMol software.

Pharmacokinetics profile prediction

The pkCSM-Pharmacokinetics (http://structure.bioc.cam.ac.uk/pkcsm) and SwissADME (http://www.swissadme.ch) tools were used to predict the pharmacokinetic profile of the selected drugs (17). The pkCSM-Pharmacokinetics is a software for the prediction of small-molecule pharmacokinetic properties using graph-based signatures while SwissADME offers a comprehensive assessment of the pharmacokinetic and physicochemical properties.

RESULTS AND DISCUSION

In silico SGLT2 docking results

The PDB 7VSI file was chosen to investigate the predicted binding mode of the ligands with the SGLT2 protein. SGLT2 is characterized by the highest resolution kinase structure available to date, at 2.95 Å, which enhances confidence in the atomic positioning within the electron density map. Additionally, the reliability factor (R-value) is below 0.15, reflecting a strong agreement between the crystallographic model and the experimental X-ray diffraction data.

The computational method employed was validated by assessing the RMSD variation (less than 2 Å) for empagliflozin, based on its affinity for the active site of SGLT2, and it was found to align with the binding cleft residues reported in the literature. The docking results further indicated that the reference inhibitor, empagliflozin,

forms polar interactions with the catalytic site of SGLT2, binding deeply through specific interactions with H80, F98, and H268. Accordingly, with previous studies, key amino acids for interaction with the SGLT2 binding pocket were identified as H80, G83, L84, F98, E99, V85, V157, K154, L283, V286, V287, Y290, W291, and Q457 (14).

Each ligand (drug) - receptor complex was thoroughly analyzed to identify the optimal docked position, determined by the lowest binding energy scores and the highest number of cluster conformations. The values of the Gibbs free energies (ΔG) are presented in first table.

TABLE I.

The Gibbs free energies (ΔG) for SGLT2 of tested drugs

Ligand	SGLT-2 ΔG (kcal/mol)	Ligand	SGLT-2 ΔG (kcal/mol)
Gliquidone	-13.79	Cetirizine	-9.06
Glipizide	-12.68	Riboflavin	-8.9
Rutin	-12.52	Ketoprofen	-8.68
Glibenclamide	-12.44	Omeprazole	-8.64
Atorvastatin	-12.33	Epalrestat	-8.61
Glimepiride	-12.05	Famotidine	-8.41
Simvastatin	-11.9	Lansoprazole	-8.14
Vitamin K	-11.69	Pantoprazole	-7.93
Canagliflozin	**-11.49**	Phenytoin	-7.8
Lovastatin	-11.41	Diclofenac	-7.74
Vitamin E	-11.31	Tolrestat	-7.53
Empagliflozin	***-11.24***	Resveratrol	-7.49
Vildagliptin	-11.07	Ibuprofen	-7.22
Saxagliptin	-10.95	Antazoline	-7.21
Gliclazide	-10.75	Ranitidine	-7.18
Desmethoxycurcumin	-10.72	Nizatidine	-7.15
Dapagliflozin	***-10.69***	Tramazoline	-7.13
Alogliptin	-10.68	Naproxen	-7.03
Bisdemethoxycurcumin	-10.63	Xylometazoline	-6.98
Ezetimibe	-10.45	Oxymetazoline	-6.91
Oleanolic acid	-10.32	Cimetidine	-6.86
Rosuvastatin	-10.18	Biotin	-6.86

Ligand	SGLT-2 ΔG (kcal/mol)	Ligand	SGLT-2 ΔG (kcal/mol)
Fluvastatin	-10.09	Naphazoline	-6.85
Pravastatin	-9.97	Tacrine	-6.85
Folic acid	-9.85	Vitamin B1	-6.71
Methotrexate	-9.84	Tetryzoline	-6.68
Linagliptin	-9.74	Dexmedetomidine	-6.46
Curcumin	-9.73	Lipoic acid	-6.08
Retagliptin	-9.68	Pantothenic acid	-6.03
Pioglitazone	-9.57	Vitamin B6	-5.59
Indomethacin	-9.54	Ascorbic acid	-5.28
Sitagliptin	-9.39	Cytarabine	-5.16
Rosiglitazone	-9.34	Adenine	-4.85
Indirubin_3_monoxime	-9.15	Metformin	-4.79
Rabeprazole	-9.11	Nicotinic acid	-4.76
Indirubin-3-monoxime	-9.09	Flucytosine	-4.28

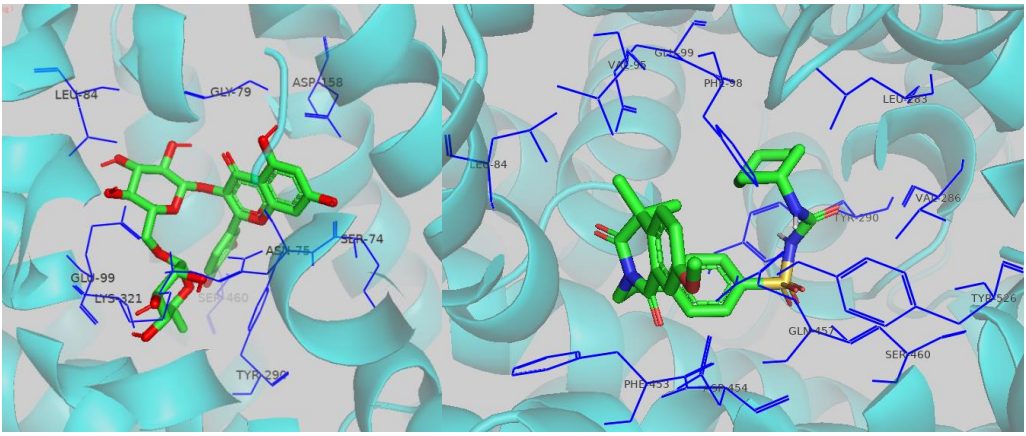
The analysis of the results showed that the estimated free binding energies (ΔG) for the reference SGLT2 inhibitors were all below -10 kcal/mol, with canagliflozin at -11.49 kcal/mol, empagliflozin at -11.24 kcal/mol, and dapagliflozin at -10.69 kcal/mol.

It is well established that ligands with the lowest docking scores require less energy to stabilize at the receptor binding site. It was observed that several drugs, belonging to different chemical structures and therapeutic classes, exhibit ΔG values lower than those of the SGLT2 reference inhibitors (canagliflozin, empagliflozin, and dapagliflozin), suggesting that these compounds have the theoretical potential to be more potent inhibitors. The most potent ligands are oral hypoglycemic drugs such as gliquidone (-13.79 kcal/mol), glipizide (-12.68 kcal/mol), glibenclamide (-12.44 kcal/mol), glimepiride (-12.5 kcal/mol), gliclazide (-10.75 kcal/mol). ΔG values less or similar to SGLT2 reference inhibitors was recorded also for rutin (-12.52 kcal/mol), statins (atorvastatin, -12.33 kcal/mol; simvastatin, -11.90 kcal/mol; lovastatin, -11.41 kcal/mol), and vitamin K (-11.69 kcal/mol).

The docking simulations revealed that the binding of the ligand to SGLT2 involves direct hydrogen bonds and van der Waals interactions with amino acids in the active site (fig. 1).

For example, rutin binds to the catalytic site of SGLT2 by forming strong hydrogen bonds with S74, N75, G79, L84, E99, Y290, and K32. Similarly, gliquidone exhibits a strong affinity for the SGLT2 active site, with hydrogen bonds formed between its sulfonylurea moiety and the amino acids Q457, S460, and Y290. Additionally, the 1,3-dioxo-2-isoquinolinyl moiety of gliquidone stabilizes the molecule through hydro-

gen bonds with Y526 and D454, as well as van der Waals interactions with amino acids from the active site, including F453, L84, F98, V95, and E99 (fig. 1).



(a) (b)

Fig. 1. The binding mode of rutin (a) and gliquidone (b) to the active site of SGLT2.

Pharmacokinetics profile prediction

The parameters related to Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADME-Tox) are crucial for understanding the pharmacokinetics, pharmacodynamics, and toxicology of drugs (18, 19). These data are vital for predicting the drug's behavior *in vivo* and assessing its safety and potential adverse effects.

The drugs with the highest docking scores were selected for pharmacokinetic predictions using computational methods. The distribution properties, including the volume of distribution at steady state (VDss), the fraction unbound to plasma proteins, the blood-brain barrier (BBB) permeability and consequently the CNS permeability were predicted, and the results are presented in second table.

TABLE II.

The pharmacokinetics profile of tested drugs, predicted using pkCSM-phamacokinetics and SwissADME

Ligand	VDss (human)	Fraction unbound (human)	CNS permeability	BBB permeability
Gliquidone	-0.34	0.23	-2.74	-0.98
Glipizide	-1.29	0.00	-2.46	-0.91
Rutin	-0.25	0.25	-3.37	-1.04
Glibenclamide	-1.92	0.09	-2.92	-1.17
Atorvastatin	0.20	0.10	-2.10	-0.27
Glimepiride	-0.22	0.00	-2.68	-1.00

Ligand	VDss (human)	Fraction unbound (human)	CNS permeability	BBB permeability
Simvastatin	1.66	0.19	-5.18	-1.90
Vitamin K	0.32	0.00	-1.11	-0.29
Lovastatin	-0.44	0.00	-2.01	-0.02
Vitamin E	-0.08	0.00	-2.46	-0.34
Vildagliptin	0.09	0.25	-2.46	-0.01
Saxagliptin	-1.01	0.06	-2.20	0.25
Gliclazide	-0.67	0.19	-2.32	0.11
Demethoxycurcumin	-1.64	0.08	-2.03	-0.57
Alogliptin	0.59	0.57	-3.13	-0.66
Bisdemethoxycurcumin	0.28	0.25	-2.62	-0.29
Ezetimibe	0.27	0.24	-2.96	-1.51
Oleanolic acid	-1.01	0.00	-1.18	-0.14

It is well established that only the unbound fraction of a drug is pharmacologically active, while high protein binding can influence both the pharmacokinetic and pharmacodynamic profiles of drugs (19).

The analysis of the results showed that the values of fraction unbound ranged from 0 (glipizide, glimepiride, vitamin K, vitamin E, lovastatin, oleanolic acid) to 0.57 (alogliptin). For VDss, the values recorded ranged from -1.92 (glibenclamide) to 1.66 (simvastatin).

To determine the statistical significance of the difference between VDss and the fraction unbound, while accounting for ligand variances in the overall error variance, a two-way ANOVA analysis was conducted. The results indicated no statistically significant difference between the two parameters ($F(0.5, 1, 16) = 11.302$, $p = 0.001$, $Fcrit = 4.358$). Thus, the lower VDss values correlate with a lower fraction of drug molecules in the plasma that are unbound (free) to proteins.

The BBB permeability is evaluated using two parameters: a qualitative measure (BBB) and a quantitative one (logBB). A value of logBB higher than 0.3 suggests that the drug crosses the BBB, having a high permeability, while a value less than -1 indicates a limited BBB permeability (19). For the tested drugs the BBB permeability values ranged between -1.90 (simvastatin) to 0.25 (saxagliptin), that means that could be classified as drugs with poor distribution to brain.

The drugs with the best pharmacokinetic predictors, in term of VDss (less than 0.5) and BBB permeability (less than -1), were oral the hypoglycemic drugs, such as glipizide (VDss = -1.29, BBB = -0.91,) glibenclamide (VDss = -1.92, BBB = -1.17) and gliclazide (VDss = -0.67, BBB = 0.11). Appropriate values were also recorded for: rutin (VDss = -0.25, BBB = -1.04), demethoxycurcumin (VDss = -1.64, BBB = -

0.57); vildagliptin (VDss = -0.09, BBB = 0.01); vitamin E (VDss = -0.08, BBB = -0.34) and vitamin K (VDss = 0.32, BBB = -0.29). The values recorded for canagliflozin, empagliflozin and dapagliflozin, used as SGLT2 references inhibitors, were VDss = -0.71, BBB = -0.99 (canagliflozin); VDss = -0.39, BBB = -1.10 (empagliflozin) and VDss = 0.33, BBB = -0.99 (dapagliflozin), respectively.

CONCLUSIONS

Drug repurposing is an emerging and promising strategy in the medical and pharmaceutical fields, focusing on discovering new therapeutic uses for drugs that have already been approved for other conditions. This approach offers a valuable alternative to traditional de novo drug development, as it is faster and more cost-effective.

Using AutoDock-GPU software, a large drug library was screened against SGLT2 using AutoDock4.2.6 scoring function, which calculate the protein-ligand binding affinity. The most promising drugs that showed SGLT2 binding affinity much better than known SGLT2 inhibitors (canagliflozin, empagliflozin, dapagliflozin) are sulfonylureas (gliquidone, glipizide, glibenclamide, glimepiride), statins (atorvastatin, simvastatin), rutin and vitamins (vitamin K and E). These drugs showed also a good pharmacokinetic profile, having a poorly blood brain barrier permeability (BBB less than −1) and a proper volume of distribution at steady state (VDss less than 0.5). Based on *in silico* results, the most favorable drugs will be considered for further investigations using *in vitro* and *in vivo* assays

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CUT-OFF VALUES OF BODY FAT PERCENTAGE AND INSULIN FOR PREDICTING LOW ADIPONECTIN / LEPTIN RATIO

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CUT-OFF VALUES OF BODY FAT PERCENTAGE AND INSULIN FOR PREDICTING LOW ADIPONECTIN / LEPTIN RATIO (Abstract): This study **aims** to establish a reliable parameter and its optimal cut-off point for accurately estimating adipose tissue dysfunctionality in apparently healthy, but abdominally obese subjects. **Materials and methods:** The 104 subjects included in the study, all with no prior chronic diseases, underwent biochemical (TG, HDL-c, glucose, insulin, adiponectin, leptin) and anthropometric measurements (waist circumference, height, adipose tissue percentage). A ROC curve was performed to assess the overall performance of these parameters to determine the dysfunctionality of the adipocytes, as assessed by adiponectin: leptin ratio (ALR) lower than 0.5. Logistic regression identified models that included 2 or 3 parameters to further increase the accuracy. The software Excel and SPSS were used for the statistical analysis. **Results:** From all individual parameters, trunk adipose tissue percentage had an AUC value of 0.812, suggesting a high accuracy for predicting a value of ALR<0.5. The optimal cut-off point for trunk adipose tissue percentage was established at 42.45% with a sensitivity of 0.725 and a specificity of 0.766. A higher AUC (0.857) was observed for the model that included whole-body adipose tissue (cut-off 41.7%) and insulin (cut-off 14.6 μU/mL), while the highest accuracy (AUC=0.889) was reported in the model for metabolically unhealthy non-obese subjects. **Conclusions:** Apparently healthy individuals, but abdominally obese, report adipocyte dysfunctionality together with high cardiometabolic risk at values for trunk adipose tissue percentage surpassing 42.45%, or combined values of 41.7% for whole-body adipose tissue and 14.6 μU/mL for insulin. **Keywords:** CUT-OFF VALUES, BODY FAT, INSULIN, ADIPONECTIN / LEPTIN RATIO.

INTRODUCTION

It is well-established that quantity and quality of adipose tissue play a critical role in metabolic health, as both excessive or insufficient adipose tissue are associated with significant health risks. In conditions such as congenital or acquired lipoatrophy insufficient adipose tissue leads to severe metabolic disturbances and conversely, obesity is also linked to similar metabolic complications (1, 2). Adipocytes have a unique secretory profile that includes adipokines like leptin and adiponectin, cytokines or various metabolites, which collectively interact to establish communication pathways between organs (3). Their bal-

ance is vital for organism physiology, therefore accurately identifying adiposopathy is pivotal in a clinical setting for prevention and diagnostics strategies, while particularly challenging to attain. Previous studies have confirmed adiponectin/leptin ratio, as opposed to single biomarker approaches, as a diagnostics tool for adipocyte metabolism dysfunctionality, with lower values than 1 being associated with cardiometabolic risk (4, 5).

This study aims to establish a reliable parameter and its optimal cut-off point for accurately estimating adipose tissue dysfunctionality defined as adiponectin/leptin ratio < 0.5. There are some key points we consider as arguments for this analysis. While adiponectin and leptin together profile the status of adiposopathy and that of cardiometabolic risk, these assays are not performed usually as screening tests in individuals and their cost is handled by the patient, therefore a balanced solution would be highly valued. Judging from an economical and practical point of view, the detailed profile of an individual that represents the core of personalized medicine is still hard to portray. However, modern technologies including artificial intelligence or advanced statistics aid in generating a more complete profile as possible. To generate algorithms solely for adiponectin and leptin with the help of hands-on and easy to obtain parameters is a challenging task. Therefore, our study focused on predicting the reduced ratio levels between the two which has a broader significance and could tell us more about the functionality of the adipose tissue, despite its limited focus on specific outcomes. We expect to have a more detailed picture of the apparently healthy individual that also has abdominal obesity, with respect to adipocyte metabolism and cardiometabolic implications.

MATERIALS AND METHODS

This observational cross-sectional study included 104 subjects, aged 35-75 years old (25.96% men and 74.04% women), with abdominal obesity and no prior known chronic disorders or acute atherosclerotic events in antecedents. All patients underwent biochemical and anthropometric measurements in the morning, with blood samples being collected after a 12 hrs. overnight fast. Serum samples for marker analysis were stored at -20ºC. Glucose, TG, HDL-c levels were determined using the spectrophotometric method, insulin using chemiluminescence, adiponectin and leptin with ELISA technique.

Visceral adiposity index was calculated based on previous validated equations (6). For adipose tissue mass and percentage, dual-energy X-ray absorptiometry (DEXA) was used, as a gold standard for this measurement.

The population included in the cohort was apparently healthy, but after investigations some subjects presented metabolic syndrome (MetS). The presence of MetS included waist circumference > 88 cm (women)/104 cm (men) and at least 2 of the following criteria: glucose ≥ 100 mg/dL, HDL < 40 mg/dL (men) / < 50 mg/dL (women), TG ≥ 150 mg/dL, SBP/DBP ≥ 130/85 mmHg (7).

Data was processed with Excel and SPSS. Distribution was assessed for all variables with the Kolmogorov-Smirnov test and confirmed visually, analyzing the histograms. Correlations between variables were performed with Spearman and Kendall's tau-b test. Logistic regression delivered models with high performance and the predicted probability calculated for these models was further used as input for the

receiver operating characteristic (ROC) curve for determining specific biomarker values to better predict a low ALR.

RESULTS

All analyzed variables are non-normally distributed, except trunk adipose tissue percentage which has a normal distribution. In this study 74.04% of the subjects were women and 25.96% were men, with a mean age of 59.51±8.18. Baseline characteristics of the study are presented in first table.

TABLE I.
Baseline characteristics of the study group

Parameter	Median (Q3-Q1)	Min	Max
Age (years)	62 (53-65)	39	75
BMI (kg/m^2)	30.99 (28.57-34.39)	21.91	53.31
BMI (kg/m^2) - women	30.23 (28.01-34.23)	21.91	49.50
BMI (kg/m^2) - men	32.43 (30.17-35.61)	22.75	53.31
Waist circumference (cm)	106 (99-115)	89	160
Insulin (µU/mL)	15 (9.38-21.45)	2.13	52
Glucose (mg/dL)	103 (94-116)	76	336
HOMA-IR	3.78 (2.38-5.99)	0.49	27.25
Adiponectin (µU/mL)	13.02 (10.33-16.77)	4.53	28.53
Leptin (ng/mL)	19.63 (11.22-39.67)	2.60	58.20
ALR	0.59 (0.33-1.36)	0.13	4.98
eVAI	4.50 (3.15-7.64)	1.38	33.66
Whole-body adipose tissue (%)	41.10 (36.32-44.27)	18.90	53.20
Parameter	**Mean±S.D.**	**C.I. (95%)**	
		Lower limit	**Upper limit**
Trunk adipose tissue (%)	40.30 ± 6.42	39.05	41.55

* S.D.= standard deviation, Q3-Q1=difference between superior quartile and inferior quartile, ALR=adiponectin/leptin ratio, eVAI=estimated visceral adipose index.

The correlation matrix between biomarkers and parametric variables describing adipose tissue health report ALR as having stronger correlations with obesity parameters than adipokines alone. The eVAI parameter reports little to no correlation with all the other variables, adipose tissue percentage presents a stronger correlation with ALR than BMI and insulin is stronger correlated with ALR than HOMA-IR. All correlation coefficients over 0.200 are statistically significant (tab. II).

Logistic regression rendered two best models presented in third table for estimating the status of ALR < 0.5. Variables with the strongest correlation were the ones that performed best, with whole-body adipose tissue percentage and insulin being the most important influencers for low ALR. In order to calculate the accuracy of each parameter and model for assessing low ALR, ROC curve analysis was performed (fig. 1).

The AUC values of factors predicting adipose tissue dysfunctionality confirms high accuracy for adipose tissue percentage, insulin and the 2 models (tab. IV). Optimal cut-off points were chosen for these parameters, preserving in the same time the highest sensitivity and specificity possible (tab. V).

TABLE II.

Correlations between biochemical and anthropometric parameters of obesity

	Insulin	Glucose	HOMA-IR	Adiponectin	Leptin	ALR	BMI	Waist circumference	eVAI	Trunk adipose tissue %	Total adipose tissue %
Insulin	1	0.336	0.946	-0.404	0.392	-0.575	0.386	0.405	0.291	0.206	0.069
Glucose	0.336	1	0.552	-0.236	-0.07	-0.047	0.13	0.38	0.248	-0.095	-0.127
HOMA-IR	0.946	0.552	1	-0.414	0.304	-0.496	0.368	0.464	0.314	0.133	0.007
Adiponectin	-0.404	-0.236	-0.414	1	0.119	0.362	-0.286	-0.308	-0.216	0.052	0.234
Leptin	0.392	-0.068	0.304	0.119	1	-0.86	0.416	0.225	0.038	0.744	0.778
ALR	-0.575	-0.047	-0.496	0.362	-0.86	1	-0.522	-0.367	-0.157	-0.674	-0.615
BMI	0.386	0.13	0.368	-0.286	0.416	-0.522	1	0.788	0.172	0.381	0.295
Waist circum-ference	0.405	0.38	0.464	-0.308	0.225	-0.367	0.788	1	0.277	0.222	0.056
eVAI	0.291	0.248	0.314	-0.216	0.038	-0.157	0.172	0.277	1	0.157	0.014
Trunk adipose tissue %	0.141	-0.061	0.086	0.034	0.557	-0.479	0.381	0.222	0.157	1	0.754
Total adipose tissue %	0.069	-0.127	0.007	0.234	0.778	-0.615	0.295	0.056	0.014	0.908	1

*Spearman test correlation was performed for all variables, except for “Trunk adipose tissue percentage” which required Kendall’s tau -b correlation test

TABLE III.

Models for estimating the status of ALR < 0.5.

Model 1				
Parameters	**B**	**Std. Error**	**Exp(B)**	**p**
Whole-body adipose tissue percentage	0.332	0.076	1.394	<0.001
Insulin	0.105	0.028	1.110	<0.001
Constant	-16.158	3.543	0.000	<0.001
Model 2				
Parameters	**B**	**Std. Error**	**Exp(B)**	**p**
Whole-body adipose tissue percentage	0.333	0.078	1.395	<0.001
Insulin	0.097	0.030	1.102	0.001
Clinical obesity phenotype				
• MHO				0.07
• MUO	1.967	0.875	7.146	0.025
• MHNO	1.988	0.804	7.302	0.013
• MUNO	1.356	1.071	3.880	0.205
Constant	-17.522	3.723	0.000	<0.001

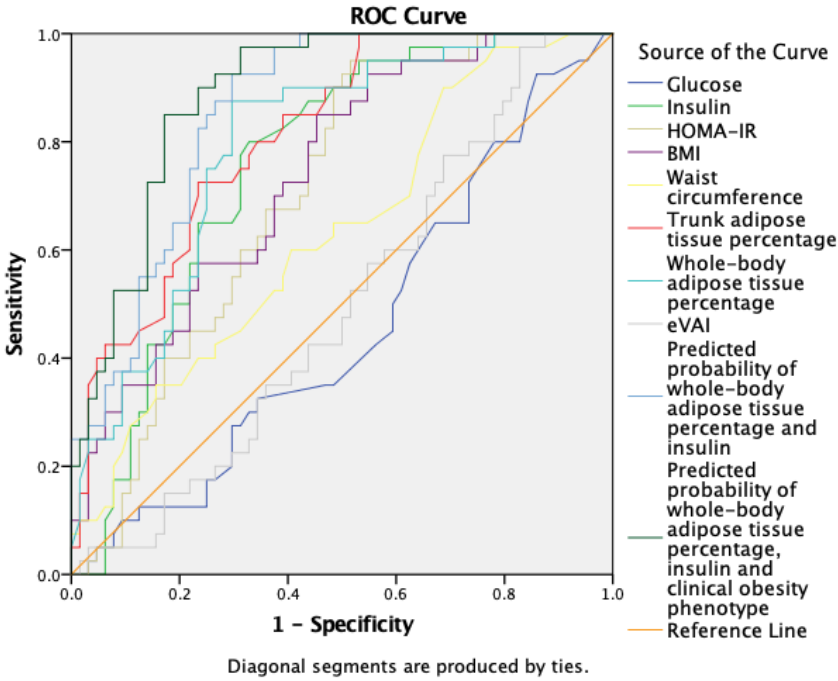

Fig. 1. Receiver operating characteristics (ROC) curve analysis on the prediction of dysfunctional adipose tissue

TABLE IV.

Accuracy estimates for factors predicting adipose tissue dysfunctionality

Parameter	AUC	Std. Error	p	Confidence Interval (95%)	
				Lower Bound	Upper Bound
Glucose	0.456	0.058	0.450	0.343	0.569
Insulin	0.758	0.047	<0.001	0.666	0.850
HOMA-IR	0.705	0.050	<0.001	0.607	0.803
BMI	0.738	0.048	<0.001	0.644	0.833
Waist circumference	0.632	0.055	0.024	0.524	0.740
Trunk adipose tissue percentage	0.812	0.041	<0.001	0.731	0.892
Whole-body adipose tissue percentage	0.795	0.044	<0.001	0.709	0.880
eVAI	0.497	0.057	0.957	0.386	0.608
Model 1*	0.857	0.035	<0.001	0.788	0.926
Model 2**	0.889	0.031	<0.001	0.828	0.950

Note: AUC = area under the curve.

*Model 1 = Predicted probability for whole-body adipose tissue percentage and insulin.

**Model 2 = Predicted probability for whole-body adipose tissue percentage, insulin and clinical obesity phenotypes.

TABLE V.

Optimal cut-off points for predicting adipose tissue dysfunctionality

Parameter	Optimal cut-off	Sensitivity	Specificity	Youden Index
Insulin (μU/mL)	14.90	0.80	0.672	0.472
Whole-body adipose tissue percentage (%)	40.85	0.875	0.703	0.578
Trunk adipose tissue percentage (%)	42.45	0.725	0.766	0.491
Model 1* - Associated variables	0.32 - Whole-body adipose tissue (%): 41.7 - Insulin (μU/mL): 14.6	0.925	0.703	0.628
Model 2** - Associated variables	0.45 - Whole-body adipose tissue (%): 39.1 - Insulin (μU/mL): 15 - Metabolically unhealthy non-obese	0.85	0.828	0.678

*Model 1 = Predicted probability for whole-body adipose tissue percentage and insulin.

**Model 2 = Predicted probability for whole-body adipose tissue percentage, insulin and clinical obesity phenotypes.

DISCUSSION

In the present study we identified predictive values of biomarkers and representative parameters of body composition for adipose tissue dysfunctionality. The main finding is that adipose tissue percent-

age together with insulin identify the presence of ALR lower than 0.5 with a high accuracy rate. The optimal cut-off point for trunk adipose tissue percentage is 42.45% and for insulin is 14.9 μU/mL. These values are referenced against other established thresholds for cardiometabolic risk in several studies. Bawasi H. *et al*. proposed sex-specific cut-off points for adipose tissue percentage in people over 40 years old, with values of 34.8% (men) and 46.3% (women) for total adipose tissue or 21.6% (men) and 23.4% (women) for trunk adipose tissue (8). The collaborative group of Macek P. reports optimal cut-off points for body fat percentage of 25.8% for men and 37.1% for women (9). These values are not standardized as they reflect a threshold for cardiovascular risk that is measured distinctly for every study, therefore it remains difficult to compare them between each other. Furthermore, to the best of our knowledge, this is the first study that calculates a cut-off point of body fat percentage for determining adiposopathy defined as low ALR and moreover, it predicts severe cardiometabolic risks since the value presents an important decrease (inferior to 0.5).

A recent comprehensive review emphasizes that besides BMI, more essential to measuring the extent of adiposity are waist circumference and body fat percentage. Together with the clinical component these are critical for a complications-centric approach to obesity care, which aligns with the Adiposity-Based Chronic Disease framework (10). A position statement on visceral and ectopic fat highlights the necessity of creating and implementing methods to detect individuals with excessive adiposity, both in clinical settings and within public health initiatives (11). Moreover, trunk adipose tissue percentage, particularly visceral fat is strongly associated with metabolic risk factors, including insulin resistance, reinforcing the utility of adipose tissue percentage as a reliable predictive marker (12), together with adiponectin and leptin as primarily derived molecules from adipose tissue (13) as promising candidates for distinguishing between insulin-sensitive and insulin-resistance obesity phenotypes (14).

The variables included in the models were depicted based on their influence on adiponectin-leptin ratio, which was assessed with logistic regression. Even though the clinical obesity phenotype variable does not have a significant p value as a whole group, model 2 outperformed model 1. Insulin is present in both models in our study, confirming its key influence on ALR, since insulin resistance likely results from the dynamic interrelationship between multiple adipokines rather than the action of a single molecule. Important pathophysiological processes take place when leptin is hyper secreted and adiponectin underproduced, like: pancreatic β-cells damage reducing insulin production, exacerbation of glucose intolerance (15), suppression of glucose transporter-4 and insulin receptor substrate-1 expression (16, 17). In our study HOMA-IR reported a lower accuracy rate for predicting adipose dysfunctionality than insulin alone, however the values demonstrated proximity. To confirm whether indeed insulin or HOMA-IR is more relevant in a clinical setting to predict adipose dysfunctionality, we would need to extend the study to a larger cohort.

The ROC analysis used in our study depicts the trade-off between sensitivity and specificity over a series of established thresholds for different obesity linked pa-

rameters. The optimal cut-off points were chosen based on the Younden index, the global measure of biomarker effectiveness (18). However, the choice could be subjective depending on the severity of the disease. In our case, identifying adiposopathy is mostly used in preventive or cardiovascular stratifying programs, therefore we can accept a balance between correctly and incorrectly classifying individuals with the disease. In severe pathologies, we would need to choose a cut-off point that provides 100% sensitivity, even if the specificity in this case would be low.

Since it is difficult in clinical settings to find the specific values for all variables in one patient, these models are difficult to implement in a day by day practice. Therefore, individual variables could be of better use to clinicians, since they provide a clear percentage of false positives or false negatives regarding adiposopathy. Since body composition analysis implies a high cost, artificial intelligence networks can be used to calculate adipose tissue percentages with high accuracy, therefore making it almost cost-free to estimate ALR. The limitation is, of course, the false positive and false negative rates that have to be considered. Our group worked on an algorithm that predicts with high accuracy trunk adipose tissue mass from easy to measure anthropometric parameters (19), while there are also other models performed on larger cohorts sustaining the need for this cost-effective prediction tool (20-22).

CONCLUSIONS

Apparently healthy individuals, but abdominally obese, report adipocyte dysfunctionality together with high cardiometabolic risk at values for trunk adipose tissue percentage surpassing 42.45%, or combined values of 41.7% for whole-body adipose tissue and 14.6 μU/mL for insulin. Adipose tissue percentages can be easily and accurately estimated based on already developed algorithms, therefore the severe cardiometabolic risk linked to ALR values lower than 0.5 can be estimated almost cost-free.

These findings have clinical relevance for patient care and contribute significantly to the advancement of personalized medicine, particularly within the context of preventive strategies and cardiovascular risk stratification.

CONFLICT OF INTEREST AND FUNDING

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EVALUATION OF ANXIETY FOR FIRST AND SECOND TRIMESTER PREGNANT WOMEN INFECTED WITH COVID-19. A CLINICAL STUDY

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EVALUATION OF ANXIETY FOR FIRST AND SECOND TRIMESTER PREGNANT WOMEN INFECTED WITH COVID-19. A CLINICAL STUDY (Abstract) **Aims:** During the COVID-19 epidemic, a widespread worsening in mental health was recorded. This study aimed to assess the psychological impact on mothers and to quantify the percentage of Romanian women infected with SARS-CoV-2 during the first and second trimesters of pregnancy who exhibited clinically significant anxiety symptoms (GAD-7 score > 10) and their associated concerns. **Materials and methods:** Between July 1st, 2021, and March 31st, 2022, 45 patients with a confirmed mild or moderate SARS-CoV-2 infection and a pregnancy less than 24 weeks of gestation were admitted to the 2nd Clinic of Obstetrics and Gynecology at the "Sf. Apostol Andrei" Clinical Emergency County Hospital Galați for treatment and investigations. Data collection was accomplished through the development of a questionnaire that comprised fourteen items. The clinical data and GAD-7 anxiety score were subsequently analyzed. **Results:** For 23 patients (51.11%), the GAD-7 score ranged from 0 to 4, indicating low anxiety. Conversely, in 4 patients (8.88%), the GAD-7 score ranged from 10 to 14, indicating moderate anxiety, while in 3 cases (6.66%), the GAD-7 score was 15, indicative of severe anxiety. **Conclusions:** Despite the large amount of literature on anxiety-depressive disorders during pregnancy, especially in the third trimester and postpartum, there is a lack of studies focusing on the mental health of pregnant women diagnosed with SARS-CoV-2 infection, particularly in the first trimester. This is especially significant considering the limited understanding of the relationship between the adverse effects of viral infection on pregnancy and maternal-fetal outcomes. **Keywords:** PREGNANCY, COVID-19, MENTAL HEALTH, GAD-7.

INTRODUCTION

In March 2020, the World Health Organization (WHO) designated COVID-19 (Coronavirus Disease 2019) as a global pandemic. Over the past five years, the pandemic has presented myriad obstacles to individuals around. Throughout the pandemic, various waves emerged, marked by intervals of rising COVID-19 cases. To mitigate the influx of new infections, numerous countries implemented social restrictions and lockdowns (1). The SARS-CoV-2 pandemic has impacted the worldwide economy, national infrastructures, healthcare systems, and the overall welfare of the global population. The Romanian authorities have adjusted to the pandemic, implementing several steps to safeguard public health (2).

The pandemic has been especially frightening for pregnant women. The majority of pregnant women infected with SARS-CoV-2 exhibit either asymptomatic or moderate symptoms (3). They frequently manifest with fever and cough, and less commonly with myalgia and chills.

The most often documented unfavorable outcomes in pregnant women with SARS-CoV-2 infection were preterm delivery and low birth weight (4). Additional obstetric consequences including stillbirth, miscarriage, preeclampsia-like syndrome, fetal growth restriction, coagulopathy, premature rupture of membranes, and postpartum hemorrhage, all of which remained infrequent (4-6).

During the COVID-19 pandemic, it was reported a general decline in mental health; however, women exhibited a 44-75% greater rise in adverse mental health outcomes compared to men (7). Fluctuations in mental health are frequently ascribed to significant life alterations, such as the implementation of remote work regulations and job loss, occurrences that may lead to heightened substance use and elevated suicide rates (7, 8). Given the impact of COVID-19 on mental health in the general population, it is highly probable that pregnant women experienced analogous elevations in the risks for mental health and substance use disorders.

In 2006, Spitzer introduced the 7-item Generalized Anxiety Disorder scale (GAD-7) as a brief measure for assessing anxiety disorders (9). Only in 2014, the National Institute for Health and Care Excellence (NICE) suggested the GAD-7 as a screening tool for identifying anxiety during routine mental health assessments in antenatal as well as in postnatal period (10). Nonetheless, the NICE requested more validation of GAD-7 within the pregnant population. This advice was mostly based on expert consensus rather than the quantity of studies (11).

Although there are numerous reports in the literature on anxiety-depressive disorders throughout pregnancy, particularly in the third trimester and postpartum, there are very few studies addressing mental health in pregnant women who have been diagnosed with Sars-Cov-2 infection, particularly in the first trimester. This is especially relevant given that little was known about the association between the detrimental impact of viral infection on pregnancy and maternal-fetal outcome.

MATERIALS AND METHODS

The aim of this study was to evaluate maternal psychological impact and to determine the proportion of women with clinically significant symptoms of anxiety (GAD-7 $\geq$ 10) among Romanian women who were infected with SARS-CoV-2 dur-

ing first and second trimester of pregnancy and their concerns.

A number of 45 patients with pregnancy less than 24 weeks of gestation, with confirmed mild or moderate SARS-CoV-2 infection (SpO2 ≥ 94% and clinical evidence of lower respiratory tract disease), were addressed and admitted to 2nd Clinic of Obstetrics and Gynecology, Clinical Emergency County Hospital" Sf. Apostol Andrei" Galați for investigations and treatment during July 1st 2021 and March 31st 2022. Pulmonary CT was not performed in our case series due to specific contraindications in pregnancy as well as the fact that none of the patients was in a severe clinical condition.

The GAD-7 questionnaire (fig. 1) was translated and used for data collection. Patients were asked to participate in the study and fill in the questionnaire at the first consultation / admission to the hospital. Clinical data along with GAD-7 anxiety score were then analyzed.

GAD-7 Anxiety

Over the last two weeks, how often have you been bothered by the following problems?	Not at all	Several days	More than half the days	Nearly every day
1. Feeling nervous, anxious, or on edge	0	1	2	3
2. Not being able to stop or control worrying	0	1	2	3
3. Worrying too much about different things	0	1	2	3
4. Trouble relaxing	0	1	2	3
5. Being so restless that it is hard to sit still	0	1	2	3
6. Becoming easily annoyed or irritable	0	1	2	3
7. Feeling afraid, as if something awful might happen	0	1	2	3

Column totals _____ + _____ + _____ + _____ =

Total score _____

Fig. 1. GAD-7 Anxiety score

The study was approved by the institutional ethics committee (No. 12534/2021), and complied with the Declaration of Helsinki. Written consent was obtained from all patients included in this study.

RESULTS

The data obtained from the study questionnaire is systematically presented first table. In all 45 cases, age varied between 19 and 42, with a mean of 28.5 ± 5.98 years, and the antigen test for SARS-CoV-2 was positive. All cases were first or second trimester pregnant women. In most cases, the pregnancy was below 12 weeks of gestation (23 patients, 51.11%). In 7 cases (15.55%) the pregnancy was between 20 and 24 weeks of gestation.

Although respiratory symptoms were mild or moderate, other obstetric manifestations were noted. Uterine contractions, resembled by patients as lower hypogastric colicky pain, were identified in 35 cases (77.77%), whereas hyperemesis was encountered in 11 cases (24.44%). In 5 cases (11.11%) genital or urinary tract infections were also present.

TABLE I.

Clinical and demographic features of the cases

Age (years): < 20 20 - 29 30 - 39 > 40	19 - 42 years, 28.5 ± 5.98 (mean ± SD) 4 (8.88%) 19 (42.22%) 21 (46.66%) 1 (2.22%)
Region	Urban: 30 (66.66%) Rural: 15 (33.33%)
Education	Primary school: 1 (2.22%) Secondary school: 4 (8.88%) High school: 29 (64.44%) University: 11 (24.44%)
Marital status	Yes: 35 (77.77%) No: 10 (22.22%)
Smoking	Yes: 12 (26.66%) No: 33 (73.33%)
Parity *	0: 30 (66.66%) 1 - 3: 14 (31.11%) > 4: 1 (2.22%)
Time from the last delivery: (parity ≠ 0)	< 2 years: 0 > 2 years: 15 (100%)
Breastfeeding (duration if any): (parity ≠ 0)	0 months: 1 (6.66%) < 6 months: 1 (6.66%) 6 - 12 months: 8 (53.33%) > 12 months: 5 (33.33%)
Abortions	0: 26 (57.77%) 1 - 3: 18 (40%) > 4: 1 (2.22%)
Gestational age (weeks)	< 12: 23 (51.11%) 12 - 15: 10 (22.22%) 16 - 19: 5 (11.11%) 20 - 24: 7 (15.55%)
Symptoms	Uterine contractions: 35 (77.77%) Hyperemesis: 11 (24.44%) Uterine/genital infection: 5 (11.11%)
SARS-CoV-2 Ag positive	45 (100%)
COVID-19 vaccination	Complete: 32 (71.11%) Incomplete: 5 (11.11%) No: 8 (17.77%)
GAD-7 score	5.08 ± 4.24 (mean GAD-7 score ± SD) 0 - 4 (minimal anxiety): 23 (51.11%) 5 - 9 (mild anxiety): 15 (33.33%) 10 - 14 (moderate anxiety): 4 (8.88%) > 15 (severe anxiety): 3 (6.66%)

Values are expressed as absolute numbers, percentages, mean ± SD

* Parity is defined as the number of times a woman has given birth to a live neonate, at 24 weeks or more, regardless of whether the child was viable or non-viable.

Analyzing the demographic features of the cases, we found that most of the patients were from urban regions (30 cases, 66.66%), married (35 cases, 77.77%) and with secondary and higher education status (high school - 29 cases, and university degree - 11 cases).

In terms of COVID-19 vaccination, 32 cases (71.11%) had completed the immunization schedule regardless of vaccine manufacturer. Just 8 cases (17.77%) had no COVID-19 vaccination at all.

All cases agreed and completed the GAD-7 questionnaire. Mean GAD-7 score was 5.08 with a standard deviation of 4.24 with values between 0 and 15, presented in histogram (fig. 2). In 23 patients (51.11%), the GAD-7 score was between 0 and 4 which stands for minimal anxiety. In contrast, in 4 patients (8.88%) the GAD-7 score was between 10 and 14 suggestive for moderate anxiety, whereas in 3 cases (6.66%) the GAD-7 score was 15, suggestive for severe anxiety.

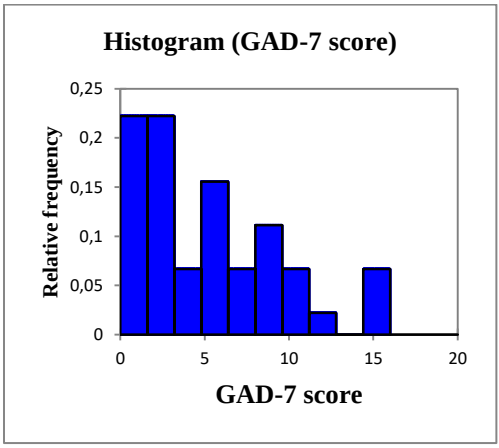

Fig. 2. GAD-7 score histogram for all patients included in the study

Analyzing the GAD-7 score in respect with the age of the patients, the correlation was only moderate (r = 0.415; p < 0,001) (fig. 3).

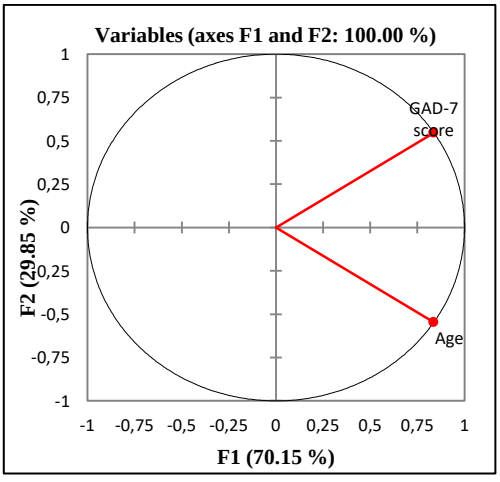

Fig. 3. Correlation between GAD-7 score and age of the patients (r = 0.415; p < 0.001)

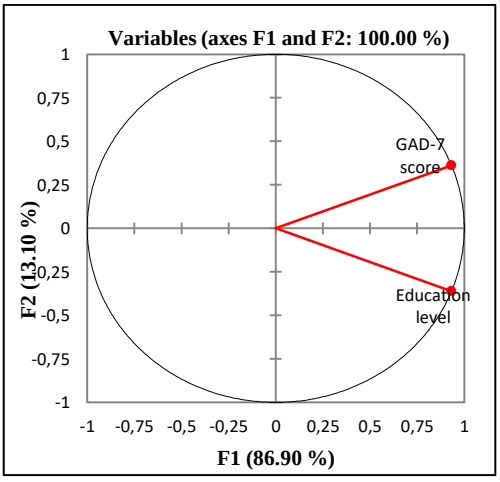

Fig. 4. Correlation between GAD-7 score (≥ 10) and level of education (r = 0.757; p < 0.001)

Pearson correlation between patients with moderate and severe anxiety (GAD-7 score ≥ 10), and the level of education showed a strong association (r = 0.757; p < 0.001) (fig. 4). In contrast, the correlation between GAD-7 score below 10 and the

educational level was not statistically significant.

Furthermore, a strong correlation was found between GAD-7 score and parity (r = 0.788; p < 0.001) (fig. 5), where an association was found between higher scores and lower parity. A strong correlation was also found between GAD-7 score and gestational age (r = 0.922; p < 0.001) (fig. 6), where an association was found between higher GAD-7 score and higher gestational age. Patients with complete immunization for COVID-19 had lower GAD-7 scores. In this respect, a strong correlation between GAD-7 score and vaccination status was noticed (r = 0.680; p < 0.001) (fig. 7).

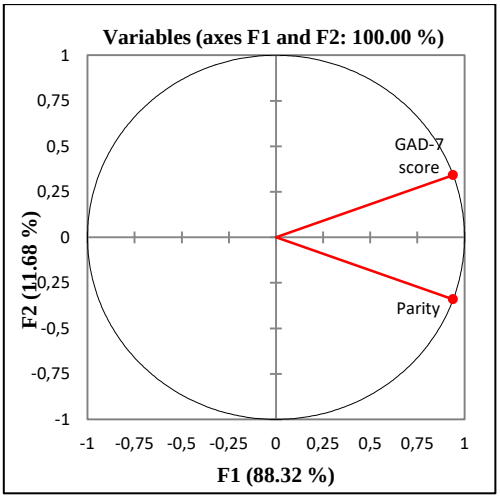

Fig. 5. Correlation between GAD-7 score and parity (r = 0.788; p < 0.001**)**

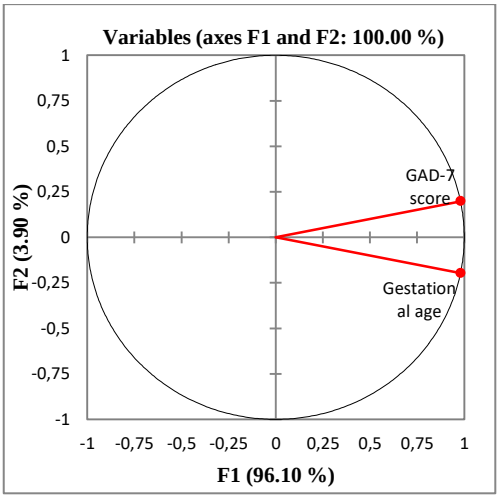

Fig. 6. Correlation between GAD-7 score and gestational age (r = 0.922; p < 0.001)

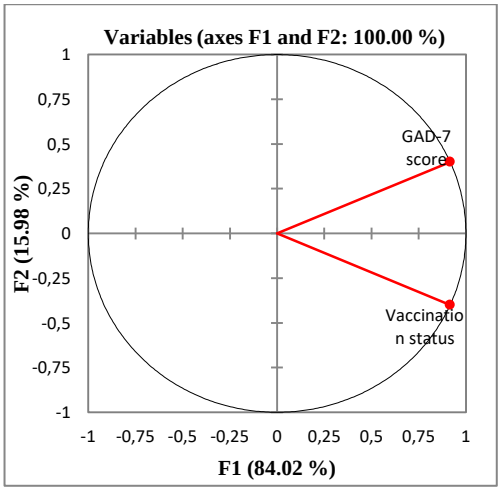

Fig. 7. Correlation between GAD-7 score and COVID-19 vaccination status (r = 0.680; p < 0.001)

DISCUSSION

Pregnancy is a phase of significant and rapid physiological transformation, during which the likelihood and repercussions of mental health disorders are heightened. Pregnant women face an increased risk of developing psychiatric disorders compared to nonpregnant women, which may continue after pregnancy ends (12, 13). Symptoms of anxiety or depression occur in 30%-58% of pregnancies, while phobias, mania, and obsessive-compulsive disorders are recorded at lesser rates (12, 13).

Anxiety is among the most prevalent psychiatric disorders during pregnancy. As many as 25% of women receive anxiety-

related diagnoses during pregnancy, with an additional 20% experiencing anxiety in the postpartum period (14). Anxiety may emerge from maternal apprehensions, such as the timing of labor, previous adverse experiences within the hospital and the health of the infant (13). Anxiety during pregnancy is frequently linked to exhaustion, physiological alterations resulting from pregnancy, and mood fluctuations that may encompass depressive symptoms (15).

Depression is a prevalent psychiatric disorder, affecting 10%-40% of women during pregnancy. Pregnant women experiencing anxiety or depression may have an elevated risk of preterm birth, cesarean delivery, and postpartum depression (PPD) (14, 16, 17). PPD is defined by depressive symptoms accompanied by anxiety, heightened arousal, and mood lability (16, 17). This issue impacts as many as 19% of mothers from 2 weeks to 1.5 years after delivery (16, 17). Moreover, postpartum depression (PPD) frequently coexists with other postpartum mental health disorders, such as anxiety disorders, and is linked to a heightened risk of obsessive-compulsive disorder or harmful thoughts towards one's newborn (13, 18).

Recent studies demonstrate that pregnant women with mental health disorders are at an increased risk of adverse pregnancy outcomes. Mental health disorders in pregnant women may elevate the risk of unfavorable outcomes, including hypertension throughout pregnancy, delivery issues, and an increased risk of alcohol consumption, recreational drug use, and smoking as coping strategies (13, 19). Poor maternal mental health in newborns may lead to consequences including preterm birth, low birth weight, and impaired cognitive and language development (20). Mental health issues in pregnant women influence not only their own health outcomes but also have a transgenerational effect on their infants.

In addition, the degree of maternal illness correlated with an increased chance of cesarean section, premature delivery, and the necessity for neonatal intensive care unit admission.

Ma and colleagues (21) performed a meta-analysis involving 19,884 subjects diagnosed with COVID-19. The aggregated percentage of asymptomatic infections was 54.1% in pregnant women, in contrast to 40.5% in the general population. Vouga and colleagues (22) documented a 9.9% incidence of severe COVID-19 in a group of 926 pregnant women who tested positive for SARS-CoV-2. In these pregnant women, the risk factors for severe COVID-19 were pulmonary comorbidities, hypertension, and diabetes. Pregnant women with symptomatic COVID-19 exhibited a higher likelihood of hospitalization (RR 5.4), immunosuppression with bacterial sepsis (23), admission to intensive care units (RR 1.5), intubation and mechanical ventilation (RR 1.7), and mortality (24). Maternal mortality from all causes was 11.3% among pregnant women, in contrast to 6.4% for non-pregnant hospitalized women (25).

Recent studies have indicated a heightened incidence of anxiety and depression among pregnant women following the commencement of the COVID-19 pandemic relative to pre-pandemic levels (26). Throughout this period, pregnant women often expressed health care concerns, including difficulties in accessing hospital services, inadequate support from partners

and family during childbirth, anxiety about transmitting COVID-19 to their fetuses or newborns, and apprehension that maternal COVID-19 infection could result in developmental defects or delays in their children (26). These concerns were also highlighted by our study revealing that higher anxiety scores were more frequently associated with low parity, higher educational level and higher gestational age. The pressures of the pandemic may have significantly impacted maternal mental health, consequently influencing the overall health of both mother and child.

GAD-7 scale is selected as a screening instrument for prenatal anxiety for several reasons. It is widely recognized that pregnant women typically encounter many somatic complaints, including fluctuations in weight and sleep disturbances. On the other hand, the GAD-7 contains limited measures that evaluate somatic symptoms, so mitigating the potential misinterpretation that these symptoms may stem from typical pregnancy experiences rather than psychological disorders (27).

The creation of COVID-19 vaccinations was deemed crucial for decreasing COVID-19-related morbidity and mortality in the general population, including pregnant women (28). The Romanian authorities advised vaccination during any trimester of pregnancy and during the breastfeeding phase as well. However, the recommendation of the COVID-19 vaccine among pregnant women was an additional source of worry and generally hardly accepted due to insufficient data on safety and efficacy, coupled with the proliferation of misinformation on social media regarding the vaccine's teratogenicity (29). Surpassing all the questions and misinformation about COVID-19 vaccine, as in our study and others from the literature, immunization against SARS-CoV-2 offered a better confidence and a lower level of anxiety in pregnant women compared with women who received no vaccination at all (30, 31).

CONCLUSIONS

The global COVID-19 pandemics was a milestone in humankind medical care. Pregnant women faced a lot of challenges and uncertainties. Social distancing, reductions in prenatal visits, lack of access in many medical facilities along with insufficient knowledge about the infection itself, the consequences on both mother and fetus, the overall prognosis, the short- and long-term effects of vaccines have created the foundation of worries, anxiety and depression. Although diagnosed more often during third trimester and postpartum period, SARS-CoV-2 infection in pregnant women led to a rise in anxiety and depression symptoms even in early gestation. Besides risks and pregnancy complications associated with COVID-19, mental health issues were also important.

The experience of COVID-19 pandemic should now serve for medical professionals and society in general as a model for preplanning the management of a future infection crisis.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no funding regarding this scientific research.

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